This electronic thesis or dissertation has been downloaded from the King's Research Portal at https://kclpure.kcl.ac.uk/portal/

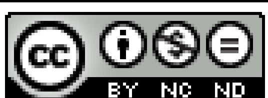
Effects of aronia berry (poly)phenols on cardiovascular health and gut microbiome

Le Sayec, Melanie

Awarding institution:
King's College London

The copyright of this thesis rests with the author and no quotation from it or information derived from it may be published without proper acknowledgement.

END USER LICENCE AGREEMENT



Unless another licence is stated on the immediately following page this work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International licence. https://creativecommons.org/licenses/by-nc-nd/4.0/

You are free to copy, distribute and transmit the work

Under the following conditions:

- Attribution: You must attribute the work in the manner specified by the author (but not in any way that suggests that they endorse you or your use of the work).
- Non Commercial: You may not use this work for commercial purposes.
- No Derivative Works - You may not alter, transform, or build upon this work.

Any of these conditions can be waived if you receive permission from the author. Your fair dealings and other rights are in no way affected by the above.

Take down policy

If you believe that this document breaches copyright please contact librarypure@kcl.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.

Download date: 25. Dec. 2024

KING'S COLLEGE LONDON

Department of Nutritional Sciences

Effects of aronia berry (poly)phenols on cardiovascular health and gut microbiome

A thesis submitted to King's College London for the degree of Doctor of Philosophy in the School of Life Course Sciences

Supervisors:
Dr Ana Rodriguez-Mateos
Prof Kennedy Cruickshank

Candidate:
Melanie Le Sayec

October 2021

To my family and friends.

PUBLICATIONS AND PRESENTATIONS

Original Research

• **Le Sayec M.**, Xu Y., Laiola M., Alvarez Gallego F., Katsikioti D., Durbidge C., Kivisild U., Armes S., Lecomte M., Fanca-Berthon P., Fromentin E., Plaza Oñate F., Cruickshank K., Rodriguez-Mateos A. *Aronia berry (poly)phenols improve arterial stiffness and modulate gut microbiome richness and composition in middle aged men and women: a randomized controlled trial.* (Submitted to AJCN)

• Serreli G.*, **Le Sayec M.***, Diotallevi C., Teissier A., Deiana M., Corona G. (2021) *Conjugated Metabolites of Hydroxytyrosol and Tyrosol Contribute to the Maintenance of Nitric Oxide Balance in Human Aortic Endothelial Cells at Physiologically Relevant Concentrations*. *These authors contributed equally to the work. Molecules 26(24), 7480.

• Rodriguez-Mateos A., Istas G., **Le Sayec M.**, Johnson S. A. (2021) Book chapter in *"Berries and Berry Bioactive Compounds in Promoting Health".* Book published by the Royal Society of Chemistry (Submitted)

• Serreli G.*, **Le Sayec M.***, Thou E., Lacour C., Diotallevi C., Arshad Dhunna M., Deiana M., Spencer J.P.E., Corona G. (2021) *Ferulic acid derivatives and avenanthramides modulate endothelial function through maintenance of Nitric Oxide balance in HUVEC cells*. *These authors contributed equally to the work. Nutrients. PMCID: PMC8231282.

• Xu Y., **Le Sayec M.**, Roberts C., Hein S., Rodriguez-Mateos A., Gibson R. (2021) *Dietary Assessment Methods to Estimate (Poly)phenol Intake in Epidemiological Studies: A Systematic Review*. Adv Nutr. PMID: 33684195.

• **Le Sayec M.**, Serreli G., Diotallevi C., Teissier A., Deiana M., Corona G. (2021) *Olive oil phenols and their metabolites modulate nitric oxide balance in Human Aortic Endothelial Cells*. Proceedings of the Nutrition Society 80.

• Mompeo O., Spector TD., Matey Hernandez M., Le Roy C., Istas G., **Le Sayec M.**, Mangino M., Jennings A., Rodriguez-Mateos A., Valdes AM., Menni C. (2020) *Consumption of Stilbenes and Flavonoids is Linked to Reduced Risk of Obesity Independently of Fiber Intake*. Nutrients. PMID: 32585900

• Istas G., Wood E., **Le Sayec M.**, Rawlings C., Yoon J., Dandavate V., Cera D., Rampelli S., Costabile A., Fromentin E., Rodriguez-Mateos A. (2019) *Effects of aronia berry (poly)phenols on vascular function and gut microbiota: a double-blind randomized controlled trial in adult men*. Am J Clin Nutr. PMID: 31152545.

Presentations

• **Le Sayec M.** (2021) Aronia: a “berry” good ally for your health*. Three-minute thesis competition (March 2021, online).* Oral presentation.

• **Le Sayec M.**, Lecomte M., Fança-Berthon P., Fromentin E., Cruickshank K., Rodriguez-Mateos A. (2020) Effects of aronia berry (poly)phenols on arterial function in prehypertensive healthy individuals*. School of Life Course Sciences Annual PGR Awards and Symposium (October 2020, online).* Poster presentation. Awarded by the “Runner Up Year 3 Poster” Prize.

• **Le Sayec M.**, Istas G., Rampelli S., Fromentin E., Rodriguez-Mateos A. (2019) Interindividual variability in vascular response to aronia berry (poly)phenols*. 9th International Conference on Polyphenols and Health (November 2019, Kobe, Japan).* Poster presentation.

• **Le Sayec M.**, Istas G., Wood E., Fromentin E., Rodriguez-Mateos A. (2019) Bioavailability of aronia berry (poly)phenols*. Frontiers in Mass Spectrometry @King's (April 2019, King’s College London, UK).* Poster presentation.

• **Le Sayec M.**, Istas G., Wood E., Rawlings C., Yoon J., Dandavate V., Fromentin E., Rodriguez-Mateos A. Fromentin E., Rodriguez-Mateos A. (2018) Bioavailability of aronia berry (poly)phenols after acute and regular consumption*. Bioavailability 2018 Conference (September 2018, Quadram Institute, Norwich, UK).* Oral presentation.

ABSTRACT

Background: *Aronia melanocarpa* is a rich source of (poly)phenolic compounds. Evidence from preclinical studies and few small-scale human intervention trials suggest that aronia berry (poly)phenols may have cardiovascular health benefits. This work aims to investigate the effect of aronia berry (poly)phenols on vascular function, bioavailability, interindividual variability in response, and gut microbiome in healthy middle-aged prehypertensive individuals.

Methods: A total of 102 prehypertensive but otherwise healthy men and women aged 40 to 70 were included in a 12-week double-blind, placebo-controlled, parallel designed study. Volunteers were randomised and allocated to consume a (poly)phenol-rich aronia berry extract (105.9 mg, equivalent to 75g of fresh berries) or a matched placebo daily for 12 weeks. Twenty-four hour and office blood pressure (BP, primary outcome), arterial stiffness (measured as aortic pulse wave velocity (PWV) and augmentation index (AIx)), flow-mediated dilation, heart rate as well as blood lipids and cortisol levels (secondary outcomes) were assessed at baseline (day 1) and after 12 weeks. Plasma and urine (poly)phenol metabolites were analysed using LC-MS. Gut microbiota composition was assessed from faecal samples via shotgun metagenomic sequencing. In addition, interindividual variability in response to aronia consumption was investigated through an exploratory analysis.

Findings: Twelve-week daily intake of the aronia extract was not associated with significant change in 24h BP, although office systolic BP decreased

significantly compared to baseline (-2.38 ± 0.92 mmHg). A significant improvement in 24h peripheral AIx (Δ= -6.8%, 95% CI [-11.2%; -2.3%], p= 0.003) and central AIx (Δ= -3.3% [-5.5; -1.0], p= 0.006) was found in the Aronia group compared with Control. A significant reduction in awake PWV was also observed in the aronia extract group compared to Control (CFC= -0.24 m/s, 95% CI [-0.79; -0.01], p< 0.05). No changes in other secondary outcomes were found. Significant increases in individual phenolic metabolites following the intervention were observed in both urine and plasma samples, some of which were significantly correlated with the improvement in arterial function in the Aronia group. Bioinformatic analysis revealed a significant improvement in microbiome gene richness, and an increase in 18 beneficial gut bacteria after consumption of the aronia extract. A high variability in the vascular response to the Aronia treatment was observed, and several factors (sex, age, BMI, physical activity, and alcohol consumption) independently influenced the overall response, with gut microbiome having an important effect in the variability in responsiveness.

Conclusion: Aronia berry (poly)phenols were bioavailable, improved some components of arterial function and gut microbiome abundance and composition in a population of healthy middle-aged individuals. Phenolic metabolites may exert their beneficial action through a bidirectional interaction with the gut microbiome, indicating that aronia berry (poly)phenol consumption may maintain cardiovascular health in individuals at low risk of cardiovascular disease.

TABLE OF CONTENT

TABLE OF FIGURES ... 12

TABLE OF TABLES ... 16

TABLE OF APPENDICES ... 21

TABLE OF SUPPLEMENTARY MATERIAL ... 22

ACKNOWLEDGMENTS ... 23

STATEMENT OF CONTRIBUTION ... 25

LIST OF ABBREVIATIONS ... 27

CHAPTER 1 – INTRODUCTION ... 32

1.1 Cardiovascular diseases (CVD) ... 33

1.1.1 Prevalence and global impact ... 33

1.1.2 Atherosclerosis: definitions and causes ... 33

1.1.3 CVD risk factors ... 52

1.1.4 Plant foods and CVD ... 54

1.2 (Poly)phenols ... 58

1.2.1 Definition ... 58

1.2.2 Absorption, distribution, metabolism, and excretion (ADME) of dietary (poly)phenols ... 62

1.2.3 Effects of PP on CVD: evidence from the literature ... 69

1.2.4 Cardiovascular mechanisms of action of PP ... 74

1.2.5 Beneficial effects of PP on gut microbiota ... 81

1.2.6 The growing interest for berry PP ... 86

1.3 Effects of aronia berry PP on cardiovascular health ... 87

1.3.1 Introduction to aronia berry ... 87

1.3.2 Effects of aronia PP on cardiovascular health ... 92

1.3.3 Effects of aronia PP on gut microbiome function and composition ... 97

1.3.4 Potential mechanisms of action of aronia PP 98
1.4 Gaps in the literature 101
1.5 Hypothesis and aims 104
CHAPTER 2 – METHODS 105
2.1 The Aronia Blood Pressure (ABP) study 106
2.1.1 Study population 106
2.1.2 Study design 107
2.2 Aronia berry extract and control capsules 110
2.3 Vascular measurements 111
2.3.1 Blood pressure (BP) and heart rate (HR) 111
2.3.2 Arterial stiffness 113
2.3.3 Endothelial function 116
2.4 Body composition measurements 118
2.5 QRISK®3 score 119
2.6 Sample collection and biochemical analysis 119
2.6.1 Processing and storage of blood samples 119
2.6.2 Processing and storage of urinary samples 121
2.6.3 Processing, storage, and analysis of faecal samples 122
2.7 Diet, health, and lifestyle questionnaires 124
2.7.1 Food frequency questionnaire 124
2.7.2 Seven-day food diaries 125
2.7.3 Assessment of dietary PP intake 126
2.7.4 Physical activity questionnaire 127
2.8 Quantification of PP metabolites in plasma and urine 127
2.8.1 Analytical standards and other materials 128
2.8.2 Sample preparation and micro-solid phase extraction 128

2.8.3 Ultrahigh performance liquid chromatography triple-quadrupole mass spectrometry 129

2.9 Power calculation and statistical analysis 130

CHAPTER 3 – EFFECTS OF ARONIA BERRY (POLY)PHENOLS ON CARDIOMETABOLIC HEALTH 133

3.1 Introduction 134

3.2 Methods 138

3.3 Results 140

3.3.1 Baseline characteristics of the study population 140

3.3.2 Background diet 144

3.3.3 Effect of aronia berry extract consumption on office and 24-hour ambulatory blood pressure 148

3.3.4 Effect of aronia berry extract consumption on office and 24-hour ambulatory arterial stiffness 152

3.3.5 Effect of aronia berry extract consumption on endothelial function and blood flow velocity 153

3.3.6 Effect of aronia berry extract consumption on office and 24-hour ambulatory heart rate 154

3.3.7 Effect of aronia berry extract consumption on QRISK®3 score 156

3.3.8 Effect of aronia berry extract consumption on blood lipids and cortisol levels 155

3.3.9 Tolerance and safety of the intervention 156

3.4 Discussion 159

CHAPTER 4 – BIOAVAILABILITY AND METABOLISM OF ARONIA BERRY (POLY)PHENOLS 174

4.1 Introduction 175

4.1.1 ADME of (poly)phenols 175

4.1.2 Bioavailability and metabolism of aronia berry PP 180

4.1.3 Previous studies investigating aronia PP bioavailability 182
4.2 Methods 185
4.3 Results 186
4.3.1 Baseline urinary and plasma phenolic metabolites................. 187
4.3.2 Urinary and plasma PP after aronia consumption 190
4.3.3 Change in urinary and plasma PP after consumption of aronia in comparison with Control group.. 194
4.3.4 Correlations between PP metabolites and clinical outcomes . 207
4.4 Discussion... 210
CHAPTER 5 – ASSESSMENT OF INTERINDIVIDUAL VARIABILITY FOLLOWING ARONIA BERRY (POLY)PHENOL CONSUMPTION 219
5.1 Introduction 220
5.2 Methods 226
5.3 Results 229
5.3.1 Analysis of the variability in response to aronia berry PP 229
5.3.2 Exploratory subgroup analysis based in biological and lifestyle parameters 237
5.4 Discussion... 259
CHAPTER 6 – IMPACT OF ARONIA BERRY CONSUMPTION ON GUT MICROBIOTA 275
6.1 Introduction 275
6.1.1 Interactions between gut microbiota and PP 273
6.1.2 Factors influencing gut microbial composition 281
6.1.3 Short-chain fatty acids and their biological role 283
6.1.4 Characterisation of the gut microbiome 284
6.1.5 Aronia berry PP and gut microbiota....................................... 286
6.1.6 Studies evaluating the effect of other berries on microbiota ... 288
6.2 Methods 292

6.3 Results ... 293

6.3.1 Quality check of homogeneity at different timepoints ... 293

6.3.2 Overall similarity and hierarchical clustering ... 293

6.3.3 Species abundance and gene richness ... 295

6.3.4 Alpha diversity ... 298

6.3.5 Beta diversity ... 298

6.3.6 Baseline gut microbiome abundance and composition ... 299

6.3.7 Effects of Aronia supplementation in gut microbiota abundance and composition ... 300

6.3.8 Function pathways related to gut microbiome composition ... 304

6.3.9 Multi-omics analysis to investigate associations between gut microbiome, cardiometabolic outcomes and circulating PP ... 305

6.3.10 Role of gut microbiota on the interindividual variability in vascular response to aronia berry PP composition ... 313

6.4 Discussion ... 327

6.4.1 Effect of aronia berry PP on gut microbiota abundance and composition ... 327

6.4.2 Role of gut microbiome in explaining variation in response to aronia consumption ... 330

6.4.3 Associations between gut microbiome, vascular outcomes, and PP metabolites ... 333

6.4.4 Strengths and weaknesses ... 334

CHAPTER 7 – DISCUSSION ... 336

7.1 Summary of main findings ... 337

7.2 Strengths of this work ... 342

7.3 Limitations ... 344

7.4 Conclusions ... 346

7.5 Future work ... 348

TABLE OF FIGURES

Figure 1.1 Structure of the arterial wall 34

Figure 1.2 Mechanisms behind physiological arterial function 39

Figure 1.3 Main flavonoids structures and food sources examples 59

Figure 1.4 Main non-flavonoids classification and structures 61

Figure 1.5 Mechanisms of ADME of (poly)phenols 67

Figure 1.6 Akt/eNOS signalling pathway 78

Figure 1.7 Summary of the signalling pathways occurring in the phenomenon of vasodilation 79

Figure 1.8 Chemical structures of major phenolic phytochemicals present in aronia berries 91

Figure 2.1 Arteriograph24™ and its cuff 112

Figure 2.2 Aortic PWV calculated by SphygmoCor® device 114

Figure 2.3 Augmentation index and pulse pressure (From SphygmoCor® user manual) 115

Figure 2.4 Flow-mediated dilation measurement and ultrasound image of the brachial artery 118

Figure 3.1 Study design of the ABP study 139

Figure 3.2 Study flow diagram 141

Figure 3.3 Twenty-four-hour, awake, and asleep peripheral SBP and DBP changes from baseline following a 12-week consumption of aronia berry extract, for both Aronia and Control groups, following BAB analysis 149

Figure 4.1 Mechanisms of ADME of (poly)phenols 177

Figure 4.2 Baseline urinary excretion (%) of PP classes analysed in both Aronia and Control groups 187

Figure 4.3 Baseline plasma concentration (%) of (poly)phenol classes analysed in both Aronia and Control groups 189

Figure 4.4 Urinary concentration (%) of (poly)phenol classes analysed in both Aronia and Control groups, following the 12-week supplementation.. 191

Figure 4.5 Pie charts representing the proportion of plasma (poly)phenol families for both Aronia and Control group and both visit 1 and 2............. 192

Figure 4.6 Urinary (poly)phenol metabolites after consumption of aronia berries for Aronia and Control groups 194

Figure 4.7 Urinary (poly)phenol metabolites after consumption of aronia extract or placebo, for Aronia and Control groups.................................... 194

Figure 4.8 Urinary PP concentrations at baseline and following a 12-week daily consumption of aronia extract, in the Aronia group 197

Figure 4.9 Plasma (poly)phenol metabolites after consumption of aronia (poly)phenols or placebo, for Aronia and Control groups 198

Figure 4.10 Families of plasma PP metabolites after consumption of aronia extract or placebo, for Aronia and Control groups.................................... 199

Figure 4.11 Individual plasma metabolites after consumption of aronia extract in the Aronia group... 204

Figure 4.12 Proposed metabolic pathways for 5-CQA and 5-FQA degradation by colonic microbiota 214

Figure 4.13 Proposed catabolic pathways of aronia (poly)phenols 215

Figure 5.1 Parameters influencing interindividual variability in cardiometabolic response to (poly)phenol consumption 221

Figure 5.2 Waterfall plots of changes from baseline for chronic changes on ambulatory and office brachial blood pressure 230

Figure 5.3 Waterfall plots of changes from baseline for chronic changes in ambulatory and office pulse wave velocity... 231

Figure 5.4 Waterfall plots of changes from baseline for chronic changes in AIx 24h brachial and aortic AIx 232

Figure 5.5 Waterfall plots of changes from baseline for acute and chronic changes in FMD.. 233

Figure 5.6 Waterfall plots of changes from baseline for chronic changes in office and ambulatory heart rate 234

Figure 5.7 Waterfall plots of changes from baseline for chronic changes in cortisol levels 235

Figure 5.8 Waterfall plots of changes from baseline for chronic changes in blood lipids 237

Figure 6.1 Potential ring cleavage sites for berry flavonoids and chlorogenic acid by gut microbiota 278

Figure 6.2 Boxplot of read counts between Aronia and Control groups at baseline (V1) and at the end of trial (V2) 293

Figure 6.3 Spearman correlation between all Aronia samples at species level 294

Figure 6.4 Spearman correlation between all Control samples at species level 295

Figure 6.5 Boxplot showing differences in species abundance and gene richness 296

Figure 6.6 Boxplot showing changes from baseline in gene count after 12-week intervention 297

Figure 6.7 PCoA plots of gut microbiome 299

Figure 6.8 Bar plot of the species significantly different between Aronia and Control groups at baseline 300

Figure 6.9 Bar plot of the species significantly different between Aronia and Control groups after considering the changes from baseline 302

Figure 6.10 Boxplot showing differently abundant species in the Aronia group 304

Figure 6.11 Heatmap showing hierarchical Ward-linkage clustering of correlations between clinical metadata and gut microbiome 311

Figure 6.12 Principal component analysis from k-means analysis based on relevant clinical parameters highlighted by the partner after k-means algorithm 315

Figure 6.13 Boxplot showing statistical differences between Aronia R, NR, and Control groups for changes in 24h and awake SBP_{br} and 24h and awake DBP_{br} following 12-week consumption 318

Figure 6.14 Boxplot showing statistical differences between Aronia R, NR, and Control groups for changes in outcomes presenting significant differences between categories of response following 12-week consumption 319

Figure 6.15 Boxplot showing statistical differences between Aronia R, NR, and Control groups for Δ gene count 321

Figure 6.16 Cuneiform plot showing bacterial taxa significantly enriched in R, NR, or Control at baseline.. 322

Figure 6.17 Spearman's correlation plot between baseline levels of *B. adolescentis* and chronic changes in 24h DBP.. 323

Figure 6.18 Species significantly different between R and NR after 12 weeks of intervention 325

TABLE OF TABLES

Table 1.1 Concentrations of the main PP present in aronia berry 89

Table 1.2 Previous studies investigating the impact of aronia berry on blood pressure 95

Table 2.1 (Poly)phenol content of intervention capsule 111

Table 3.1 Baseline characteristics of the study population 142

Table 3.2 Average macro-, micronutrient and (poly)phenol intakes taken from 7-day diet diaries at baseline and during the 12-week intervention ... 145

Table 3.3 Comparison of Eatwell guide dietary recommendations with the baseline intake of micro- and macronutrients of the ABP study population 147

Table 3.4 Effects of aronia berry extract after 12-week consumption on 24-hour, awake and asleep peripheral SBP and DBP, for both ITT and PerP populations and each intervention group, following BAB analysis 149

Table 3.5 Effects of aronia berry extract after 12-week consumption on 24-hour, awake and asleep central SBP in the ITT population and each intervention group, following BAB analysis 150

Table 3.6 Effects of aronia berry extract after 12-week consumption on office peripheral and central SBP and DBP in the ITT population and each intervention group, following BAB analysis 151

Table 3.7 Effects of aronia berry extract after 2-hour and 12-week consumption on office PWV and AIx in the ITT population and each intervention group, following BAB analysis 152

Table 3.8 Effects of aronia berry extract after 12-week consumption on 24-hour, awake and asleep central PWV and peripheral and central AIx in the ITT population and each intervention group, following BAB analysis 153

Table 3.9 Effects of aronia berry extract after 2-hour and 12-week consumption on FMD and blood flow velocity in the ITT population and each intervention group, following BAB analysis 154

Table 3.10 Effects of aronia berry extract after 2-hour and 12-week consumption on office heart rate (seated and supine) in the ITT population and each intervention group, following BAB analysis 155

Table 3.11 Effects of aronia berry extract after 12-week consumption on 24-hour, awake and asleep heart rate in the ITT population and each intervention group, following BAB analysis 155

Table 3.12 Effects of aronia berry extract after 12-week consumption on QRISK®3 score in the ITT population and each intervention group, following BAB analysis 156

Table 3.13 Effects of aronia berry extract after 12-week consumption on blood lipids and cortisol levels in the ITT population and each intervention group, following BAB analysis 157

Table 3.14 Safety parameters assessed during the ABP study at baseline and after 12-week daily consumption of the aronia extract, for both Aronia and Control groups 158

Table 4.1 Urinary PP levels at baseline and following a 12-week daily consumption of aronia extract, and changes with respect to Control 196

Table 4.2 Acute, chronic, and acute on chronic plasma changes (μM) of total PP for both Aronia and Control group 198

Table 4.3 Significant changes from Control (CFC) acutely on the first and the last day, following the intake of aronia berry PP extract or placebo 202

Table 4.4 Urinary (poly)phenols correlation with significant outcomes in Aronia group 207

Table 4.5 Plasma (poly)phenols correlation with significant outcomes in Aronia group 209

Table 5.1 Subgroup analysis conducted in the ABP study (n= 97) for sex, age, BMI, habitual total PP and fruits and vegetables consumption, blood pressure at baseline, habitual alcohol consumption and habitual physical activity level 228

Table 5.2 Parameters analysed in the response analysis and coefficient of variation associated 236

Table 5.3 Results from the subgroup analysis based on the sex of participants ... 238

Table 5.4 Significant changes from baseline (CFB) in the different sex subgroups of the Aronia group ... 239

Table 5.5 Results from the subgroup analysis based on the age of participants with a limit of 56.2 years ... 240

Table 5.6 Significant changes from baseline (CFB) in the different age subgroups of the Aronia group ... 241

Table 5.7 Results from the subgroup analysis based on the BMI of participants ... 242

Table 5.8 Significant changes from baseline (CFB) in the different BMI subgroups of the Aronia group ... 243

Table 5.9 Distribution of the participants for each tertile of the total daily PP intake ... 244

Table 5.10 Subgroup analysis of the main outcomes based on the distribution by tertile of the total PP intake ... 244

Table 5.11 Results from the subgroup analysis based on the baseline (poly)phenol (PP) intake of volunteers, with a cut-off limit of 700 mg/day, calculated based only on the data collected 7DD ... 245

Table 5.12 Significant changes from baseline (CFB) in the different baseline PP intake subgroups of the Aronia group ... 246

Table 5.13 Results from the subgroup analysis based on the baseline intake of fruits and vegetables, with a cut-off limit of 400 g of fruits and vegetables (FV) per day ... 247

Table 5.14 Significant changes from baseline (CFB) in the different baseline fruits and vegetables intake subgroups of the Aronia group ... 248

Table 5.15 Results from the subgroup analysis based on the baseline SBP of volunteers, with a cut-off limit of 120 mmHg ... 249

Table 5.16 Significant changes from baseline (CFB) in the different baseline SBP subgroups of the Aronia group ... 251

Table 5.17 Results from the subgroup analysis based on the baseline office DBP of volunteers, with a cut-off limit of 80 mmHg 252

Table 5.18 Significant changes from baseline (CFB) in the different baseline DBP subgroups of the Aronia group 253

Table 5.19 Results from the subgroup analysis based on weekly alcohol consumption of participants 254

Table 5.20 Significant changes from baseline (CFB) in the different alcohol consumption subgroups of the Aronia group 255

Table 5.21 Results from the subgroup analysis based on the physical activity level of participants 257

Table 5.22 Significant changes from baseline (CFB) in the different physical activity level subgroups of the Aronia group 258

Table 5.23 Alcohol intake of the ABP population 268

Table 5.24 Summary of the changes from Control observed in subjects classified as "very healthy" and “less healthy” for the according parameters used in the subgroup analysis 271

Table 6.1 Summary of animal intervention investigating the impact of berry PP on gut microbiome... 289

Table 6.2 Summary of human intervention investigating the impact of berry PP on gut microbiome... 291

Table 6.3 Summary of the relative treatment effect upon gene count 297

Table 6.4 Characteristics of Shannon-Wiener and Simpson indices at baseline and after 12-week daily supplementation, in Aronia and Control groups 298

Table 6.5 Changes from baseline in Aronia group and changes from Control of Shannon-Wiener and Simpson indices following the 12-week intervention 298

Table 6.6 Species significantly different between Aronia and Control groups at baseline 301

Table 6.7 Species significantly different between Aronia and Control groups after considering the changes from baseline 303

Table 6.8 Functional pathways related to Aronia and Control groups when considering changes from baseline.. 305

Table 6.9 Summary of positive and significant correlations between Aronia PP-related plasma metabolites and gut microbiome................................. 306

Table 6.10 Summary of negative and significant correlations between Aronia PP-related plasma metabolites and gut microbiome................................. 308

Table 6.11 Summary of positive and significant correlations between Aronia PP-related urinary metabolites and gut microbiome 308

Table 6.12 Summary of negative and significant correlations between Aronia PP-related urinary metabolites and gut microbiome 310

Table 6.13 Summary of negative and significant correlations between clinical outcomes and significantly different species in gut microbiome 312

Table 6.14 Summary of positive and significant correlations between clinical outcomes and significantly different species in gut microbiome 313

Table 6.15 Summary of the parameters included in the cluster analysis, with detail of their cut-off limit 314

Table 6.16 Background baseline parameters for R and NR. 316

Table 6.17 Statistical differences between Aronia R, NR, and Control groups for chronic changes in primary and secondary outcomes 320

Table 6.18 Species significantly different between R and NR after 12 weeks of intervention. 326

TABLE OF APPENDICES

Appendix A: Poster displayed in Clapham Junction station 351

Appendix B: Advertisement published in Evening Standard and Metro newspapers 351

Appendix C: Participant information sheet of the ABP study 352

Appendix D: Case report form of the ABP study 357

Appendix E: Consent form of the ABP study 392

Appendix F: Example of food-frequency questionnaire section 394

Appendix G: Example of 7-day food diary section (EPIC-Study) 395

Appendix H: International Physical Activity Questionnaire (IPAQ) 396

TABLE OF SUPPLEMENTARY MATERIAL

Supplementary Figure S1: Heatmap showing hierarchical Ward-linkage clustering of correlations between targeted Aronia-related compounds and significantly different features in gut microbiome and urinary and plasma metabolite 403

Supplementary Table S1: Multiple reaction monitoring (MRM) parameters of validated compounds and internal standards optimized by LC-ESI-MS/MS 406

Supplementary Table S2: Number of volunteers included in the BAB and BACO analysis for each parameter and each timepoint, in the ITT population 409

Supplementary Table S3: Contribution of food groups and some specific foods to the intake of total (poly)phenols and (poly)phenol classes in the ABP study 410

Supplementary Table S4: Effects of aronia berry extract after 12-week consumption on 24-hour, awake and asleep central PWV and peripheral and central AIx in the ITT population and each intervention group, following BACO analysis 411

Supplementary Table S5: Post-hoc analysis of the power calculation of the main vascular parameters of the ABP study 412

Supplementary Table S6: Urinary (poly)phenol metabolites at baseline and following acute, chronic, and acute on chronic consumption of aronia berries for Aronia and Control groups, and associated changes from Control. 413

Supplementary Table S7: Plasma (poly)phenol metabolites at baseline and following acute, chronic, and acute on chronic consumption of aronia berries, showing data for Aronia and Control groups 415

Supplementary Table S8: Plasma (poly)phenol metabolites' changes from Control following acute, chronic, and acute on chronic consumption of aronia berries 417

ACKNOWLEDGEMENTS

First and foremost, I would like to thank my primary supervisor and mentor, Dr Ana Rodriguez-Mateos, for her constant guidance, patience, support, and enthusiasm through the years. Ana, you are a superhero who manages to fit two days in one, and you truly are an example for all scientists. I feel privileged to have worked with you during this PhD.

I would also like to thank my second supervisor, Prof Kennedy Cruickshank, for his guidance, encouragements, and expertise. It was an honour to have such an estimable expert to follow me and my research through these years.

Thank you to all BSc and MSc students who helped me through the trial: Thamarat Ahmed, Victoria Lau, Abinayah Jayanthan, Ashwa Saeed, Wenxue Pan, Sarah Armes, Fabiola Alvarez Gallego, Dafni Katsikioti, Chandler Durbidge, and Uku Kivisild. This trial would have not been the same without you and your unvaluable help.

I also would like to thank all my office colleagues and members of my team: Yifan, Ellie, Wafa, Paul, Alex, Sabine, Caroline, Zicheng, and Yong. We shared way more than an office, between small victories and, more often, challenging moments, but mostly we share our passion for research. Thank you for your support guys!

Thanks also to Dr Geoffrey Istas, who supported us at the beginning of the thesis and taught us unvaluable knowledge related to the various aspects of a clinical trial.

I would like to thank all the technician and staff from the Nutritional Sciences department, and in particular Rosie Calokatsia and Rose-Marie Minaisah, our irreplaceable “Rosies” who assisted and guided me through the PhD, and Tim Wingham, for his availability and patience during study days.

Thanks go to Dr Émilie Fromentin, Dr Manon Lecomte and Dr Pascale Fança-Berthon, whose help and support during the PhD was greatly appreciated.

Thank you to my close friends, in particular Manon, Justine, Élodie, Maëlle, Cédric, Geoffrey, Ludovic, Vincent and Yann. You are my everlasting support but mostly the best friends someone can dream of.

Above all, I am forever grateful to my family and my parents, who always believed in me and helped me go through difficult moments. Your unconditional support and love kept me going through the challenges of these recent years. I could not have done it without you, I love you more than anything.

STATEMENT OF CONTRIBUTION

The author, Dr Ana Rodriguez-Mateos and Dr Emilie Fromentin designed the study.

The author developed all study materials, including study protocol, case report form, ethics application, study registration in ClinicalTrials.gov and preparation of all the volunteer-related documents and material.

The author coordinated the recruitment, screening visits, follow-up, and the day-to-day running of the study, along with the help of BSc and MSc students Thamarat Ahmed, Victoria Lau, Abinayah Jayanthan, Ashwa Saeed, Wenxue Pan, Sarah Armes, Fabiola Alvarez Gallego, Dafni Katsikioti, Chandler Durbidge and Uku Kivisild. These students also occasionally collected and analysed anthropometric data and blood pressure. Their role also sometimes included the processing and storage of 24-hour urine and faecal samples.

The author was solely responsible for FMD analysis and blood sample collection. The author, along with Uku Kivisild and Fabiola Alvarez Gallego, performed the analysis of arterial stiffness data.

Fellow PhD student Yifan Xu supervised the data entry and analysis of (poly)phenol and nutrient content retrieved from 7-day food diaries and food frequency questionnaires.

The author, along with Yifan Xu, conducted sample preparation for plasma and urine (poly)phenol analysis in ultra-high-performance liquid chromatography triple-quadrupole mass spectrometry and performed all data quantification analysis.

Microbiome analysis of faecal samples via shotgun sequencing was performed by CosmosID (Rockville, MD, USA). Bioinformatic analysis and modelling of the gut microbiome data was conducted by INRAe-Metagenopolis (Paris, France).

Statistical analysis and data interpretation were done by the author and Dr Ana Rodriguez-Mateos.

Naturex part of Givaudan provided the test capsules used in this work, along with detailed nutritional and (poly)phenol content.

This work was funded by an unrestricted research grant by Naturex, part of Givaudan.

LIST OF ABBREVIATIONS

7DD	7-day food diary
95% CI	95% confidence interval
ABP study	Aronia Blood Pressure study
ACN	Anthocyanins
ADME	Absorption, distribution, metabolism, excretion
AIx	Augmentation index
AIx_{ao}	Aortic/central augmentation index
AIx_{br}	Brachial/peripheral augmentation index
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
ao	Aortic
AST	Aspartate aminotransferase
BAB	Baseline, age, and BMI as covariate
BACO	Baseline as covariate only
βG	β-glucosidase
BMI	Body mass index
BMR	Basal metabolic rate
BP	Blood pressure
BPM	Beats per minute
BPV	Blood pressure variability
br	Brachial
CFB	Change from baseline
CFC	Changes from Control

Cf-PWV	Carotid-femoral pulse wave velocity
cGMP	Cyclic guanosine 3′,5′-monophosphate
COMT	Catechol-O-methyltransferase
COX	Cyclooxygenases
CoV	Coefficient of variation
CPT	Cell preparation tubes
CRP	C-reactive protein
CVD	Cardiovascular disease
DASH	Dietary approaches to stop hypertension
DBP	Diastolic blood pressure
DBP_{ao}	Central diastolic blood pressure
DBP_{br}	Peripheral diastolic blood pressure
DP	Degree of polymerisation
EFSA	European Food Safety Authority
eGFR	Estimated glomerular filtration rate
eNOS	Endothelial-nitric oxide synthase
EPIC	European Prospective Investigation into Cancer and Nutrition
ET	Ellagitannins
FETA	FFQ EPIC Tool for Analysis
FFQ	Food frequency questionnaire
FMD	Flow mediated dilation
FRS	Framingham Risk Score
FV	Fruits and vegetables
GGT	Gamma-glutamyl-transpeptidase
GLUT2	Glucose transporter 2

HDL	High density lipoprotein
HPLC	High-pressure liquid chromatography
HR	Heart rate
hs-CRP	High sensitivity C-reactive protein
IPAQ	International physical activity questionnaire
ITT	Intention to treat
LC-MS	Liquid chromatography / Mass spectrometry
LDH	Lactate dehydrogenase
LDL	Low density lipoprotein
LOX	Lipoxygenases
LPH	Lactase phlorizin hydrolase
MCHC	Mean corpuscular haemoglobin concentration
MCV	Mean corpuscular volume
MET	Metabolic equivalent task
MRP	Multidrug-resistance-associated protein
MS	Mass spectrometer
MSP	Metagenomic Species Pangenome
NDNS	National Diet and Nutrition Survey
NO	Nitric oxide
NOX	NADPH oxidase
Nrf2	Nuclear E2-related factor 2
NS	Not significant
NR	Non-responders
oxLDL	Oxidised LDL
PA	Physical activity

PBS	Phosphate buffered saline
PCoA	Principal Coordinates Analysis
PI3K	Phosphatidylinositol 3-kinase
PKG	Protein kinase G
PP	(Poly)phenol
PPAR-γ	Peroxisome proliferator-activated receptor gamma
PerP	Per protocol
PSBP	Peripheral SBP
PWV	Pulse wave velocity
R	Responders
RCT	Randomised controlled trial
ROS	Reactive oxygen species
SBP	Systolic blood pressure
SBP_{ao}	Central systolic blood pressure
SBP_{br}	Peripheral systolic blood pressure
SCFA	Short-chain fatty acids
SCORE	Systematic Coronary Risk Evaluation
SD	Standard deviation
SEM	Standard error of mean
sGC	Soluble guanylate cyclase
SGLT1	Sodium-dependent glucose transporter 1
SMC	Smooth muscle cells
SOD	Superoxide dismutase
μ-SPE	Micro-solid phase extraction
SS	Safety set

SST	Serum separator tubes
SULT	Sulfotransferase
TBARS	Thiobarbituric acid reactive substances
TG	Triglycerides
UGT	Uridine-5′-diphosphate glucuronosyltransferase
USDA	US Department of Agriculture
WHO	World Health Organization

CHAPTER 1

Introduction

1.1 Cardiovascular diseases (CVD)

1.1.1 Prevalence and global impact

CVD, also called heart diseases, is an umbrella term used to refer to conditions affecting the heart or circulation, and includes coronary artery disease, stroke, and hypertension. CVDs are the first cause of mortality worldwide (Roth et al. 2017), responsible for an estimated 31% of all deaths globally (World Health Organisation 2017). Each year, CVD accounts for almost 27% of the deaths in the UK and the whole healthcare cost related to CVD in the UK is estimated at £9 billion per year (British Heart Foundation).

1.1.2 Atherosclerosis: definitions and causes

Atherosclerosis refers to the progressive disease characterized by the build-up of lipids and fibrous plaques in arteries, leading to hardening and narrowing of these large vessels. Atherosclerosis occurs in the most internal of the 3 layers of the artery: the tunica intima. This layer is constituted of a monolayer of endothelial cells surrounded by some elastin fibres and an extracellular matrix. This tunica intima is directly in contact with the middle layer of the artery called the tunica media, which is composed of smooth muscle cells (SMC). The external layer, called the tunica externa, accounts for the flexibility and structure of the artery thanks to the elastin and collagen fibres present in this layer (Wolinsky and Glagov 1967). The structure of the arterial wall is represented in Figure 1.1 below. The word atherosclerosis takes its origin in the Greek work for “porridge”, suggesting the irregular appearance of the fatty deposit found at the heart of the atherosclerotic plaque (also called atheroma) found in the intima (Libby et al. 2019). Cardiovascular events can occur due

to an accumulation of atherosclerotic plaques obstructing or narrowing the arterial lumen (Libby 2012). Optimising lifelong cardiovascular health limits both the initiation and progression of atherosclerosis, leading to a reduction in future CVD risk (Napoli et al. 2012). Atherosclerosis-based CVD is one of the main causes of vascular disease worldwide. Indeed, after a slow progression during lifetime, the disease can eventually lead to peripheral vascular diseases and/or stroke among the older general population (Otsuka et al. 2015). Some risk factors have been related to the onset and development of atherosclerosis, such as hypercholesterolemia, hypertension, cigarette smoking and diabetes mellitus (Hackam and Anand 2003).

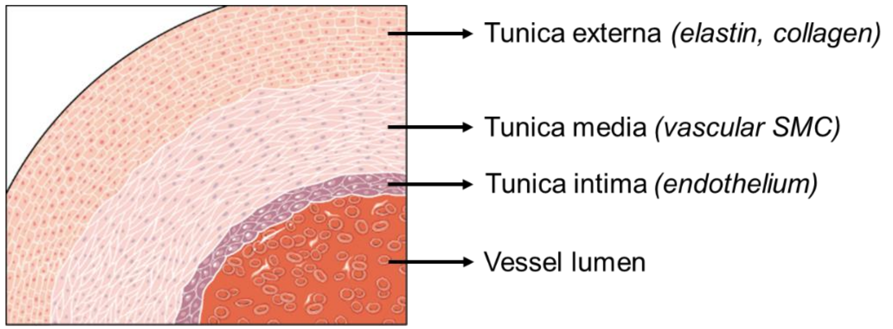


Figure 1.1: Structure of the arterial wall.

1.1.2.1 Endothelial dysfunction and activation

Atherosclerosis has been studied for decades, and the oxidation of lipids and proteins has been detected in various vascular pathologies, suggesting the role of reactive oxygen species (ROS) in atherogenesis (Stocker and Keaney 2004). Early stages of atherosclerosis can begin during childhood and adolescence (Strong et al. 1999). Even though the process behind atherosclerosis is still not fully elucidated, it is now accepted and understood

that low-density lipoproteins (LDL) play a major role in the development of the disease (Ference et al. 2012).

The primary event taking place in the process of atherosclerosis is the increased transport and accumulation of LDL in the vascular wall, and more precisely between the endothelium and the tunica intima (Libby et al. 2019). This phenomenon is due to two mechanisms. First, some vascular regions such as bifurcations can present a disturbed flow which can impact the structure of the vessel by increasing the fragility of the elastin fibres and thus enhancing the permeability of the arterial wall (Kwon et al. 2008). Then, this disturbed flow has been shown to increase the expression of chemotactic proteoglycan proteins responsible for the LDL binding and retention (Steffensen et al. 2015).

Once LDL are accumulated within the arterial wall, they are accessible to various enzymatic and non-enzymatic entities which will then oxidise the LDL into highly reactive products. This process is called lipid peroxidation and results in the formation of oxidised LDL called ox-LDL (Binder, Papac-Milicevic, and Witztum 2016). This oxidation can be done by enzymes such as cyclooxygenases (COX), lipoxygenases (LOX) or cytochrome P450 but also by non-enzymatic mechanisms involving reactive oxygen species (ROS) generated from NADPH oxidases (NOX) or endothelial nitric oxide synthases (eNOS) (Bochkov et al. 2010). More than just an oxidation, this step will lead to the modification of the LDL structure and the generation of new epitopes called oxidation-specific epitopes (Binder, Papac-Milicevic, and Witztum 2016).

The third step of the atherosclerosis process is the endothelial cells activation. During this stage the oxidation-specific epitopes are recognised by the endothelium which is then taking up these oxLDL via specific receptors called LOX-1, TLR2 and TLR4 (Leopold and Loscalzo 2009). This leads to various toxic biological effects, including the synthesis of SMC mitogenic factors and the stimulation of cytokines secretion as well as the upregulation of adhesion molecules expression on the endothelium (McIntyre and Hazen 2010). This leads to the attachment of the surrounding immune cells such as monocytes to the endothelium (Wu, Wang, et al. 2018). Monocytes can then migrate into the artery wall and differentiate into macrophage under the action of mediators such as macrophage colony-stimulating factor. The newly formed macrophages can take up oxLDL via their scavenger receptor and secrete cytokines themselves which thus stimulates the inflammatory process of the intima (Weber and Noels 2011). Contrary to the regular LDL receptors, scavenger receptors are not inhibited by an increased cellular cholesterol. As a result, macrophages present a swollen cytoplasm full of lipid droplets, which gives them the appearance and the name of “foam cells”. The supersaturation of lipids in these macrophages can lead to their death by apoptosis (Tabas 2010). Lipid-laden cells are the earliest detectable lesion appearing in the process of atherosclerosis, and can further lead to the generation of a fatty plaque (Hopkins 2013).

1.1.2.2 Mechanisms of plaque formation and rupture

Even though the development of lipid build-up is considered as an early stage of atherosclerosis, the disease formally starts once the accumulation appears as confluent extracellular cholesterol clusters (Insull 2009) called the atheroma

plaque. The leads to cell necrosis and, as a result, an alteration of the normal tunica intima structure, until its full disruption. These clusters of lipid-rich necrotic cells can ultimately occupy up to 50% of the arterial volume.

Progressively, the atherosclerotic plaque can turn into a more rigid structure, with the generation of a fibrous cap right under the endothelium, which can obstruct the lumen of the vessel (Libby et al. 2019). This is due to the secretion of collagen by vascular SMC around the atheroma plaque in response to inflammation (Kragel et al. 1989). This can lead to a decrease in blood flow and potentially an ischemia of the surrounding tissues. Fibrous atheroma plaque can occur in teenagers and early 20s individuals (Virmani et al. 2000). The fibrous atheroma can also detach itself from the endothelium wall, causing a thrombosis in the coronary arteries and ultimately a myocardial infarction (Libby et al. 2019). This phenomenon is called “plaque rupture”. Briefly, the fibrous cap can become fragile due to proteolytic enzyme action which causes the dissolution of the fibrous material (Insull 2009). This phenomenon is more likely to be observed among an older population, generally aged 55 years and above (Cheruvu et al. 2007), which coincide with the peak of myocardial infarction incidence.

1.1.2.3 Atherosclerosis and nitric oxide

Because of its direct contact with blood and its importance for the vascular system integrity, the endothelium is targeted by numerous physical and chemical factors ensuring its homeostasis (Furchgott and Zawadzki 1980). This endothelial layer is a key player in the regulation of the vessel tone and diameter and is able to generate some vasoactive molecules to achieve this

role (Schechter and Gladwin 2003). Nitric oxide (NO) is a vasodilating gas synthesised by the endothelial cells via the action of the enzyme eNOS on L-arginine, and which can disperse itself to the adjacent vascular SMC (Forstermann and Munzel 2006). Under normal physiological conditions, the stable laminar shear stress observed in the healthy arteries maintains the NO concentrations at a sufficient level to prevent any dysfunction which could lead to inflammation or thrombosis (Moncada and Erusalimsky 2002; Gimbrone 1999). As mentioned earlier, atherosclerosis is more likely to happen around bifurcations and arterial branches. This is due to the rupture of the laminar flow caused by this kind of structures, creating a turbulent flow recognised by mechanoreceptors from the endothelium. These receptors are then able to induce modification of the gene expression which ultimately leads to a diminution of the NO availability (Wentzel et al. 2012).

This decrease in bioavailable NO is linked to a diminution of the activity of eNOS as well as the degradation of NO via the generation of superoxide anions ($O_2^{\circ-}$) (Torres et al. 2015). The depletion in NO also promotes the infiltration and adhesion of leucocytes into the arterial wall (Badimón, Vilahur, and Padró 2009). The diminution in NO concentration has also been shown to lead to the activation of the pro-inflammatory transcription factor NF-κB and to the downregulation of adhesion molecules (Forstermann and Munzel 2006). Reduced NO can also enhance the expression of high LDL affinity proteoglycan on the surface of the endothelium, leading to an increase in the uptake of oxLDL by the arterial wall (Williams and Tabas 1995). All these phenomena induce endothelial dysfunction by decreasing the stock of NO, helping the adhesion of leucocytes, and increasing inflammation status, while

ultimately leads to and aggravation of the atherosclerosis process (Goncharov et al. 2015) (Figure 1.2).

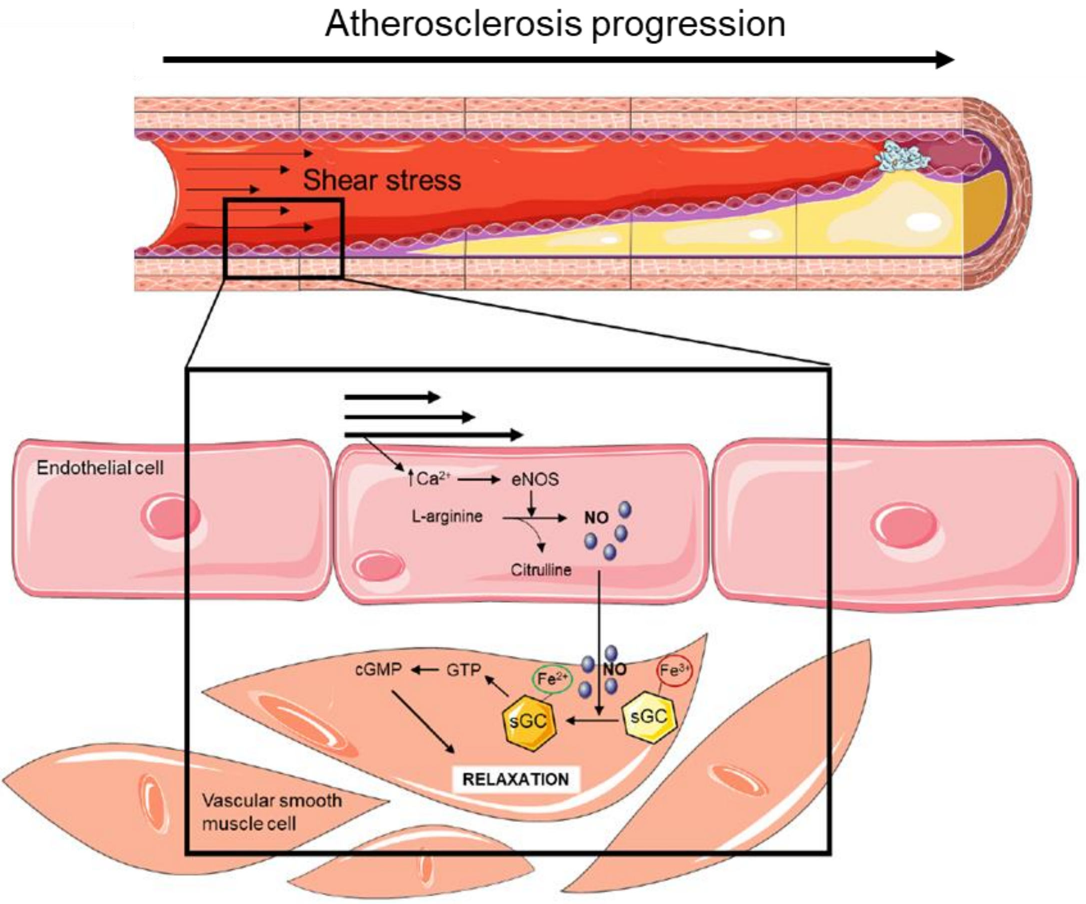


Figure 1.2: Mechanisms behind physiological arterial function.

1.1.2.4 Clinical assessment and applications

By definition, a functional surrogate marker is a measurement or physical evidence which may be used as a substitute for hard clinical endpoints, such as mortality or morbidity (Katz 2004). These biomarkers are expected to predict clinical benefit or lack of benefit following an intervention and are of interest in the context of CVD for various reasons (Albert 2011). Indeed, their use involve a substantial decrease in the number of subjects included in the trial as well as the study duration, which drops considerably the costs and efforts. Moreover, these CVD surrogate markers are non-invasive and

applicable in all populations including young subjects who will not present vascular events for decades (Peters, Grobbee, and Bots 2011). Back in 1989, Prentice developed a list of criteria to characterize surrogate markers, including a good reproducibility, comparison with a “gold standard”, ability to change over time or a well-established relationship with existing risk factors (Prentice 1989). In nutrition research, randomised controlled trials investigating the effect of diet or dietary components on cardiovascular health have used several surrogate markers of CVD such as blood pressure, arterial stiffening (measured as pulse wave velocity and augmentation index), flow-mediated dilation, carotid intima-media thickness, CVD risk calculators, total, HDL and LDL cholesterol levels, (Rodriguez-Mateos, Heiss, et al. 2014; Wierzbicki 2008). In this section, the surrogate markers used in the current PhD thesis will be discussed.

1.1.2.4.1 Blood pressure

- ***Blood pressure and CVD risk***

Blood pressure (BP) has been shown to be a robust surrogate marker of CVD risk (Giles 2013) and a decrease of 3 mmHg in systolic BP (SBP) was associated with a decrease in the risk of CVD mortality by 5% (Whelton et al. 2002). In a longitudinal study including participants from the Framingham Heart Study and National Health and Nutrition Examination Survey II (NHANES II), Cook and colleagues reported that 2 mmHg drop in diastolic BP (DBP) was associated with a 17% reduction in the prevalence of hypertension, a 6% reduction in the risk of coronary heart disease and a 15% decrease in risk of stroke in white individuals aged 35 to 64 years (Cook et al. 1995). More

recently, a meta-analysis including more than 600 000 subjects demonstrated that a 10-mmHg reduction in SBP could reduce the risk of main CVD events by 20%, and all-cause mortality by 13% (Ettehad et al. 2016). This has been confirmed by a recent meta-analysis stating that for every 10 and 5 mmHg increase in SBP and DBP, respectively, the risk of cardiovascular events was raised by 4-5% (Luo et al. 2020).

- ***Blood pressure measurements***

Which type of BP measurement is optimal is still a matter of debate, and many studies have compared office versus ambulatory BP assessment, peripheral BP versus central BP, and 24-hour versus night-time BP. Overall, several meta-analyses of clinical and epidemiological studies suggest that 24-hour ambulatory blood pressure is a better tool to assess CVD risk than office BP (Kidambi et al. 2020; Bakogiannis et al. 2020). However, a study revealed that 10-year prediction of cardiovascular events calculated from ambulatory and office BP measurements did not differ (Mortensen et al. 2017). Nevertheless, ambulatory BP monitoring presents some limitations, as it cannot be used in all settings and present some burden for the subjects (e.g., disturbance at night, risk of error when moving, limitation in the movements, etc.).

Moreover, some studies have compared central versus peripheral BP. For example, a study based on 1014 healthy individuals concluded that office central BP was more efficient than office peripheral BP to predict the occurrence of CVD-based mortality (Huang et al. 2011). As internal organ damage is a potent predictor of cardiovascular events, this may explain the findings by Huang et al. (Laurent, Sharman, and Boutouyrie 2016).

Yet, measurement of central BP can be limited by the cost of the equipment, as well as the complexity of the calibration which may leads to potential errors (Mitchell et al. 2016).

Lastly, the potential of 24-hour and night-time BP measurement for the prediction of cardiovascular events was underlined in a prospective study including more than 11 000 subjects as authors linked a 10-mmHg increase in night-time and 24-hour BP to 23% and 22% increased CVD risk, respectively (Yang et al. 2019).

- ***Blood pressure variability***

More recently, studies have highlighted the specific relationship between BP variability (BPV) and CVD risk prevention. BPV stands for the fluctuations in BP levels that can occur over time periods varying from seconds to years, and thus highlight a dynamic aspect of cardiovascular health (Parati et al. 2018). Another longitudinal study found that a 4.7% increase in BPV corresponded to a 13% and 7% rise in mortality and CVD risk, respectively (Dai et al. 2018). A meta-analysis of 23 observational studies investigating the impact of BP variability on the occurrence of CVD events. Increases in long-, mid- and short-term variability all showed to be associated to an augmentation of the risk of all-cause mortality, CVD mortality, CVD events, coronary heart disease and stroke, by 15, 18, 18, 10 and 15%, respectively (Stevens et al. 2016). This highlights that BPV may be a potent predictor of CVD outcomes. However, this parameter is still rarely assessed in medical practice and trials, mostly due to the lack of method standardization for an accurate and reliable measure of BPV (Mena et al. 2017).

- ***Blood pressure and atherosclerosis***

Although mechanisms behind the link between BP and atherosclerosis have not been fully identified yet, the importance of the endothelium and the process of oxidative stress occurring at its level seem to be the main explanation (Alexander 1995). However, while NO exerts a key role in the regulation of vascular tone and BP, it has also been observed that compromised NO activity is an essential element of the onset of hypertension (Hermann, Flammer, and Lüscher 2006). The pulsatile factor of BP has been shown to induce atheroma instability and eventually, plaque rupture, indicating the mediating role of BP on atherosclerosis (Safar and Jankowski 2009).

1.1.2.4.2 Pulse wave velocity assessment

Arterial stiffness is a phenomenon occurring in the arterial wall and involving an increased hardness of the vessel, which mainly results of a structural change associated to elasticity loss, collagen accumulation, and elastin degradation (Lusis 2000). This physiological event, also called arteriosclerosis, is associated to normal vascular aging but can be correlated to different risk factors such as smoking habits, unhealthy diet, hypercholesterolemia, or low physical activity level (Mikael et al. 2017).

Pulse wave velocity (PWV) is the most used technique to assess arterial stiffness. Briefly, PWV uses applanation tonometry technology to measure the pressure waves speed traveling in the arterial system. PWV, expressed in m/s, is calculated based on the division of the distance between the two points of measurement by the pulse pressure wave transit time at these two locations (Cavalcante et al. 2011). In common practice, carotid and femoral artery are

the preferred sites for the assessment of PWV, which is then designated as Cf-PWV.

Numerous trials have shown the link between arteriosclerosis and the development of atherosclerosis as well as the incidence of CVD events. Indeed, Cf-PWV has been strongly and positively associated to coronary atherosclerosis (Oberoi et al. 2013), arterial calcifications (Tsao et al. 2014; McEniery et al. 2009), as well as carotid thickness and atheroma development (van Popele et al. 2001; Zureik et al. 2003).

The link between atherosclerosis and arteriosclerosis is extremely narrow. In fact, the increase in shear stress and arterial pressure caused by the vessel stiffening leads to endothelial dysfunction and development of the plaque, which promotes the advancement of atherosclerosis (Kim and Kim 2019; Zieman, Melenovsky, and Kass 2005). Several mechanisms have been suggested to explain these events. First, increased BP occurring with the development of arterial stiffness could induce a vascular remodelling and stimulate collagen excessive production and accumulation in the arterial wall (Dao et al. 2005). Moreover, the surge in pulse pressure may be linked to atheroma growth and to a further extent, its rupture (Witteman et al. 1994).

Furthermore, relationship between Cf-PWV and CVD events risk have been widely reported in the literature (Boutouyrie et al. 2002). Cf-PWV was found to predict CVD events independently from usual CVD risk factors in two large prospective study populations followed-up for 9.4 and 7.8 years, respectively (Mitchell et al. 2010; Willum-Hansen et al. 2006). In another study, Cf-PWV has been shown to be an independent predictive measurement for

cardiovascular and all-cause mortality among hypertensive and diabetic individuals (Laurent et al. 2001; Cruickshank et al. 2002). More recently, a meta-analysis involving 17 635 volunteers demonstrated that, after adjustment for sex, age and hazard factors, an increase of 1 standard deviation of $\log_{\text{Cf-PWV}}$ was associated with an increased risk for CVD, coronary heart disease and stroke of 30%, 23% and 28%, respectively (Ben-Shlomo et al. 2014). After adjustments, authors also showed that an increase of 1 m/s increase in PWV was associated with a 7% augmented risk of cardiovascular event for a 60-year-old man. Another meta-analysis of longitudinal studies including almost 16 000 individuals and an average follow-up of nearly 8 years has shown that an augmentation of 1 m/s in Cf-PWV was related to an increased risk of total CVD events by 14% (Vlachopoulos, Aznaouridis, and Stefanadis 2010). These findings have been confirmed by a recent meta-analysis of 19 studies which demonstrated that individuals with a higher Cf-PWV by 1 m/s had an elevated risk of both CVD events and mortality (Zhong et al. 2018).

To sum up, PWV has been shown to be a valuable tool for defining CVD risk and atherosclerosis. This is a reproductible and non-invasive measurement which is now proven to be a major risk factor for the future development of CVD (Kim and Kim 2019).

1.1.2.4.3 Augmentation index measurements

Augmentation index (AIx) assessment is another useful tool for the measurement of arterial stiffness which has been developed over the last decades. Using the same applanation tonometry technique as for PWV, AIx is

a non-invasive method estimating the pulse wave reflections of the arteries which is expressed as a percentage (Janner et al. 2012). PWV and AIx represents two different measures of arterial stiffness which are not interchangeable (Laurent et al. 2001).

Although AIx measurement is not as widely used in common practice, some studies have been investigated the impact of AIx increase on the development of CVD events. The clinical significance of AIx was investigated in a meta-analysis including 11 longitudinal studies which demonstrated that a 10% increase in central AIx was associated with an increase in the risk of cardiovascular events and all-cause mortality by 32% and 38%, respectively (Vlachopoulos, Aznaouridis, and Stefanadis 2010). This is in line with a previous study that showed an association between a 10% increase in AIx and a 48% increased risk for cardiovascular mortality (London et al. 2001).

However, some limitations have been found in the assessment of AIx. Indeed, a few studies raised the concern about the legitimacy of the technique among older individuals (Fantin et al. 2007; McEniery et al. 2005) as a result of the association existing between age and AIx (Janner et al. 2010). Another study evaluated the gender differences related to the amplitude of arterial wave reflections in a hypertensive population. After matching men and women for age, BP, height and BMI, researchers found that arteries of older women were stiffer than those of men (Gatzka et al. 2001).

Similarly, contradictory results have been observed concerning AIx measurement as a CVD risk predictor (Mitchell et al. 2010). Indeed, while AIx percentage has been linked to an increase of CVD risk in individuals at high

risk (Vlachopoulos, Aznaouridis, and Stefanadis 2010; Weber et al. 2010) and in men (Weber et al. 2005), findings related to the predictions among women and general population have been more debatable (Dart Anthony et al. 2006; Wang et al. 2010).

The mechanisms behind AIx estimation and its relation to CVD and atherosclerosis have not been fully elucidated yet. AIx has been shown to increase significantly with age, following a curvilinear pattern (McEniery et al. 2006; Namasivayam, Adji, and O'Rourke 2010). However, it has been suggested that the increase in AIx could be related to an elevation of the ROS level, which constitutes one of the early stages of the development of atherosclerosis. Indeed, a study showed AIx was associated to serum ROS concentration in a population of smokers. This study also observed that older age and hypertension were associated with elevated AIx (Sugiura et al. 2017).

1.1.2.4.4 Flow mediated dilation

Endothelial dysfunction relates to the decreased production or availability of NO around the endothelium, leading to the impairment of the blood vessel. As explained earlier, NO presents a wide range of vasoprotective effects towards the process of atherosclerosis. As a result, it is comprehensible that the diminution of NO concentration is commonly described as endothelial dysfunction. This phenomenon is the first stage of the atherosclerosis pathogenesis and as a result pay an important part in its progression and the development of cardiovascular diseases (Ross 1999). Endothelial dysfunction is described as an independent predictor for CVD (Hadi, Carr, and Al Suwaidi 2005)

Several techniques have been established to evaluate endothelial function *in vivo* in humans. These methods assess the ability of an artery to dilate as a response to various stimuli, such as an injection of vasodilator or a reactive hyperaemia through the release of NO (Maruhashi, Kihara, and Higashi 2018). In other words, these approaches can indirectly estimate the ability of the endothelial cells to produce NO after a stimulus (Ludmer et al. 1986). Most invasive methods use acetylcholine injections, a potent vasodilator, or various antagonists such as adenosine and bradykinin (Okumura et al. 1992; Nabel, Selwyn, and Ganz 1990). However, this kind of procedures are not appropriate for large trials or patients with poor vasculature and have been mainly replaced with non-invasive methods. The main technique used as a gold standard to assess endothelial function non-invasively is known as flow-mediated dilation (FMD) (Celermajer et al. 1992; Grover-Paez and Zavalza-Gomez 2009). FMD uses ultrasound technology to estimates the variations in brachial artery diameter, following a 5-minute ischemia caused by the inflation of a sphygmomanometric cuff to a fixed pressure around the forearm (Thijssen et al. 2019). Some studies use a more proximal cuff, upper on the arm. However, a meta-analysis including 14 trials concluded that the position of the cuff (distal on the forearm versus proximal on the arm) did not impact the result of the FMD measurement (Green et al. 2011).

Impairment in FMD values has been linked a predisposition to atherosclerosis and CVD (Charakida et al. 2010; Yeboah et al. 2007; Rossi et al. 2008). A prospective study with a 3-year follow-up indicated that FMD assessment was a sensible predictor of targeted organ damage –a marker of high CVD risk–, in a hypertensive population (Yang et al. 2014). Importantly, several

meta-analyses have shown that a 1% increase in FMD was associated with 8 to 13% reduction in CVD events (Inaba, Chen, and Bergmann 2010; Xu et al. 2014). These findings have been observed independently of the risk level of the population but were more significant in individuals with a previous history of CVD (Ras, Streppel, et al. 2013; Matsuzawa et al. 2015; Shechter et al. 2014). FMD measurement of the brachial artery has also been shown to be a relevant marker of NO bioavailability (Green et al. 2014; Heiss et al. 2005)

Numerous randomised controlled trials have investigated the temporality of FMD changes, from a couple of hours to a few months (Lüscher et al. 2012; Bruno et al. 2018; Green et al. 2014; Istas et al. 2019). An increase in FMD can be interpreted by an improvement in endothelial function as well as an enhancement of the protective effect against atherosclerosis, which proves the relevance of the method in primary prevention strategies.

The main limitation of FMD assessment is the low reproducibility related to the technique, which implies the requirement of a highly trained and skilled operator (Higashi 2015).

1.1.2.4.5 CVD risk calculators

CVD risk calculators are algorithms developed by health specialists and academics to assess an individual's risk of developing a cardiovascular event (stroke, heart disease, etc.) over a 10-year period. They are usually based on large databases and cohorts and present the average risk of people from the database who entered the same risk factors.

The Framingham Risk Score (FRS) is by far the most used CVD risk calculator. It has been developed in 1987 in the US and was based on a sample of people originated from North-East America (Wilson et al. 1998). The algorithm used in the last update from 2008 applies the values of blood pressure, age, sex, smoking status, presence of diabetes as well as LDL-, HDL- and total cholesterol. One of the limitations of this FRS is the homogeneity of the original database cohort, which was mostly composed of a middle-aged white population.

The SCORE (for Systematic Coronary Risk Evaluation) project has been developed by the European Society of Cardiology at the beginning of the century and pooled datasets from 12 European cohorts, including in total more than 200 000 people (Conroy et al. 2003). The main advantage of the SCORE calculator is the ability to be adapted to various countries and cultures.

The QRISK®3 score is the most used calculator in the UK among general practitioners. It is based on the same equation than the FRS, but the algorithm includes far more parameters in the analysis. For example, body mass index (BMI), ethnicity, family history, as well as the presence of mental illnesses or the intake of specific medication are included in the calculation of the 10-year CVD risk. The QRISK®3 score has been shown to sightly underestimate the CVD risk, but this bias was less important than the overestimation that can occur with the FRS (Collins and Altman 2009).

1.1.2.4.6 LDL cholesterol

In 2010, LDL cholesterol has been classified as a surrogate marker for CVD by the US Institute of Medicine, concluding that LDL cholesterol lowering was “one of the best biomarkers for CVD” (Institute of Medicine Committee on Qualification of and Surrogate Endpoints in Chronic 2010). Indeed, as described in section 1.1.2., LDL is closely related to the process of atherosclerosis and its relationship with coronary artery diseases has been clearly established for decades (Goldstein et al. 1973; Castelli et al. 1986).

Meta-analysis from the Cholesterol Treatment Trialists Collaboration demonstrated that statin therapy, –the most common treatment for hypercholesterolaemia–, was associated with a substantial reduction in men, women, older adults, and subjects with low risk of CVD, by 16, 22, 21 and 21%, per 1.0 mmol/L decrease, respectively. A recent meta-analysis involving 327 037 subjects from 52 studies revealed that 1 mmol/L decrease in LDL cholesterol was linked to 19% reduction of cardiovascular events (Wang et al. 2020). As a result of the strong evidence available, the American College of Cardiology and American Heart Association as well as Canadian Cardiovascular Society recommend a threshold of 1.8 mmol/L of LDL cholesterol to reduce the risk of atherosclerotic CVD (Grundy et al. 2019; Anderson et al. 2016). In Europe, guidelines are stricter with a cut-off of 1.4 mmol/L recently publishes by the European Society of Cardiology ('2019 ESC/EAS guidelines for the management of dyslipidaemias: Lipid modification to reduce cardiovascular risk' 2019).

1.1.3 CVD risk factors

Traditional CVD risk factors can be divided into modifiable and unmodifiable risk factors. While unmodifiable parameters such as age, sex, ethnicity, hereditary characteristics cannot be changed, most CVD risk factors can be influenced and modulated by lifestyle choices, dietary improvement, or treating any pre-existing physiopathological disorders (Omura et al. 1996). Main modifiable risk factors include diet, smoking status, alcohol consumption, low physical activity, obesity, dyslipidaemia, hypertension and the presence of diabetes mellitus (Francula-Zaninovic and Nola 2018).

For example, smokers have been shown to be at higher risk of developing CVD compared to non-smokers (Rigotti and Clair 2013). Alcohol consumption in excess (21-30g per day) has also been correlated to a higher risk of CVD, as described in a meta-analysis (Briasoulis, Agarwal, and Messerli 2012). Hypertension (defined as blood pressure > 140/90 mmHg) is one of the most prevalent risk factors, with around 30-45% of hypertensive individuals among the population worldwide (Kjeldsen 2018). A randomised controlled trial including more than 9000 individuals demonstrated that adults with grade 1 hypertension have twice as much risk of CVD compared to normotensive individuals (Wright et al. 2015). Dyslipidaemia is another largely prevalent risk of CVD, and a decrease of the plasmatic LDL concentration has been shown to attenuate this risk (Wadhera et al. 2016). Sedentary lifestyle is become increasingly prominent and problematic as the World Health Organization (WHO) estimated that around 80% of the worldwide population is not sufficiently active (World Health Organization 2008). Recommendations and

consensus for a better management of this risk factor have been booming in the last years and scientific working groups are now elaborating some guidance and prescriptions adapted to specific populations (Hansen et al. 2018).

Diet is one of the most important modifiable CVD risk factors (Piepoli et al. 2016). Indeed, in 2017 it has been estimated that 11 million deaths were associated to risks factors linked to dietary intake worldwide (Afshin et al. 2019). The link between dietary intake and chronic diseases has been widely studied, with a plethora of epidemiological evidence and meta-analysis highlighting the substantial association between dietary patterns and specific diseases such as CVD, diabetes, or cancer (Micha et al. 2012; Micha et al. 2017; Norat et al. 2010; Fund 2018). A recent meta-analysis estimated the association between dietary risk factors and disease-specific burden at a global scale (Afshin et al. 2019). The authors reported that leading risks factors for death worldwide were high sodium consumption, low intake of fruits and low consumption of wholegrains, and were associated to 3 million, 2 million and 3 million of global deaths, respectively. This is in line with the main current dietary guidelines recommending the reduction of salt in the diet, as well as an increased consumption of fibre and fruits and vegetables (FV) as effective measures for the prevention of CVD (Jenkins et al. 2017; Joshipura et al. 2001; Anand et al. 2015).

1.1.4 Plant foods and CVD

In the last 20 years, several observational studies have shown positive association between a higher intake of FV and a decrease of the CVD risk, as well as a reduction of all-cause mortality (Bazzano et al. 2002; Bendinelli et al. 2011; Takachi et al. 2008; Yu et al. 2014; Wang et al. 2014; Kim et al. 2019; Angelino et al. 2019). Recent meta-analysis including almost 1.5 million volunteers from 47 epidemiological studies showed a dose-dependent association between increased intake of FV and reduction in the risk of CVD. Compared with participants whose FV consumption was the lowest, individuals with the highest FV intake (800g) presented a 17% decrease in CVD risk (Zhan et al. 2017). Another study found similar findings with a reduction of 28% in CVD risk up associated with the 800g threshold (Aune et al. 2017). This study also reported the association of a daily intake of 200g of FV with 8% and 10% decreased risk for CVD and all-cause mortality, respectively.

Nowadays, most developed countries share the same guidelines in terms of daily FV intake. The WHO, as well as UK and US recommend a minimum of 400g of FV per day, representing 5 portions of FV per day (Krauss et al. 2000; World Health Organization 1990; Public Health England 2016; U.S. Department of Health 2015 – 2020). In other countries in Europe, recommendations tend to vary according to the country, with some divergence. For example, while Germany encourage to consume at least 650g of FV per day, Hungary advise a daily intake of “at least 4 servings” of FV (European Commission). A report by WHO estimated that increasing intake of

FV above 600g per day could decrease the risk of heart disease by 31% and reduce the incidence of stroke by 19% (Lock et al. 2005).

Despite the strong evidence on the numerous health benefits of FV consumption, most of the population worldwide is not meeting the recommendations. A recent review focused on the dietary habits of teenagers from North America, Europe and Oceania revealed that the intake of FV was clearly below the recommended 400g per day in almost all the populations studied (Rosi et al. 2019). A few years earlier the European Health Interview Survey reported that only 14% of the European population aged 15 years and above was consuming 400g of FV per day, and 51% were not reaching that cut-off (Eurostat 2016). Recently, an analysis based on various British studies including a total of 557 722 participants highlighted than only 26% of the UK population is adhering to the dietary recommendations related to the intake of FV (Scheelbeek et al. 2020).

The consumption of FV is depending on various factors such as culture or the accessibility of such foods. However, socio-economic status has been found to be a very limiting factor regarding the intake of healthy plant-based diets. A review of 33 studies published in 2001 highlighted that FV were consumed more frequently among people with higher education (Roos et al. 2001). Almost 20 years later, these findings were confirmed by a cross-sectional study including nearly 38 000 European adults which highlighted that higher level of education was associated with increased dietary intake of FV (Stea et al. 2020). Another study based on data from 10 European countries revealed that the improvement of FV intake in low-educated groups to reach the

consumption level of higher educated populations would have a positive effect on both life expectancy and disability-free life expectancy (Baars et al. 2019). The impact of individual incomes and global wealth on FV intake has also been studied.

A meta-analysis involving more than 143 000 subjects in 18 countries showed that mean consumption of FV in low- versus high- income countries was 2.14 versus 5.42 servings, respectively, indicating that limited wealth induces a poor adherence to WHO recommendation of 5 servings per day (Miller et al. 2016). Another recent meta-analysis enrolling 193 606 individuals from 28 countries was in line with those results and presented that the proportion of subjects meeting the reference intake of 400g per day significantly increased with the gross domestic product of the country (Frank et al. 2019). Authors also revealed that subjects with secondary or greater education were 61% more likely to achieve the dietary guidelines compared with individuals without formal education.

Based on the promising results highlighted from the epidemiological studies, a number of relatively large scale RCTs have investigated the effect of various plant-rich dietary patterns including the Mediterranean diet (investigated by the PREDIMED study), Dietary Approaches to Stop Hypertension (DASH) diet, the Portfolio diet or vegetarian diets (Challa, Ameer, and Uppaluri 2020; Kargin, Tomaino, and Serra-Majem 2019; Chiavaroli et al. 2018; Estruch et al. 2018). While vegetarian diets exclude meat, fish and poultry and may or may not include eggs and dairy, DASH diet is based on the consumption of FV, whole grains, nuts, legumes and low-fat dairy and limits the intake of saturated

fat processed food and added sugar (Sacks et al. 1995). Additionally, the Portfolio diet combines known cholesterol-lowering foods such as nuts, viscous fibres or plant sterols (Kendall and Jenkins 2004) and the Mediterranean diet promotes a high consumption of FV, cereals, beans, nuts, unprocessed foods, olive oil as the main source of fat, low intake of red meat, high intake of fish and low to moderate amount of red wine (Willett et al. 1995). A meta-analysis including 7 prospective studies showed an association of a vegetarian dietary pattern with a reduction of coronary heart disease by 22% (Glenn et al. 2019). Furthermore, an umbrella review of meta-analysis and reviews including 15 prospective studies and 31 randomised controlled trials (RCTs) reported an association between adherence to the DASH diet and a decrease in CVD and coronary heart disease of 20 and 21%, respectively (Chiavaroli et al. 2019). This review also reported that DASH diet was associated with a decrease of 5.2 and 2.6 mmHg in SBP and DBP, respectively, based on results from the RCTs. Moreover, another meta-analysis of 29 observational studies has shown some convincing evidence for an inverse association between Mediterranean diet and a 10% reduction in the risk of all-cause mortality (Soltani et al. 2019). This has been confirmed by the results of a recent meta-analysis involving 30 RCTs and exploring the effectiveness of Mediterranean diet reporting a 40% decrease in the number of strokes and a reduction of SBP and DBP of 3.0 and 2.0 mmHg, respectively, following a Mediterranean diet pattern intervention (Rees et al. 2019). Likewise, adherence to the Portfolio Diet was associated with a decrease in the estimated 10-year risk of coronary heart disease by 13%, in a meta-analysis grouping 7 studies (Chiavaroli et al. 2018). While the above findings

regarding the beneficial effects of FV and plant-based foods are very promising, there is still a need for well-designed and robust RCTs to confirm the real benefit of fruits, vegetables, and overall plant food consumption on chronic diseases and especially CVD. Among the various bioactive components present in plant foods, (poly)phenols seem to be good candidates which may be responsible for their cardioprotective effects (Liu 2013).

1.2 (Poly)phenols

1.2.1 Definition

(Poly)phenols (PP) are secondary plant metabolites containing one or more phenolic rings in their structure, and with a mass ranging from 300 to 3000 Da, and even up to 20 000 Da for large compounds (Mena et al. 2015). More than 8000 different types of PP have been identified in plants (Cheynier 2005). They are very abundant in fruits and vegetables, and plant foods and beverages such as coffee, tea, nuts, olive oil, soy products or cocoa (Harborne and Williams 2000). In recent years, these compounds have attracted a lot of attention and have been widely investigated due to their potential health benefits (Del Rio et al. 2013). Epidemiological and clinical studies have shown that PP have the potential to modulate physiopathological conditions and thus reduce the risk of chronic diseases such as CVD and dementia (Vauzour et al. 2010; Corona, Spencer, and Dessì 2009; Zheng et al. 2017).

PP are one of the most abundant and major groups of phytochemicals, which also includes terpenoids –such as carotenoids–, alkaloids and sulphur compounds. They are synthesized by plants to protect them against UV-mediated oxidative stress and strengthen the cell wall, to repel herbivores and

infections, and to attract pollinators (Gunnaiah et al. 2012; Harborne and Williams 2000). PP are naturally found on their glycoside form and less frequently as an aglycone or genin (i.e., without the glycosyl moiety). They are classified into different groups based on the number of phenyl rings and their structure. Thus, PP can be divided in two major groups: flavonoid and non-flavonoids (Harborne 1995).

Flavonoids are chemically related to a 15-carbon skeleton structure, which consists of two phenyl rings, A and B, and one heterocyclic ring containing oxygen named C. This structure is also known as “C6-C3-C6”. Usually the B ring is bound to position n°2 of the C ring, but it can also be found in position n°3, as is the case of isoflavones (Del Rio et al. 2013). Flavonoids are divided in 7 sub-classes: flavones, flavanones, anthocyanins, flavonols, flavan-3-ols, isoflavones, and dihydrochalcones (Manach et al. 2004) (Figure 1.3).

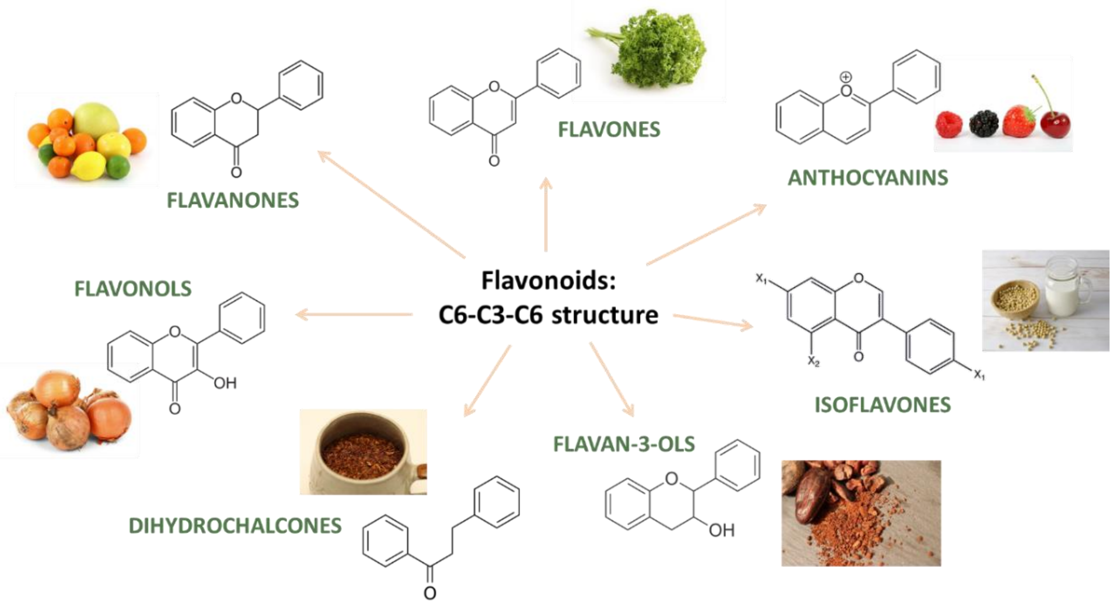


Figure 1.3: Main flavonoids structures and food sources examples.

Among flavonoids, flavones such as luteolin, apigenin or wogonin can be found in parsley and celery (Manach et al. 2004). Flavanones (naringenin,

hesperidin) are more specific to citrus fruits (Tomás-Barberán and Clifford 2000). Regarding isoflavones (genistein, daidzein), their sources are widely related to Asian culture, as a result of their abundance in products such as soya, miso or tofu (Franke et al. 1999). Furthermore, anthocyanins such as cyanidin, pelargonidin or malvidin derivatives are characteristic of blue/red fruits and vegetables, for example berries, red grapes or aubergines (Clifford 2000). Flavonols, such as quercetin, myricetin, isorhamnetin or kaempferol, can be found in onions, broccoli, but also in red wine, berries or tea (Crozier et al. 1997). Flavan-3-ols are found in cocoa, tea, berries or wine, where they can be found as monomers (catechin, epicatechin, epigallocatechin, or epigallocatechin gallate) but also as oligomers (proanthocyanidins) (Lakenbrink et al. 2000). Finally, dihydrochalcones are less represented in the diet compared with the other subfamilies. The main compounds are known as phloretin and asphalathin and have been identified in a few plant sources such as rooibos tea or apples (Stalmach, Mullen, Pecorari, et al. 2009).

The non-flavonoids family include stilbenes, lignans, phenolic acids, and other PP, such as tyrosols, pyrogallols, or hydroxycoumarins (Manach et al. 2004). Phenolic acids are divided into five subgroups, i.e. hydroxyphenylpropanoic-, hydroxyphenylacetic-, hydroxyphenylpentanoic-, hydroxycinnamic- and hydroxybenzoic- acids (Phenol Explorer) (Figure 1.4).

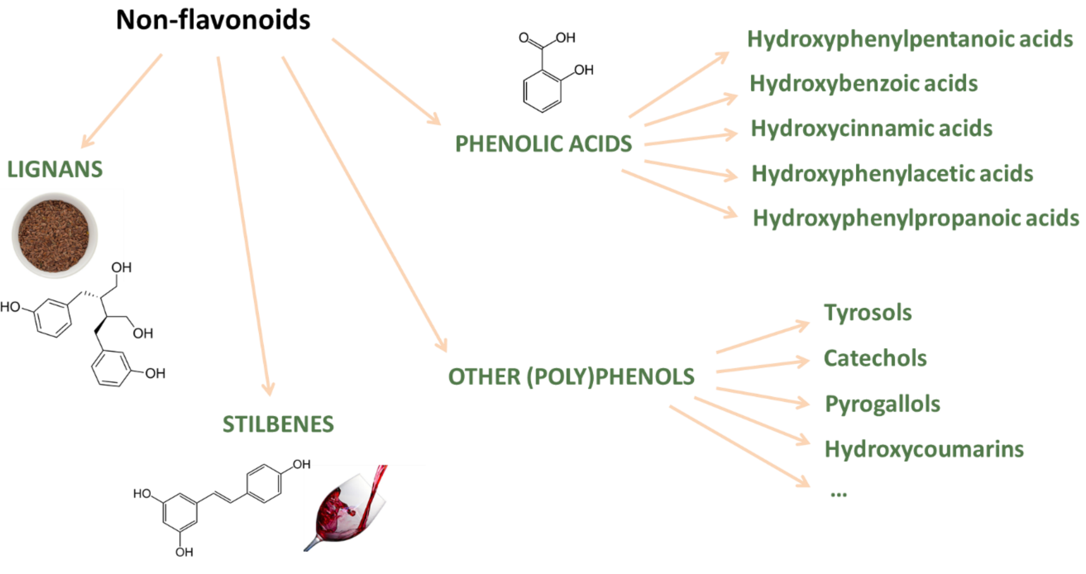


Figure 1.4: Main non-flavonoids classification and structures.

Stilbenes such as resveratrol or pterostilbene can be found in small amounts in grape, and as a result in wine, but also in peanuts (Vitrac et al. 2002). Lignans, and their precursor secoisolariciresinol diglucoside, can be identified in olive oil, sesame, pumpkin seeds or flaxseeds (Adlercreutz and Mazur 1997). Likewise, hydroxycinnamic acids, for example caffeic or ferulic acid, are found in various products, from coffee to wine, and from blueberries to wholegrains (Pérez-Jiménez et al. 2010). Similarly, the main hydroxybenzoic acid representative in plants, gallic acid, has been identified in many fruits, nuts, vegetables and seeds (Oroian and Escriche 2015). Chlorogenic acid, major compound of the hydroxycinnamic acids subgroup, is found in significant quantity in coffee, wine, and berries, which makes it one of the main phenolic acid consumed in the population (Naveed et al. 2018). Finally, ellagic acid is a monomer of hydroxybenzoic acid which polymerises into polymers called ellagitannins. Those ones are widely identified in raspberries, pomegranates and nuts (Landete 2011).

1.2.2 Absorption, distribution, metabolism, and excretion (ADME) of dietary (poly)phenols

Human feeding studies investigating the fate of PP in the body suggested that PP have a low bioavailability, with only 1 to 10% of the total amount ingested being excreted in urine (D'Archivio et al. 2007). However, recent human trials using isotopically labelled compounds and more sensitive analytical platforms indicate that the bioavailability of PP has been underestimated and that metabolites can remain in the circulation for more than 48h after consumption, before being excreted in urine, breath or via the bile (Czank et al. 2013). Recoveries in urine as high as 30-70% have been recently reported (Crozier, Del Rio, and Clifford 2010; Del Rio, Borges, and Crozier 2010; Stalmach et al. 2010). These findings are in line with new discoveries related to the role of the gut microbiota in catabolizing PP, leading to a wider range of absorbed metabolites which were previously not accounted for (Kay et al. 2017).

1.2.2.1 Absorption and distribution

Although highly variable depending on the compound, maximal concentrations of PP in plasma can be reached 30 minutes to 2-3 hours post-consumption for compounds absorbed in the small intestine (Del Rio et al. 2013; Beattie, Crozier, and Duthie 2005). PP not absorbed in the small intestine reach the colon and are metabolised by the gut microbiota, leading to a second wave of metabolites appearing at around 4 to 6 hours post-consumption and able to stay in the circulation for 48 hours or longer (Feliciano, Boeres, et al. 2016; Pimpao et al. 2015). Additionally, PP levels observed after the intake highly differ according to the nature of the compound and the food source. The

plasma concentrations of intact flavonoids rarely exceed 1 µM (D'Archivio et al. 2007), but gut microbial metabolites can reach higher concentrations (Kay et al. 2017).

Following their intake, PP are following the same metabolism pathway than xenobiotics and start their transformation when in contact with saliva (Zanotti et al. 2015). Going down the gastrointestinal tract, conjugated O-glycoside PP undergo the action of phase I metabolism enzymes and sugar moieties are cleaved enzymatically by the lactase phlorizin hydrolase (LPH), located at the brush border of the enterocytes, or the β-glucosidase (βG), located in the cytosol of epithelial cells of the small intestine (Del Rio et al. 2013; Spencer et al. 1999; Arora, Nair, and Strasburg 1998). LPH is known to present a strong affinity for flavonoid-O-β-D-glucosides, and the resulting aglycones present a raised lipophilicity which allow them to cross the enterocyte passively (Day et al. 2000). The second route operated by the cytosolic βG involves the active sodium-dependent glucose transporter 1 (SGLT1) to carry the PP in the enterocyte (Crozier, Del Rio, and Clifford 2010; Gee et al. 2000).

To sum up, two routes allow the hydrolysation of PP conjugates and the passage of their aglycones in enterocytes: passive diffusion with LPH and active transport via βG (Figure 1.5). However, not all PP follow this pathway, with the example of flavonoids bound to a rhamnose which must reach the colon to be hydrolysed by α-rhamnosidases enzymes secreted by the microbiota before being absorbed (Marín et al. 2015). Moreover, some PP of high molecular weight, such as proanthocyanidins or ellagitannins, cannot be absorbed in the small intestine due to their large structure. In fact, those

compounds can only be absorbed after being metabolised by the gut microbiota. While the majority of PP go through the process of deglycosylation, a few exceptions were found in the literature, e.g., for anthocyanins and flavonols, which have been found in urine and plasma in their glycoside form (Kay, Mazza, and Holub 2005; Hollman et al. 1997).

Phenolic compounds' bioavailability is also associated with some multidrug-resistance-associated proteins (MRP-1, MRP-2) which are active ATP-dependent transporters and sometimes called "phase III enzymes" (Leonarduzzi et al. 2010). While MRP-2 is located on the apical membrane of enterocytes and facilitate the entrance of PP in the lumen of intestinal cells, MRP-1 is located closer to the enterocyte capillaries to favour the access of the metabolites in blood (van Zanden et al. 2007; Singh et al. 2008). Moreover, literature reports that glucose transporter 2 (GLUT2) and multidrug-resistance-associated proteins 3 (MRP-3) are both implicated in the efflux of metabolites from the basolateral side of intestinal cells to the portal vein to then reach the hepatocytes of the liver (Manzano and Williamson 2010).

1.2.2.2 Phase II metabolism

After their absorption in the intestinal cells, PP metabolites undergo phase II metabolism, which will produce various conjugated compounds, namely methyl, sulfate and glucuronide metabolites. These phase II enzymes, located in the cellular endoplasmic reticulum, are known as catechol-O-methyltransferases (COMT), sulfotransferases (SULT) and uridine-5′-diphosphate glucuronosyltransferases (UGT), respectively (Manach et al. 2004; Del Rio et al. 2013). Indeed, COMT catalyses the transfer of a methyl

group ($-CH_3$) from S-adenosyl-L-methionine to the aglycone (poly)phenol on the C3 or the C4 position of the B ring of the PP. Moreover, while SULT catalyse the transfer of a sulfate unit from 3'-phosphoadenosine-5'-phosphosulfate to a hydroxyl (-OH) group on PP, UGT enzymes catalyse the reaction of glucuronidation, by transferring a glucuronic acid from UDP-glucuronic acid to the aglycone, more likely on the carbon number 7 of the A ring of flavonoids, although it can also go in other available positions (Manach et al. 2004). Several transformations can also occur at the same time for a specific compound, as it has been confirmed by the discovery of sulfo-glucuronide metabolites of quercetin in the rat (Kawai et al. 2009).

Hepatic compounds can then be distributed via the systematic circulation to tissues, where they will exert their biological action. There is very little known regarding the metabolic pathways involved in the liver. However, it has been reported that plasma PP are diverse, mostly hydrophilic, and rarely found under their aglycone form (Santhakumar, Battino, and Alvarez-Suarez 2018). Hepatic metabolites can use the biliary excretion to be secreted *de novo* in the intestine, where they will undergo another deconjugation by the intestinal enzymes and reabsorbed another time. This phenomenon, called enterohepatic circulation, may contribute to the high half-life of some PP found in plasma (Adlercreutz et al. 1987).

1.2.2.3 Gut microbial metabolism

Only 5 to 10% of the total dose of PP ingested are absorbed by the enterocytes following deglycosylation process (Cardona et al. 2013). The remaining fraction (90-95%) and the native conjugated compounds will cross the gastrointestinal conduct to end up accumulating in the colon, where they will undergo the action of microbiome's bacterial enzymes which results in the creation of a new variety of catabolites (Bowey, Adlercreutz, and Rowland 2003). These enzymes can cleave large and complex structures into smaller molecules which eases their absorption (Santhakumar, Battino, and Alvarez-Suarez 2018). Indeed, gut microbiome enzymes can break the glycosidic linkages as well as the heterocyclic backbone of metabolites which creates a whole different range of low molecular weight compounds such as phenolic acids and hydroxycinnamic acids (Aura et al. 2005). Microbiome enzymes include hydrolases, reductases, decarboxylases, demethylases, and act to reduce the size and the complexity of the (poly)phenolic metabolites (Zanotti et al. 2015; Selma, Espín, and Tomás-Barberán 2009). The majority of the colonic catabolites will be able to reach the systemic circulation where they can be distributed to various peripheral organs and tissues before being eliminated by the kidneys via the urine (Rodriguez-Mateos, Vauzour, et al. 2014).

A summary of the different steps involved in the absorption, distribution and metabolism can be found in Figure 1.5 below.

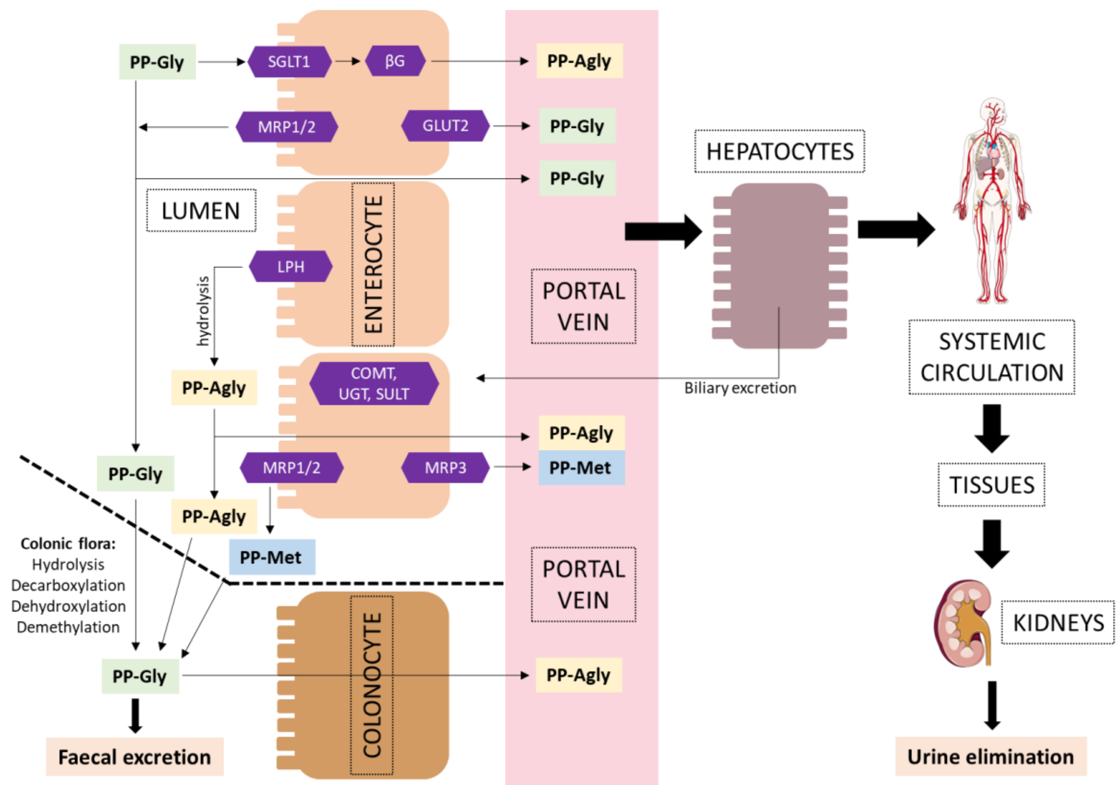


Figure 1.5: Mechanisms of ADME of (poly)phenols.
βG, cytosolic β-glucosidase; COMT, catechol-O-methyl transferase; GLUT2, glucose transporter; LPH, lactase phlorizin hydrolase; MRP1-2–3, multidrug-resistant proteins; PP-Agly, (poly)phenol aglycone; PP-Gly, (poly)phenol glycoside, PP-Met, (poly)phenol sulfate/glucuronide/methyl metabolites; SGLT1, sodium-dependent glucose transporter; SULT, sulfotransferase; UGT, uridine-5'-diphosphate glucuronosyltransferase. Adapted from Santhakumar and colleagues (2018).

1.2.2.4 Excretion

After many cycles of metabolism along the intestinal tract, unabsorbed PP are excreted from the body in faeces (Scalbert and Williamson 2000). This constitutes the faecal excretion of PP, which is one of the exit routes of phenolic metabolites along with the urinary elimination, and which mainly apply to high molecular weight compounds. On the contrary, the urinary pathway will be followed by smaller metabolites which, after being distributed to peripheral tissues, will be excreted via the kidneys (Crozier, Del Rio, and Clifford 2010). Maximal urine PP concentration following small intestine metabolism is often reached within 2 to 4 hours after ingestion (Jin et al. 2011), while for gut microbial metabolites can be 6 to 48 hours (Parkar, Trower, and

Stevenson 2013; González-Sarrías, Espín, and Tomás-Barberán 2017). Percentages of urinary excretion based on the intake vary according to the nature of the compound, from 0.005 to 0.1% of intake for intact anthocyanins to 16-66% of intake for the isoflavone daidzein (Manach et al. 2004). As PP are metabolised in a similar manner compared to xenobiotics, they will not accumulate in the body but will instead undergo a turnover. This indicates the importance of regular intake of phenolic compounds to maintain a high plasma concentration of PP.

1.2.2.5 Next steps in the understanding of PP ADME

As explained earlier, the bioavailability of PP appear be to higher than previously thought (Kay et al. 2017). For instance, a human study using isotopically labelled (–)-epicatechin proved that 82 ± 5% of ingested epicatechin was absorbed and highlighted gut microbiome as a key driver of the metabolism of epicatechin, as around 70% of the ingested isotopes were found absorbed in the lower intestine (Ottaviani et al. 2016). Moreover, when quantifying metabolites' excretion following acute consumption of orange juice, Pereira-Caro and colleagues reported that all urinary catabolites that significantly increased following the intervention were products of the gut microbial breakdown and that these metabolites represented 88% of the initial PP intake (Pereira-Caro et al. 2014).

Understanding the metabolism and bioavailability of PP is key when studying the potential physiological benefits of these molecules. Indeed, many metabolites may still be unknown and, as a result, not targeted by the metabolite analysis on biosamples. The development of studies focusing on

PP bioavailability using radiolabelled compounds in human will help us to expand our knowledge of plant bioactives' ADME.

1.2.3 Effects of PP on CVD: evidence from the literature

There is strong epidemiological evidence showing that PP-rich foods, such as fruit and vegetables, nuts, and wholegrains present beneficial effects on CVD risk, as discussed in section 1.1.4., prospective studies have also investigated the impact of PP consumption on CVD risk. For example, a study conducted on 100 000 elderly men and women found an association between total flavonoid intake and a 18% reduction of the risk of CVD-related mortality (McCullough et al. 2012). Another 16-year follow-up prospective study including more than 35 000 post-menopausal women indicated that increased consumption of anthocyanins was associated to a reduction by 10% of the CVD mortality (Mink et al. 2007). Similarly, higher consumption of anthocyanins was associated with a 32% reduction risk of myocardial infarction in 100 000 young and middle-aged women followed-up after 18 years (Cassidy et al. 2013).

Several systematic reviews have focused on the association between flavonoid intake and CVD risk and CVD mortality. For example, a systematic review including 15 prospective studies demonstrated a CVD risk reduction of 14% which was linked to the participants having the highest daily flavonoid intake (Kim and Je 2017). The authors also performed a subgroup analysis and revealed that flavones, flavan-3-ols, anthocyanidins, flavanones and proanthocyanidins were inversely associated with CVD mortality by 11 to 14%. Similarly, another systematic review of 10 prospective studies investigating the

association between flavonoid consumption and CVD- and all cause-mortality revealed that subjects with the higher intake were presenting a 18% decreased risk for all-cause mortality, as well as a 15% reduced risk for death from CVD (Liu et al. 2017). Authors reported that a dose-response effect was present for studies providing 200 mg of flavonoids per day, as this dose was associated with the lowest risk of all-cause mortality. This was in line with the meta-analysis by Grosso and colleagues which reported a reduced risk of 26% of all-cause mortality associated with a high flavonoid intake (Grosso et al. 2017), or the review by Jiang et al suggesting that high flavonoid consumption was related to a 15% decrease in CVD risk (Jiang, Wei, and He 2014).

Some systematic reviews based on epidemiological studies have also focused on specific families of flavonoids or individual compounds. For instance, a systematic review summarizing the findings of 23 prospective studies evaluated the association between soy isoflavones intake and CVD risk and risk of mortality. Authors reported that subjects with the highest intake of soy isoflavones presented a 10% reduction in risk of all-cause mortality compared with participants with low consumption (Nachvak et al. 2019). Another recent review by Kimble et al reported a reduction in CVD mortality of 8% following anthocyanin supplementation but did not show any impact on the risk of myocardial infarction or total CVD (Kimble et al. 2019). Finally, a systematic review of 23 studies investigating the impact of flavan-3-ol consumption on CVD risk reported an association of the intake with a 13% decrease in CVD risk (Raman et al. 2018). However, all these promising findings have to be considered with caution, as a few epidemiological studies have not been able to show a significant association between the intake of PP and a potential

reduction of the CVD risk (Hertog, Feskens, and Kromhout 1997; Lin et al. 2007; Rimm et al. 1996) (Sahebkar 2017). These discrepancies can be due to the difficulty behind the assessment of the general population's diet, and in particular the assessment of PP intake.

Meta-analysis of RCTs also reported the importance of PP supplementation on CVD risk, using well established surrogate markers of CVD risk such as BP, endothelial function, or blood lipid levels. Indeed, a recent meta-analysis investigating the effects of PP on cardiovascular disorders in 34 studies noted a significant improvement of SBP, DBP, LDL cholesterol and FMD (-1.01 mmHg, -2.04 mmHg, -4.39 mg/dL and 0.89%, respectively) following the PP administration (Potì et al. 2019). However, this analysis did not consider the different classes of PP that could potentially be related to the significance of the effect. In their investigation of the influence of flavan-3-ol consumption on CVD risk, Raman and colleagues studied the strength of the relationship in a meta-analysis of 156 RCTs and showed significant improvement in BP (-1.46 and -0.99 mmHg for SBP and DBP, respectively), FMD (1.21%) and blood lipid levels (-0.07 and 0.03 mmol/L for LDL and HDL cholesterol, respectively) (Raman et al. 2019). Another meta-analysis reporting on the effect of PP-rich olive oil on CVD risk observed some significant improvement in oxLDL and total cholesterol levels, of -0.44 μmol/L and -4.5 mg/dL, respectively (George et al. 2019).

Regarding meta-analysis focusing on specific PP families, we can cite a meta-analysis by Yang and colleagues reporting the effects of anthocyanins on lipid profiles in 32 RCTs and showing a significant reduction of total and LDL

cholesterol following supplementation, of -0.33 and -0.35 mmol/L, respectively (Yang et al. 2017). Similarly, meta-analysis investigating the impact of cocoa flavan-3-ol consumption on cardiometabolic biomarkers and including 19 RCTs revealed a significant improvement in blood lipids levels (triglycerides and HDL cholesterol) and C-reactive protein (CRP) after daily consumption for 2 to 52 weeks (-0.10 nmol/L for triglycerides, 0.06 mmol/L for HDL cholesterol and -0.83 mg/L for CRP levels) (Lin et al. 2016). Furthermore, the effects of quercetin on BP were investigated in a meta-analysis by Serban and colleagues. Authors showed significant improvement in SBP and DBP, of -3.04 and -2.63 mmHg respectively, following supplementation with quercetin (Serban et al. 2016). It was reported that there was a BP-reducing dose effect in RCTs involving supplementation of doses higher than 500 mg/day, which was not found in trials designed with a dose lower than 500 mg/day. Finally, a recent meta-analysis of 28 RCTs observing the effects of isoflavone consumption on CVD risk reported some significant improvement in FMD, SBP and DBP, of 1.98%, -5.47 mmHg and -2.03 mmHg, respectively following the intake of the soy PP (Li, Wu, et al. 2020). The effect of anthocyanins on BP is currently unclear, as 2 meta-analysis did not report any significant improvement in SBP or DBP (Ellwood et al. 2019; Zhu et al. 2016). Authors mentioned that this inconclusive result might be related to the small sample sizes and single-centre trials involved in the analysis.

Two large databases have been created to standardize and report the PP content of plant-based foods and to avoid any bias caused by the high variability of reporting flavonoid content. Phenol-Explorer (Neveu et al. 2010) and the US Department of Agriculture (USDA) database for flavonoid content

in foods (USDA 2014) both gather published and recognized data related to PP content and based on specific analytical methods such as high-pressure liquid chromatography-mass spectrometry (HPLC-MS). Nevertheless, these databases are only approximate as the PP content of fruits and vegetable is greatly associated to the differences in varieties and processing, to the season of harvest to even storage conditions. These two databases are also limited by the accuracy of the reporting. Indeed, the items listed in the various food frequency questionnaires based on Phenol-Explorer and USDA lack in precision, which can induce a high degree of error in the measurement and thus the outcomes of nutritional studies (Zamora-Ros et al. 2014).

In addition to these risks of bias induction present in the current methods of PP quantification, it is important to note that other approaches have started to develop in the recent years to estimate more accurately the intake and metabolism of phenolic compounds. This is the case for the use of biomarkers of PP consumption in biosamples, such as plasma and urine. This technique is thought to be more objective as it reflects directly the “bioavailable” PP exposure levels and is not impacted by the inaccuracy of self-reporting. A biomarker of PP intake is a useful tool to validate a dietary assessment method (Krogholm et al. 2010). However, this new technique presents some limitations such as the necessity of using complex and expensive analytical techniques and the very few validated accessible and efficient methods available (Zamora-Ros et al. 2014). Results from this analytical methodology also depend greatly on the reliability and accessibility of authentic standards. Furthermore, PP present a very short half-life which could obstruct the capacity to represent the usual diet (Guasch-Ferré, Bhupathiraju, and Hu

2018). On a larger picture, another limitation of nutritional biomarkers can be attributed to the difficulty of food source identification as PP are rarely specific to a single fruit or vegetable (Kuhnle 2012). Nevertheless, this new analytical approach for dietary assessment might, along with a better and more accurate use of food databases and recalls, represent the future of nutritional science and are paramount to correlate the health benefits with the intake of phenolic compounds.

1.2.4 Cardiovascular mechanisms of action of PP

1.2.4.1 Empirical antioxidant capacity of PP

The expression “oxidative stress” appeared for the first time in a publication in 1970 (Paniker, Srivastava, and Beutler 1970). Fifteen years later, it was defined as a source of cell and tissue damage caused by the discrepancy between oxidant and antioxidant concentrations (Sies and Cadenas 1985). More recently, the literature has characterised this concept as a an imbalance between cellular oxidoreduction signalling and control (Jones 2006).

As a result of findings emanating from epidemiological studies in the 90s highlighting the associations between flavonoid-rich diets and decreased mortality (Hertog et al. 1993), an over-simplification of the oxidative stress theory was used as the potential mechanism of action regarding the biological effects of PP (Ghezzi et al. 2017). Indeed, as PP demonstrated antioxidant effects *in vitro*, it was believed that beneficial physiological effects in humans were related to this antioxidant hypothesis (Dragsted 2003; Rice-Evans 1995). This concept was followed by the development of several assays to assess the antioxidant capacities of beverages and foods *in vitro*, such as the

2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, the Oxygen Radical Absorbance Capacity (ORAC) assay, or the Trolox Equivalent Antioxidant Capacity (TEAC) assay (Litescu, Eremia, and Radu 2010).

However, with the advancement of the knowledge related to oxidative stress, as well as the growing scientific literature linked to the complex ADME and bioavailability of phenolic compounds *in vivo*, the idea of PP beneficial effects of health to be associated merely to their antioxidant potential has been abandoned over the years (Ruskovska, Maksimova, and Milenkovic 2020). Hollman and colleagues nicely summarized in their reviews that, based on the evidence available, the hypothesis that the direct antioxidant properties of PP can explain their positive health effects has to be rejected due to the poor absorption of phenolic compounds and the extensive metabolism of these ones into derivatives inducing the invalidation of potential antioxidant entities (Hollman 2014). Due to the low concentration of PP in plasma compared to well-known antioxidants such as vitamin C or uric acid, the relevance of PP as direct antioxidant to improve cardiovascular health is likely to be irrelevant.

Nowadays, a more plausible explanation is that the biological activity of PP is exerted through the modulation of several pathways mostly expressed through transcription genes modulation, leading to an enhanced antioxidant protection against the development of CVD (Yahfoufi et al. 2018). In three decades, PP went from simple antioxidant molecules to complex metabolites and signalling bioactive compounds (Croft 2016).

Although the literature related to the antioxidant activity of PP *in vitro* is abundant, RCTs with relevant biomarkers of oxidative stress have failed to

demonstrate such an action *in vivo* (Halliwell, Rafter, and Jenner 2005; Hollman et al. 2011). One reason could be that phase II metabolism occurring *in vivo* leads to a decrease of the antioxidant capacity of metabolites (Pollard et al. 2006). Another reason behind this divergence in findings could be, along with the common use of non-metabolised PP, the high and non-physiologically relevant doses employed in these *in vitro* studies, which can be up to 100 000 times higher than biological plasma concentrations (Manach et al. 2004). Moreover, animal studies do not account for the differences in metabolism between the investigated species and humans, which are substantial. For example, Borges et al demonstrated that the metabolism of epicatechin in rats is very different to humans as they reported a profile of structural-related (–)-epicatechin metabolites widely divergent between the 2 species (Borges et al. 2018). The next section describes the latest findings regarding the biological effect of PP on vascular health.

1.2.4.2 Indirect antioxidant activity in the endothelium

1.2.4.2.1 The Akt/eNOS pathway

One of the many mechanisms linked to the potential of PP to improve cardiovascular health is the ability to modulate the levels and activity of nitric oxide (NO). PP can interact with signalling pathways involving kinases such as phosphatidylinositol 3-kinase (PI3-kinase)/Akt and eNOS, leading to an increase in NO production (Vauzour et al. 2010) (Figure 1.6). NO is implicated in signalling pathways and can mediate various physio-pathological functions such as neuromodulation, cytotoxicity and, especially, vasodilation. Indeed,

this efficient vasodilator leads to the relaxation of vessels both *in vivo* and *in vitro* (Faraci and Heistad 1998).

As stated earlier, NO produced by eNOS is an essential actor of cardiovascular homeostasis which promotes, among others, blood flow and vascular smooth muscle cells' relaxation (Ghimire et al. 2017). Additionally, FMD as a surrogate marker of CVD risk is widely dependent on eNOS activity (Sies 2010). After its release by the endothelium, NO stimulates the soluble guanylate cyclase (sGC) in vascular muscle cells, leading to a rise of the intracellular concentration of cyclic guanosine 3′,5′-monophosphate (cGMP) and to relaxation. In fact, cGMP can activate the protein kinase G (PKG), which can then induce the phosphorylation of other proteins to finally induce vasodilation (Bian and Murad 2014). NO may also react with a superoxide anion to form peroxynitrite ($ONOO^-$), a strong oxidant that can lead to protein and DNA damage. As NO reacts with superoxide anion $O_2^{\circ-}$ faster than the rate of dismutation and inactivation of $O_2^{\circ-}$ by superoxide dismutase (SOD), this concentration of SOD can be a significant determinant of the NO bioactivity (Faraci and Didion 2004).

The endothelial NOS is stimulated via the PI3-kinase pathway and can be induced by various factors such as insulin-like growth factor-1 (IGF-1) or vascular endothelial growth factor (VEGF). The serine/threonine protein kinase Akt, also known as PKB, is an important target of PI3-kinase. Furthermore, Akt can directly phosphorylate eNOS, which activates the enzyme and leads to NO production (Shen et al. 2012). Some publications have shown that (poly)phenolic compounds can lead to a modulation of

endothelial NO production via kinases signalling pathways such as this PI3-kinase/Akt pathway (Fairlie-Jones et al. 2017; Varela et al. 2016). It has been shown that resveratrol, a stilbene found in small amounts in grapes, is able to increase the eNOS expression in human endothelial cells by upregulating its expression and stimulating its enzymatic activity (Li et al. 2019). However, the amounts used in this cell work (μM range) are not physiologically relevant as resveratrol is mainly metabolised and found in its native form in ranges lower than 40 nM. Another publication highlighted that S-(–)equol, a gut microbial metabolite of daidzein, can activate the PI3-kinase/Akt pathway (Zhang et al. 2013). Similar effects were observed for genistein (Grossini et al. 2015).

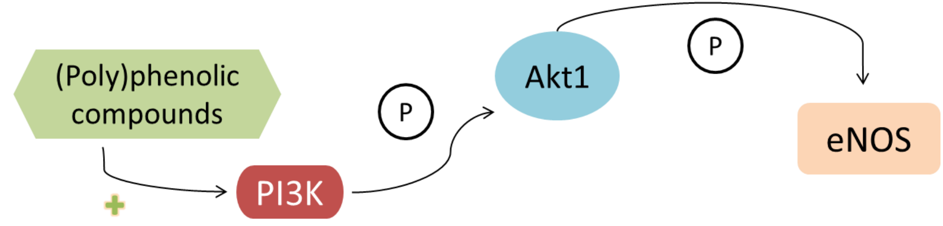


Figure 1.6: Akt/eNOS signalling pathway. P represents the process of phosphorylation. PI3K, phosphatidylinositol 3-kinase; Akt, protein kinase B; eNOS, endothelial nitric oxide synthase.

1.2.4.2.2 The NOX pathway

NADPH oxidases (NOX) family is composed of five isoforms and two connected enzymes. Only three isoforms are expressed in the vascular system: NOX1, NOX2 and NOX4 (Katsuyama 2010). NOX enzymes generate $O_2^{\circ -}$ from O_2 using NADPH as an electron donor. NOX2 is the predominant isoform in endothelial cells (Schröder 2010). Indeed, some experiments have shown that when NOX2 is mutated and inactivated, it leads to some impairment in oxidative stress markers as well as improved endothelial vasodilation (Violi et al. 2009). These data suggest that NOX2, when

expressed normally, is associated to an altered vascular function. In fact, when superoxide radicals produced by NOX2 interact with NO to generate peroxynitrite, it decreases the stock of NO present in the cell. As a result, NO is not able to exert its vasodilative properties (Steffen, Schewe, and Sies 2007). To illustrate, a schematic representation of the link between NOX and NO pathway is presented in the Figure 1.7 below.

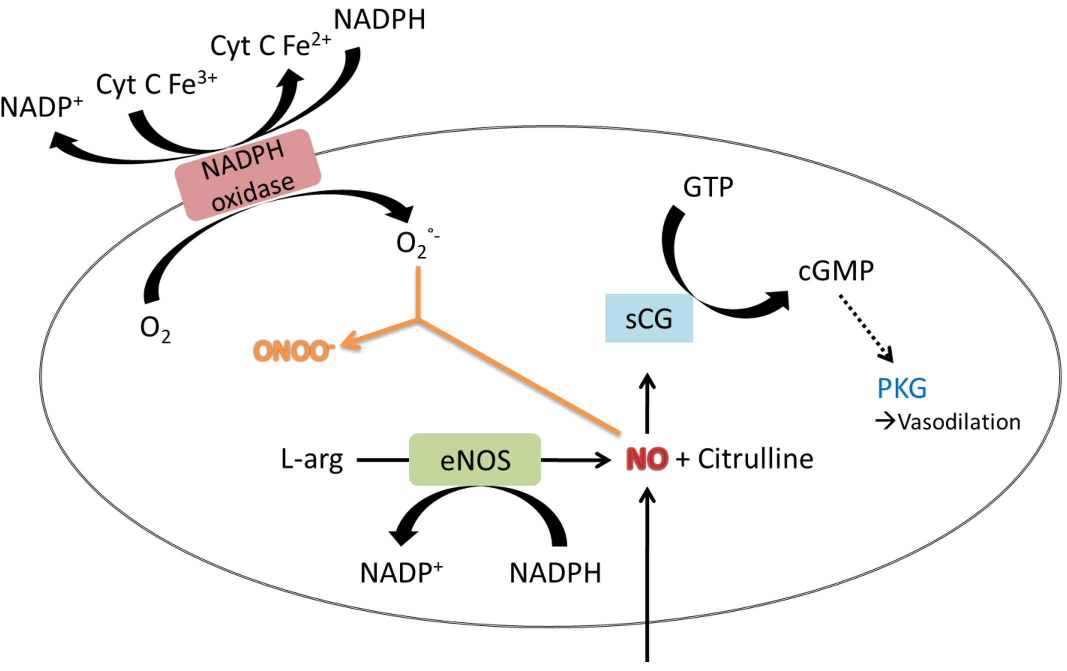


Figure 1.7: Summary of the signalling pathways occurring in the phenomenon of vasodilation. L-arg, L-arginine; NO, nitric oxide; eNOS, endothelial nitric oxide synthase; sCG, soluble guanylyl cyclase; cGMP, guanosine 3',5'-cyclic monophosphate; GTP, Guanosine triphosphate; PKG, phosphokinase G.

1.2.4.2.3 The Nrf2 pathway

The activation of the nuclear E2-related factor 2 (Nrf2) receptor has arisen as a potential target for PP in the recent literature (Satta et al. 2017). The endothelial Nrf2 can be triggered via increased reactive oxygen species production and PI3K-Akt signalling induced by shear stress (Chen et al. 2003).

When laminar shear stress is perturbed at curved sections of arteries, the bioavailability of NO decreases, leading to an increase of the $O_2^{\circ-}$ levels (Hosoya et al. 2005) and a decrease of Nrf2-activated genes, which results in a predisposition of the endothelium to atherogenesis (Cheng, Siow, and Mann 2011). The Nrf2 pathway is thus considered as a key modulator of the oxidant/antioxidant balance and it has been shown that (poly)phenolic compounds could induce a response in targeted cells, leading to the activation of several defence enzymes such as glutathione-S-transferase, haem oxygenase 1, quinone oxidoreductase 1, and glutamyl-cysteine ligase (Satta et al. 2017).

1.2.4.2.4 Concluding remarks on molecular mechanisms

In the last decades, our knowledge of the molecular mechanisms behind the biological action of PP has substantially grown. While the Akt/eNOS, NOX and Nrf2 pathways are the main models studied in the literature, it has been shown that phenolic compounds can modulate various cellular functions indirectly related to the redox balance regulation, such as inflammation or endothelial dysfunction (Ruskovska, Maksimova, and Milenkovic 2020). On top of their action at a transcriptional level, literature recently highlighted the potential of PP to exert some post-transcriptional regulation of genes through the modulation of non-coding RNA's expression, called microRNAs, and which behave as gene regulators (Milenkovic et al. 2012; Milenkovic, Jude, and Morand 2013). Indeed, PP have been shown to modulate more than 100 microRNA (Krga et al. 2016). However, the few studies focusing on this aspect have used cellular models and, more rarely, animal models, which indicates

the need for more studies to validate the impact of phenolic compounds on gene targets.

1.2.5 Beneficial effects of PP on gut microbiota

Often considered as a "second brain", the microbiome is a large ecosystem composed of trillions of bacteria which interacts with the whole organism during all the life of an individual (Vaiserman, Koliada, and Marotta 2017). As explained previously, gut microbiota exerts an important role in the bioavailability of PP as the presence of high molecular weight compounds associated with the relatively low absorption of phenolic compounds in general favour the interaction of the latter with colonic bacteria (Marchesi et al. 2016). When reaching the large intestine, a bidirectional relationship will develop between PP and microbiota. Indeed, while PP can modulate the composition and diversity of the microbiome, intestinal bacteria catabolize PP to produce smaller compounds that are usually more active and which present a better absorption than the original metabolite (Espin, Gonzalez-Sarrias, and Tomas-Barberan 2017). In the following section, we will only discuss the effects of PP on gut microbiome, as gut microbial metabolism has already been discussed in section 1.2.2.3.

1.2.5.1 Prebiotic effect of PP

By definition, a prebiotic is "*a non-digestible compound that through its metabolization by microorganisms in the gut, modulates the composition and/or activity of the gut microbiota, thus conferring a beneficial physiological effect on the host*" (Bindels et al. 2015). As a result of this bidirectional connection between gut microbiota and PP, these latter have the potential to

modify the human colonic microflora to exert a “prebiotic-like” action (Kennedy 2014; Fogliano et al. 2011; Oteiza et al. 2018; Tomas-Barberan, Selma, and Espin 2016). For example, tea PP, including catechin and its derivates, were shown to inhibit the development of detrimental bacteria such as *Staphylococcus aureus*, *Salmonella typhimurium*, *Helicobacter pylori* or *Escherichia coli* (Duda-Chodak et al. 2015). Some other compounds on the other hand, such as anthocyanins or hydroxybenzoic acids, we able to promote the development of some beneficial bacteria such as *Lactobacillus* spp., *Bifidobacterium* spp., or *Faecalibacterium prausnitzii* which is responsible for the production of the short chain fatty acid butyrate, in human clinical trials (Vendrame et al. 2011; González-Sarrías et al. 2018). Improvement in the microbiome composition has also been correlated to beneficial changes in cardiovascular markers in human subjects. For instance, 4-week consumption of cocoa flavan-3-ols significantly increased the abundance of *Lactobacillus* spp. and *Bifidobacterium* spp., while significantly decreased the counts of *Clostridium histolyticum* (Tzounis et al. 2011). Authors also reported that these changes in microbiome were correlated with reductions in plasma triglycerides and CRP. These results are in line with another study evaluating the effect of red wine PP consumption for 4 weeks on microbiome and clinical biomarkers and which observed a significant improvement in cholesterol and CRP which was correlated with the changes in *Bifidobacterium* count (Queipo-Ortuno et al. 2012). Moreover, a previous RCT led by our team and studying the impact of 12-week consumption of aronia extract in healthy young men showed a significant increase in the

butyrate-producer *Anaerostipes* and a correlation between FMD and *Dialister*, *Phascolarctobacterium* and *Roseburia* (Istas et al. 2019).

Furthermore, in a recent systematic review investigating the prebiotic effect of PP and including 22 animal studies, abundances of *Akkermansia*, *Roseburia, Bifidobacterium* and *Faecalibacterium* were significantly increased following the intake of tea flavan-3-ols, soy isoflavones, naringenin, lignans, stilbenes and phenolic acids by mice and rats (Gwiazdowska et al. 2015; Jin et al. 2012; Koutsos et al. 2017; Derrien, Belzer, and de Vos 2017; Eppinga et al. 2016; Alves-Santos et al. 2020)

Evidence suggests that specific PP, at certain concentrations, can exert their "prebiotic-like" effect to influence the *Firmicutes/Bacteroidetes* balance (Stoupi et al. 2010; Lee et al. 2006; Hervert-Hernández et al. 2009; Remely et al. 2015). Indeed, this ratio has been observed to be an indicator of health, as a higher ratio has been associated with type 2 diabetes and obesity (Cani and Everard 2016). However, recent findings contradict this theory as it has been reported that the interindividual variability in relative abundance of *Bacteroidetes* and *Firmicutes* is high and influenced by lifestyle factors such as physical activity or diet, which could explain the contradiction in the results observed in normoweight and obese participants (Magne et al. 2020). Another study investigating the variations of microbiome composition according to age highlighted the substantial modulation of phyla abundance through life, with a *Firmicutes/Bacteroidetes* ratio increasing with age independently from gender (Vaiserman et al. 2020).

1.2.5.2 *Effects of gut microbiota on PP metabolism*

As previously stated, the gut microbiota can transform the intact PP reaching the colon into smaller compounds with an enhanced bioavailability. The main reactions occurring in the large intestine are reductions, cleavage, and hydrolysis (Espin, Gonzalez-Sarrias, and Tomas-Barberan 2017). As bioavailability of phenolic compounds is tightly related to their range of biological effects, the wide spectrum of compounds generated by the microbiome catabolism has been considered more deeply by bioavailability studies in the last decade. This is how some metabolites have been identified to be products of the microbial metabolism, with the examples of urolithins (derived from ellagitannins), enterolactones and enterodiols (produced from lignans), equol (coming from isoflavones catabolism) or valerolactones (derived from catechin and proanthocyanidins) (Tomas-Barberan, Selma, and Espin 2016). In this context, the notion of metabotype has been developed, referring to a "*metabolic phenotype with specific gut microbiome-derived metabolites that characterize the metabolism of the parent compound*" (Espin, Gonzalez-Sarrias, and Tomas-Barberan 2017).

This implies that a gut metabotype is characterized by both composition and activity of the microbiome and neo-metabolised compounds. This concept of metabotypes is an important discovery in the field of personalised nutrition and is knowing an increasing interest (Palmnäs et al. 2020; Espin, Gonzalez-Sarrias, and Tomas-Barberan 2017). For instance, 3 urolithin metabotypes of ellagitannins can be classified in the general population and have been related to the presence of specific bacteria such as *Gordonibacter urolithinfaciens* and

Ellagibacter isourolithinfaciens (Selma et al. 2014; Selma et al. 2018; Tomas-Barberan et al. 2014). Metabotyping has also been reported for isoflavones as the presence of equol producer and equol non-producer metabotypes among the population has been recently discovered, while only equol producers appear to undergo the beneficial cardiometabolic effects following the intake of isoflavones (Frankenfeld 2017). Indeed, some metabotypes have been found more frequently in patients with specific diseases leading to a microbiome dysbiosis such as metabolic syndrome or obesity (Tomas-Barberan, Selma, and Espin 2016). Moreover, dysbiosis of intestinal microbiome has been linked to the development of various chronic diseases, such as CVD (Ridaura et al. 2013; Masumoto et al. 2016). As a result, investigating the relationship between PP metabolites and gut microbiota could lead to significant beneficial findings in the field of health management (Oteiza et al. 2018; Tomas-Barberan, Selma, and Espin 2016; Fraga et al. 2019).

It has been shown that interindividual variability in PP intake as well as in gut microbiome quality can lead to variation in the bioavailability and the biological efficacy of PP (Bolca et al. 2010; van Dorsten et al. 2010). The interindividual variations observed in the gut can be related to environmental factors (diet, lifestyle, exercise, physiopathological status), but also to genetic factors (Arumugam et al. 2011).

To sum up, gut microbiota plays a very important part in the bioavailability of PP and ultimately, in their biological outcome. However, the evidence to support these beneficial effects of phenolic compounds derived from the gut

microbiota metabolism and the mechanisms involved is still unclear. There is a need for larger trials investigating the impact of PP supplementation on the composition and diversity of the gut microbiota, but also extensive bioavailability studies to look into the potential of bacterial metabolism-derived compounds for cardiovascular health. The relationship between interindividual variability in response to PP intake and microbiome metabolism needs to be further investigated.

1.2.6 The growing interest for berry PP

A growing number of human studies and meta-analysis are showing the health benefits associated to the consumption of berries, especially because of their high flavonoid content, in particular in anthocyanins (Rodriguez-Mateos, Heiss, et al. 2014; Wood et al. 2019; Miller, Feucht, and Schmid 2019). More recently, the relationship between berry PP consumption and gut microbiome benefits has been investigated (Lavefve, Howard, and Carbonero 2020). Indeed, as described in section 1.2.5., although berry PP often present a poor bioavailability and a complex structure, the products of their extensive metabolism by the intestinal bacteria are thought to vastly increase their ability to reach the systemic circulation and their bioactivity.

1.3 Effects of aronia berry PP on cardiovascular health

1.3.1 Introduction to aronia berry

1.3.1.1 Plant characteristics

Aronia melanocarpa (aronia) is a berry belonging to the *Rosaceae* family (Kokotkiewicz, Jaremicz, and Luczkiewicz 2010). Also known as “black chokeberry” due to its astringent taste, the berry is originally native from Northern America. However, aronia is nowadays found and cultivated in Central and Eastern Europe (USDA), and, since its introduction, various cultivars have been created, such as Nero, Viking or Aron (Ireneusz Dariusz, Jozef, and Milosz 2012) which present bigger berries and a better resistance compared to the original cultivar (Leonard 2013). Aronia shrub can measure up to 2 meters, and presents white flowers during spring, before developing berries during summer, which will mature as small dark berries in autumn. Aronia berries are slightly smaller than blueberries, with a weight of up to 2 grams (Popescu 2018). The bush is easy to cultivate and can produce up to 6 kg of berries each year (Sidor and Gramza-Michałowska 2019; SkupieD, Ochmian, and Grajkowski 2008). Due to the strong astringent taste of aronia, berries are more commonly consumed in a processed from such as jam, juice, or fruit wine (Oszmianski and Wojdylo 2005).

Aronia berries have been ancestrally used to treat various ailments. Indeed, Native Americans commonly used them to relieve the symptoms of cold (Kokotkiewicz, Jaremicz, and Luczkiewicz 2010) and Russians employ them

in the treatment of hypertension (Buda et al. 2020). Its interest has been blooming over the last decade, notably after the listing of aronia with the “antioxidant effect” health claim by the European Food Safety Authority (EFSA) in 2010 (Efsa Panel on Dietetic Products and Allergies 2010). While many studies have investigated the health benefits of *Aronia melanocarpa*, no toxicity or unwanted adverse events have been reported following the consumption of aronia berry products in the few studies focusing on this aspect of food safety (Kokotkiewicz, Jaremicz, and Luczkiewicz 2010; Chrubasik, Li, and Chrubasik 2010).

1.3.1.2 Micro- and macro-nutrients content

Dry matter of aronia berries represents between 15.3 and 30.8% of the berry’s weight (Sidor and Gramza-Michałowska 2019). For 100g of fruit was reported a protein content of 3.7g, a carbohydrate content of around 14g, 0.09 to 0.17g of fat, up to 1g of soluble fibres and 4 to 5.25g of insoluble fibres (Skrede et al. 2012). Although the sugar content of aronia is average when comparing with other berries, it is notably high in sorbitol, an alcohol sugar with a laxative effect which is more slowly absorbed and metabolised than glucose (Efsa Panel on Dietetic Products and Allergies 2011). The sugar to acid ratio of the berry is similar to the one of cranberry and blackberry, and aronia berry pH is positioned around 3.6 (Lancrajan 2012). Aronia berries contain substantial concentrations of calcium, potassium, and magnesium, which are minerals considered as essential for human health (Juranović Cindrić et al. 2017). Indeed, the consumption of 100g of fresh berries could cover up to 20% of the daily recommended intake for those 3 minerals. The berries also present an important amount of vitamin C, with a concentration of 13.7 mg per 100g of

fresh fruit, which covers more than 25% of WHO's daily recommended intake for that vitamin (World Health Organization 2004). Aronia berries also contain close to 5 mg of carotenoids for 100g (Razungles, Oszmianski, and Sapis 1989). Finally, relevant amounts of vitamin K, B1, B2, B3, B5, B6 and B9 are also reported (Kulling and Rawel 2008).

1.3.1.3 PP content

Aronia berries are a rich source of PP and are considered one of the highest source of PP among berries in general (Mikulic-Petkovsek et al. 2012). Main PP found are in the berry are represented by procyanidins, anthocyanins, and phenolic acids (Wangensteen et al. 2014; Najda and Aabuda 2013). However, their concentration varies according to the cultivar and environmental conditions (Skrede et al. 2012; Taheri et al. 2013).

As there is no standard procedure for the assessment and analysis of PP content among food, divergences and variations can be found in the findings among studies. The most common techniques for the determination of total PP concentration are liquid chromatography and spectrophotometry (Sidor and Gramza-Michałowska 2019). Even if still widely used nowadays, spectrophotometry technique is less accurate than chromatography as it does not use authentic standards for the assessment of the concentrations. Chromatographic assays noted a concentration of PP of 247-2773 mg gallic acid equivalent per 100g of fresh fruit and 6351-7849 mg gallic acid equivalent per 100g of dry matter (Table 1.1).

Table 1.1: Concentrations of the main (poly)phenols present in aronia berry. Adapted and modified from Sidor et al.

	Concentrations (mg/100g of fresh weight)	Concentrations (mg/100g of dry matter)
Total PP (chromatography)	247 - 2773	6351 - 7849
Procyanidins	664 - 1646	4646 - 5182
Cyanidin-3-O-galactoside	101 - 636	221 - 1612
Cyanidin-3-O-arabinoside	94 - 299	460 - 591
Cyanidin-3-O-xyloside	9.9 - 38	90 - 95
Cyanidin-3-O-glucoside	3.4 - 46	78 - 90
Chlorogenic acid	61 - 218	301 - 642
Neochlorogenic acid	38 - 116	312 - 346
Flavonols	21 - 71	274

The main anthocyanins present in aronia berries are cyanidin-3-O-galactoside followed by cyanidin-3-O-arabinoside (Ochmian, OszmiaDski, and SkupieD 2009; Ireneusz Dariusz, Jozef, and Milosz 2012) (Table 1.1). The most abundant PP subfamily found in *Aronia melanocarpa* is represented by procyanidins (SkupieD, Ochmian, and Grajkowski 2008; Kulling and Rawel 2008). Those molecules are composed of (–)-epicatechin monomers, polymerised at a high degree (Kulling and Rawel 2008). Indeed, around 80% of the procyanidins found in the berry have a degree of polymerisation of 10 and above (Wu et al. 2004).

The main phenolic acids reported in aronia are chlorogenic- and neochlorogenic acid (Jakobek et al. 2012). Aronia also contains a small amount of flavonols, mainly represented by quercetin glycosides (Ochmian, OszmiaDski, and SkupieD 2009). The highest concentration of PP has been observed in the pomace, which contains the skin, seeds and pulp of the berry resulting after a process of press (Kapci et al. 2013). The harvest and process of the berries lead to an important degradation of PP, and especially

anthocyanins, which can degrade even at room temperature (Wilkes et al. 2014). Structures of the main PP found in aronia are presented in Figure 1.8.

The large inter-study variation in terms of concentration can be related to the nature of the raw product, but also to the differences in methodology between laboratories. This aspect highlights the need for the development of standardized and accurate techniques using authentic standards for the analysis of aronia PP.

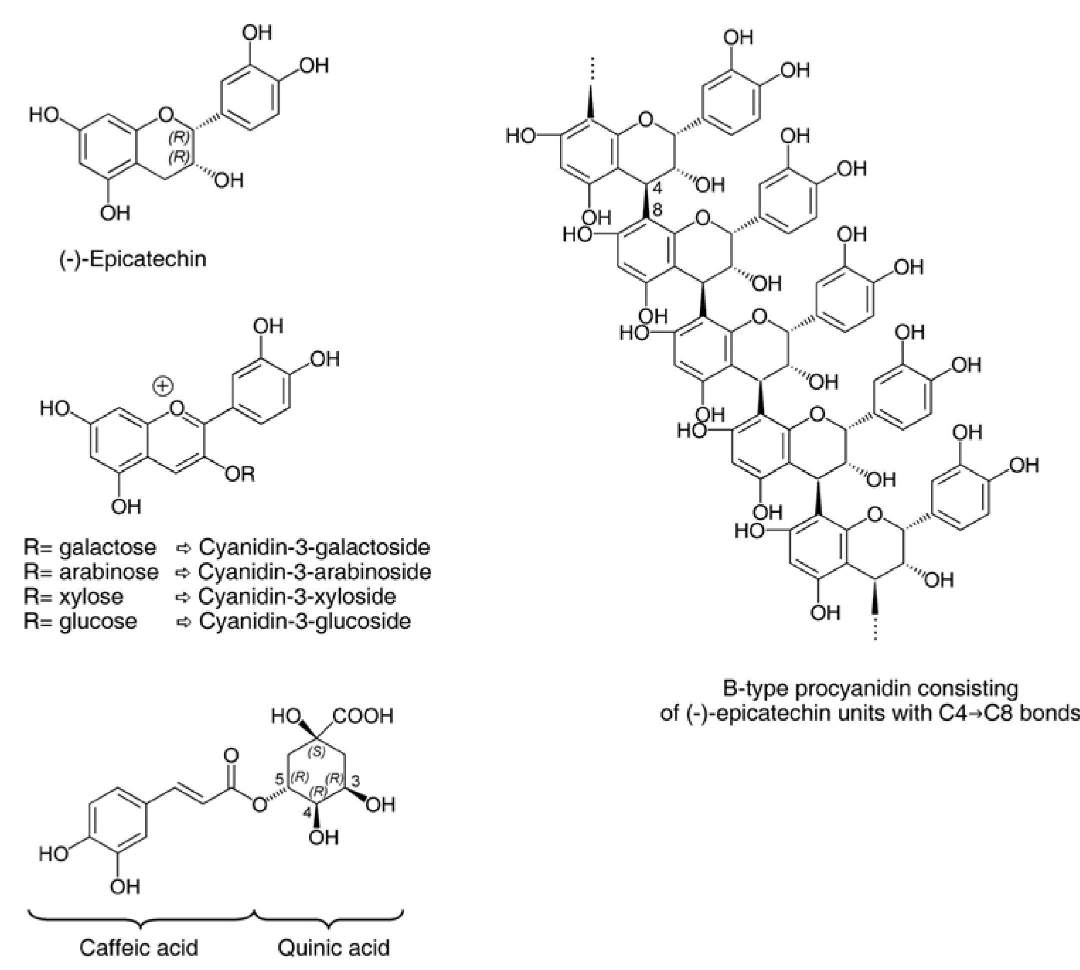


Figure 1.8: Chemical structures of major phenolic phytochemicals present in aronia berries (Kulling and Rawel 2008).

1.3.2 Effects of aronia PP on cardiovascular health

Few studies have investigated the effect of aronia berry consumption on cardiovascular health, which are discussed below.

1.3.2.1 Effect on blood pressure

To date, a total of 13 studies have investigated the effects of aronia berry PP consumption on BP, including 8 RCTs and 5 uncontrolled quasi-designed studies (Table 1.2). Among these studies, 5 trials did not find any significant differences in BP following daily intake of aronia-rich products for 4 to 24 weeks (Xie et al. 2017; Istas et al. 2019; Pokimica et al. 2019; Milutinovic et al. 2019; Ahles et al. 2020), while 3 observed a significant decrease in both office SBP and DBP after the aronia berry product supplementation (Naruszewicz et al. 2007; Broncel et al. 2010; Skoczyńska et al. 2007). Precisely, Naruszewicz and colleagues observed a decrease of 11 and 7.2 mmHg in office SBP and DBP in 44 middle-aged survivors of myocardial infarction following 6 weeks of aronia extract supplementation. Similar magnitude of effect was reported in the uncontrolled quasi-designed studies led by Broncel and Skoczyńska. In fact, while the first one described a decrease in SBP and DBP of 11.6 and 5.0 mmHg, respectively, in a population of 58 men with CVD risk, the second study involving subjects with mild hypercholesterolaemia showed a drop in SBP and DBP of 13.5 and 7.0 mmHg, respectively.

Additionally, 3 studies have found an improvement in office SBP but not in DBP following the intervention (Tjelle et al. 2015; Kardum et al. 2014; Sikora, Broncel, and Mikiciuk-Olasik 2014). In brief, Tjelle and colleagues reported a

significant decrease in office SBP (-6.7 mmHg) in 46 middle-aged hypertensive participants with a mean baseline SBP of 141 mmHg following a 6-week daily intake of mixed berry juices including aronia. Another trial (Kardum et al. 2014) including middle-aged females with obesity noted a significant reduction in office SBP of 11.2 mmHg following a crossover intervention with a 4-week supplementation of aronia juice.

Most studies used office BP, while only two trials have investigated the impact of aronia on 24-hour ambulatory BP, showing a significant decrease in BP during daytime (Loo et al. 2016; Kardum et al. 2015). Indeed, in prehypertensive individuals with elevated baseline BP (140.8 ± 9.3 and 86.6 ± 7.0 mmHg for 24-hour SBP and DBP, respectively) Kardum et al observed a reduction in 24h SBP of 8.5 mmHg and a decrease in awake SBP and DBP of 10 and 5.9 mmHg, respectively, following a 4-week daily intake of 200 mL of aronia juice containing 386 ± 9.7 mg of PP per 100g. The other trial (Loo et al. 2016) showed a significant decrease of 1.64 mmHg in awake DBP following daily intake of 300 mL of aronia juice and 3g of aronia powder containing a total of 2194 mg of PP.

A large variation can be noted in the study design of these studies, as per the nature of the interventional product (juice or extract), the duration of the intervention (from 4 to 24 weeks) or the type of population included (young individuals versus middle-aged participants, healthy versus at risk of CVD). A recent and small meta-analysis including 4 of the studies described above concluded that aronia berry extract supplementation for 2 to 3 months led to a significant mean size effect of -0.331 for SBP, while the analysis did not find

any significant effect on DBP (Hawkins et al. 2020). Authors highlighted that the effect on SBP was even stronger when considering subjects aged over 50 years only. More studies will be needed to confirm the findings of this meta-analysis which, due to the small number of studies included, should be interpreted with caution.

Table 1.2: Previous studies investigating the impact of aronia berry on blood pressure.

Authors	Year	Country	Design	n (M/F)	BP type	Mean age	Duration (weeks)	Intervention	Daily quantity	Health status	Findings
Naruszewicz et al.	2007	Poland	RCT	44 (33/11)	Office BP	66	6	Extract	255 mg	Myocardial infarction + statins treatment	↓ SBP and DBP
Skoczynska et al.	2007	Poland	UQD	58 (58/0)	Office BP	54.1	2x6	Juice	250 mL	Mild HC without treatment	↓ SBP and DBP
Broncel et al.	2010	Poland	UQD	47 (15/32)	Office BP	42-65*	8	Extract	300 mg	HC + Elevated BP > 130/85 mmHg	↓ SBP and DBP
Kardum et al.	2014	Serbia	RCT	20 (0/20)	Office BP	53	4	Juice	100 mL	Central obesity	↓ SBP
Sikora et al.	2014	Poland	UQD	25 (11/14)	Office BP	50-69*	8	Extract	300 mg	MS	↓ SBP
Kardum et al.	2015	Serbia	UQD	23 (12/11)	24-hour ABPM	47.5	4	Juice	200 mL	Elevated BP > 130/85 mmHg	↓ 24-hour and awake SBP and DBP
Tjelle et al.	2015	Norway	RCT	46 (30/16)	Office BP	62	2x6	Berry juice (14.5% aronia)	500 mL	Elevated BP > 130/85 mmHg	↓ SBP after 6 weeks
Loo et al.	2016	Finland	RCT	38 (14/24)	24-hour ABPM	55.8	8 (CO)	Juice + dried powder	300 mL + 3g	Elevated BP > 130/85 mmHg	↓ daytime DBP
Xie et al.	2017	USA	RCT	49 (24/25)	Office BP	35	12	Extract	500 mg	Healthy former smokers	NSD
Istas et al.	2019	UK	RCT	66 (66/0)	Office BP	24	12	Extract	116 mg	Healthy	NSD
Milutinović et al.	2019	Serbia	UQD	35 (12/23)	Office BP	56.3	12	Juice	150 mL	T2DM	NSD
Pokimica et al.	2019	Serbia	RCT	84 (32/52)	Office BP	40.6	4	Juice	100 mL	Subjects at CVD risk (HC, BP > 120/80 mmHg)	NSD
Ahles et al.	2020	Holland	RCT	101 (36/65)	Office BP	53	24	Extract	150 / 90 mg	Healthy, BMI between 25 and 35 km/m^2	NSD

* These studies provided age information as an age range and not an average.

ABPM, ambulatory blood pressure monitoring; BMI, body mass index; BP, blood pressure; CO, crossover; DBP, diastolic blood pressure; HC, hypercholesterolemia; MS, metabolic syndrome; NSD, no significant difference; RCT, randomised controlled trial; SBP systolic blood pressure; T2DM, type 2 diabetes mellitus; UQD, uncontrolled quasi-design.

1.3.2.2 *Effect on PWV and AIx*

While almost 30 trials investigating the biological effects of aronia berry can be reported in the literature, only one RCT reported the impact of the berry on PWV and AIx so far. Indeed, Istas et al measured changes in arterial stiffness after 12-week daily aronia berry extract or whole fruit powder consumption (Istas et al. 2019). No significant differences in PWV or AIx were reported after acute or chronic consumption of the extract or the whole fruit powder (containing 116 and 12 mg of PP, respectively) when compared with placebo. This could be due to the young age of the population investigated (average age 24 ± 5.3 years old).

1.3.2.3 *Effect on endothelial function*

To date, only 2 studies have reported the effect of daily chronic aronia PP consumption on endothelial function. As mentioned previously, the gold standard to assess this parameter non-invasively is the FMD technique (Celermajer et al. 1992; Grover-Paez and Zavalza-Gomez 2009). A first study by Poreba et al including 35 middle-aged men with mild hypercholesterolemia (mean age 53.9 ± 5.8 years) studied the effect of a 6-week aronia juice daily intake on endothelial function. Following the intervention, a significant increase of 4.5% in FMD was found at the end of the intervention (Poreba et al. 2009). However, this study was uncontrolled so findings must be taken with caution. Recently, a RCT led by our team including 66 healthy young men (mean age 24 ± 5.3 years) explored the impact of acute and 12-week daily consumption of an aronia extract on various vascular outcomes including FMD. Significant increase in FMD of 1.0%, 1.4% and 1.5% were observed chronically, acutely

(2-hour post-consumption of aronia extract) on day 1 and week 12, respectively (Istas et al. 2019).

1.3.3 Effects of aronia PP on gut microbiome function and composition

To date, only 1 human trial and 2 preclinical studies have investigated the effect of aronia berry PP on gut microbiome diversity and composition.

In our previous study mentioned in the previous section (Istas et al 2019), no change in microbiome diversity following 12-week daily intake of aronia berry extract was found. However, a significant increase in the concentration of *Anaerostipes* and *Bacteroides* has been reported following the 12-week intake of aronia extract and whole fruit powder, respectively. In the same trial, microbiome was also correlated with FMD and plasma metabolites. *Anaerostipes* are known to be butyrate producers, a short chain fatty acid (SCFA) associated with positive effects in cardiometabolic diseases such as stroke or hypercholesterolaemia (Muñoz-Tamayo et al. 2011). Additionally, *Bacteroides* have been described as polysaccharide A producers, a metabolite reported to prevent inflammatory bowel disease in mice (Mazmanian, Round, and Kasper 2008). A study investigating the impact of red wine consumption for 1 month also showed a significant increase in the *Bacteroides* abundance in healthy subjects (Queipo-Ortuno et al. 2012).

Moreover, another study using an animal model (high-fat fed rats fed with aronia berry PP for 40 days) also reported a change in microbiome composition. A decrease in the *Firmicutes/Bacteroidetes* ratio was observed, as well as an increase in the concentration of *Bacteroides*, *Prevotella* and

Akkermansia, suggesting that these bacteria enhance lipid metabolism in rats fed with a high-fat diet (Zhu et al. 2020).

Finally, a study using a simulation of the human intestinal microbial ecosystem and testing the effect of aronia juice (6.5 g/L) for 2 weeks showed significant differences in the composition of the microbiome. In fact, the juice led to a change in the composition of the SCFA with a decrease in the concentration of acetate in favour of an increase in propionate and butyrate (Wu, Grootaert, et al. 2018). SCFA are gut microbiota-derived metabolites which have been shown to exert a role in the maintenance of a healthy cardiometabolic function (Anselmi et al. 2020). Besides, Wu and colleagues also observed a significant increase in the concentration of *Firmicutes* and *Akkermansia* bacteria following the treatment with aronia juice, in agreement with the *in vivo* study on rats. The increase in the abundance of *Firmicutes* could result from the bidirectional relationship between (poly)phenols and microbiome, as the main bacteria involved in the catabolism of flavonols and flavan-3-ols belong to this phylum (Cueva et al. 2016). Moreover, *Akkermansia* has been noted as a beneficial genus implicated in the regulation of cardiometabolic diseases by producing SCFA (Lyu et al. 2017).

Although promising findings, more human studies are needed to investigate the impact of aronia berry PP on the gut microbiome.

1.3.4 Potential mechanisms of action of aronia PP

Mechanisms surrounding the beneficial effects of aronia berry PP are not yet fully understood. One way how aronia PP could exert beneficial effects could be through modulating the antioxidant enzyme activity and expression

(Fernández-Pachón et al. 2009). Indeed, when the endogenous system of antioxidant protection is more performant, fighting against oxidative damage becomes easier. Among our defence system targeting radicals several enzymes are found, such as catalase (preventing the production of hydroxyl radicals), SOD (catalysing the transformation of radical superoxide in H_2O_2), or glutathione peroxidase, which prevents lipid peroxidation (Dubois-Deruy et al. 2020). These enzymes play an important role in the cellular protection against reactive oxygen species, which increase in concentration in specific physiopathological conditions such as atherosclerosis.

Research focused on the potential mechanism of actions of aronia berry PP *in vivo* on humans. This is the case of a study including 16 young males who consumed 240 mg of aronia extract for 30 days. Following the intervention, a significant increase in glutathione peroxidase and catalase was observed (Kowalczyk et al. 2005). Another trial shown the potential of anthocyanins from aronia berry to be potent inhibitors of lipid peroxidation occurring at the early stage of atherosclerosis. Indeed, following the daily consumption of 300 mg of aronia extract for 2 months, a decrease of the lipid peroxidation and oxidative status among the 47 participants included has been detected (Broncel et al. 2010). Finally, a recent study involving 16 young handball players who were instructed to consume 30 mL of aronia extract for 12 weeks detected a significant drop in the concentration of pro-oxidants thiobarbituric acid reactive substances (TBARS, end-product of lipid peroxidation) as well as a rise of the catalase activity following the intervention (Cikiriz et al. 2020).

However, as it is now understood that PP do not likely exert their beneficial effect on health through an antioxidant action, the potential of aronia berry towards modulating CVD risks factors has also been studied. Investigation of aronia extract on TNF-α human aortic endothelial cells revealed an inhibition of intracellular and vascular cell adhesion molecules –associated with CVD risk–, an inhibition of the phosphorylation of NF-κB –a key player in the development and progression of inflammation–, as well as a decrease in the concentration of ROS (Zapolska-Downar et al. 2012; Kunutsor, Bakker, and Dullaart 2017). Some *in vitro* work on porcine and bovine coronary arteries revealed that aronia extract led to an enhancement of the NO synthesis, which was modulated by an upregulation of the phosphorylation of Akt and eNOS (Varela et al. 2016). It has been shown than the effect was especially modulated by chlorogenic acids and cyanidins (Kim et al. 2013). Furthermore, anti-platelet effects has also been observed in porcine coronary arteries (Olas et al. 2008).

Nevertheless, concentrations used in *in vitro* studies (1-50 µg/mL) are supra-physiological as plasma levels of aronia PP are very low, and as usual doses of commercial extract would not get to these concentrations (Zapolska-Downar et al. 2012). Therefore, conducting mechanistic studies using physiologically relevant doses of compounds and using circulating metabolites rather than the parent compounds present in the fruit is paramount to reveal fully the mechanisms enfolding the beneficial effects of aronia berry PP.

1.4 Gaps in the literature

While studies continue to demonstrate the beneficial effect of dietary PP for the prevention of CVD (Ivey et al. 2015), literature is mixed regarding the magnitude of effects attributed to the consumption of phenolic compounds (Dower et al. 2015).

Following recent ADME studies (Feliciano, Istas, et al. 2016; Feliciano et al. 2017; Istas et al. 2018), the importance of compounds' bioavailability appears to be key in the understanding of metabolites' bioactivity. In the case of vascular function assessment, it was reported that plasma PP concentration and magnitude of the biological effect are directly related (Rodriguez-Mateos et al. 2013). Moreover, understanding the ADME of PP is essential for understanding health benefits. There is a need for the development of time courses and dose-response analysis to assess the optimal daily intake of aronia PP to exert beneficial effects. As a result, human studies involving state-of-the-art techniques to assess both bioactivity and bioavailability are required.

Mixed results reported from the literature could be related to the interindividual variability in response to PP intervention. The work of the European scientific cooperative COST Action POSITIVe highlighted that, along with gut microbiota and background genetics, age, sex, ethnicity, physical activity, smoking status, and physiopathological status could be linked to the variability in the biological response to PP supplementation (Gibney et al. 2019). The importance of subgroup examination based on these factors and analysis of the responders

versus non-responders' responsiveness is thus key and could explain the inconclusive or divergent results of some RCTs.

As described earlier, very few double-blind RCTs have investigated the effect of aronia berry PP on cardiovascular health, in particular endothelial function, and arterial stiffness, which very scarce data existing. Regarding BP, it would be important to conduct studies using the gold standard 24-hour ambulatory BP as this measure was found to be more accurate to assess CVD risk (Bakogiannis et al. 2020). This could lead to new findings and resources for the primary prevention of CVD.

Besides, most trials focusing on the effect of aronia supplementation were quasi-experimental studies, with a low number of participants included, which is prone to bias (Hawkins et al. 2020). Future research on the health effects of aronia berries will need to use a strong methodology, including larger sample sizes justified by power calculation, detailed criteria for the inclusion of volunteers as well as the consideration of cofounding factors such as sex, BMI, age, or baseline PP intake. Six to eight weeks have been shown to be sufficient to induce significant improvement in SBP, based on 4 RCTs (Hawkins et al. 2020). Nevertheless, accounting for the possibility of bias described above, future RCTs should investigate the potential of longer durations of supplementation to induce greater vascular enhancements.

Recently, microbiome composition and diversity has been gaining attention in the field of nutritional sciences as the number of studies demonstrating the importance of the microbiota metabolism of PP have been growing (Kawabata, Yoshioka, and Terao 2019; Tomas-Barberan, Selma, and Espin 2016;

Cassidy and Minihane 2017; Chambers et al. 2018). However, the impact of gut microbiota on PP metabolites is not fully elucidated yet and will need further investigations. The use of well-designed RCTs involving "omics" technologies such as targeted and untargeted nutrigenomics, metabolomics or microbiomics will be useful tools to uncover these aspects (Bayram et al. 2018).

Finally, the use of aglycone standards in analytical methods to quantify phase II metabolites in plasma or urine is now known to lead to inaccurate results (Feliciano, Boeres, et al. 2016). Indeed, this technique –widely used in PP research– does not reflect well the presence and abundance of metabolites generated by phase II and gut microbiota metabolism. For instance, a recent study revealed that the usage of unmetabolised standards for the quantification of conjugated metabolites was resulting in under- and over-estimations of -94% and +250%, respectively (Ottaviani et al. 2018). As a result, the use of authentic standards of PP metabolites is needed to accurately assess the bioavailability and metabolism of phenolic compounds from aronia in biosamples.

Based on the current literature, aronia berry PP are promising candidates to improve cardiovascular health. Using dietary strategies to improve overall health status is a low-risk and non-invasive way which should be considered as an option by health care providers to delay the onset of CVD in healthy populations. As a result, we need stronger evidence of the power of PP supplementation, with reliable RCTs involving gold standard techniques as well as adequate and representative populations.

1.5 Hypothesis and aims

The main objective of this PhD was to investigate the effects of aronia berry PP on cardiometabolic health. The central hypothesis of this thesis can be summarized as follows: Aronia berry PP are bioavailable, and their consumption can exert a beneficial effect on vascular function in healthy subjects via modulation of the gut microbiome.

Specific objectives were:

Objective 1: To investigate the effect of daily aronia berry supplementation on cardiometabolic health (Chapter 3).

Objective 2: To investigate the bioavailability and metabolism of aronia berry PP (Chapter 4).

Objective 3: To investigate the interindividual variability in the vascular response to aronia berry PP (Chapters 5 and 6).

Objective 4: To investigate the effect of daily aronia berry supplementation on gut microbiome composition and diversity (Chapter 6).

CHAPTER 2

Methods

2.1 The Aronia Blood Pressure (ABP) study

2.1.1 Study population

One hundred and two healthy prehypertensive males and females aged 40 to 70 years were recruited in London (UK) over a period of 17 months. Recruitment strategies included local and national newspaper advertisement, radio advertisement, professional flyer distribution, professional poster display in train stations, Facebook page and paid advertisement, dedicated website (www.abpstudy.com, not accessible anymore), as well as posters and flyers in local public establishments surrounding the university (museums, coffee shops, supermarkets, schools, gyms, hospitals and libraries) (examples and participant information sheet in Appendix A, B and C).

All volunteers attended a screening visit during which their health was assessed by a routine clinical examination of their blood pressure and anthropometric parameters (height, weight) as well as a specific lifestyle questionnaire including sections related to medical history, concomitant medication, as well as drinking and smoking status (Appendix D). Participants were eligible for the study if their blood pressure was in the prehypertensive range at the screening visit assessment: SBP comprised between 120-140 mmHg and/or DBP comprised between 80-90 mmHg. Other inclusion criteria included: being between 40 to 70 years of age, willing to maintain a normal eating and drinking routine and avoid changes in body weight over the course of the study, being able to understand the nature of the study, and being willing to sign the informed consent form (Appendix E).

Volunteers presenting CVD (including coronary artery disease, cerebrovascular disease, or peripheral artery disease) were excluded from the trial. Further exclusion criteria included hypertension (≥ 140 mmHg systolic blood pressure and/or ≥ 90 mmHg diastolic blood pressure), obesity (defined as BMI ≥ 30 kg/m^2), acute inflammation, diabetes mellitus and metabolic syndrome, terminal renal failure, abnormal heart rate (< 50 or > 100 bpm) and malignancies. Additionally, volunteers were excluded if they had allergies to berries or other significant foods, were taking specific medication related to or affecting the cardiovascular function, smoked an irregular daily number of cigarettes or were planning to quit smoking in the next 6 months. Subjects fulfilling the inclusion criteria requirement but taking dietary supplements or herbal remedies at the screening visit were instructed to stop them for a minimum of one month before being able to attend the following pre-visits.

2.1.2 Study design

This study was a 2-arm, double-blind, parallel, randomised, controlled trial. Once participants fulfilled all inclusion criteria at the screening visit, they attended 4 visits, defined as follows: pre-visit 1, visit 1, pre-visit 2 and visit 2. Pre-visit 1 and visit 1 represent the baseline measurements of the study while pre-visit 2 and visit 2 were conducted after 12 weeks of daily consumption of the interventional product. Both pre-visits 1 and 2 were short visits (30 minutes) and performed 24 hours prior visit 1 and visit 2, respectively. During the first pre-visit, office blood pressure was measured again to confirm that participants had a blood pressure within the range of the eligibility criteria. If not, they were excluded from the study. If the blood pressure reading was

consistent with the assessment of the screening visit, participants were included in the study and allocated randomly to one of the treatments. On both pre-visits, participants were given a bottle to collect their urine for 24 hours, and 24-hour ambulatory BP monitors were fitted on the non-dominant arm of the subject, and the first measurement was taken. To capture participants habitual diet and physical activity, and any changes that could occur within the intervention period, 7-day food diaries were completed by the subjects a week prior each pre-visit and a validated physical activity questionnaire as well as a food-frequency questionnaire were filled by all participants at visit 1 to assess the related baseline characteristics. Details related to these questionnaires can be found in section 2.7 of this chapter.

All subjects were instructed to avoid caffeine, alcohol, strenuous exercise and tobacco at least 1h prior screening and each study visit. Participants were also asked to fast for 12 hours before each visit 1 and visit 2. Measurements of peripheral office BP, FMD, PWV, and AIx, as well as blood samples, were all taken at baseline (0h), then again 2h post-acute consumption of the interventional product, on visit 1 and visit 2. Faecal samples were collected during both visits 1 and 2 and stored immediately at -80°C.

Participants were instructed to take 1 capsule of the interventional product every morning with a glass of water, ideally with food. The first capsule was delivered after the first round of measurements on visit 1, marking the start of the 12 weeks of consumption. The last capsule was also taken within the research unit on the last day (visit 2), following the first set of vascular measurements. Subjects were followed-up every month throughout the 12-week period via email to ensure good compliance and record any potential

adverse event. For clarity, the following designations will be used throughout the manuscript: acute effects (2h compared with 0h on visit 1), chronic effects (0h on visit 2 compared with 0h on visit 1), and acute on chronic effects (2h compared with 0h on visit 2). The primary outcome of this study was the effect of aronia berry extract (Aronia) versus placebo (Control) on ambulatory 24-hour SBP and DBP at 12 weeks post-consumption. Second endpoints included the effect of the extract on office BP, office and 24-hour heart rate, arterial stiffness (measured as PWV and AIx), blood lipids (total, HDL and LDL cholesterol, triglycerides), blood cortisol levels compared with placebo after 12 weeks of daily consumption, as well as the safety and tolerability of the aronia berry extract.

Secondary objectives also included the investigation of the effect of aronia extract versus placebo on FMD and blood flow velocity at 2h and 12 weeks post-consumption. Tertiary outcomes comprised the analysis of blood samples taken at all timepoints to assess aronia PP metabolites, the analysis of 24-hour urine samples to investigate excretion of PP metabolites, as well as the analysis of the gut microbiome on faecal samples 12 weeks after consumption of the aronia berry extract or placebo.

Qualified and trained researchers enrolled participants on the trial and allocated the interventional treatments. Both participants and researchers leading the study were blinded to the intervention groups. An independent external investigator generated the randomised treatment allocation sequence using an arbitrary number generator. The aim of this process was to attribute a specific and unique number to each participant to guarantee a complete safeguard of the allocation information. Once study visits and analysis of the

primary, secondary, and tertiary objectives of the trial were achieved the external independent investigator rendered the codes needed to unblind the interventional treatment groups. The trial was conducted in agreement to the guidelines stated in the current revision of the Declaration of Helsinki.

All procedures were approved by King's College London Ethics Committee (RESCM-17/18-5283) and the trial was registered at ClinicalTrials.gov under the reference NCT03434574. Participants were assessed and data was collected and reported in comprehensive case report forms (Appendix D) between February 2018 and September 2019 in the Metabolic Research Unit of the Department of Nutritional Sciences of King's College London.

2.2 Aronia berry extract and control capsules

The aronia berry extract, manufactured by Naturex-DBS (Massachusetts, USA), was supplied in capsules. Interventional capsules contained PP-rich concentrated aronia berry extract. Thirteen compounds or families of phenolic compounds were analysed with different analytical methods and quantified using liquid chromatography and spectrophotometry. The process to obtain the content of one berry extract capsule implied the removal of fibres and organic acids from 75g aronia berries and resulted in 105.9 mg of total PP quantified by HPLC (146.2 mg via Folin-Ciocalteu). The control capsules, identical in appearance to the treatment capsules, contained coloured maltodextrin and no PP. All capsules were matched in weight, carbohydrates and calories and were stored in plastic bottles displaying both the unique treatment allocation number and patient randomization number. Details of the PP composition of the different intervention capsules is detailed in Table 2.1.

Table 2.1: (Poly)phenol content of intervention capsule.

	Average	CoV (%)
Procyanidins (>DP10) (%)	5.50	7.56
Total anthocyanins (%)	4.65	0.44
Cyanidin-3-galactoside (%)	*3.06*	*0.50*
Cyanidin-3-glucoside (%)	*0.14*	*3.67*
Cyanidin-3-arabinoside (%)	*1.05*	*0.59*
Cyanidin-3-xyloside (%)	*0.15*	*1.09*
Cyanidin (%)	*0.25*	*1.34*
Neochlorogenic acid (%)	3.52	0.92
Chlorogenic acid (%)	3.43	0.45
Caffeic acid (%)	1.67	1.13
Quercetin-O-glycosides (%)	1.16	2.47
Procyanidins (DP2-DP10) (%)	0.86	1.19
Rutin (%)	0.28	1.20
Quercetin (%)	0.12	1.06
Total PP (Folin-Ciocalteu) (%)	29.2	5.19
Total PP quantified individually (%)	21.2	2.08
Total PP quantified individually (mg/capsule of 500 mg)	**105.9**	

CoV, coefficient of variation; DP, degree of polymerisation; PP, (poly)phenols

2.3 Vascular measurements

2.3.1 Blood pressure (BP) and heart rate (HR)

2.3.1.1 24-hour ambulatory BP and HR

Twenty-four-hour ambulatory peripheral (brachial) and central (aortic) BP, as well as heart rate (HR), were measured using an Arteriograph24^{TM} (TensioMed, Budapest, Hungary) (Figure 2.1). Twenty-four-hour SBP, DBP and HR were measured every 30 minutes over the 24 hours separating the fitting of the cuff and the retrieving of the data. Three sizes of cuffs were available, and the best fit was attributed based on the volunteer's arm circumference. Participants were instructed to continue with their daily routine and were asked to fill a 24-hour activity record to track their actions throughout the day and assess their awake and asleep time. It was decided that a

minimum of 10 measurements of blood pressure taken during the volunteer's usual waking hours and 4 recordings taken during the person's usual recorded sleeping hours was required to calculate the average value for the 24-hour ambulatory blood pressure. Otherwise, the values were inputted as missing values. These cut-offs are in line with the recommendations of the European Society of Hypertension which requires at least 2 valid daytime and 1 valid night time measurements for research purposes (Parati et al. 2014).

Figure 2.1: Arteriograph24™ and its cuff.

2.3.1.2 Clinical peripheral ambulatory BP and HR

Supine office peripheral (brachial) BP and heart rate were measured using an automated clinical digital sphygmomanometer OMRON M3 (OMRON Healthcare UK Ltd, Milton Keynes, UK) at the upper right arm in supine position, after 10 min of rest in a quiet room with the arm rested at heart level, legs uncrossed and supported, back supported and an empty bladder, according to the recommendations of the American Heart Association (Muntner et al. 2019). Participants were instructed to stay silent during the measurement as an active conversation can increase the values of the reading. Three measurements were taken, 1 minute apart, and the last 2

measurements were averaged and considered as final result for statistical analysis.

2.3.2 Arterial stiffness

2.3.2.1 Twenty-four-hour ambulatory PWV and AIx

Twenty-four-hour PWV and AIx were measured every 30 minutes over the 24 hours separating the pre-visits and the visits using Arteriograph24™. Participants were asked to fill a 24-hour activity record to indicate their daily activities day and evaluate their awake and asleep period.

2.3.2.2 Office PWV

Volunteers were instructed to lie down and remain silent for the duration of this measurement. PWV (expressed in m/s) was assessed via applanation tonometry using SphygmoCor® (Smart Medical, Gloucestershire, UK). Briefly, PWV is derived from the difference between transit time from the heart to femoral artery pulse and that from the heart to carotid artery pulse. The sensor of the device captures the time lapse between pulse waves at both arteries and is thus able to determine the wave velocity, as the carotid-to-heart and heart-to-femoral distances were measured beforehand with a tape measure. The SphygmoCor® Manual of Procedures requires a standard deviation of the Pulse Wave Velocity measurement to be less than or equal to 10% (Supiano et al. 2018). In the present trial, results with a standard deviation of less than 8% were included in the analysis and considered reliable (Figure 2.2). The average standard deviation of all measurements performed as part of the ABP study was 6.9 ± 2.3% for visit 1 and 6.9 ± 2.2% for visit 2.

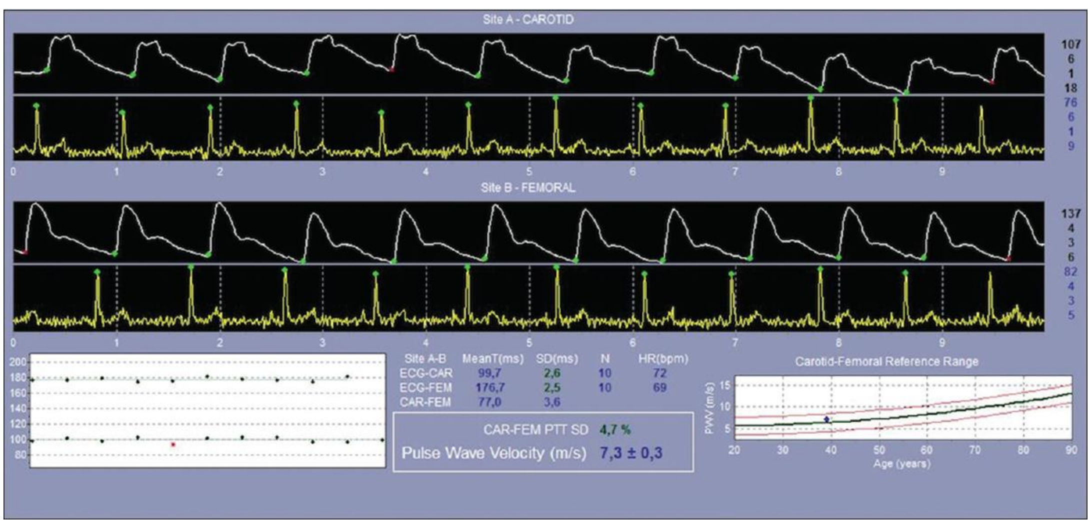


Figure 2.2: Aortic PWV calculated by SphygmoCor® device.

2.3.2.3 Office AIx

Ascending pressure waveforms are generated at the aorta level and involve a reflected wave which is dependent on the vessels structure and general peripheral resistance. As this latter is reduced at the body periphery, pressure is decreased from the aorta to the wrist. The waveform thus has a different shape when measured at the aorta level compared with the wrist. AIx is measured as the ratio of the central pulse pressure at aorta level and the reflected pulse pressure (called augmentation pressure) (Figure 2.3). AIx is a relevant indicator of the arterial stiffness status of the vascular system of an individual.

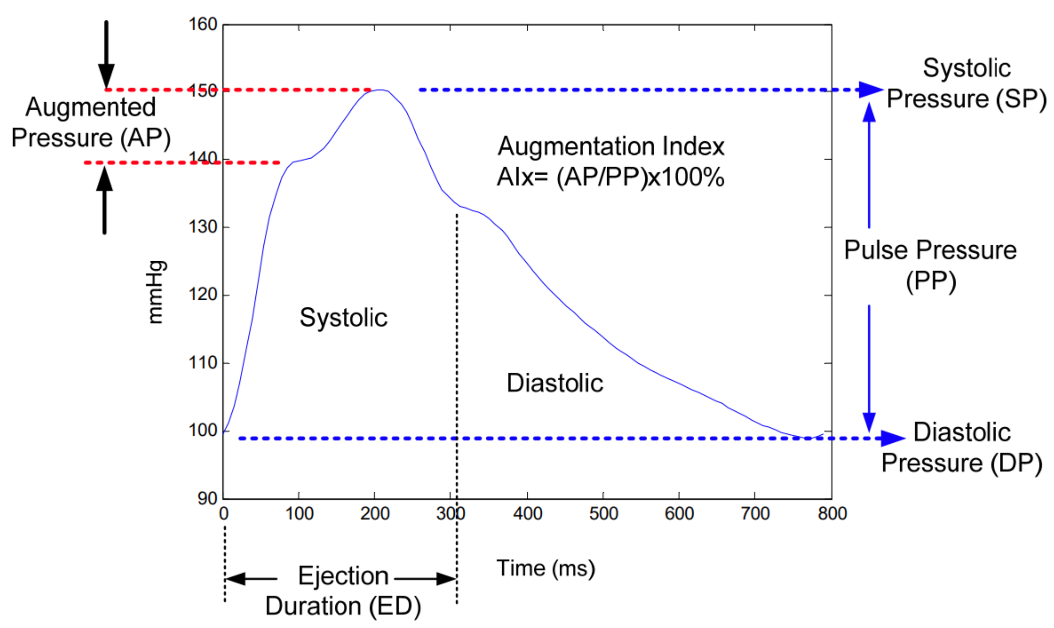


Figure 2.3: Augmentation index and pulse pressure (from SphygmoCor® user manual).

The measure of pressure waves at the peripheral brachial artery, located in the wrist, was performed using applanation tonometry (SphygmoCor®, Smart Medical, Gloucestershire, UK). After a Fourier transform (Fetics et al. 1999), the central aortic pressure waveform was calculated (Wilkinson et al. 2000). AIx is expressed in percentage and standardized to a heart rate of 75 beats per minute (AIx@HR75). An operator index value was given by the device to represent the accuracy of the measurement. The SphygmoCor® software computes an "operator index" from electrocardiographic and tonometric data variability (diastolic variation, shape deviation, etc.) and is used as a quality control marker (Dawson et al. 2009; Frimodt-Møller et al. 2008). Only waveforms with an operator index >88 were considered as good quality and were included in this study. The average of the operator indexes of all measurements completed during the ABP study was 94 ± 5.5% for visit 1 and 94 ± 5.3% for visit 2.

2.3.3 Endothelial function

Endothelial function is assessed by the measurement of FMD. In brief, FMD represents the endothelium-dependent relaxation of an artery due to an increased blood flow (hyperaemia). This non-invasive measure indicates the nitric oxide-dependent reactivity of the artery's endothelium as a response to shear stress. FMD is also a pertinent marker of increased CVD risk.

FMD assessment was conducted according to established protocols in our laboratory (Rodriguez-Mateos et al. 2013; Rodriguez-Mateos et al. 2015; Heiss et al. 2003). Participants were instructed to lie down and a sphygmomanometric cuff was placed on their forearm below the elbow pit. Electrodes patches were placed on the volunteers to measure their electrocardiogram. Using some electrode gel, a 12 MHz transducer (Vivid I, GE healthcare, Buckinghamshire, UK) was applied 2 cm proximal to the elbow and a baseline brachial artery diameter was then measured (Figure 2.4).

The doppler mode of the device helped the operator to locate the artery by identifying the blood flow with a pulsative red colour. Landmarks of the vessel were identified by the operator to ensure the image to stay at the exact same location after deflation of the cuff. After the baseline image recording, a temporary ischemia of the lower arm was initiated by a 5-min occlusion subsequent the inflation to 180 mmHg of the sphygmomanometric cuff. Artery diameter and blood flow were subsequently measured at 20-, 40-, 60- and 80-second post-deflation. While 20-, 40- and 80- seconds diameters were recorded as back-up in case the quality of the 60-second image was not optimal, only the 60-second measure was used to assess the FMD

percentage. This protocol was developed by Professor Christian Heiss and fellow cardiologists from the Division of Cardiology, Pulmonology and Vascular Medicine of the University of Dusseldorf, and it was used in multiple studies over the last 20 years in patients with cardiovascular disease and healthy individuals showing very reproducible results and in agreement with current knowledge (Heiss et al. 2007; Heiss et al. 2005; Heiss, Rodriguez-Mateos, and Kelm 2015). Moreover, this protocol was used in several RCTs demonstrating significant improvement in FMD performed by our research team in the past years (Istas et al. 2019; Rodriguez-Mateos et al. 2016; Rodriguez-Mateos et al. 2013). After storage of the images, data analysis was achieved using an automatic edge-detection software (Brachial Analyser, Medical Imaging Applications, Iowa City, USA). FMD is expressed in percentage and calculated using the following formula: $\frac{(diameter\ 60\ sec - diameter\ baseline)}{diameter\ baseline} * 100$. To assess the reproducibility of the FMD measurements, which are highly dependent on the operator, intra and interindividual variability was evaluated as part of a training prior the start the study using repeated measures between subjects and on the same subjects, respectively. Based on the guidelines established by the International Brachial Artery Reactivity Task Force in 2002 regarding the assessment of the FMD reproducibility (Corretti et al. 2002), 20 individuals working in the department volunteered to have their FMD measurements assessed 5 times each, on 4 different days, for a total of 100 measures. The intraindividual variability was calculated using the coefficient of variation of the 5 repeated measures for 1 volunteer whereas interindividual variability was evaluated via the calculation of the coefficient of variation accounting for all measurements performed.

Interindividual variability in FMD percentage was evaluated at 13.2% while the intra-subject variation was 6.7%. Our results were in line with the technical standards proposed by Thijssen and colleagues and suggesting that an interindividual variability in FMD< 15% should be considered as good reproducibility (Thijssen et al. 2019).

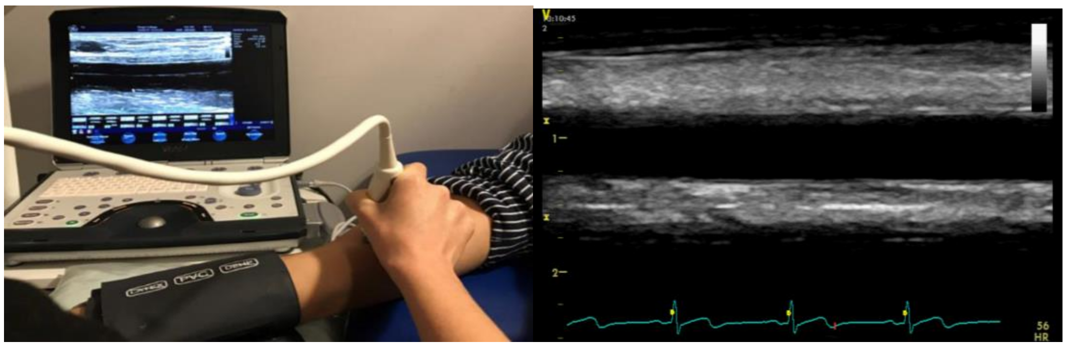


Figure 2.4: Flow-mediated dilation measurement and ultrasound image of the brachial artery.

2.4 Body composition measurements

Height was measured using a mechanical wall-mounted stadiometer platform (CMS Weighing Equipment Ltd., London, UK) and was rounded to the closest centimetre. Body composition was assessed using the BC-418 Segmental Body Composition Analyzer Tanita (TANITA Corporation, Sportlife Tokyo, Japan). Briefly, participants were undergoing the measurement fasted, in the morning of the study visit, after emptying their bladder and removing any unnecessary layer of clothes. Their age and height were entered, and the sex was selected. The BC-418 scale offers two choices of body type for each gender: “athletic” and “standard”. To ensure the normalization of the results over the study population the “standard” body type was chosen for all participants. Participants were then instructed to step on the weighing platform barefoot and to grab both hand grips to ensure the correct measure of body

weight and impedance. The analyser calculated the values of weight (in kg), body mass index (BMI, in kg/m^2), basal metabolic rate (BMR, in kcal) and body fat percentage.

2.5 QRISK®3 score

The collection of plasma lipid data and blood pressure allowed the calculation of QRISK®3 score. The QRISK®3-2018 risk calculator (https://qrisk.org/three/) is a validated tool developed by doctors and academics working in the UK National Health Service and widely used in general practice in the UK. It is based on the same equation than the Framingham Risk Score (Wilson et al. 1998), but the algorithm includes far more parameters in the analysis, such as BMI, ethnicity, and family history. The QRISK®3 score algorithm evaluates a person's risk of developing a heart attack or stroke over the next 10 years. The volunteer's age, sex, smoking status, cholesterol/HDL ratio, BMI and baseline systolic blood pressure were parameters included in the calculation of the 10-year QRISK®3 score.

2.6 Sample collection and biochemical analysis

2.6.1 Processing and storage of blood samples

Following vascular measurements, venepuncture was performed to collect blood samples from the participants, using a safety-lock 21G butterfly needle and various vacutainers (Beckton Dickinson Biosciences, Plymouth, UK): 8.5 mL yellow top serum separator tubes (SST, blood lipids and liver function), 3 mL grey top fluoride oxalate tubes (fasted blood glucose), 3 mL pink EDTA K3 tubes (full blood count), 6 mL green top heparin tubes (plasma PP

metabolites), 10 mL purple top EDTA tubes (plasma PP metabolites), 8 mL sodium citrate mononuclear cell preparation tubes (CPT tubes, targeting peripheral blood monocytes) and 2.5 mL glass PAXgene blood RNA tubes (intracellular RNA). The EDTA K3 vacutainers were sent without further processing to an independent biochemistry laboratory for the analysis of the full blood count (Affinity Biomarker Laboratories, London).

Blood samples collected in EDTA, heparin, SST and fluoride oxalate tubes were inverted several times and centrifuged at 3000 rpm for 15 min at 4°C immediately after collection. Full blood was collected from a separate heparin tube, which was not centrifuged, and aliquoted in a 2 mL safe-lock tube (Eppendorf, Stevenage, UK). Serum and plasma from SST and fluoride oxalate tubes were then aliquoted in 2 mL safe-lock tubes and shipped rapidly for further analysis. All clinical chemistry parameters, including total cholesterol, LDL and HDL cholesterol, high sensitivity C-reactive protein (hs-CRP), cortisol, liver function, glucose, and full blood count were analysed according to standard procedures in an independent laboratory (Affinity Biomarker Laboratories, London). Plasma samples from EDTA and heparin tubes were aliquoted in 2 mL safe-lock tubes and stored at -80°C until further analysis. Some EDTA plasma collected for the analysis of (poly)phenols were spiked with formic acid (99.5% UPLC-grade, Fisher Scientific, Loughborough, UK) to obtain a final concentration of 2% before storage.

CPT glass vacutainers were inverted 8 times before being centrifuged at 2700 rpm for 30 min at room temperature. The top half of the supernatant was then discarded, and the layer of mononuclear cells was collected with a 5 mL

pipette and transferred to a 50 mL Greiner centrifuge tube. Phosphate buffered saline (PBS) 1X was added to adjust the volume to 15 mL and the tube was gently inverted 5 times. The tube was then centrifuged at 1400 rpm for 15 min at room temperature. After that step, the supernatant was discarded, and the cells were resuspended in 1 mL of PBS 1X before being transferred to a 2 mL safe-lock tube. Finally, the mononuclear cells were centrifuged at 2100 rpm for 15 min at room temperature. The supernatant was removed without disturbing the pellet of cells and the tube was stored at -80°C until further utilisation. Following their collection, PAXgene tubes were resting for 2 hours at room temperature before being stored at -80°C in an upward position.

2.6.2 Processing and storage of urinary samples

Participants were given a 3L urine bottle containing 3g of ascorbic acid powder and were instructed to collect all their urine from the time they left the research unit on the pre-visit until they came back 24 hours after for the actual study day. Subjects were provided a cooling bag with some ice packs to keep the sample at a low temperature and hence avoid the degradation of PP metabolites. Once the collected urine was returned, the volume was measured precisely using a volumetric cylinder. A representative sample was saved from the bottle and centrifuged (3000 rpm; 15 min; 4°C) immediately after collection. Samples were then aliquoted with and without 2% formic acid (99.5% UPLC-grade, Fisher Scientific, Loughborough, UK) and stored at -80°C until further analysis.

2.6.3 Processing, storage, and analysis of faecal samples

Faecal samples were collected as close as possible of each study visit in OMNIgene GUT self-collection tubes (DNA Genotek Inc., Ottawa, Canada) and stored at -80°C until further analysis. Participants were asked to record the collection date.

Microbiome shotgun metagenomics analysis was performed by CosmosID, Inc. (Rockville, MD, USA). Briefly, extracted DNA samples were quantified using Qubit 4 fluorometer and Qubit™ dsDNA HS Assay Kit (Thermofisher Scientific). DNA libraries were then prepared using the Nextera XT DNA Library Preparation Kit (Illumina) and Nextera Index Kit (Illumina) following the manufacturer's protocol with slight modifications. The standard protocol was used for total DNA input of 1 ng. Genomic DNA was fragmented using a proportional amount of Illumina Nextera XT fragmentation enzyme. Combinatory dual indexes were added to each sample followed by 12 cycles of PCR to construct libraries. DNA libraries were purified using AMpure magnetic Beads (Beckman Coulter) and eluted in QIAGEN EB buffer and were quantified using Qubit 4 fluorometer and Qubit™ dsDNA HS Assay Kit. DNA libraries were finally pooled together for sequencing on the Illumina HiSeqX. Raw reads from metagenomics samples were analysed by CosmosID metagenomic software (CosmosID Inc., Rockville, MD) to reveal strain level identification of microbes in the specimen as described elsewhere (Ponnusamy et al. 2016; Hasan et al. 2014; Ottesen et al. 2016). In short, the system utilizes a high-performance data mining k-mer algorithm and highly

curated dynamic comparator databases that rapidly disambiguate millions of short reads into the discrete genomes or genes engendering the particular sequences. This query of reads enables the sensitive yet highly precise detection and taxonomic classification of microbial reads. The resulting statistics were analysed to return the fine-grain taxonomic and relative abundance estimates for the microbial datasets.

Bioinformatic analysis and statistical modelling of gut microbiome was then performed with the help of INRAe-Metagenopolis (Paris, France). Metagenomic Species Pangenome (MSP) are repertoires of genes composed of genes presents in all strains (“core genes”) and genes present in only some of them (“accessory genes”) (Medini et al. 2005). The clustering tool MSPminer, developed by Plaza Oñate and colleagues (Plaza Oñate et al. 2019) can group co-abundant genes into MSP, based on the largest gene abundance table of the human gut microbiota available and containing 10.4 million genes isolated from 1267 faecal samples (Li et al. 2014). MSP abundance profiles were calculated as the mean abundance of 100 markers genes, defined as the robust base of each MSP cluster.

The association of multiple omics’ data was carried out through a parallel and vertical integration scheme (Wu et al. 2019). The variable selection was performed in per single omic level and more precisely, for each omic dataset a univariate statistical analysis (Wilcoxon test) was performed to investigate the differences of individual molecular levels between the given phenotypes of interest (Aronia versus Control). The parallel integration treats each type of omics measurements equally and the integration has been identified

simultaneously in a joint model by collecting and correlating all the per-single significantly different features. Spearman correlations were calculated between concentrations of plasma and urine metabolites, clinical outcomes, and gut functions, and microbial species (Supplementary Figure 1).

2.7 Diet, health, and lifestyle questionnaires

2.7.1 Food frequency questionnaires

Usual dietary intake was assessed using a detailed 217-item food-frequency questionnaire (FFQ) developed from the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort (Bingham et al. 2001). Participants were asked to choose their frequency of consumption for each food listed in the FFQ by answering the question “how often have you eaten these foods in the last 12 months?” using one of nine response categories ranging from “never or less than once a month” to “6+ per day”. An amount was described for each food listed, such as a "medium serving" or a common household unit such as a slice or teaspoon. An example can be found in Appendix F. FFQ data was then inputted into an Access software file and analysed by Food Frequency Questionnaire EPIC Tool for Analysis (FETA) software (Mulligan et al. 2014). FETA generates the average daily nutrient, food group intakes and the amount of food consumed per individual, for each food code and each FFQ line. FETA output was then exported to Microsoft Excel for analysis, using the method stated in 2.7.3.

2.7.2 Seven-day food diaries

Dietary intake were assessed using the validated European Prospective Investigation into Cancer and Nutrition 7-day food diary (EPIC 7DD, University of Cambridge, UK) which was completed by participants before the first visit (baseline) and after 11 weeks, before attending the second visit, to ensure their habitual diet did not change during the study period (Day et al. 1999). Volunteers were instructed to record all food and beverages consumed over a 7-day period in as much detail as possible, including cooking methods, type of fat or oil used, brand names of commercial products and details of recipes used (Appendix G). Participants were told to record portion sizes of foods and beverages in the appropriate measurement, such as household measures. The 7DD also included photographs of 17 foods, with three different portion sizes each. Participants could choose which photograph represented the portion size or indicate if they consumed more or less than the amount shown in the photograph.

After being returned by the participants, the 7DDs were coded into Nutritics (Nutritics 5.6 Research Edition, Nutritics Ltd, Dublin, Ireland) to analyse volunteers' nutrient and food intake. All coders undertook supervised training and used a standard coding protocol developed in our research group to reduce coding error rate. Participant intake was coded using McCance and Widdiwson's Composition of Foods Integrated Dataset 2015 and the Irish food composition database (Roe et al. 2015). Some "Nutritics database codes" were also used if detailed composition information was available, based on databases from diverse countries and including a wide range of sports

supplements, health food products, supplier, and restaurant data (Nutritics 2020). Any food with missing information regarding the portion size was coded into medium portion size option on Nutritics or estimated by coders. Multi-level items such as sandwiches were coded into their different constituent. When a simple recipe was provided, all ingredients were coded with the reported amount consumed. When no recipe was provided a usual equivalent recipe was set up in Nutritics. To ensure the coding quality, 5% of the diaries coded by each operator were randomly selected for quality check by an external researcher and 3 arbitrary days of each selected diary were checked against the written diet diary. A total of 1328 and 1397 foods were extracted from the 7DD in the ABP trial, for visit 1 and visit 2 respectively. Nutritics output was exported into Microsoft Excel.

2.7.3 Assessment of dietary PP intake

Both EPIC FFQ and 7DD were used to analyse participant's habitual PP intake. The phenolic compound content of each food was calculated using the PP composition database Phenol Explorer (www.phenol-explorer.eu), which contains 502 different types of PP, and includes 452 foods (Neveu et al. 2010). For the 7DD, references from the US Department of Agriculture (USDA) database (Nutrient Data Laboratory 2015b, 2016, 2015a) was also used to increase the accuracy of the analysis as Phenol Explorer does not contain all the foods of interest. The PP content from each food was calculated in aglycones by multiplying the content of each PP by the reported consumption of each food item. Five different classes of PP were identified, including flavonoids (and their different subclasses), phenolic acids, stilbenes, lignans, and other PP. To account for the weight changes mostly due to water

movement during processing, an additional coefficient included in Phenol Explorer called “yield factor” was applied to the content value of each PP in foods (Phenol Explorer).

2.7.4 Physical activity questionnaire

The International Physical Activity Questionnaire (IPAQ) was developed in 1998 to facilitate surveillance of physical activity (PA) based on a global standard (Craig et al. 2003). The long form version of the questionnaire, containing 31 items, was used to assess the usual activity and general fitness of volunteers participating in the study. Detailed information regarding the domains of household-, occupational- and leisure-time physical activity was collected for all participants (Appendix H). They were asked to assess the frequency and duration of various activities (walking, moderate- and vigorous-intensity exercise), per week. After applying a specific scoring protocol, the questionnaire evaluated the total weekly PA energy expenditure in Metabolic Equivalent Task minutes per week (MET-minutes/week) (International Physical Activity Questionnaire 2005). Subjects were then classified into 3 groups of physical activity level based on the value of MET-minutes/week: low PA (< 600 MET-minutes/week), moderate PA (≥ 600 MET-minutes/week) and high PA (≥ 3000 MET-minutes/week).

2.8 Quantification of PP metabolites in plasma and urine

Plasma and urinary PP were analysed according to a method validated by our research group (Domínguez-Fernández et al. 2020), which uses micro-solid

phase extraction (µ-SPE) followed by ultra-high performance liquid chromatography and mass spectrometry (HPLC-MS).

2.8.1 Analytical standards and other materials

A total of 128 analytical standards were included in the method for quantification of PP in biological samples. The list of PP metabolites with their retention time as well as the molecular weight and the manufacturing information can be found in Supplementary Table S1. Acetic and formic acids were sourced from Thermo Fisher Scientific (Loughborough, UK), while phosphoric acid was obtained from Yorlab (Fluka, York, UK). All other reagents and solvents were obtained from Sigma-Aldrich Co (Steinheim, Germany) unless otherwise stated. The standards' selection was based on the most abundant and relevant PP present in the human diet and the availability of authentic standards. Micro-elution plates (Oasis HLB, 2 mg sorbent per well, 30 µm) were sourced from Waters (Waters, Eschborn, Germany).

2.8.2 Sample preparation and micro-solid phase extraction (µ-SPE)

The µ-SPE was performed based on the protocol of a published method (Domínguez-Fernández et al. 2020). Briefly, 600 µL of plasma or urine samples were thawed on ice and centrifuged at 15 000*g* at 4°C for 15 min. Urine samples were diluted 5-fold with HPLC grade water. The supernatant (350 µL) was transferred to a microtube and diluted (1:1) with 4% phosphoric acid. The mixture (600 µL) was loaded onto Oasis 96-well reversed-phase HLB (hydrophilic-lipophilic balanced) sorbent µ-SPE plates (Waters, Eschborn, Germany) and spiked with internal standard Taxifolin

(concentration 0.25 mg/ml) prior to extraction. Isotope labelled Ferulic acid-1,2,3-$^{13}C3$ (FA-$^{13}C3$) (0.99 mg/ml, Sigma-Aldrich, Steinheim, Germany) was spiked after μ-SPE extraction as internal standard for matrix effects calculation in samples. Plates were then washed with water (200 μL) and 0.2% acetic acid (200 μL). Targeted compounds were eluted 3 times with 30 μL of methanol containing 0.1% formic acid and 10 nM ammonium formate into a 96-well collection plate. Forty microliters of water or standard mix with isotope labelled internal standard were added afterwards to the collection plate, making a 130 μL final volume. Thus, targeted compounds in samples were concentrated 2.3 times. An adhesive seal was applied to the 96-well collection plate to prevent samples from evaporation.

2.8.3 Ultrahigh performance liquid chromatography triple-quadrupole mass spectrometry

The analysis of plasma and urine PP metabolites was performed on a SHIMADZU Triple Quadrupole Mass Spectrometer (MS) (LC-MS8060, SHIMADZU, Kyoto, Japan) with an electro-spray interface source after separation on a UHPLC system. Samples (5 μL) were injected through a Raptor Biphenyl column 2.1 x 50 mm, 1.8 μm (Restek, Bellefonte, USA) with a compatible Raptor Biphenyl Guard Cartridges 5 x 2.1 mm (Restek, Bellefonte, USA). Mobile phases were water (HPLC grade, Sigma-Aldrich, Steinheim, Germany) with 0.1% formic acid (LC-MS grade, Thermo Fisher Scientific, Loughborough, UK) as solvent A, and acetonitrile (HPLC grade, Sigma-Aldrich, Steinheim, Germany) with 0.1% formic acid as solvent B. A 14 min gradient was applied to the run under a flow rate of 0.5 mL/min at 30°C. The gradient started at 1% solvent B, held constant for 1 min before risen to

12% at 4 min and kept for another 4 min. The percentage of B increased to 15% after 0.1 min, then held constant for 2.9 min before rising to 30% at 11.5 min, then 99% at 12 min and kept constant for 2 min. Finally, a 2-minute equilibration was applied to revert the percentage of B to 1%. Specific parameters were set for the SHIMADZU Triple Quadrupole Mass Spectrometer. The interface temperature was 300°C, the DL temperature was 250°C, nebulizing gas flow was 3.0 L/min, heating gas flow was 10.0 L/min, heat block temperature was 400°C, and drying gas flow was 10.0 L/min. Identification of metabolites was based on comparisons of the retention time of each analyte with its correspondent standard and considering the quantifier and two qualifier transitions, when possible, as well as their relative ion abundance based on the MS optimizations. The data acquisition and analysis were performed using the LabSolutions software (SHIMADZU, Kyoto, Japan). The calibration curves linked to the quantification of plasma and urine metabolites were developed using the ratio of the peak area of each individual PP to the peak area of the internal standard Taxifolin for each concentration. For each analytical run one full calibration curve was included. Plasma concentrations were expressed as nM and 24-hour urine concentrations were converted to nmoles based on the volume of the 24-hour urine collection.

2.9 Power calculation and statistical analysis

Power calculations were performed for the primary endpoint: changes in 24h BP response after chronic consumption. Based on literature and internal data, the minimum relevant differences expected in the change of SBP and DBP versus baseline between Aronia and Control are 6 and 4.5 mmHg,

respectively, and the standard deviations expected are 9 and 6.5 mmHg respectively (Broncel et al. 2010; Naruszewicz et al. 2007; Ras, Zock, et al. 2013; Draijer et al. 2015; Istas et al. 2019). Therefore, a total of 90 and 82 evaluable patients was necessary to ensure an 80% power to detect a significant difference in change of SBP and DBP, respectively, between treatment groups for two-sided test at the 2.5% level (alpha correction to control of the type I error rate due to multiple primary endpoint). Assuming a 10% drop out, 100 subjects were required into this two-treatment parallel-design study (n= 50 per arm).

The analysis of the outcomes was conducted using an ANCOVA with baseline as a covariate (BACO analysis) to compare responses due to either aronia berry extract or the placebo capsule (fixed factors). An additional analysis using ANCOVA with baseline, age, and baseline BMI as covariate (BAB analysis) was also performed for the primary outcome in order to see the influence of these factors. Response to treatment was calculated as change from baseline (CFB). CFB for the chronic analysis was calculated between 0h timepoint on week 12 versus 0h baseline on day 1. For outcomes measured at 0h and 2h at each visit, response to treatment was also calculated as CFB between 2h timepoint on day 1 versus 0h baseline on day 1 for the acute analysis and between 2h timepoint on week 12 versus 0h baseline on week 12 for the acute over chronic analysis.

The data analysis included the exploration of 3 different populations:

• Intention to treat (ITT) population, including all randomised participants who fulfilled all entry criteria and have been randomised.

• Per protocol (PerP) population, including ITT participants without any major protocol deviations and who have taken at least 80% of the study treatment.

• Safety Set (SS) population, including all participants who consumed at least one dose of the study product.

All statistical analyses of primary, secondary, and exploratory outcomes will be performed on ITT population and PerP population. All statistical analyses of safety outcomes will be performed on SS population.

The normality of dependent variable was verified using Shapiro Wilk for normality testing. If the normality could not be validated, a log-transformation of dependent variable and baseline value of dependent variable was modelled with the same model characteristics. If the normality of the log-transformed dependent variable could not be validated a non-parametric analysis using Mann Whitney U test without adjustment variable was performed. The control of the Type I error rate for the primary endpoint family was performed using Bonferroni method.

Correlations are presented as Pearson's r for nonnormal distribution and as Spearman's ρ for normal distribution. Statistical analysis was performed with the use of IBM SPSS Statistics 26.0 (Statistical Product and Service Solutions; IBM Corp.) and GraphPad Prism version 8 for Windows (GraphPad Software). Statistical significance is accepted at $p< 0.05$.

CHAPTER 3

Effects of aronia berry (poly)phenols on cardiometabolic health

3.1 Introduction

Cardiovascular diseases (CVD) are one of the main causes of death in Europe and other developed countries with an estimated 31% of all deaths worldwide (World Health Organization 2017). Diet has been shown to influence the risk of CVD and chronic diseases (Piepoli et al. 2016). Indeed, many large epidemiological studies such as the Global Burden of Disease study showed that diet is one of the most important modifiable risk factors for CVD, accounting for 11 million deaths and 255 million disability-adjusted life-years in 2017 (Afshin et al. 2019). The leading dietary risk factors were a high intake of sodium, low intake of wholegrain foods and low intake of fruits. Fruits and wholegrains are foods rich in bioactive compounds such as (poly)phenols (PP) (Zhan et al. 2017), which has been highlighted as one of the main reasons behind their health benefits (Grassi et al. 2015; Del Rio et al. 2013; Zhao et al. 2017; Hartley et al. 2013).

Berries have been recognized as an important source of PP and seem to have a role in the modulation of vascular health when consumed at physiologically relevant doses (Martini et al. 2020; Rodriguez-Mateos, Heiss, et al. 2014). Over the last decades, *Aronia melanocarpa* held researchers' attention due to its high anthocyanin concentration, which is the one of the highest among berries (Zheng and Wang 2003). In preclinical studies, this berry has revealed a protective effect towards chronic diseases such as gastrointestinal diseases, diabetes, and CVD (Jurikova et al. 2017).

Although a limited number of RCTs have investigated the effect of aronia berry consumption on cardiovascular health, some promising findings have been

shown, with some positive effects on inflammatory markers (Naruszewicz et al. 2007; Loo et al. 2016), blood lipids (Duchnowicz et al. 2018; Broncel et al. 2010), blood pressure (Kardum et al. 2015; Kardum et al. 2014; Broncel et al. 2010; Skoczyńska et al. 2007) and endothelial function (Istas et al. 2019). However, results were mixed, and several studies have not concluded on cardiometabolic changes following the consumption of aronia PP. So far, all existing RCTs investigating the effect of aronia berry consumption on blood pressure (BP) have included a relatively small number of volunteers (n< 55), and were mainly conducted with participants at high risk of CVD, i.e., hypertensive, with hypercholesterolemia, diabetes or taking statins (Pokimica et al. 2019; Loo et al. 2016; Simeonov et al. 2002; Xie et al. 2017; Naruszewicz et al. 2007).

Moreover, some of these trials did not include BP as a primary outcome, implying that statistical power was not calculated based on this parameter. Only one trial included healthy volunteers, more specifically young men, and office blood pressure was measured as a secondary outcome (Istas et al. 2019). Importantly, only 2 of these RCTs have used the gold standard 24-hour monitoring of BP (Loo et al. 2016; Kardum et al. 2015), which is a better predictor of CVD mortality and global CVD events than office BP (Yang et al. 2019; Kidambi et al. 2020; Huang et al. 2011; Bakogiannis et al. 2020). Both RCTs included healthy middle-aged prehypertensive participants (133/83 mmHg for Loo et al. and 134/83 mmHg for Kardum et al.), however duration (4 versus 8 weeks) and PP daily intake of the aronia juice administered (386 mg/day versus 2194 mg/day, respectively) were different. Interestingly, the trial with shorter duration and lower PP content showed a stronger effect, with

an overall decrease in awake ambulatory BP of 10 and 5.9 mmHg in SBP and DBP, respectively, compared with placebo, while a more modest decrease of 1.6 mmHg in DBP for the longer trial with higher PP content aronia juice was reported. Kardum and colleagues were the only ones to observe a significant decrease in 24-hour BP (changes in SBP= -8.5 mmHg). Both studies included a small number of subjects (n= 12 and 37 for Kardum and Loo, respectively). Based on these findings, there is a need for well-designed RCTs, involving a larger population of healthy individuals and using gold-standard techniques for the assessment of cardiometabolic outcomes, and in particular BP.

It is hypothesized that aronia berry intake improves vascular function in healthy individuals and that the effect may be partly imputed to the aronia-derived metabolites present in the bloodstream. The primary focus of this study was to evaluate the effects of 12-week daily consumption of an aronia berry extract on 24-hour ambulatory SBP and DBP (primary endpoint) in a prehypertensive population, compared with placebo. Secondary outcomes included office BP, heart rate (HR), flow-mediated dilation (FMD) and blood flow velocity, blood lipids, blood cortisol levels, safety and tolerability of the treatment, ambulatory and office heart rate (HR), ambulatory and office arterial stiffness measured as pulse wave velocity (PWV) and augmentation index (AIx), and QRISK®3 score.

Moreover, plasma and urinary concentrations of aronia PP were analysed and quantified to explore the relationship between circulating metabolites and vascular outcomes (this will be detailed in Chapter 4).

Other exploratory objectives of the present study included the collection of faecal samples on day 1 and day 84 to investigate gut microbiota composition and microbiome changes following the intake of aronia berry extract or placebo (results described in Chapter 6).

3.2 Methods

Detailed information about the methodology used in the study can be found in Chapter 2, including study population, inclusion and exclusion criteria, study design, investigational product, vascular and body composition measurements, QRISK®3 score calculation, sample collection and biochemical analysis, diet, health, and lifestyle questionnaires, as well as power calculation and statistical analysis.

A summary of the ABP study design is shown in Figure 3.1. Briefly, this study was a 2-arm, double-blind, parallel randomised-controlled trial. The primary objective was to investigate the effect of an aronia berry extract on ambulatory 24h SBP and DBP after 12 weeks daily consumption compared with placebo. Secondary objectives of the study included effects of 12-week aronia extract or placebo consumption on office SBP and DBP, office and ambulatory heart rate and arterial stiffness, endothelial function (FMD), blood flow velocity, blood lipids, and blood cortisol levels. The safety and tolerability of the extract was also a secondary outcome. The acute effects of the interventional product on FMD and blood flow velocity at 2h post-consumption were also included in the secondary outcomes of the study (Figure 3.1).

The number of volunteers included in the analysis of each parameter investigated in this trial can be found in Supplementary Table S2.

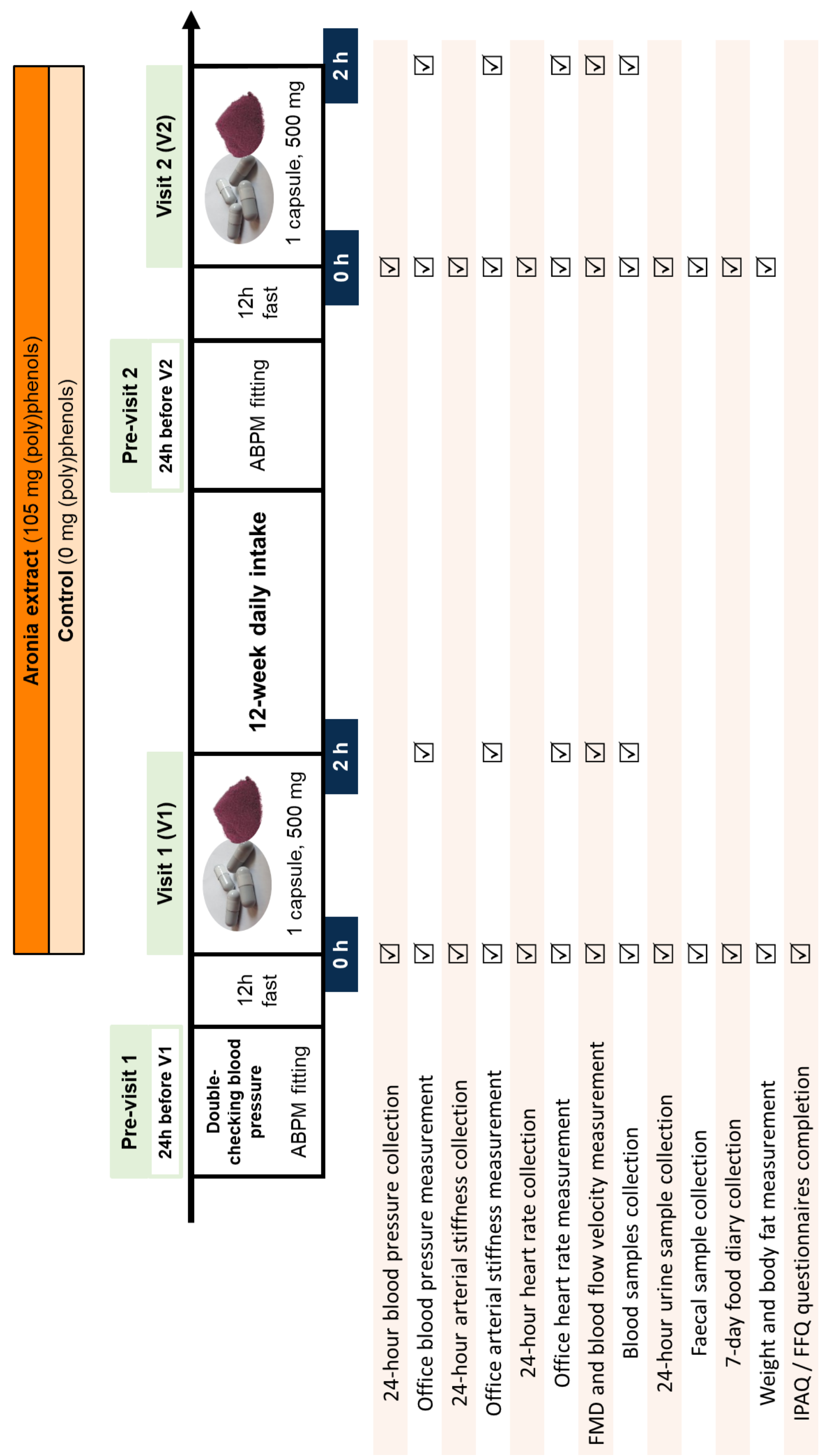


Figure 3.1: Study design of the ABP study.
ABPM, ambulatory blood pressure monitor; FMD, flow mediated dilation; FFQ, food frequency questionnaire; IPAQ, International physical activity questionnaire.

3.3 Results

3.3.1 Baseline characteristics of the study population

A total of 323 volunteers visited the unit and were screened to be part of the study, among whom 221 were excluded and 102 (47 men, 55 women) were enrolled and assigned randomly to one of the 2 intervention groups to constitute the intention-to-treat (ITT) population (Figure 3.2). Two participants discontinued the intervention for personal reasons and 3 were lost to follow-up, accounting for a total 5% drop-out. Ninety-seven participants completed all visits and were thus included in the acute, chronic, and acute on chronic analysis. As well as these 5 dropouts, 5 participants were removed from the per protocol (PerP) population due to low compliance (< 80%, n= 1), history of aortic stenosis (n= 1), abnormal increase in GGT, ALT and TG at visit 2 along with a high alcohol consumption at screening visit (n= 1), abnormal rise of cholesterol level associated with thyroid deficiency (n= 1) and suspicion of flu at visit 2 (n= 1).

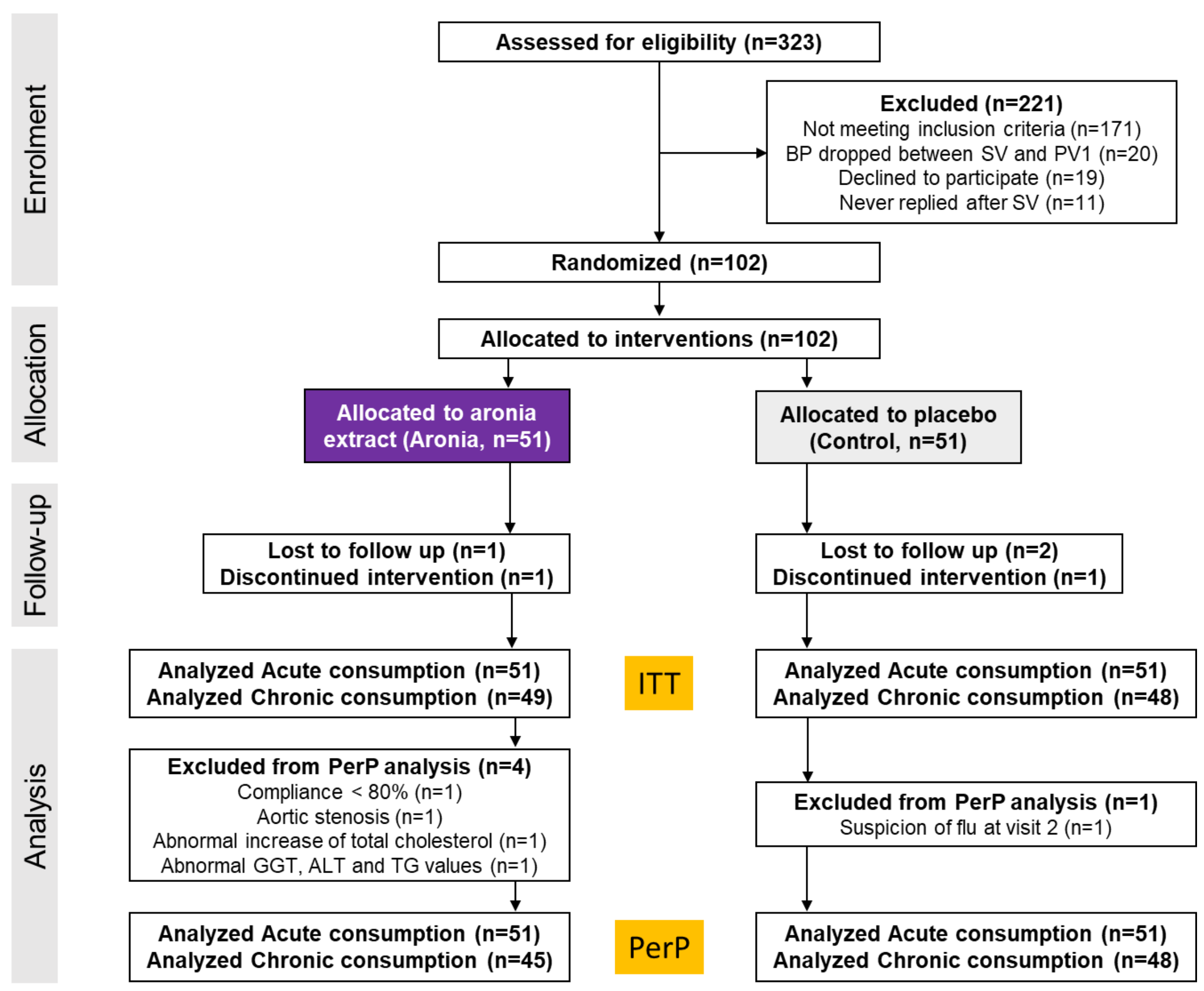


Figure 3.2: Study flow diagram.
ALT, Alanine aminotransferase; BP, Blood pressure; GGT, γ-glutamyl transferase; ITT, Intention to treat; PerP, Per protocol; PV1, Previsit 1; SV, Screening visit; TG, Triglycerides.

Baseline characteristics of both intervention groups can be found in Table 3.1. No statistically significant differences between Control and Aronia group were found, except the asleep heart rate which was higher in the Aronia group (p= 0.031).

Table 3.1: Baseline characteristics of the study population.

Population characteristics	All (n=102)		Aronia (n=51)		Control (n=51)		$P^{\dagger}$
	Mean	SD	Mean	SD	Mean	SD	
Sex (male/female, *n*)	47/55		23/28		24/27		0.840
Age (years)	56.2	8.8	56.2	8.7	56.2	9.0	0.980
Height (cm)	169.6	8.7	168.4	8.1	170.8	9.2	0.160
Weight (Kg)	70.9	10.7	71.0	9.9	70.8	11.6	0.949
BMI (Kg/m^2)	24.7	3.2	25.1	3.2	24.3	3.1	0.201
Body fat (%)	26.5	8.6	27.1	8.2	25.9	8.9	0.466
Basal metabolic rate (Kcal)	1528	280	1518	257	1538	303	0.721
IPAQ (MET-min/week)	5574	3819	5360	3663	5789	3996	0.590
Smokers (*n*)	5		1		4		0.173
24h SBP_{br} (mmHg)	120.2	9.7	120.5	9.3	119.9	10.1	0.740
24h DBP_{br} (mmHg)	69.6	7.0	69.7	5.5	69.5	8.4	0.904
24h heart rate (bpm)	69.7	8.5	70.0	7.2	69.5	9.7	0.775
24h SBP_{ao} (mmHg)	116.5	12.2	116.6	13.1	116.4	11.4	0.941
24h AIx_{ao} (%)	29.9	11.2	30.1	12.0	29.7	10.4	0.864
24h AIx_{br} (%)	-15.1	21.7	-14.5	23.2	-15.7	20.5	0.803
24h PWV (m/s)	9.3	1.1	9.4	1.1	9.2	1.1	0.504
Asleep SBP_{br} (mmHg)	108.1	11.9	107.6	11.3	108.5	12.7	0.726
Asleep DBP_{br} (mmHg)	60.3	7.9	59.9	6.6	60.6	9.1	0.681
Asleep heart rate (bpm)	61.8	8.1	63.6	7.2	60.0	8.7	**0.031***
Asleep SBP_{ao} (mmHg)	106.8	14.9	106.1	15.3	107.6	14.6	0.638
Asleep AIx_{ao} (%)	32.9	12.8	31.9	13.1	33.7	12.6	0.514
Asleep AIx_{br} (%)	-9.4	25.4	-11.3	26.0	-7.7	25.0	0.514
Asleep PWV (m/s)	8.8	1.3	8.9	1.3	8.7	1.3	0.415
Awake SBP_{br} (mmHg)	126.0	9.6	126.8	9.7	125.2	9.4	0.382
Awake DBP_{br} (mmHg)	73.9	7.2	74.4	5.7	73.5	8.4	0.509
Awake heart rate (bpm)	73.7	9.7	73.5	8.4	73.9	11.0	0.864
Awake SBP_{ao} (mmHg)	121.7	11.7	122.4	12.8	121.0	10.7	0.567
Awake AIx_{ao} (%)	27.8	11.6	28.3	12.8	27.4	10.6	0.719
Awake AIx_{br} (%)	-19.0	22.6	-17.9	24.7	-20.1	20.7	0.638
Awake PWV (m/s)	9.5	1.1	9.6	1.1	9.4	1.1	0.329
Office SBP (mmHg)	121.7	9.3	122.8	10.3	120.6	8.2	0.248
Office DBP (mmHg)	80.4	5.8	81.0	5.6	79.9	6.1	0.349
Office peripheral heart rate (bpm)	66.5	9.3	67.1	9.4	66.0	9.3	0.544
Office PWV (m/s)	7.4	1.5	7.4	1.3	7.5	1.6	0.675
Office AIx (%)	21.6	10.8	22.5	11.7	20.7	9.8	0.427
Office central SBP (mmHg)	114.5	9.3	115.3	10.5	113.6	7.9	0.361
Office central DBP (mmHg)	81.1	6.6	81.4	7.1	80.9	6.1	0.665
Office central heart rate (bpm)	61.5	9.0	62.5	8.2	60.5	9.8	0.252
Flow mediated dilation (%)	5.1	1.4	5.1	1.4	5.1	1.4	0.986
Brachial artery diameter at 0 sec (mm)	3.7	0.6	3.7	0.5	3.7	0.6	0.996
Brachial artery diameter at 60 sec (mm)	3.9	0.6	3.9	0.5	3.9	0.7	0.999
Blood flow velocity (cm/sec)	89.6	23.8	92.3	24.4	86.8	23.0	0.246
10-year QRISK®3 score (%)	5.5	4.0	5.6	3.8	5.3	4.2	0.709
Cortisol (mmol/L)	241.9	82.7	245.3	77.1	238.8	88.2	0.712
Fasted plasma glucose (mmol/L)	5.1	0.5	5.2	0.4	5.1	0.6	0.637
Sodium (mmol/L)	141.6	4.7	142.2	5.3	141.1	4.2	0.269
Potassium (mmol/L)	4.2	0.7	4.1	0.4	4.3	0.8	0.277
Urea (mmol/L)	5.2	1.3	5.3	1.2	5.1	1.3	0.468
Creatinine (umol/L)	78.8	21.7	75.0	15.5	82.3	25.8	0.098
eGFR (mL/min)	75.7	15.9	76.5	15.6	74.9	16.4	0.621

Total protein (g/L)	66.1	6.1	65.4	4.5	66.7	7.3	0.314
Albumin (g/L)	43.4	2.6	43.3	3.0	43.5	2.2	0.694
Globulins (g/L)	22.7	5.2	22.1	3.9	23.2	6.3	0.327
Total bilirubin (umol/L)	13.9	6.8	13.5	6.2	14.4	7.4	0.504
ALP (IU/L)	60.4	21.3	63.0	21.8	57.9	20.8	0.237
AST (IU/L)	25.8	7.0	26.0	7.1	25.7	7.0	0.858
ALT (IU/L)	21.7	10.6	23.7	13.4	19.8	6.9	0.074
GGT (IU/L)	23.0	24.3	24.7	27.9	21.4	20.5	0.513
LDH (IU/L)	174.7	28.7	174.3	28.0	175.1	29.5	0.891
hs-CRP (mg/L)	1.5	2.3	1.1	1.3	1.9	2.9	0.088
Urate (umol/L)	264.9	85.1	277.2	82.3	253.4	86.8	0.170
Cholesterol (mmol/L)	5.4	0.9	5.4	0.9	5.4	1.0	0.950
Triglycerides (mmol/L)	0.9	0.4	0.9	0.4	0.9	0.4	0.490
HDL (mmol/L)	1.7	0.4	1.7	0.5	1.7	0.4	0.828
LDL (mmol/L)	3.5	1.0	3.5	1.1	3.5	0.8	0.837
Non-HDL cholesterol (mmol/L)	3.7	1.0	3.7	1.2	3.7	0.8	0.769
Total cholesterol/HDL ratio	3.4	1.0	3.4	1.0	3.4	1.0	0.964
Total white blood cells (10^9/L)	5.0	1.3	5.0	1.3	5.1	1.4	0.659
Total red blood cells (10^{12}/L)	4.4	0.5	4.4	0.6	4.4	0.4	0.684
Hemoglobin (g/L)	134.8	15.9	135.0	12.0	134.6	19.0	0.917
Mean corpuscular value (fL)	89.8	5.3	89.8	4.9	89.9	5.7	0.952
MCV (pg)	30.9	3.1	30.9	2.9	30.9	3.3	0.973
MCHC (g/dL)	343.4	24.4	343.7	24.8	343.0	24.2	0.894
Red cell distribution width (%)	13.5	1.1	13.5	1.0	13.5	1.1	0.983
Platelets (10^9/L)	234.6	54.4	234.5	53.7	234.6	55.7	0.992
Mean platelet value (fL)	9.2	1.4	9.1	1.4	9.2	1.4	0.823
Neutrophils (10^9/L)	2.9	1.1	2.9	1.1	2.9	1.1	0.957
Lymphocytes (10^9/L)	1.4	0.5	1.4	0.4	1.5	0.6	0.610
Monocytes (10^9/L)	0.4	0.2	0.4	0.2	0.4	0.2	0.582
Eosinophils (10^9/L)	0.1	0.1	0.1	0.1	0.2	0.1	0.080
Basophils (10^9/L)	0.1	0.0	0.1	0.0	0.1	0.0	0.820

† *P* value from the unpaired t test. * P < 0.05.

AIx, augmentation index; ALP, alkaline phosphatase; ALT: alanine aminotransferase; ao, aortic; AST, aspartate aminotransferase; BMI: Body mass index; br, brachial; DBP, diastolic blood pressure; GGT, γ-glutamyl transferase; HDL, high density lipoprotein; hs-CRP, high-sensitivity C-reactive protein; IPAQ, international physical activity questionnaire; LDH, lactate dehydrogenase; LDL, low density lipoprotein; MCHC, mean corpuscular haemoglobin concentration; MCV, mean corpuscular volume; PWV, pulse wave velocity; SBP, systolic blood pressure.

The average age was in the middle of our age range and was similar for both Aronia and Control groups (56.2 years). Overall, the population was healthy, with a BMI in the normal range (24.7 kg/m^2), a hs-CRP of 1.5 mg/L, corresponding to an average risk of CVD and a QRISK®3 score of 5.5 which stands for a low risk of developing CVD in the next 10 years. Only 5 smokers were included in the trial, with 4 of them allocated to the Control group. Participant were very active, with an average IPAQ score of 5574 MET-

min/week, which is way above the cut-off limit of 3000 MET-min/week defining the high physical activity level range. Participants were in the very low range of prehypertension as average office SBP and DBP for the overall population were 121.7 and 80.4 mmHg, respectively. Averages of metabolic variables were universally within our central laboratory's ranges.

3.3.2 Background diet

The analysis of 7-day food diaries completed by participants at baseline (1 week prior the start of the study) showed no significant differences between the diet of participants in the Aronia and Control groups in terms of micro-, macronutrients and PP at baseline, apart from vitamin B3 (p= 0.047) which was higher in the Control group (36.2 versus 40.8 mg). During the last week of the study, when participants completed another 7-day diary, no difference between the diet of the groups was found except for flavonols (p= 0.042), which were consumed in higher amounts in the Control group (85 versus 74 mg) (Table 3.2). The average intake of fruits and vegetables in the overall population was 385 and 183g, respectively (data not shown).

Table 3.2: Average macro-, micronutrient and (poly)phenol intakes taken from 7-day diet diaries at baseline and during the 12-week intervention.

	Aronia				Control				*V1 A vs C (P)*	*V2 A vs C (P)*	*Aronia V1 vs V2 (P)*	*Control V1 vs V2 (P)*
	Visit 1 (n=50)		Visit 2 (n=48)		Visit 1 (n=51)		Visit 2 (n=48)					
	Mean	SD	Mean	SD	Mean	SD	Mean	SD				
Energy (kcal)	2021	586	1832	436	1938	581	1925	656	0.473	0.416	0.029*	0.688
Protein (g)	82.0	28.1	74.0	21.2	85.8	25.9	76.4	24.3	0.484	0.605	0.084	0.006*
Carbohydrates (g)	223	64.7	204	53.9	206	66.9	209	78.6	0.181	0.722	0.007*	0.924
Fibres (g)	25.8	11.1	23.4	9.0	23.5	9.7	22.8	9.6	0.266	0.724	0.023*	0.468
Fat (g)	80.5	32.9	71.3	27.6	79.1	33.5	78.3	33.1	0.840	0.266	0.179	0.811
Saturated fat (g)	28.6	13.6	24.9	12.4	26.9	11.3	27.4	11.5	0.502	0.306	0.197	0.832
Monoinsaturated fat (g)	28.9	13.2	26.2	11.0	30.3	13.7	29.5	14.8	0.611	0.221	0.425	0.694
Polyinstaurated fat (g)	12.1	5.9	11.5	4.9	13.3	9.5	13.0	6.3	0.434	0.194	0.648	0.755
Omega-3 (g)	1.5	1.1	1.3	1.0	1.5	1.1	1.5	1.4	0.868	0.583	0.296	0.766
Omega-6 (g)	7.3	5.2	6.2	3.7	8.2	7.9	7.3	4.2	0.518	0.175	0.199	0.326
Trans fats (g)	1.3	1.8	0.9	0.6	1.8	5.8	1.0	0.6	0.537	0.285	0.223	0.320
Vitamin A (µg)	1114	720	917	616	1137	1323	915	693	0.913	0.989	0.031*	0.286
Retinol (µg)	358	394	276	164	481	1190	388	473	0.486	0.124	0.259	0.572
Carotene (µg)	3945	3231	3260	3040	3468	2953	2703	2446	0.440	0.325	0.009*	0.125
Vitamin D (µg)	3.2	3.0	2.7	2.0	3.0	2.2	2.9	1.9	0.704	0.668	0.172	0.834
Vitamin E (mg)	9.9	4.9	9.7	4.4	9.6	5.4	10.8	5.9	0.772	0.304	0.958	0.104
Vitamin K1 (µg)	78.2	64.1	58.3	45.1	66.3	83.5	76.8	97.7	0.423	0.238	0.011*	0.375
Vitamin B1 (mg)	1.7	0.6	1.8	1.9	1.9	1.1	1.6	0.7	0.310	0.583	0.702	0.033*
Vitamin B2 (mg)	1.6	0.5	1.5	0.4	1.9	0.9	1.7	0.7	0.085	0.050	0.597	0.161
Vitamin B3 (mg)	36.2	11.0	33.1	9.9	40.8	12.1	36.5	13.4	0.047*	0.163	0.144	0.009*
Vitamin B5 (mg)	5.6	1.7	5.2	1.7	6.0	2.4	5.6	2.0	0.266	0.207	0.332	0.144
Vitamin B6 (mg)	1.8	0.5	1.7	0.5	2.4	3.9	2.1	2.1	0.246	0.194	0.529	0.181
Vitamin B7 (mg)	44.2	20.2	42.0	21.9	48.6	21.9	44.6	20.8	0.302	0.562	0.808	0.134
Vitamin B9 (µg)	305	100	261	90	296	125	293	120	0.678	0.143	0.002*	0.632
Vitamin B12 (µg)	5.2	4.1	4.7	2.6	5.5	5.2	4.7	2.8	0.747	0.928	0.594	0.321
Vitamin C (mg)	118	66.7	104	71.7	109	68.8	114	74.2	0.490	0.511	0.049*	0.790
Sodium (g)	2074	788	1967	703	2105	908	2014	853	0.855	0.767	0.498	0.222
Potassium (mg)	3489	1092	3153	837	3432	1139	3314	1241	0.801	0.459	0.030*	0.302
Calcium (mg)	918	301	860	295	882	305	893	332	0.556	0.603	0.426	0.985
Magnesium (mg)	352	124	335	103	348	144	344	140	0.875	0.740	0.266	0.792
Phosphorus (mg)	1399	406	1303	335	1440	443	1370	476	0.632	0.429	0.232	0.173
Iron (mg)	12.8	4.3	11.8	3.7	12.2	5.0	12.0	4.9	0.522	0.872	0.091	0.524
Copper (mg)	1.6	0.6	1.5	0.6	1.9	1.9	2.4	5.2	0.210	0.219	0.246	0.586
Zinc (mg)	9.8	4.5	9.2	2.8	10.1	4.3	9.2	3.9	0.790	0.988	0.732	0.096
Chloride (mg)	3081	998	2916	760	3224	1243	3108	1306	0.527	0.381	0.374	0.302
Manganese (mg)	4.7	2.4	4.6	3.2	4.6	2.2	4.9	4.9	0.773	0.680	0.868	0.556
Selenium (µg)	53.9	22.2	51.7	22.0	55.8	26.2	53.9	28.2	0.692	0.667	0.730	0.557
Iodine (µg)	135	64.4	131	66.9	323	1321	132	69	0.319	0.893	0.889	0.302
Caffeine (mg)	151	114	133	98.9	201	155	165	120	0.069	0.159	0.573	0.015*
Anthocyanins (mg)	77.0	62.1	61.2	56.9	59.2	68.9	73.3	85.7	0.177	0.416	0.137	0.354
Flavan-3-ols (mg)	272	367	232	233	334	281	322	283	0.336	0.093	0.971	0.698
Proanthocyanins (mg)	211	153	241	184	308	424	320	281	0.131	0.109	0.239	0.979
Flavanones (mg)	20.4	27.7	17.8	30.0	20.9	24.2	17.0	25.6	0.925	0.890	0.355	0.184
Flavones (mg)	4.5	4.5	6.8	7.3	5.0	7.5	5.8	11.6	0.699	0.621	0.016*	0.693
Flavonols (mg)	74.0	53.2	56.7	34.5	85.1	73.4	72.8	41.9	0.385	0.042*	0.028*	0.140
Isoflavonoids (mg)	5.5	13.4	5.8	11.3	1.9	4.0	3.7	9.3	0.071	0.336	0.904	0.203
Lignans (mg)	16.9	38.7	9.4	16.8	9.6	22.5	21.3	51.5	0.248	0.132	0.175	0.045*
Stilbenes (mg)	0.4	0.6	0.5	0.6	0.3	0.4	0.3	0.6	0.197	0.145	0.350	0.806
Phenolic acids (mg)	583	365	581	547	691	585	695	706	0.273	0.380	0.873	0.887
Other (poly)phenols† (mg)	99.1	266	46.2	84.1	222	916	55.4	70.7	0.363	0.564	0.182	0.186
Total (poly)phenols (mg)	1364	705	1258	652	1738	1287	1586	998	0.074	0.060	0.608	0.350

Average macro-, micronutrient and (poly)phenol intakes taken from 7-day diet diaries at baseline and during the 12-week intervention. Comparisons Aronia versus Control (A vs C) calculated using an independent t-test. Comparisons visit 1 (V1) versus visit 2 (V2) calculated using a paired t-test. *Significant difference at $p < 0.05$. †Other (poly)phenols: Chalcones, Dihydrochalcones, Hydroxphenylpropanoic acids and Other as defined in the Phenol Explorer classification (http://phenol-explorer.eu/compounds/classification). P, p-value.

The analysis of food diaries completed by subjects at baseline allowed us to compare the intake in micro- and macronutrients from the study population with the UK recommendation from the Eatwell guide (Public Health England 2016) (Table 3.3). A coefficient of variation (CoV) was calculated for each parameter and ranged from 1 to 74%, indicating a high adherence of our population to the UK guidelines. The variability between Eatwell guide and baseline ABP intake was low for the macronutrients, with the biggest divergence being associated to protein intake (CoV= 36%).

In terms of vitamins, the ABP population systematically presented a higher intake compared to the Eatwell guide's recommendations, except for vitamin D, which was lower than the recommended intake. However, vitamin D levels were similar to those found in a recent study investigating vitamin D levels in various cohorts of the National Diet and Nutrition Survey (NDNS) and reporting an average level of 3.7 μg/day for the population aged 19 to 74 years (Calame, Street, and Hulshof 2020).

Regarding micronutrients, the intakes recorded in the ABP population was also higher than the Eatwell recommendations, except for sodium, potassium, and selenium, which were slightly lower than the guidelines. The higher coefficient of variation was observed for phosphorus (CoV= 62%).

Table 3.3: Comparison of Eatwell guide dietary recommendations with the baseline intake of micro- and macronutrients of the ABP study population.

	Eatwell guide (19-74 y/o)	**ABP study** (average 56.2 y/o)	Coefficient of variation (%)
Energy (kcal/day)	2189	1979	7
Protein (g/day)	50.1	83.9	36
Carbohydrate (g/day)	292	214	22
Fibres (g/day)	30	24.7	14
Fat (g/day)	85	79.8	4
Saturated fat (g/day)	26.8	27.8	3
Monounsaturated fat (g/day)	31.8	29.6	5
Polyunsaturated fat (g/day)	15.8	12.7	15
Vitamin A (µg/day)	650	1126	38
Vitamin D (µg/day)	10	3.1	74
Vitamin B1 (mg/day)	0.9	1.8	49
Vitamin B2 (mg/day)	1.2	1.7	26
Vitamin B3 (mg/day)	14.5	21.4	27
Vitamin B6 (mg/day)	1.3	2.1	34
Vitamin B9 (µg/day)	200	301	28
Vitamin B12 (µg/day)	1.5	5.4	80
Vitamin C (mg/day)	40	114	68
Sodium (g/day)	2.4	2	9
Potassium (mg/day)	3500	3460	1
Calcium (mg/day)	700	900	18
Magnesium (mg/day)	285	350	14
Phosphorus (mg/day)	550	1420	62
Iron (mg/d)	8.7	12.5	25
Copper (mg/day)	1.2	1.8	27
Zinc (mg/day)	8.3	10.0	13
Chloride (mg/day)	2500	3153	16
Selenium (µg/day)	67.5	54.9	15
Iodine (µg/day)	140	230	34

y/o: years old.

Some differences were found between the diet of volunteers at baseline and at the end of the study in both Aronia and Control group (Table 3.2). Indeed, energy levels, carbohydrates, fibres, carotene, vitamin A, K1, B9 and C, potassium, and flavonols intakes all significantly decreased from visit 1 to visit 2 for the Aronia group. Only flavone intake was significantly increased following the intervention in the Aronia group. Regarding Control group,

protein, caffein, vitamin B1 and B3 intakes were all significantly reduced after the intervention, based on the self-reporting 7-day food diary completed by participants. On the contrary, subjects from the Control group had a significantly increased intake of lignans on study day 2 compared to the first visit. However significant, these differences seem to be minor as the total PP intake was not different between visits for any of the intervention groups.

Daily average baseline PP intake was 1364 ± 705 mg in the Aronia group and 1738 ± 1287 mg in the Control group. The main contribution of food products to the intake of total PP at baseline was for coffee (29%), tea (27%), fruits (11%), herbs and spices (9%), nuts and seeds, tubers and roots, alcoholic drinks and chocolate (2% each) (Supplementary Table S3). The main fruits contributing were predominantly apples (44%), followed by berries (strawberries, blueberries and raspberries, 32%) and grapes (6%). Vegetables accounted for 1% of the total PP intake.

3.3.3 Effect of aronia berry extract consumption on office and 24-hour ambulatory blood pressure

The primary outcome of the study was to determine the effect of Aronia versus Control on average brachial 24h SBP and DBP (SBP_{br} and DBP_{br}), after 12-week daily consumption. These two parameters were measured using the Arteriograph24^{TM} (TensioMed, Budapest, Hungary). No significant differences were observed for the primary outcome for neither ITT nor PerP population, and neither for BAB analysis (with baseline, age, and BMI as covariate) (Table 3.4) nor BACO (baseline as covariate only) analysis (data not shown). However, a trend for a bigger decrease for both SBP_{br} and DBP_{br} was found

in Aronia group (Figure 3.3). Moreover, changes from baseline were significant for 24-hour and awake SBP and DBP for both intervention groups.

Table 3.4: Effects of aronia berry extract after 12-week consumption on 24-hour, awake and asleep peripheral SBP and DBP, for both ITT and PerP populations and each intervention group, following BAB analysis.

Parameter (mmHg)	ITT population				PerP population			
	CFB Aronia	CFB Control	CFC	p-value	CFB Aronia	CFB Control	CFC	p-value
24-hour SBP_{br}	-2.05 ± 0.66*	-1.77 ± 0.67*	-0.3	0.763	-1.74 ± 0.74*	-1.57 ± 0.73*	-0.18	0.867
Awake SBP_{br}	-1.97 ± 0.80*	-1.80 ± 0.81*	-0.2	0.177	-1.95 ± 0.85*	-1.75 ± 0.83*	-0.20	0.240
Asleep SBP_{br}	-1.29 ± 1.06	-1.34 ± 1.06	0.05	0.725	-1.16 ± 1.03	-1.57 ± 1.01	0.41	0.973
24-hour DBP_{br}	-1.71 ± 0.51*	-1.27 ± 0.51*	-0.4	0.543	-1.77 ± 0.55*	-1.07 ± 0.55*	-0.70	0.373
Awake DBP_{br}	-1.78 ± 0.59*	-1.35 ± 0.60*	-0.4	0.613	-1.83 ± 0.62*	-1.27 ± 0.61*	-0.56	0.522
Asleep DBP_{br}	-1.02 ± 0.79	-.50 ± 0.79	-0.5	0.643	-1.04 ± 0.83	-.58 ± 0.82	-0.46	0.693

Values expressed as mean ± SD. Changes from baseline (CFB) and changes from Control (CFC) were calculated using ANCOVA (Bonferroni) with age, baseline BMI and baseline parameter value as covariates. br, brachial; DBP, diastolic blood pressure; ITT, intention to treat; PerP, per protocol; SBP, systolic blood pressure. *Significance at p< 0.05 between baseline and 12 weeks.

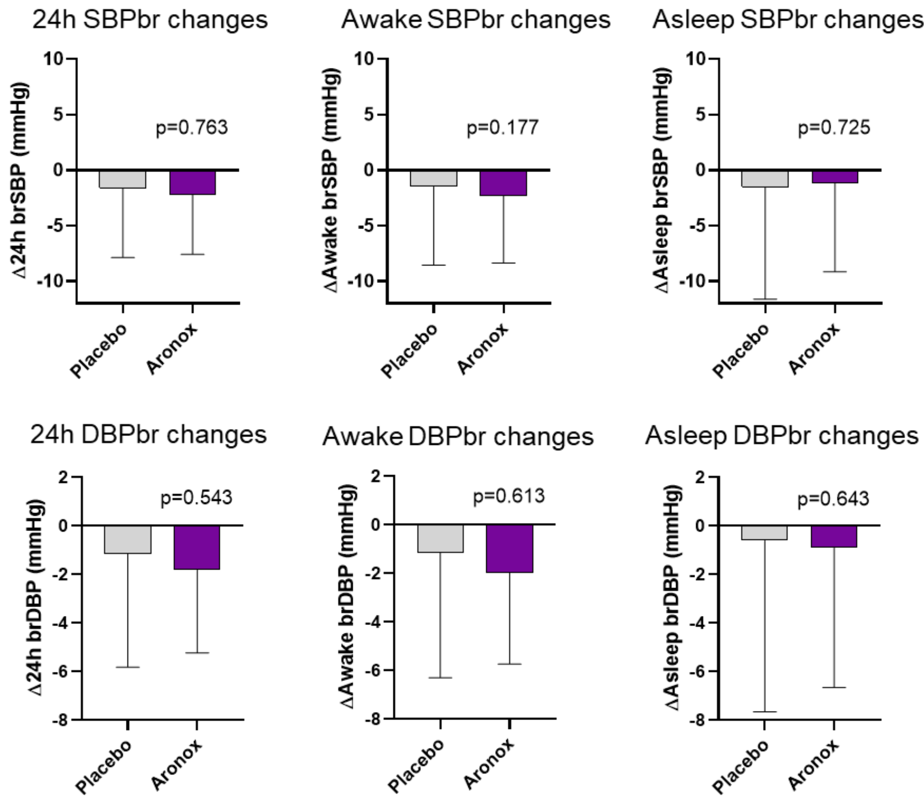


Figure 3.3: Twenty-four-hour, awake, and asleep peripheral SBP and DBP changes from baseline following a 12-week consumption of aronia berry extract, for both Aronia and Control groups, following BAB analysis. P-value represents the change from Control.

Among the secondary objectives, changes in ambulatory central SBP (SBP_{ao}) were investigated (Table 3.5). No significant difference was observed between the treatment groups. Nonetheless, the changes from baseline of the Aronia group appeared lower than those of the Control group for 24-hour, awake and asleep SBP_{ao}.

Table 3.5: Effects of aronia berry extract after 12-week consumption on 24-hour, awake and asleep central SBP in the ITT population and each intervention group, following BAB analysis.

Parameter	*Unit*	CFB Aronia	CFB Control	CFC	p-value
24-hour SBP_{ao}	*mmHg*	-1.44 ± 0.89	-0.28 ± 0.87	-1.17	0.433
Awake SBP_{ao}	*mmHg*	-1.69 ± 1.02	-1.03 ± 1.00	-0.65	0.652
Asleep SBP_{ao}	*mmHg*	-1.57 ± 1.40	0.02 ± 1.38	-1.58	0.421

Values expressed as mean ± SD. ao, aortic; CFB, changes from baseline; CFC, changes from Control; DBP, diastolic blood pressure; SBP, systolic blood pressure.

Secondary outcomes of this trial also included the investigation of PP-rich aronia extract's effect on office peripheral and central SBP and DBP, measured using OMRON and SphygmoCor®, respectively (Table 3.6) No significant changes have been found following the 12-week daily consumption of the investigational product, even though chronic central SBP values were the same magnitude as results found using the ambulatory BP monitor (24h SBP_{ao}= -1.4 ± 0.9 and -0.3 ± 0.9 mmHg for Aronia and Control, respectively while office SBP_{ao}= -1.8 ± 0.9 and -0.2 ± 1.0 mmHg for Aronia and Control, respectively), which is a good indicator of the reliability of the results. Moreover, while chronic office SBP_{br} changes from Control were close to significance (CFC= -2.4 mmHg, p= 0.074), changes from baseline were significant for the same parameter and timepoint in the Aronia group (CFB= -2.4 ± 0.9 mmHg, 95% CI= [-4.195; -0.561 mmHg]) (Table 3.6).

Acute peripheral and central office BP changes on the first and last study day present some significant increases from baseline for both treatment groups. This rise is significantly higher in Aronia group for office SBP_{ao} two hours post-consumption on the last visit (CFC= 2.2 mmHg, p= 0.017). Similar results were found for BACO analysis (data not shown).

Table 3.6: Effects of aronia berry extract after 12-week consumption on office peripheral and central SBP and DBP in the ITT population and each intervention group, following BAB analysis.

Parameter	*Unit*	CFB Aronia	CFB Control	CFC	p-value
Acute changes					
Office SBP_{br}	*mmHg*	5.97 ± 1.11*	6.82 ± 1.11*	-0.85	0.590
Office DBP_{br}	*mmHg*	1.33 ± 0.65*	1.45 ± 0.65*	-0.12	0.898
Office SBP_{ao}	*mmHg*	6.66 ± 1.12*	7.05 ± 1.13*	-0.40	0.788
Office DBP_{ao}	*mmHg*	1.35 ± 0.97	1.39 ± 0.98	-0.04	0.908
Chronic changes					
Office SBP_{br}	*mmHg*	-2.38 ± 0.92*	-0.01 ± 0.92	-2.37	0.074
Office DBP_{br}	*mmHg*	-0.91 ± 0.70	-1.02 ± 0.71	0.11	0.912
Office SBP_{ao}	*mmHg*	-1.80 ± 0.93	-0.17 ± 0.95	-1.63	0.227
Office DBP_{ao}	*mmHg*	-0.21 ± 0.81	-1.04 ± 0.81	0.83	0.466
Acute on chronic changes					
Office SBP_{br}	*mmHg*	7.15 ± 1.12*	5.10 ± 1.14*	2.05	0.153
Office DBP_{br}	*mmHg*	1.73 ± 0.60*	0.27 ± 0.61	1.46	0.060
Office SBP_{ao}	*mmHg*	7.45 ± 1.18*	5.26 ± 1.21*	2.19	**0.017**
Office DBP_{ao}	*mmHg*	1.39 ± 0.63*	0.17 ± 0.64	1.22	0.181

Values expressed as mean ± SD. ao, aortic; br, brachial; CFB, changes from baseline; CFC, changes from Control; DBP, diastolic blood pressure; SBP, systolic blood pressure. *Significance at p< 0.05 between baseline and 12 weeks.

3.3.4 Effect of aronia berry extract consumption on office and 24-hour ambulatory arterial stiffness

One of our second objectives was to assess the effect of the intervention on arterial stiffness, defined as PWV and AIx. These parameters were measured using gold standard applanation tonometry technology with SphygmoCor®. No significant differences were found between groups following acute and chronic consumption of aronia extract or placebo. Similar results were found for both BAB (Table 3.7) and BACO (data not shown) analysis.

Table 3.7: Effects of aronia berry extract after 2-hour and 12-week consumption on office PWV and AIx in the ITT population and each intervention group, following BAB analysis.

		Acute changes			
Parameter	*Unit*	CFB Aronia	CFB Control	CFC	p-value
PWV	*m/sec*	0.63 ± 0.23*	0.46 ± 0.24	0.17	0.623
AIx	*%*	0.83 ± 1.00	0.45 ± 1.02	0.38	0.529
		Chronic changes			
Parameter	*Unit*	CFB Aronia	CFB Control	CFC	p-value
PWV	*m/sec*	0.33 ± 0.20	0.30 ± 0.20	0.03	0.910
AIx	*%*	-0.90 ± 1.06	-0.56 ± 1.09	-0.34	0.823
		Acute on chronic changes			
Parameter	*Unit*	CFB Aronia	CFB Control	CFC	p-value
PWV	*m/sec*	-0.27 ± 0.19	0.17 ± 0.19	-0.44	0.089
AIx	*%*	1.05 ± 0.98	-0.10 ± 1.04	1.15	0.425

Values expressed as mean ± SD. AIx, augmentation index; CFB, changes from baseline; CFC, changes from Control; PWV, pulse wave velocity. *Significance at p< 0.05 between baseline and 12 weeks.

Twenty-four-hour, awake, and asleep PWV, central AIx (AIx_{ao}) and peripheral AIx (AIx_{br}) were measured prior both visit 1 and 2 using Arteriograph24™. Aronia extract consumption for 12 weeks led to a significant decrease in 24h and awake peripheral and central AIx in comparison with Control (Δ24h AIx_{br}= -6.8%, p= 0.003; Δ24h AIx_{ao}= -3.3%, p= 0.006, ΔAwake AIx_{br}= -6.1%,

p= 0.020; ΔAwake AIx_{ao}= -2.9%, p= 0.034) (Table 3.8). A non-significant trend for a decrease in Aronia was also observed for asleep AIx_{ao} and AIx_{br}. A significant reduction in awake PWV was also observed in Aronia compared to Control (ΔPWV= -0.24 m/s, $p < 0.05$). Similar results were found for BACO analysis (Supplementary Table S4).

Table 3.8: Effects of aronia berry extract after 12-week consumption on 24-hour, awake and asleep central PWV and peripheral and central AIx in the ITT population and each intervention group, following BAB analysis.

Parameter	*Unit*	CFB Aronia	CFB Control	CFC	p-value
24-hour PWV_{ao}	*m/s*	-0.05 ± 0.11	0.11 ± 0.11	-0.15	0.336
Awake PWV_{ao}	*m/s*	-0.13 ± 0.14	0.11 ± 0.14	-0.24	**0.049**
Asleep PWV_{ao}	*m/s*	-0.11 ± 0.13	0.18 ± 0.13	-0.29	0.128
24-hour AIx_{ao}	*%*	-0.99 ± 0.81	2.28 ± 0.79*	-3.26	**0.006**
Awake AIx_{ao}	*%*	-1.05 ± 1.01	1.85 ± 0.99	-2.89	**0.034**
Asleep AIx_{ao}	*%*	-0.97 ± 1.28	1.80 ± 1.26	-2.76	0.134
24-hour AIx_{br}	*%*	-2.33 ± 1.58	4.45 ± 1.54*	-6.78	**0.003**
Awake AIx_{br}	*%*	-2.62 ± 2.03	3.51 ± 2.01	-6.13	**0.020**
Asleep AIx_{br}	*%*	-1.91 ± 2.52	3.54 ± 2.49	-5.45	0.134

Values expressed as mean ± SD. AIx, augmentation index; ao, aortic; br, brachial; CFB, changes from baseline; CFC, changes from Control; PWV, pulse wave velocity.
* Significance at $p < 0.05$ between baseline and 12 weeks.

3.3.5 Effect of aronia berry extract consumption on endothelial function and blood flow velocity

No significant change was observed following the 12-week daily intake of aronia extract or placebo for endothelial function, measured as FMD, and blood flow velocity (Table 3.9). FMD and blood flow velocity did not significantly change in any of the groups or timepoints, with the exception of a significant increase in FMD from baseline in the Control group at 12 weeks post-consumption but did not reach significance when compared with the aronia group which also had a small non-significant increase from baseline. Similar results were found for both BAB and BACO analysis (data not shown).

Table 3.9: Effects of aronia berry extract after 2-hour and 12-week consumption on FMD and blood flow velocity in the ITT population and each intervention group, following BAB analysis.

Acute changes

Parameter	*Unit*	CFB Aronia	CFB Control	CFC	p-value
FMD	*%*	0.30 ± 0.20	0.22 ± 0.19	0.08	0.777
Blood flow velocity	*cm/sec*	-4.7 ± 3.4	-6.6 ± 3.3	1.92	0.785

Chronic changes

Parameter	*Unit*	CFB Aronia	CFB Control	CFC	p-value
FMD	*%*	0.13 ± 0.21	0.58 ± 0.21*	-0.44	0.144
Blood flow velocity	*cm/sec*	-1.3 ± 3.5	3.7 ± 3.5	-4.93	0.070

Acute on chronic changes

Parameter	*Unit*	CFB Aronia	CFB Control	CFC	p-value
FMD	*%*	-0.21 ± 0.21	-0.42 ± 0.22	-0.02	0.997
Blood flow velocity	*cm/sec*	-4.6 ± 3.6	-4.6 ± 3.6	0.21	0.500

Values expressed as mean ± SD. CFB, changes from baseline; CFC, changes from Control; FMD, flow mediated dilation. * Significance at $p< 0.05$ between baseline and 12 weeks.

3.3.6 Effect of aronia berry extract consumption on office and 24-hour ambulatory heart rate

Office heart rate (HR) was monitored at each timepoint and using 2 different devices. The automatic sphygmomanometer (OMRON M3) used to perform the BP measurement was also giving the information regarding seated HR. Additionally, supine HR was recorded during at each timepoint during the assessment of arterial stiffness using SphygmoCor®. No significant difference was found for seated and supine office HR (Table 3.10), although a significant decrease in acute HR from baseline was found in both Aronia and Control groups, and in chronic HR in the Control group only.

Table 3.10: Effects of aronia berry extract after 2-hour and 12-week consumption on office heart rate (seated and supine) in the ITT population and each intervention group, following BAB analysis.

Acute changes

Parameter	*Unit*	CFB Aronia	CFB Control	CFC	p-value
Office HR (seated)	*bpm*	-7.68 ± 0.66*	-8.44 ± 0.66*	0.42	0.756
Office HR (supine)	*bpm*	-6.35 ± 0.73*	-5.97 ± 0.74*	-0.38	0.700

Chronic changes

Parameter	*Unit*	CFB Aronia	CFB Control	CFC	p-value
Office HR (seated)	*bpm*	-0.47 ± 0.92	-2.23 ± 0.93*	1.76	0.186
Office HR (supine)	*bpm*	-0.72 ± 0.87	-1.06 ± 0.89	0.34	0.787

Acute on chronic changes

Parameter	*Unit*	CFB Aronia	CFB Control	CFC	p-value
Office HR (seated)	*bpm*	-7.62 ± 0.70*	-6.91 ± 0.71*	-0.71	0.175
Office HR (supine)	*bpm*	-5.51 ± 0.71*	-5.41 ± 1.04*	-0.10	0.924

Values expressed as mean ± SD. CFB, changes from baseline; CFC, changes from Control; HR, heart rate. * Significance at $p < 0.05$ between baseline and 12 weeks.

Twenty-four-hour, awake, and asleep ambulatory HR were measured prior both visit 1 and 2 using Arteriograph24^{TM}. No significant difference was observed between Aronia and Control group, for any of the parameters measured (Table 3.11). The changes observed for 24-hour and awake HR were the same magnitude as the chronic changes observed with the assessment of office HR. Similar results were found for both BAB and BACO (data not shown) analysis.

Table 3.11: Effects of aronia berry extract after 12-week consumption on 24-hour, awake and asleep heart rate in the ITT population and each intervention group, following BAB analysis.

Parameter	*Unit*	CFB Aronia	CFB Control	CFC	p-value
24-hour HR	*bpm*	-0.16 ± 0.90	-1.61 ± 0.91	1.45	0.330
Awake HR	*bpm*	-0.09 ± 1.03	-1.86 ± 1.04	1.77	0.352
Asleep HR	*bpm*	0.17 ± 0.94	-0.29 ± 0.94	0.46	0.961

Values expressed as mean ± SD. CFB, changes from baseline; CFC, changes from Control; HR, heart rate.

3.3.7 Effect of aronia berry extract consumption on QRISK®3 score

No significant change was observed following the analysis of QRISK®3 score after 12 weeks daily consumption of the aronia extract or control treatment (Table 3.12). Similar results were found for both BAB and BACO analysis (data not shown).

Table 3.12: Effects of aronia berry extract after 12-week consumption on QRISK®3 score in the ITT population and each intervention group, following BAB analysis.

Parameter	*Unit*	CFB Aronia	CFB Control	CFC	p-value
QRISK®3 score	*points*	-0.24 ± 0.18	-0.25 ± 0.18	0.01	0.855

Values expressed as mean ± SD. CFB, changes from baseline; CFC, changes from Control.

3.3.8 Effect of aronia berry extract consumption on blood lipids and cortisol levels

No significant difference was reported between treatment groups following 12-week consumption of investigational product for blood lipid or cortisol levels. Similar results were found for both BAB (Table 3.13) and BACO (data not shown) analysis. However, a significant decrease in cortisol levels was found 12 weeks after the consumption of aronia when compared with baseline ($CFB_{cortisol}$= -22.5 mmol/L, 95% CI= [-42.4; -2.6 mmol/L]). Those changes did not reach significance when compared with the Control group (Δcortisol= -16.8 mmol/L, p= 0.111). Changes from baseline were also significant for HDL cholesterol in the Aronia group only (CFB_{HDL}= 0.07 mmol/L, 95% CI= [0.004; 0.141 mmol/L]).

Table 3.13: Effects of aronia berry extract after 12-week consumption on blood lipids and cortisol levels in the ITT population and each intervention group, following BAB analysis.

Parameter	*Unit*	CFB Aronia	CFB Control	CFC	p-value
Cortisol	*mmol/L*	-22.5 ± 10.0*	-5.70 ± 9.57	-16.8	0.111
Total cholesterol	*mmol/L*	0.04 ± 0.10	-0.12 ± 0.10	0.16	0.304
Triglycerides	*mmol/L*	-0.00 ± 0.04	-0.02 ± 0.04	0.02	0.634
HDL-cholesterol	*mmol/L*	0.07 ± 0.03*	0.05 ± 0.03	0.02	0.971
LDL-cholesterol	*mmol/L*	0.18 ± 0.10	0.02 ± 0.09	0.16	0.223

Values expressed as mean ± SD. CFB, changes from baseline; CFC, changes from Control; HDL, high density lipoprotein; LDL, low density lipoprotein. * Significance at $p < 0.05$ between baseline and 12 weeks.

3.3.9 Tolerance and safety of the intervention

Overall, the aronia extract was well tolerated by the participants when consumed daily for 12 weeks, with no serious adverse event reported, and only 2 adverse events in 2 participants out of 102 found to be “possibly” related to the intervention. One participant experienced various symptoms including bad taste in the mouth, nausea, and change in skin texture, while another felt unwell after the intake of the first capsule and decided to drop out of the study. The other adverse events were found to be “unlikely” related or “unrelated” to the intervention, and included ad-hoc nausea, migraines, cold, or joint pain.

Regarding safety, a total of 38 parameters were measured in blood of the participants at both baseline and 12-week timepoints, all of them remaining in the normal range after the 12-week intervention with no significant changes reported following the intervention (Table 3.14).

Table 3.14: Safety parameters assessed during the ABP study at baseline and after 12-week daily consumption of the aronia extract, for both Aronia and Control groups.

Safety parameter *(unit)*	Normal range	Aronia		Control		Changes at 12 weeks (Aronia - Control)*
		Baseline	12 weeks	Baseline	12 weeks	
Cortisol *(mmol/L)*	130-580	245 ± 77	222 ± 69	239 ± 88	234 ± 80	-15.58 (-42.77, 11.61)
Fasted plasma glucose *(mmol/L)*	4.16-6.38	5.2 ± 0.4	5.1 ± 0.5	5.1 ± 0.6	5 ± 0.5	0.07 (-0.10, 0.23)
Sodium *(mmol/L)*	136-146	142 ± 5.3	141 ± 6.1	141 ± 4.2	142 ± 3.7	-0.92 (-3.05, 1.20)
Potassium *(mmol/L)*	3.5-5.1	4.1 ± 0.4	4.2 ± 0.4	4.3 ± 0.8	4.3 ± 0.3	-0.08 (-0.22, 0.06)
Urea *(mmol/L)*	1.7-8.3	5.3 ± 1.2	5.4 ± 1.5	5.1 ± 1.3	5.2 ± 1.5	0.23 (-0.32, 0.78)
Creatinine *(umol/L)*	44-97	75 ± 16	77 ± 20	82 ± 26	81 ± 28	2.01 (-5.24, 9.26)
eGFR *(mL/min)*	> 60	77 ± 16	77 ± 14	75 ± 16	76 ± 16	2.49 (-6.87, 11.85)
Total protein *(g/L)*	64-83	65 ± 4.5	66 ± 3.9	67 ± 7.3	65 ± 5.7	1.23 (-0.67, 3.13)
Albumin *(g/L)*	38-44	43 ± 3	44 ± 2.2	44 ± 2.2	44 ± 2.1	-0.23 (-1.03, 0.58)
Globulins *(g/L)*	20-35	22 ± 3.9	22 ± 3	23 ± 6.3	21 ± 4.8	1.45 (-0.11, 3.00)
Total bilirubin *(umol/L)*	< 17	14 ± 6.2	13 ± 5.5	14 ± 7.4	15 ± 7.6	-1.55 (-3.59, 0.48)
ALP *(IU/L)*	30-90	63 ± 22	63 ± 16	58 ± 21	60 ± 20	-0.12 (-4.20, 3.97)
AST *(IU/L)*	< 31	26 ± 7.1	27 ± 7.1	26 ± 7	27 ± 7.3	-0.04 (-2.61, 2.54)
ALT *(IU/L)*	< 31	24 ± 13	25 ± 11	20 ± 6.9	21 ± 6.6	1.10 (-1.37, 3.58)
GGT *(IU/L)*	< 32	25 ± 28	24 ± 39	21 ± 21	21 ± 17	-1.84 (-5.08, 1.41)
LDH *(IU/L)*	100-210	174 ± 28	180 ± 36	175 ± 30	175 ± 29	6.3 (-3.42, 16.02)
hs-CRP *(mg/L)*	1.0-3.0	1.1 ± 1.3	1 ± 1.3	1.9 ± 2.9	2.7 ± 6.7	-1.45 (-3.55, 0.64)
Urate *(umol/L)*	142-416	277 ± 82	291 ± 78	253 ± 87	280 ± 86	-7.29 (-27.27, 12.70)
Cholesterol *(mmol/L)*	< 5.5	5.4 ± 0.9	5.5 ± 1.2	5.4 ± 1	5.3 ± 1	0.15 (-0.12, 0.43)
Triglycerides *(mmol/L)*	< 1.69	0.9 ± 0.4	0.9 ± 0.5	0.9 ± 0.4	0.8 ± 0.3	0.03 (-0.09, 0.14)
HDL *(mmol/L)*	> 1.04	1.7 ± 0.5	1.7 ± 0.6	1.7 ± 0.4	1.7 ± 0.4	0.02 (-0.08, 0.11)
LDL *(mmol/L)*	< 4.90	3.5 ± 1.1	3.7 ± 1.3	3.5 ± 0.8	3.5 ± 0.9	0.16 (-0.10, 0.42)
Non-HDL cholesterol *(mmol/L)*	< 4	3.7 ± 1.2	3.9 ± 1.3	3.7 ± 0.8	3.7 ± 0.9	0.15 (-0.12, 0.42)
Total cholesterol/HDL ratio	-	3.4 ± 1	3.4 ± 1.2	3.4 ± 1	3.2 ± 0.8	0.12 (-0.07, 0.30)
Total white blood cells *(10^9/L)*	4.50-10.37	5 ± 1.3	5 ± 1.2	5.1 ± 1.4	5.1 ± 1.7	-0.07 (-0.54, 0.40)
Total red blood cells *(10^{12}/L)*	3.86-5.62	4.4 ± 0.6	4.4 ± 0.5	4.4 ± 0.4	4.5 ± 0.5	-0.02 (-0.12, 0.09)
Hemoglobin *(g/L)*	118-169	135 ± 12	136 ± 12	135 ± 19	136 ± 12	-1.23 (-5.82, 3.39)
Mean corpuscular value *(fL)*	81.6-97.7	90 ± 4.9	90 ± 4.7	90 ± 5.7	90 ± 6.1	-0.12 (-0.90, 0.66)
MCV *(pg)*	26.8-33.8	31 ± 2.9	31 ± 2.9	31 ± 3.3	31 ± 3	-0.02 (-0.95, 0.91)
MCHC *(g/dL)*	311-355	344 ± 25	342 ± 22	343 ± 24	342 ± 21	1.09 (-7.75, 9.93)
Red cell distribution width *(%)*	12.8-16.8	13.5 ± 1	13.5 ± 1.2	13.5 ± 1.1	13.6 ± 1.1	-0.20 (-0.44, 0.05)
Platelets *(10^9/L)*	151-361	235 ± 54	233 ± 49	235 ± 56	239 ± 54	-3.93 (-15.14, 7.27)
Mean platelet value *(fL)*	6.1-14.1	9.1 ± 1.4	9.2 ± 1.4	9.2 ± 1.4	9.2 ± 1.6	0.05 (-0.38, 0.48)
Neutrophils *(10^9/L)*	2.43-7.42	2.9 ± 1.1	2.9 ± 1	2.9 ± 1.1	3 ± 1.5	-0.11 (-0.55, 0.33)
Lymphocytes *(10^9/L)*	1.08-3.17	1.4 ± 0.4	1.4 ± 0.4	1.5 ± 0.6	1.4 ± 0.5	0.02 (-0.15, 0.18)
Monocytes *(10^9/L)*	0.2-0.91	0.4 ± 0.2	0.5 ± 0.2	0.4 ± 0.2	0.5 ± 0.2	0.01 (-0.05, 0.08)
Eosinophils *(10^9/L)*	0.01-0.53	0.1 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	-0.00 (-0.04, 0.04)
Basophils *(10^9/L)*	0.01-0.13	0.1 ± 0	0.1 ± 0	0.1 ± 0	0.1 ± 0	-0.01 (-0.03, 0.01)

Values expressed as Mean ± SD or Mean (95% CI). *Differences were calculated using ANCOVA (Bonferroni) with baseline values as covariate, comparing Aronia with Control changes from baseline. ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; eGFR, estimated glomerular filtration rate; GGT, gamma-glutamyl-transpeptidase; HDL, high density lipoprotein; hs-CRP, high-sensitivity C-reactive protein; LDH, lactate dehydrogenase, LDL, low density lipoprotein; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume.

3.4 Discussion

The present study investigated the effects of 12-week aronia berry PP consumption on BP and several biomarkers of CVD risk including arterial stiffness, HR, FMD, blood flow velocity, blood lipids, blood cortisol levels, as well as QRISK®3 score. The present study demonstrates for the first time that consumption of aronia PP resulted in no changes in BP, endothelial function, or blood lipids. However, a significant improvement in 24-hour and awake arterial function was found in healthy prehypertensive middle-aged men and women.

No significant changes in the primary outcome of the study, 24h ambulatory BP (24h, awake or asleep), were found, although a trend for a decrease was observed, with average changes from baseline in the Aronia group of -2.1 ± 0.7 and -1.7 ± 0.5 mmHg for 24-hour SBP_{br} and DBP_{br}, respectively. These findings disagree with 2 studies that investigated the effects of aronia berry juice consumption on 24-hour BP (Kardum et al. 2015; Loo et al. 2016). Kardum and colleagues observed a decrease of 8.5 mmHg in 24h SBP and 10 and 5.9 mmHg in awake SBP and DBP, respectively, following a 4-week daily intake of 200 mL of aronia juice (containing 386 mg of total phenolics per 100g) in prehypertensive individuals (uncontrolled quasi-designed study). No changes in 24-hour ambulatory BP but a modest significant decrease of 1.6 mmHg in awake DBP was found in another study (Loo et al. 2016), despite the intervention having a much higher PP content (300 mL of aronia juice and 3g of aronia powder, containing 2194 mg of total

PP per day, including 1024 mg of anthocyanins, versus 105.9 mg of total PP in the present study).

One possible reason for the lack of effect in BP of our intervention, beside the low PP content compared with previous studies, was that our volunteers were in the lower range of prehypertension with an average of 121.7 ± 9.3 mmHg for SBP and 80.4 ± 5.8 mmHg for DBP. The baseline BP in the study of Kardum and colleagues was much higher, with an average baseline BP in the hypertension range (140.8 ± 9.3 / 86.6 ± 7.0 mmHg for SBP and DBP, respectively). The study by Loo et al. also presented a higher baseline BP (129 ± 9.8 / 85 ± 6.6 mmHg for SBP and DBP, respectively). In a meta-analysis of 35 RCTs investigating the effect of cocoa PP on blood pressure, the BP lowering effect of cocoa flavanols was significant in hypertensive but not in normotensive populations (Ried, Fakler, and Stocks 2017), which agrees with the lack of effects on blood pressure in our aronia intervention in comparison with the other studies showing an effect.

Although no significant differences were observed for office BP following a 12-week daily consumption of aronia PP, a within-treatment effect was seen in the Aronia group, with a significant decrease of -2.4 mmHg at 12-week consumption compared with baseline levels for office SBP_{br} in the Aronia group (CFB= -2.4 mmHg, 95% CI= [-4.2; -0.6 mmHg]) with no changes in DBP.

A meta-analysis showed that a decrease of 10 mmHg in SBP reduced the risk of CVD events by 20% and all-cause mortality by 13% (Ettehad et al. 2016). Another recent one highlighted that for every 10- and 5-mmHg increase in SBP and DBP, respectively, the risk of cardiovascular events was raised by 4-5%

(Luo et al. 2020). Therefore, even a modest reduction in BP such as the one seen in the Aronia group may still be relevant, with potentially a 5% decrease in risk of CVD events.

A novel finding of this work is the observed significant decrease in 24h and awake AIx_{ao} (6.8 and 6.1%, respectively) and 24h and awake AIx_{br} (3.3 and 2.9%, respectively), as well as a decrease in awake PWV of 0.24 m/s following 12-week consumption of aronia berries in comparison with placebo. PWV and AIx are gold standard techniques for the assessment of arterial stiffness, which is an evaluation of both arterial structure and function (Laurent et al. 2006). Both techniques are known to predict CVD strongly, independently of other CVD risk factors including BP (Mitchell et al. 2010; Ben-Shlomo et al. 2014). These two parameters increase significantly with age, in a non-linear relationship (McEniery et al. 2006). The clinical significance of AIx has been assessed among volunteers with renal failure (London et al. 2001). The results showed an association between a 10% increase in AIx and a decrease of 48% of the risk of cardiovascular mortality. The significant decrease in awake PWV found in the present study could also be related to a decrease in CVD risk of around 4%, as previous meta-analysis reported that an increase in PWV of 1 m/s was associated with a 14% increase in the risk of CVD incidence (Vlachopoulos, Aznaouridis, and Stefanadis 2010). This was confirmed by a more recent meta-analysis including 19 studies and showing that 1 m/s rise in PWV was associated with 12 and 9% increased risk for CVD events and CVD mortality, respectively (Zhong et al. 2018).

While the results on ambulatory AIx and PWV are promising, no changes were observed for PWV and AIx measured with applanation tonometry using

SphygmoCor®. One possible reason is that, while Arteriograph24™ captures a large number of measurements during subject's daily life every 30 minutes, SphygmoCor® measurements are based on one single measurement, in a clinical setting and during the study visit. As per BP measurements, a 24-hour average of multiple measurements may be more reliable and accurate measurement of arterial stiffness (Reino-Gonzalez et al. 2017). The devices differ, in that SphygmoCor® uses applanation tonometry to assess AIx, a technique that consists on the recording of radial artery pulse calibrated against BP measurements from the upper arm; Arteriograph24™ estimates AIx and PWV using an oscillometric measure after brachial flow occlusion (Rezai et al. 2011). Indeed, differences in values between both devices were found, with significant higher value in AIx measured with Arteriograph24™ compared with SphygmoCor® (17.6 ± 15.0% versus 10.3 ± 18.1%, respectively). However, no differences were observed in PWV values between both devices (Baulmann et al. 2008; Rajzer et al. 2008; Nürnberger et al. 2011). A plausible reason for the discrepancy found between devices is that the assessment of arterial stiffness via applanation tonometry was performed 24h after the intake of the last capsule ingested, while the Arteriograph24™ captured the effects from the moment volunteers consumed the capsule to 24 hours after the ingestion. Considering the kinetics of aronia derived plasma (poly)phenols, the level of circulating metabolites present after 24 h post-consumption will likely be much lower than the amount present within the first 24 hours after consumption of the aronia extract.

This study was powered for the main outcome, ambulatory SBP and DBP. However, we did not power it for the secondary vascular outcomes. A post-hoc power calculation analysis was conducted, and it was found that we did not have enough power to see statistically significant differences in office augmentation index measured via applanation tonometry, while ambulatory AIx and PWV were fully powered based on the outcomes of the present study (Supplementary Table S5).

Furthermore, although a trend towards a decrease was found in night-time changes in ambulatory central and peripheral AIx, these results did not reach significance. A possible explanation could be related to the self-recorded “sleep time” of the volunteers. Indeed, participants recorded the time they went to bed, which is different to the actual time they fell asleep. This could induce a bias in the accuracy of the sleep time division of the data. Another reason behind the difference in results between night and daytime could be linked to the time of consumption of the aronia berry extract capsule. Participants were instructed to consume the capsule at breakfast. Several RCTs focusing on the pharmacokinetics of berry PP have shown that most flavonoids reach their maximum absorption 1 to 8 hours post-consumption, which could explain why night-time results were not significant (Feliciano et al. 2017; Rodriguez-Mateos et al. 2016; Rodriguez-Mateos et al. 2019; Rodriguez-Mateos et al. 2013).

Several studies have shown improvements in arterial stiffness after consumption of berries and berry products. An RCT investigating the effects of 6-week daily blueberry consumption on BP and arterial stiffness found a significant decrease of 4.25% in AIx ($p= 0.024$, measured with SphygmoCor®) in the blueberry group compared to the placebo (McAnulty et al. 2014).

However, the PP content of the intervention was not stated, so we cannot extrapolate these findings to the present study. Another trial performed by our research team investigating the effect of cranberry juice intake on vascular function showed no significant difference for BP or arterial stiffness compared to placebo but a significant decrease in AIx compared to baseline (-14% vs -4.2%, p= 0.031) after 1.5h following the consumption of the cranberry juice (Rodriguez-Mateos et al. 2016). Nonetheless, the PP dose administered in this trial was much higher than the one used here (1534 mg), and no chronic effects were investigated.

Regarding QRISK®3 score, based on a calculation using BP and cholesterol/HDL ratio, no change was observed in the population following the intervention. The baseline score was 5.5 for the overall population, implying a low average risk of developing a CVD in the next 10 years of 5.5%, or "1 in 20" chance. To date, no other nutritional RCT involving PP has examined this parameter.

No significant improvements in endothelial function measured as FMD were found in the present study. A previous trial led by our team (Istas et al. 2019) with a similar study design and the same aronia interventional product consumed daily for 12 weeks demonstrated a significant increase in FMD following acute, chronic and acute on chronic consumption of the extract (1.1 ± 0.3, 1.2 ± 0.4, and 1.5 ± 0.4%, respectively) in an homogenous population of healthy young males (mean age = 24 ± 5.3 years). Differences in the results may be due to the differences in age and sex of both study populations, as most acute studies that have shown improvements in FMD after berry PP consumption were conducted in a small number

(n< 25) of healthy young men (mean age ≈ 25-30 years) (Martini et al. 2020). Regarding chronic RCTs involving FMD assessment, a few studies involving middle-aged men and women have also shown improvements in FMD (Dohadwala et al. 2011; Khan et al. 2014). However, the BMI of these populations was close to the obese range (BMI ≈ 29 kg/m^2), the proportion of women was low (32-35%), and the number of participants included did not exceed n= 66. Our study was constituted of a more heterogenous population of middle-aged healthy men and women, including predominantly female participants (55 out of 102 subjects), with a normal BMI.

Another important difference is the habitual PP consumption of population. In Istas et al (2019), daily PP intake measured using 7-day food diaries was 531 ± 357 mg, whereas in our study was 1537 ± 989 mg per day, around 3 times higher. As a result, the relatively low concentration of aronia berry PP present in the interventional product (105.9 mg of phenolic compounds per capsule), does not represent a significant addition to the diet of the participants. The low intake of coffee and fruits and vegetables in the previous study seemed to be the biggest difference in terms of dietary intake which could lead to the divergence in the results. We therefore hypothesize that the high consumption of PP by the volunteers in the study may explain the lack of effects of the aronia extract consumption on FMD when compared with the previous study. In addition, volunteers in the present study had an overall higher level of physical activity (PA), with an IPAQ score of 5574 ± 3819 MET-min/week, with 74%, 25% and 1% for high, moderate, and low PA level, respectively, in comparison with Istas et al. (IPAQ score of 4205 ± 3422 MET-min/week, with 61%, 30% and 9% for high, moderate, and low PA level, respectively

[internal data]). Higher levels of PA as part of usual life were significantly associated to a better FMD (Payvandi et al. 2009; Franzoni et al. 2005), which could explain why subjects from the present study did not present an improvement of their vascular function.

The “healthy diet” rich in plants foods and the high PA, combined with the baseline BP close to the normotensive range could explain the very modest effects on vascular function shown here. It also indicates that our study population was not highly representative of the usual UK population, as a recent report stated that 22% of adults were classified as “inactive” (National Health Service 2018), while individuals with low PA level only represents 1% of the total ABP study population. Studies investigating PP intake in the UK also showed rather lower levels of intake that the present study. For example, a study including 103 English women aged 18 to 50 years and recording the diet of participants using a 7-day food diary showed that the daily total PP intake was 808 ± 680 mg, which represents almost half the intake of our population (Yahya et al. 2016). A more recent and much larger cross-sectional analysis on 9374 individuals using the database from the UK National Diet and Nutrition Survey (NDNS) Rolling Programme (2008-2014) and segmented by age range reported a total daily PP intake of 1035 ± 545 mg for participants ages 50 to 64 years (Ziauddeen et al. 2019), which is still lower than the present study. Additionally, Zamora-Ros and colleagues found similar PP intakes within a sample of 309 “UK health-conscious” volunteers (1521 mg per day) (Zamora-Ros, Knaze, et al. 2016), which fit with the characteristics of our study population. Moreover, the fruits and vegetables average intake of the overall population of the present study was 469 ± 159g per day, which is above

the daily 400g recommended by Public Health England and WHO (Krauss et al. 2000; World Health Organization 1990; Public Health England 2016). Participants were also close to the UK fibre intake guideline of 30g as the overall average was 24.7 ± 10.4g of fibres per day. Moreover, the average calorie intake of the study population was 1980 ± 584 kcal per day, which is close to the 2000 to 2500 kcal recommended for an adult every day (Public Health England 2016).

Finally, another plausible explanation is that in the previous RCT by Istas et al volunteers followed a 24-hour low PP diet before starting the study, whereas in the present study volunteers did not have any restriction other than fasting for 12 hours prior study visits. It is possible that the low PP diet may have caused a decrease in FMD baseline values and a subsequent increase after consumption of the Aronia berry, both 2h post-consumption and 12 weeks later. In this study, due to the high PP baseline levels, which were confirmed when plasma and urine metabolites were measured, it is possible that FMD levels were already high and therefore did not increase further. Previous studies have shown that improvements in FMD after berry PP consumption reach a plateau at around 766 mg of berry PP. Other secondary outcomes that did not change significantly after Aronia berry consumption were HR, blood lipids, cortisol and QRISK®3 risk prediction score.

It is important to note that the power calculation's post-hoc analysis shows that the present study was fully powered for FMD (Supplementary Table S5). Thus, a lack of statistical power cannot explain the discrepancy in results.

The present study showed no significant change for ambulatory and office HR 2-hour and 12-week post-consumption of the interventional capsules, compared with Control. This is in line with the findings from the previous RCTs investigating the impact of aronia berry PP on heart rate (Istas et al. 2019; Kardum et al. 2015).

In terms of blood lipids, considering that participants had a lipid profile within the health range and were all normocholesterolemic (5.4, 0.9, 1.7 and 3.5 mmol/L for total cholesterol, triglycerides, HDL, and LDL cholesterol), no changes were expected. Previous meta-analyses have shown that consumption of anthocyanins or berry interventions improved the blood lipid profile of hypercholesteraemic individuals but not in subjects with normal levels (Heneghan et al. 2018). A small but significant increase in HDL cholesterol was found in the Aronia group at 12 weeks in comparison with baseline (CFB= 0.07 mmol/L, 95% CI= [0.004; 0.141 mmol/L], which was of the same magnitude than a meta-analysis by Huang and colleagues showing that berry intervention improved non-significantly serum HDL cholesterol levels by 0.06 mmol/L. A meta-analysis of observational studies observed that an increase of 1% in HDL cholesterol was associated with a 1% decreased risk of cardiovascular event (Brown, Stukovsky, and Zhao 2006). In the present study, based on a baseline HDL cholesterol level of 1.69 mmol/L and a change from baseline of 0.07 mmol/L, we saw an increase from baseline of 5%, which could be associated to a 5% decreased risk of cardiovascular events according to the findings of Brown and colleagues. Our data is also in line with another RCT investigating the effects of berry consumption on cardiovascular factors which also showed an improvement of HDL cholesterol

by 5% (Erlund et al. 2008). As a result, the small but significant change from baseline we demonstrated is meaningful.

In terms of cortisol levels after 12-week consumption of Aronia berries, no significant changes were found, although a trend for a decrease was observed in the Aronia group compared to Control (16.8 mmol/L, p= 0.111). Additionally, changes from baseline were significant in the Aronia group (CFB= -22.4 mmol/L, 95% CI= [-42.4; -2.6 mmol/L]), but not in the Control group. Cortisol concentration is positively correlated with elevated BP and cardiovascular comorbidities (Kelly et al. 1998; Rosmond 2005). A recent meta-analysis highlighted a positive association between plasma cortisol and CVD risk (OR= 1.18 per 1 SD higher cortisol), confirming the status of CVD risk factor of this biological parameter (Crawford et al. 2019). Although no study has presented cut-offs to assess the clinical relevance of serum cortisol levels yet, we could hypothesize on the potential of aronia PP consumption to reduce this parameter and decrease the risk of CVD.

The mechanisms behind AIx reduction and its relation to CVD have not been fully elucidated yet. However, it was suggested that an increase in AIx could be related to an elevation of the reactive oxygen species (ROS) level, which constitutes one of the early stages of the development of arterial dysfunction. Indeed, a study showed AIx was associated to serum ROS concentration in a population of smokers. This study also observed that older age and hypertension were associated with elevated AIx (Sugiura et al. 2017). When McAnulty and colleagues observed a significant decrease of AIx in their population but no change in BP, they explained their results via the hypothesis of blueberry PP to be able to increase the production of eNOS, leading to a

greater arterial distensibility (Suzuki et al. 2006; Kappus et al. 2011). This enhanced elasticity would thus be able to produce longer pulse wave transit time which could decrease the magnitude of returning aortic pulse wave. The reason behind BP to be unchanged following this process may be related to the fact aorta contains elastic nodules which present very high eNOS potential, and as a result these nodules are better up-regulated by NO to increase the distensibility. Blood pressure on the contrary is linked to peripheral vasculature, which might require a higher dosage of PP to present the same effect (McAnulty et al. 2014). Moreover, gut microbial diversity has been associated to lower PWV in women (Menni et al. 2018) and aronia PP have shown to have a beneficial effect on microbiota composition and the enhancement of some species such as *Anaerostipes* (Istas et al. 2019). As PWV and AIx are both factors characterizing arterial stiffness, it may be interesting to explore the gut microbiota diversity of the present study population to assess a potential link with the significant increase in AIx observed in the Aronia group. Chapter 6 will focus on the microbiome analysis of the faecal samples collected during the present RCT.

Blood pressure, arterial stiffness, and endothelial function, although all related to the arterial system, present some distinct mechanisms and regulation pathways. Indeed, increases in blood pressure relate to an increased peripheral resistance to blood flow, primarily attributable to structural narrowing of small diameter arteries (Andersson et al. 2004). This increase in peripheral resistance is believed to be associated to various factors such as the alteration of the renal renin-angiotensin system, an increase in cytokine levels (interleukins, TNF-α), but also the inflammation of the vascular system

and an alteration of endothelial function (Navar 2010; Esler, Lambert, and Schlaich 2010; Versari et al. 2009; Marchesi, Paradis, and Schiffrin 2008; Gooch and Sharma 2014). Endothelial dysfunction targets primarily small diameter arteries and arterioles and is characterised by the inability of these vessels to dilate entirely in response to a stimulus via the impairment of NO production (Flammer et al. 2012; Sitia et al. 2010). In fact, NO helps to maintain a contractibility (also called compliance) in the small diameter arteries. As a result, endothelial dysfunction leads to the mechanical and functional modifications in the microcirculation (Gilani et al. 2007; Duprez et al. 2011). On the contrary, arterial stiffness relates to the rise in stiffening occurring in large and central arteries and is imputed to two main arterial structural changes, i.e., the depletion of the elastin layer due to mechanical stress and an increase in collagen level (Díez 2007). While those mechanisms are not entirely independent, there are many reports showing that decreases in arterial stiffness can occur without changes in blood pressure or endothelial function (Mills et al. 2020; Karalliedde et al. 2008; Nakamura et al. 2005; Verhaar et al. 1999). It was suggested that discrepancy may be related to the potential of NO to modulate the mean arterial pressure of large vessels, decreasing arterial stiffness, while peripheral blood pressure and endothelial function would remain unchanged (Wilkinson, Franklin, and Cockcroft 2004).

Several limitations to the present study should be taken into consideration. Participants included middle-aged individuals, so findings cannot be extrapolated to a younger or older segment of the general population. Moreover, as discussed, the volunteers participating in this study had a high physical activity level and a high consumption of PP, as well as a healthy diet

with a high consumption of plants foods and fibres. Another drawback of the study is that the interventional product was composed of an extract of the berry rather than fresh whole fruits, containing only PP and no fibre or other nutrient, and therefore cannot compare with commonly used foods such as berries. Another limitation could be related to the protocol used for FMD assessment. Indeed, although validated by several publications (Corretti, Plotnick, and Vogel 1995; Uehata et al. 1997), this protocol may be outdated compared to more recent trials involving continuous automated computerised monitoring of the brachial diameter for a minimum of 3 minutes (Dobbie et al. 2020; Thijssen et al. 2019). This continuous assessment of the artery diameter may lead to a finer characterisation of the maximal FMD percentage for each individual. Hence, it may be possible that the capture time used in the present study (up to 80 seconds post-deflation only) may not be optimal for all subjects, potentially missing the maximum peak of dilation in some individuals. Finally, although validated, the questionnaires used for the assessment of the participants' diet and physical activity could have been replaced by alternative methodology such as wrist accelerometers that would quantify more accurately and reliably the active and sedentary time of the volunteers, or mobile applications to assess more thoroughly and accurately the nutrient and PP intake of the participants.

This study also presents some strengths. The use of gold standard techniques such as FMD, 24h BP and arterial function monitoring, as well as a robust double-blind RCT design are one of those. The recruitment of a heterogenous healthy population constituted of males and females, from 40 to 70 years old is another one. The number of participants recruited (n= 102) is above all of

those from previous RCTs focusing on the impact of aronia berry on BP (Pokimica et al. 2019; Loo et al. 2016; Naruszewicz et al. 2007; Xie et al. 2017; Simeonov et al. 2002; Istas et al. 2019).

Safety and tolerance analysis showed an absence of serious adverse events and revealed the treatment was well tolerated by all volunteers. This is in line with the literature as no side effect was, to our knowledge, reported following the consumption of aronia berry PP extract.

To conclude, the present findings suggest that daily consumption of aronia berry extract was found to be tolerable and safe and led to a significant improvement in arterial function in healthy middle-aged individuals. No other significant changes in BP, FMD and other biomarkers of CVD risk investigated were found, although improvements in SBP, HDL-cholesterol and cortisol were found 12 weeks after the consumption of Aronia extract when compared with baseline.

This indicates that daily intake of dietarily achievable amounts of aronia berry PP, along with a healthy diet and lifestyle, could help maintain cardiovascular health and good circulatory function in a population of middle-aged individuals at low risk of CVD, which represents a promising approach for the prevention of chronic disease and as well as the promotion of public health.

CHAPTER 4

Bioavailability and metabolism of aronia berry (poly)phenols

4.1 Introduction

4.1.1 ADME of (poly)phenols

The dietary intake of (poly)phenols (PP) varies among populations, countries, and cultures, but the average intake in the UK has been reported to fluctuate with age between 636 mg per day for the 19-34 years to 1035 mg per day for the population aged 65 years and above (Ziauddeen et al. 2019). Research conducted over the last 20 years reported a low bioavailability of PP, with only 1 to 10% of the total amount ingested being excreted in urine in human feeding studies (Rodriguez-Mateos, Vauzour, et al. 2014; D'Archivio et al. 2010; Del Rio et al. 2013). However, with the progress in analytical techniques recent human trials using isotopically labelled compounds have indicated that the bioavailability of phenolic compounds may have been underestimated and that metabolites can remain in the circulation for more than 48h after consumption, before being excreted in urine, breath or via the bile (Czank et al. 2013). Recoveries in urine as high as 30-70% have been recently reported (Crozier, Del Rio, and Clifford 2010; Del Rio, Borges, and Crozier 2010; Stalmach et al. 2010), suggesting the importance of gut microbiota metabolism to catabolize PP, leading to a wider range of absorbed metabolites which were previously not accounted for (Kay et al. 2017). An overview of the absorption, distribution, metabolism, and elimination (ADME) of PP will be presented in this section.

4.1.1.1 Absorption and distribution

Maximal concentrations of phenolic compounds in plasma are reached from 30 minutes to 2-3 hours post-consumption for PP absorbed in the small intestine (Del Rio et al. 2013; Beattie, Crozier, and Duthie 2005). Those not absorbed in the small intestine will reach the large intestine where they will be metabolised by the gut microbiota. Bacterial metabolism generates a second wave of metabolites appearing at around 4 to 6 hours post-consumption and able to stay in the circulation for 48 hours or longer (Feliciano, Boeres, et al. 2016; Pimpao et al. 2015). While plasma concentrations of intact PP rarely exceed 1 μM (D'Archivio et al. 2007), gut microbial metabolites can reach higher concentrations (Kay et al. 2017).

After being ingested, PP follow the same metabolism pathway than xenobiotics and start their transformation when in contact with saliva (Zanotti et al. 2015). They will then go down the gastrointestinal tract, where they will undergo the action of phase I metabolism enzymes and see their sugar moieties cleaved enzymatically by the lactase phlorizin hydrolase (LPH), located at the brush border of the enterocytes, or the β-glucosidase (βG), located in the cytosol of epithelial cells of the small intestine and involving the active sodium-dependent glucose transporter 1 (SGLT1) so PP can enter the enterocytes (Del Rio et al. 2013; Spencer et al. 1999; Arora, Nair, and Strasburg 1998) (Figure 4.1). While the majority of (poly)phenols go through the process of deglycosylation, a few exceptions was found in the literature such as anthocyanins and flavonols which were identified in urine and plasma on their glycoside form (Kay, Mazza, and Holub 2005; Hollman et al. 1997).

High molecular weight PP, such as proanthocyanidins, cannot be absorbed in the small intestine and are absorbed to some extent after being metabolised by the gut microbiota.

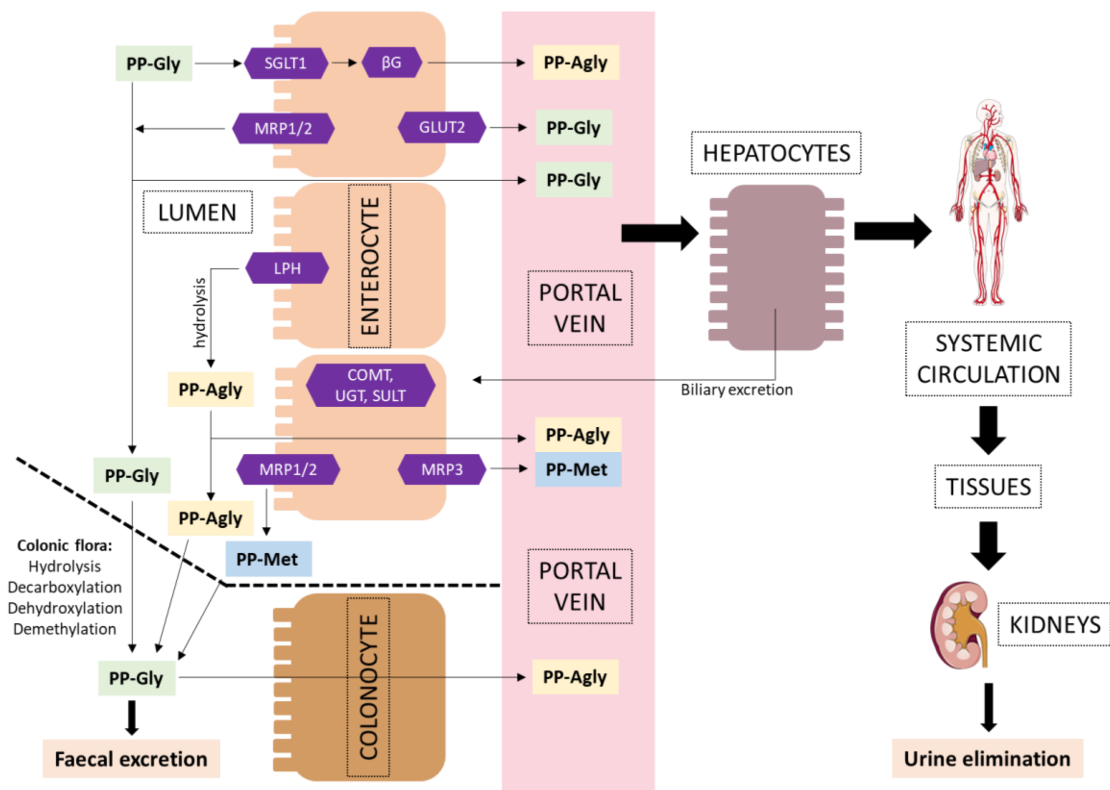


Figure 4.1: Mechanisms of ADME of (poly)phenols. βG, cytosolic β-glucosidase; COMT, catechol-O-methyl transferase; GLUT2, glucose transporter; LPH, lactase phlorizin hydrolase; MRP1-2–3, multidrug-resistant proteins; PP-Agly, (poly)phenol aglycone; PP-Gly, (poly)phenol glycoside, PP-Met, (poly)phenol sulfate/glucuronide/methyl metabolites; SGLT1, sodium-dependent glucose transporter; SULT, sulfotransferase; UGT, uridine-5'-diphosphate glucuronosyltransferase. Adapted from Santhakumar and colleagues (2018).

4.1.1.2 Phase II metabolism

Once absorbed in the enterocytes, PP undergo phase II metabolism, located in the cellular endoplasmic reticulum, which can also take place in other sites such as in the liver or large intestine (Murota, Nakamura, and Uehara 2018). Catechol-O-Methyltransferase (COMT), sulfotransferases (SULT) and uridine-5′- diphosphate glucuronosyltransferases (UGT) will respectively catalyse the

transfer of a methyl group (-CH_3), sulfate unit and glucuronic acid to the aglycone PP (Manach et al. 2004; Del Rio et al. 2013) (Figure 4.1). This phase II metabolism also involves the presence of multidrug-resistance-associated proteins (MRP-1, MRP-2 and MRP-3) which are active ATP-dependent transporters (Leonarduzzi et al. 2010). MRP-1 and MRP-2 are located on the apical membrane of enterocytes and facilitate the entrance of PP in the lumen of intestinal cells. On the contrary, MRP-3, along with glucose transporter 2 (GLUT2), are both implicated in the efflux of metabolites from the basolateral side of intestinal cells to the portal vein (Manzano and Williamson 2010).

Once they enter the liver, PP can be further metabolised through oxidations and demethylations operated by a variety of cytochrome P450 monooxygenase families, as well as further phase II metabolism, leading to the creation of new conjugates, which can be then distributed to tissues via the systematic circulation (Otake and Walle 2002). Plasma concentrations depend on the nature of the compounds as well as the food matrix, food processing, age and gender of the individual, as well as genetic polymorphisms, among others (Cifuentes-Gomez et al. 2015). Through the enterohepatic circulation, metabolites can be secreted *de novo* in the intestine and reabsorbed another time, which accounts for the high half-life of some PP found in plasma (Adlercreutz et al. 1987). The complexity and the size of phenolic compounds highly impacts their absorption. For instance, the large molecular weight and extensive polymerisation of proanthocyanidins limits their intestinal absorption, and it was shown that they are instead metabolised by the gut microbiota in the colon, though their major part is not but is rather excreted in faeces (Tao et al. 2019; Ottaviani et al. 2012).

4.1.1.3 Gut microbial metabolism

Only a small fraction of the phenolic compounds ingested are absorbed by the enterocytes (Cardona et al. 2013), leading to 90 to 95% of the total PP ingested to reach the colon, where they undergo the action of the gut microbiota bacterial enzymes which results in the creation of a wide variety of catabolites (Bowey, Adlercreutz, and Rowland 2003). These enzymes, including hydrolases, reductases, decarboxylases, and demethylases can cleave large and complex structures into smaller molecules such as phenolic acids which eases their absorption (Santhakumar, Battino, and Alvarez-Suarez 2018; Aura et al. 2005; Zanotti et al. 2015; Selma, Espín, and Tomás-Barberán 2009). Most colonic catabolites will reach the systemic circulation and be distributed to peripheral organs and tissues before being eliminated by the kidneys through the urine (Rodriguez-Mateos, Vauzour, et al. 2014).

4.1.1.4 Excretion

After many cycles of metabolism along the intestinal tract, unabsorbed PP are excreted from the body in faeces, which mainly apply to high molecular weight compounds (Scalbert and Williamson 2000). In a study using radiolabelled epicatechin, Ottaviani and colleagues have reported an accumulated radioactivity in faeces representing 12% of the ingested dose (Ottaviani et al. 2016). The urinary pathway is instead followed by smaller metabolites which, after being distributed to peripheral tissues, are excreted through the kidneys (Crozier, Del Rio, and Clifford 2010). Maximal urinary PP concentration following metabolism in the small intestine is often reached within 2 to 4 hours after ingestion (Jin et al. 2011). As PP are metabolised in a similar manner as

xenobiotics, they will not accumulate in the body but will instead undergo a rapid turnover, highlighting the importance of a regular intake to maintain a high plasma concentration of PP.

4.1.2 Bioavailability and metabolism of aronia berry PP

Most abundant PP found in *Aronia melanocarpa* are procyanidins of high degree of polymerisation (SkupieD, Ochmian, and Grajkowski 2008). Indeed, around 80% of the procyanidins found in the berry have a degree of polymerisation of 10 and above (Wu et al. 2004). In second position of abundance are the anthocyanins, with cyanidin-3-O-galactoside and cyanidin-3-O-arabinoside being the most abundant in the berry (Ochmian, OszmiaDski, and SkupieD 2009; Ireneusz Dariusz, Jozef, and Milosz 2012). Aronia also contain a non-negligible quantity of phenolic acids, and in particular, chlorogenic and neochlorogenic acid (Jakobek et al. 2012). Finally, a small amount of flavonols are also found in the berry, mainly represented by quercetin glycosides (Ochmian, OszmiaDski, and SkupieD 2009).

Very little is known on the overall metabolism of aronia berry PP. However, it is possible to look at what is known in terms of ADME of the main individual compounds present in the berry, i.e., proanthocyanidins and anthocyanidins. Procyanidins are proanthocyanidins built from flavan-3-ols (+)-catechin and (−)-epicatechin (Bittner, Rzeppa, and Humpf 2013). Proanthocyanidins (also called "condensed tannins") show a very low bioavailability as polymers with a degree of polymerization superior to 4 are not absorbed in their original form and depolymerisation of these compounds in the upper intestinal tract is negligible (Ou and Gu 2014). As a result, most of these PP are metabolised

by the colonic microbiota into derivative metabolites such as valerolactones, phenylacetic acids, benzoic acids, phenylpropanoic acids and other smaller phenolic compounds (Donovan et al. 2002; Rios et al. 2002; Déprez et al. 2000). Besides requiring the help of the microbiota for their catabolism, proanthocyanidins also present direct beneficial action on the mucosa of the intestine by applying a protective action against carcinogens and oxidative stress (Manach et al. 2005).

The absorption of intact anthocyanins is very low, leading them to be rarely detected in plasma and usually found in urine at a concentration representing 0.05% of the intake (Crozier, Jaganath, and Clifford 2009; Garcia-Alonso et al. 2009; Mertens-Talcott et al. 2008). Anthocyanins' C_{max} is usually found at 1.5h post consumption in plasma and 2.5h post-consumption in urine (Manach et al. 2005). There is strong evidence of an early absorption of intact anthocyanins glycosides in the stomach as cyanidin glucosides have been observed increased in plasma 30 minutes following the intake of an aronia berry extract (Talavera et al. 2003; Xie et al. 2016). Passamonti and colleagues indicated that bilitranslocase, an enzyme acting as an anion carrier and present in the epithelium of the stomach, may be implicated in the absorption of anthocyanins (Passamonti et al. 2003). However, it is now believed that most anthocyanins are degraded into phenolic metabolites after breakdown in the small intestine due to their low stability at neutral pH, and after metabolism by the gut microbiota in the colon. Indeed, when considering gut microbial metabolites, bioavailability of proanthocyanidins and anthocyanins can go up to 18% (Czank et al. 2013; Gonthier et al. 2003). Several key intermediates are found related to aronia PP catabolism, such as

protocatechuic acid, ferulic acid, hippuric acid and phenylpropanoic acid (El Mohsen et al. 2006; Monagas, Quintanilla-López, et al. 2010; Stalmach et al. 2013; De Ferrars et al. 2014).

4.1.3 Previous studies investigating aronia PP bioavailability

To our knowledge, only 5 studies have reported the bioavailability of aronia PP in humans, based on analysis of plasma or urine human samples.

A first study by Wiczkowski and colleagues, involving 13 healthy young men and women, determined the ADME of cyanidin glycosides consumed through the intake of aronia juice, at a physiologically relevant dose (Wiczkowski, Romaszko, and Piskula 2010). Eight metabolites were identified in plasma and urine (4 cyanidin and 4 peonidin metabolites), with a predominance of cyanidin-3-galactoside (66%). The maximum anthocyanin concentration in plasma was 32.7 ± 2.9 nM and was reached after 1.3 hour following the intake of the juice, indicating the ability of anthocyanins to be metabolised in the upper gastrointestinal tract. Only 0.25% of the ingested dose was excreted in the urine over the 24 hours following the consumption. The results of this study were in line with another trial exploring the metabolism of aronia anthocyanins in men (Kay, Mazza, and Holub 2005)

Moreover, a RCT led by Xie and colleague on 6 young healthy men and women investigated the bioavailability of anthocyanins and gut microbial PP metabolites in urine and plasma after the consumption of 500 mg of aronia berry extract (Xie et al. 2016). Nine metabolites were identified and quantified

in urine and plasma. Hippuric acid accounted for 85 and 98.5% of the total PP identified in plasma and urine, respectively. Gut microbial catabolites represented 95.8% and ≈ 100% of total increased compounds in plasma and urine, respectively. Authors reported that the maximal concentration of 3,4-dihydroxybenzoic acid was reached as early as 1h post-consumption, which was in line with other studies (Vitaglione et al. 2007; Keane et al. 2016).

Another study by Loo et al showed the potential of a PP-rich aronia intervention (juice + powder) to significantly increase the concentration of 14 out of 17 urinary compounds analysed in their population of 38 middle-aged men and women (Loo et al. 2016). Among them, no glucuronide, methyl or sulfate compounds were present.

Finally, a study led by our team and involving the same investigational product as the study presented in this thesis performed a detailed metabolomic analysis on plasma samples, collected from 22 healthy young men, at baseline and after 12-week consumption of the capsule (Istas et al. 2019). A total of 63 metabolites were quantified, and the consumption of the aronia extract resulted in increases in total PP concentration of 166 ± 171 and 30 ± 156 μM following 2-hour and 12-week consumption, respectively. Forty-eight compounds increased significantly compared to baseline 2 hours following the consumption of the first capsule in the aronia extract group, 18 significantly improved chronically after 12 weeks of daily consumption, and 22 increased on week 12, 2 hours after the intake of the last capsule. In terms of abundance, and compared with baseline, the main classes of PP reported after the

12-consumption of the aronia extract were hippuric acids (38%), phenylacetic acids (30%) and benzoic acids (19%).

These 5 studies considered dietarily relevant doses of aronia PP in their investigation, which is a real strength. However, these studies also present some limitations. The biggest limitation is that no metabolite could be exclusively attributed to aronia berry PP, as all metabolites are common to a wide number of PP, that are part abundant in the daily diet, such as coffee, tea, fruits, and vegetables. In addition, all existing studies were conducted in a relatively low number of people on healthy young subjects with an average age below 30 years. Studies led by Wiczkowski and Kay only considered anthocyanin metabolites in the analysis, which does not represent the whole diversity of phase I, II and gut microbial metabolites, and other PP present in *Aronia melanocarpa*. The study by Xie investigated few of these important metabolites but the study was very small and only 9 metabolites were quantified. Moreover, the RCT by Loo et al showed impressive rises in metabolites' concentrations but involved the consumption of more than 2g of PP per day as part of their intervention. Finally, Istas and colleagues did not study the bioavailability of aronia PP in urine, and only young men were included in this trial.

The aim of this chapter is to investigate the bioavailability and the metabolism of aronia berry PP after acute and daily sustained consumption of aronia berry PP, using the most comprehensive method used so far in both plasma and urine, and including more than 100 metabolites and a large population of middle-aged men and women.

4.2 Methods

Urine samples (24-hour urine) were collected from the participants prior to each visit, on day 1 and 12 weeks later. Blood was collected during study days at baseline and 2 hours post-consumption on both visit 1 and 2.

Detailed information regarding the collection and processing of urine and plasma samples can be found in Chapter 2, section 2.6. Regarding the analytical protocol used for the quantification, more information can be found in Chapter 2, section 2.8. Finally, explanatory details on the statistical analysis performed with the data is presented in Chapter 2, section 2.9.

4.3 Results

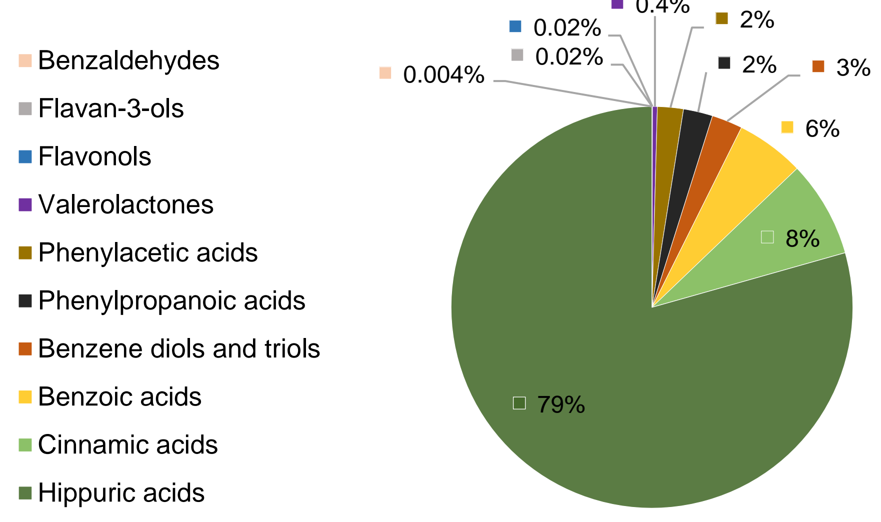
A total of 128 metabolites were identified in plasma and 24-hour urine samples collected at baseline and after 12-week daily consumption of the interventional or placebo product. From those 128 metabolites, a total of 36 were discarded as they are known to be unrelated to the aronia berry intervention and rather part of the habitual consumption of PP, including citrus flavanones (naringenin and hesperitin derivatives), soy isoflavones (equol derivatives and daidzein), wholegrains and flaxseed lignans (secoisolariciresinol, enterolactones and enterodiols), stilbenes (resveratrol derivatives), methylxanthine alkaloids from coffee, hydroxycoumarins from raspberries (ellagic acid and urolithin derivatives), tyrosols, flavones and dihydrochacones. The other 92 metabolites included here could also come from the background diet, as previously discussed, but they are metabolites that were found to derive from anthocyanins, proanthocyanidins, flavonols, and phenolic acids, and therefore at least to some extent could come from aronia berry PP.

A total of 48 and 18 of them were found significantly increased after acute and chronic consumption of aronia berry extract in our previous study, respectively (Istas et al. 2019)

Supplementary Table S1 includes a list of all these 92 metabolites identified and included derivatives of flavonoids (flavonols, n= 7; flavan-3-ols, n= 5), cinnamic acids (n= 21), benzoic acids (n= 22), hippuric acids (n= 5), benzene diols and triols (n= 7), benzaldehydes (n= 3), phenylacetic acids (n= 4), phenylpropanoic acids (n= 16) and valerolactones (n= 1).

4.3.1 Baseline urinary and plasma phenolic metabolites

The total urinary excretion of the main classes of PP compounds quantified in 24h urine at baseline in both Aronia and Control groups is shown in Figure 4.2 below. Overall, phenolic profiles are very similar between groups, with hippuric acids being the most abundant type of compounds present in urine, representing 79% and 82% of total PP for Aronia and Control, respectively. The concentration of hippuric acids was 10 times higher than the second most represented type of compounds, cinnamic acids (8% and 7% of the total concentration in Aronia and Control, respectively). The third most abundant class of PP was benzoic acids (6% and 4% of the total concentration in Aronia and Control, respectively), followed by benzene diols and triols, phenylpropanoic acids, phenylacetic acids, valerolactones, flavonols, flavan-3-ols and benzaldehydes.



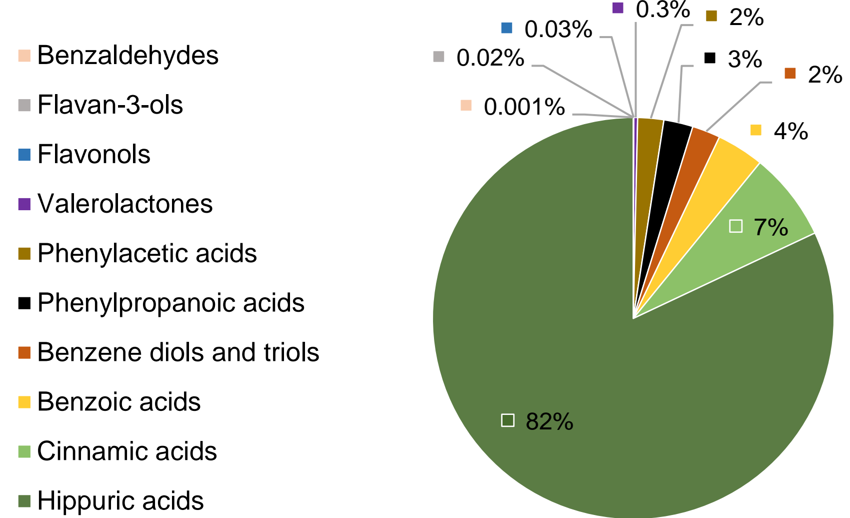


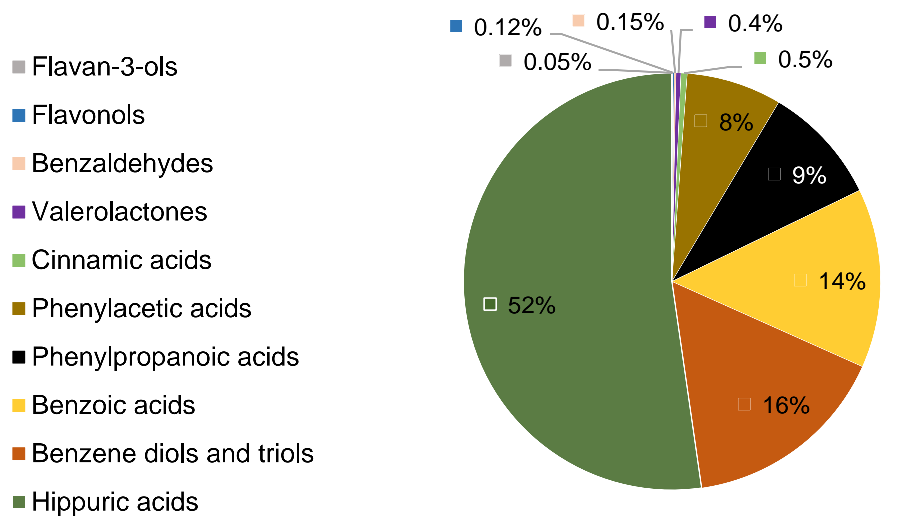
Figure 4.2: Baseline 24 h urinary excretion (%) of PP classes analysed in both Aronia and Control groups.

In terms of individual compounds, a total of 85 metabolites were quantified in urine. Most urinary metabolites were present at nmol and µmol concentration, while hippuric acid was found at mmol concentration, even at baseline (3.5 mmol) (Supplementary Table S5).

No significant differences in total urine PP metabolites were found between the two groups at baseline, and only 1 individual urinary metabolite, 2-hydroxybenzene-1-glucuronide, was found to be significantly higher in the Control group (p= 0.038).

Regarding plasma, the main classes of PP compounds quantified at baseline in both groups is reported in Figure 4.3 below. As it was the case in urine, hippuric acids were the most abundant compounds in plasma (52% and 55% of the total PP concentration for Aronia and Control, respectively). However, benzene diols and triols were more represented in plasma than in urine, as this was the second most abundant class with 16% and 17% of the overall

total PP quantified in Aronia and Control, respectively. Benzoic acids were third in the order of abundance, followed by phenylpropanoic acids, phenylacetic acids, cinnamic acids, valerolactones, benzaldehydes, flavonols and flavan-3-ols.



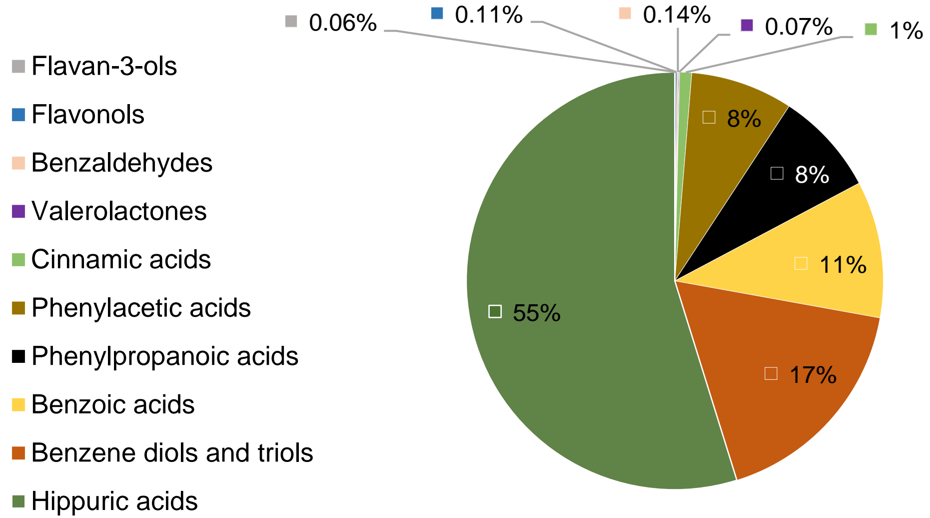


Figure 4.3: Baseline plasma concentration (%) of (poly)phenol classes analysed in both Aronia and Control groups.

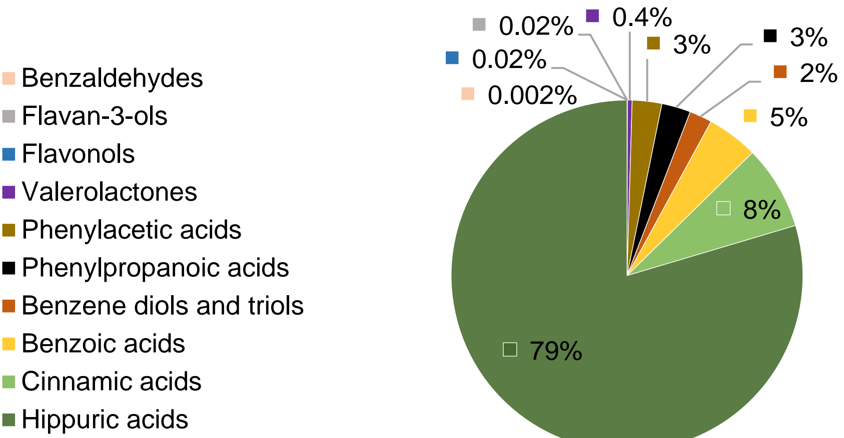
In plasma, the same phenolic metabolites quantified in urine were found, except for morin and 3,4-dihydroxybenzaldehyde that were only found in plasma. As a result, a total of 87 compounds were quantified in plasma. No significant differences were found in baseline total plasma PP, or individual metabolites between Aronia and Control groups.

Most abundant baseline plasma metabolites were hippuric acid, 2-hydroxy-4-methylbenzene-1-sulfate and 2-hydroxy-3-(4'-hydroxyphenyl)propanoic acid, found at concentrations of 176.4 µM, 46.9 µM and 17.0 µM, respectively (Supplementary Table S6).

4.3.2 Urinary and plasma PP after aronia consumption

4.3.2.1 Urinary PP metabolism after their acute and chronic consumption

The relative proportion of each PP families in urine at both visit 1 and 2 and for both Aronia and Control group is presented in Figure 4.4 below. Profiles are very similar for both treatment groups and both visits, with hippuric acid accounting for around 80% of the overall total PP.



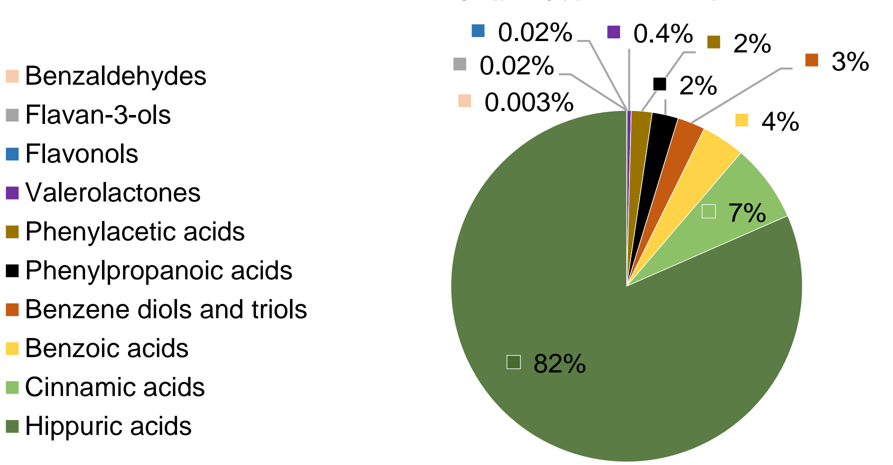


Figure 4.4: Urinary concentration (%) of (poly)phenol classes analysed in both Aronia and Control groups, following the 12-week supplementation.

4.3.2.2 Plasma PP metabolism after their acute and chronic consumption

The relative proportion of PP in plasma for both investigational groups following the supplementation is presented in Figure 4.5, graphs A to F. Two hours after the consumption of the first capsule on visit 1 a shift towards an increase of the proportion of benzene diols and triols and a decrease of the hippuric acids importance is seen for both Aronia and Control, but the

difference with baseline is more dramatic for the Control group. This indicates that aronia extract consumption led to a higher increase in the concentration of hippuric acid metabolites, compared with the placebo intake.

Regarding the ratio of abundance of PP with respect to total PP in plasma for the baseline of visit 2, the main difference can be found in the proportion of hippuric acids and benzoic acids between the 2 treatment groups. Indeed, the proportion of benzoic acids is lower in the Aronia group compared to Control (8% versus 14%), which leads to a larger portion of hippuric acids for the Aronia group (56% versus 51% in the Control group). Once again, this shows the higher concentration of hippuric acids in plasma following the 12-week daily intake of aronia berry PP, compared with Control.

Finally, the phenolic compounds profile for the last timepoint (2-hour post-consumption of the last capsule on visit 2) is similar between Aronia and Control. However, it differs from the baseline of visit 2 for the treatment group as a bigger proportion of benzoic acids, phenylacetic acids and benzene diols and triols can be observed.

Hippuric acids
Benzene diols and triols
Benzoic acids
Phenylpropanoic acids
Phenylacetic acids
Cinnamic acids
Valerolactones
Benzaldehydes
Flavonols
Flavan-3-ols

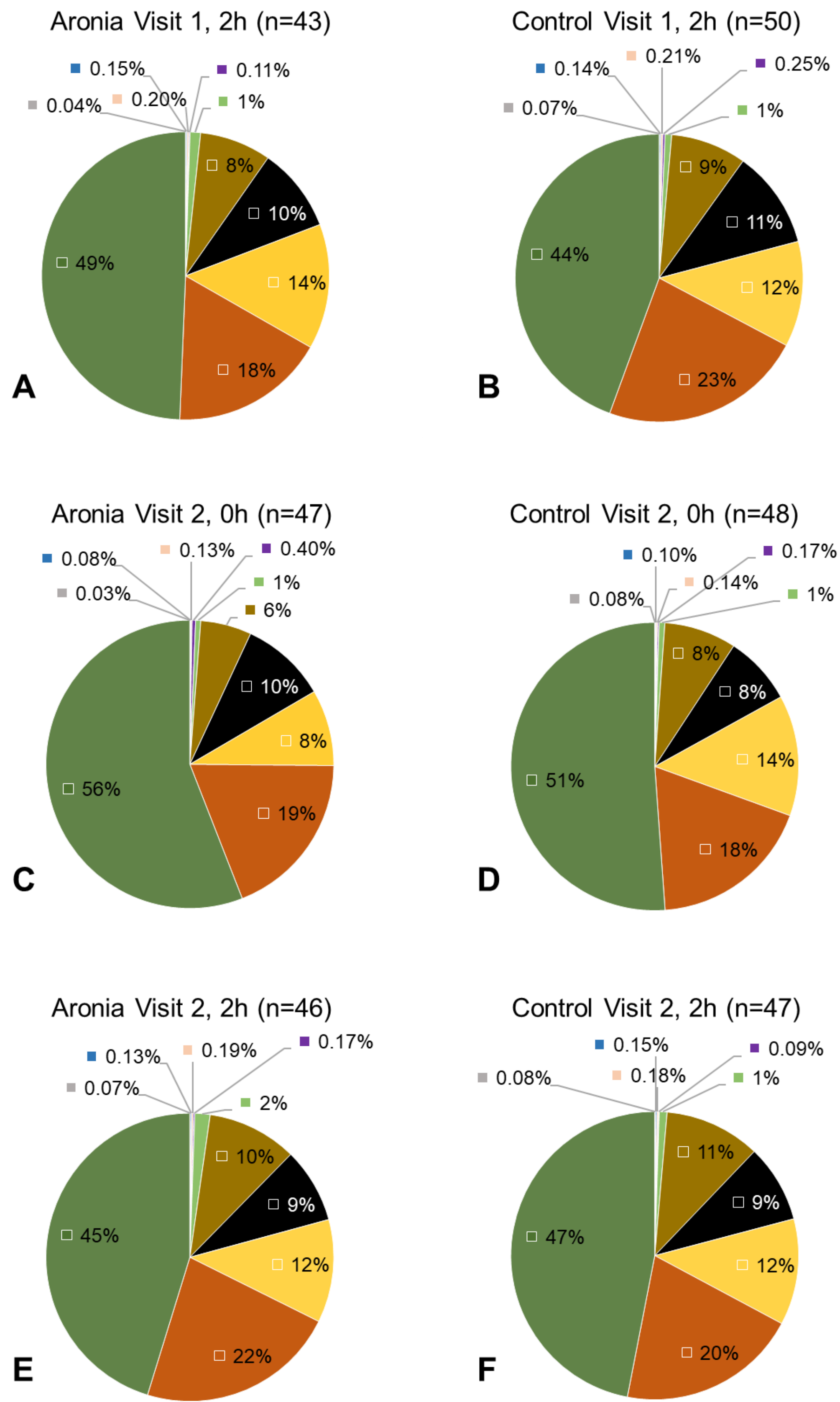


Figure 4.5: Pie charts representing the proportion of plasma (poly)phenol families for both Aronia and Control group and both visit 1 and 2.

4.3.3 Change in urinary and plasma PP after consumption of aronia in comparison with Control group

4.3.3.1 Changes in urinary PP metabolism after consumption of aronia in comparison with Control

Following 12-week daily consumption, no significant changes were found in total 24h excreted urinary phenolic compounds in Aronia and Control groups (-859 ± 2672 and -41.3 ± 4745 µmol, respectively (Figure 4.6). No significant changes were observed between groups or when compared with baseline levels when the main families were compared (Figure 4.7).

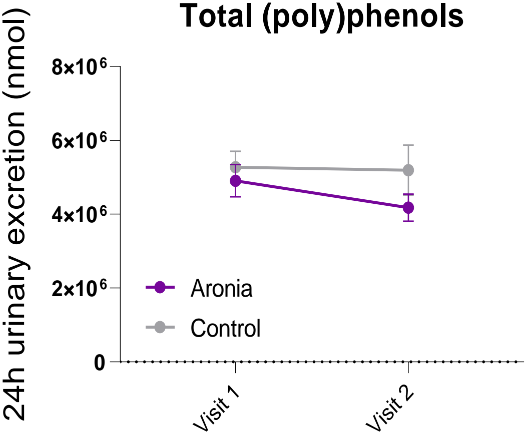
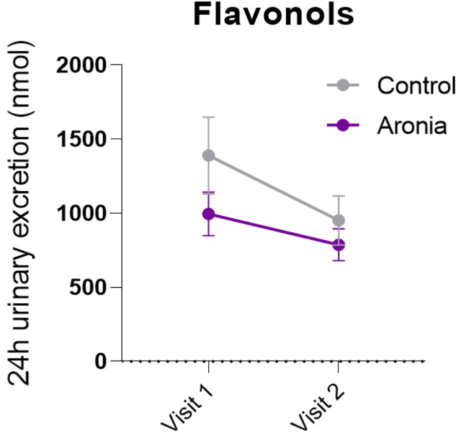
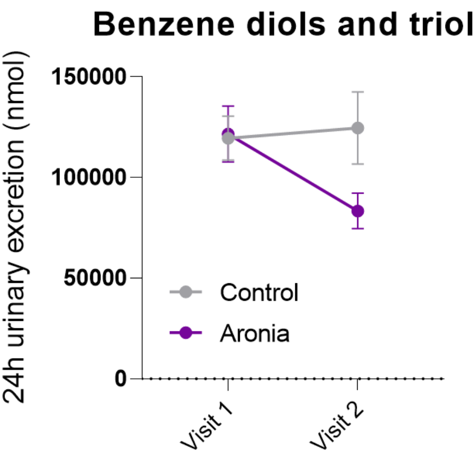


Figure 4.6: 24h Urinary (poly)phenol metabolites after consumption of aronia berries for Aronia and Control groups. Values expressed as mean ± SEM.





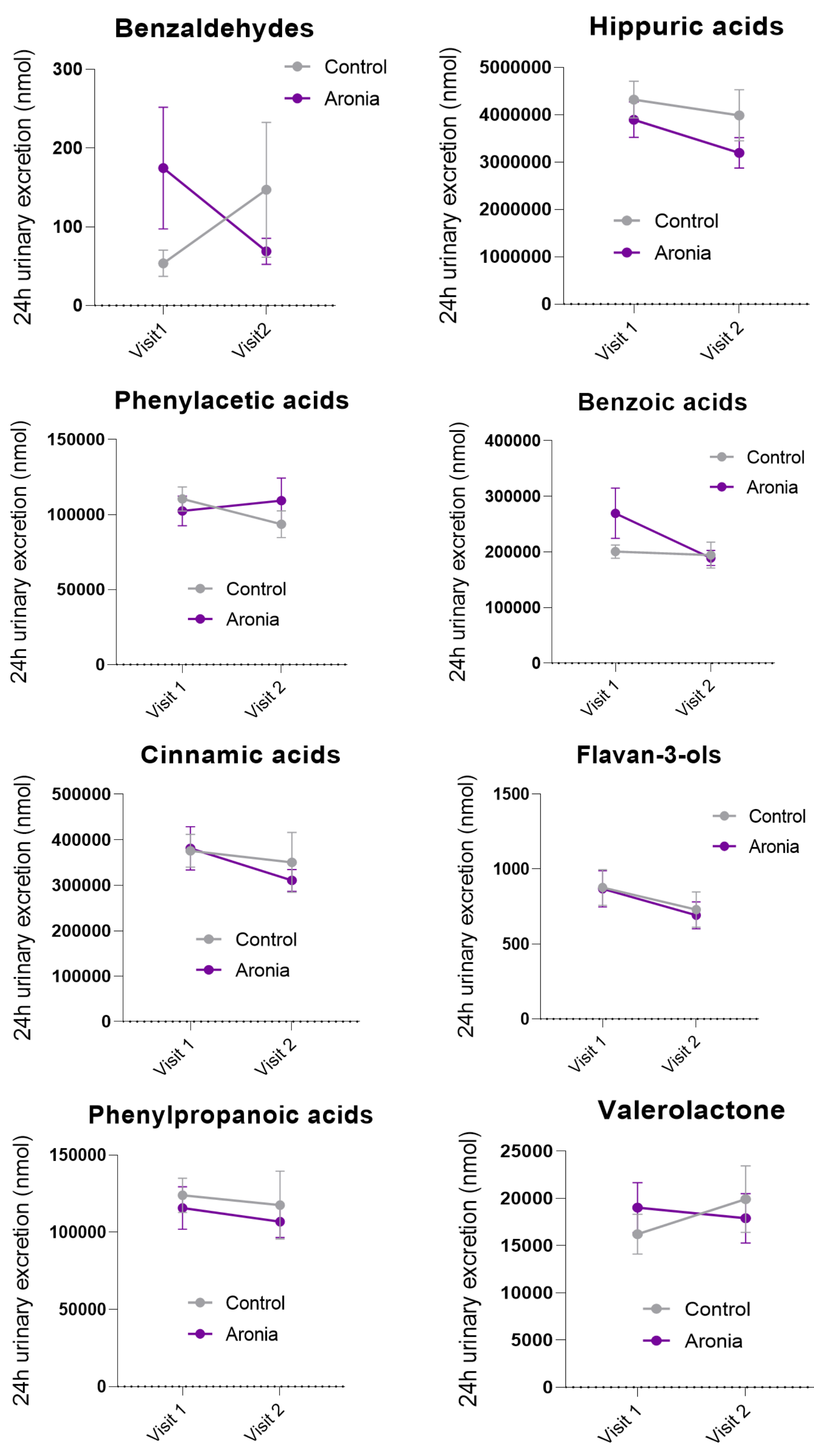


Figure 4.7: Urinary (poly)phenol metabolites after consumption of aronia extract or placebo, for Aronia and Control groups. Values expressed as mean ± SEM.

Regarding individual metabolites, a total of 19 urinary PP showed a trend for an increase after the 12-week intervention compared to Control in the Aronia group (8 cinnamic acids, 5 benzoic acids, 4 phenylpropanoic acids, 1 flavan-3-ol and 1 phenylacetic acid), including 5 compounds that significantly increased with respect to Control (Table 4.1 and Supplementary Table S5).

Table 4.1: Urinary PP levels at baseline and following a 12-week daily consumption of aronia extract, and changes with respect to Control. In bold are the p-values (P) of the 5 compounds significantly increased compared to Control.

	Aronia				Control				Chronic CFC (Aronia - Control)				
	Baseline (n= 51)		12 weeks (n=48)		Baseline (n= 51)		12 weeks (n=49)		Mean diff.	SEM	95% CI		P
	Mean	SD	Mean	SD	Mean	SD	Mean	SD			Lower	Higher	
Flavan-3-ols													
EC-3'-S	712	797	655	598	663	710	622	698	14.9	131	-245	275	0.823
Benzoic acids													
BA	16744	35288	10740	11975	10886	11962	7462	7058	1825	1547	-1246	4897	0.537
2,3-diOH-BA	134653	224300	94401	50583	84247	46896	89442	79441	2637	13657	-24479	29753	**0.040**
2,5-diOH-BA	30330	56479	20472	11155	18270	10693	19642	18878	326	3176	-5980	6632	1.000
3,4-diOH-BA	1289	825	1310	684	1286	628	1174	1195	122	185	-246	490	0.948
3,4-diOH-BA-3-GlcUA	113	85.4	116	64.5	121	82.8	109	104	9.4	16.5	-23.4	42.1	0.215
Cinnamic acids													
CA	-	-	27.1	190	110	555	204	1086	33.9	58.7	-82.8	150.5	0.548
4-CQA	165	225	224	245	266	388	217	459	46	71	-96	187	**0.005**
4/5-CQA*	1021	1306	1340	1448	1607	2289	1315	2716	250	422	-588	1088	**0.005**
3-FQA	2967	6380	3106	4139	3086	3645	2942	5964	138	1018	-1882	2159	**0.020**
4-FQA	1875	2530	2234	3282	2755	3169	2799	6190	82	906	-1717	1881	**0.012**
4'-OH-CA	1376	1031	1394	864	1372	984	1307	1098	74	196	-315	462	0.403
2'-OH-CA	125	150	131	169	153	135	128	134	16	28	-40	72	0.382
CA-4'-GlcUA	680	703	632	469	503	433	598	560	7	105	-203	216	0.566
Phenylacetic acids													
3'-OH-PA	83213	61864	95992	102238	88972	57731	79667	51751	18552	15468	-12160	49263	0.391
Phenylpropanoic acids													
2-(4'-OH-ph-O)-PrA	4960	5480	5539	5228	6368	5609	5734	8911	916	1133	-1335	3166	0.051
3-(4'-OH-ph)-PrA-3'-GlcUA	1573	2704	2243	4211	1570	1493	1550	2300	649	677	-694	1992	0.395
3-(4'-OH-ph)-PrA-3'-S	18812	15384	22342	16715	21571	17359	22541	39765	1325	5105	-8812	11461	0.170
3-(4'-MeO-ph)-PrA-3'-GlcUA	7660	8374	8573	10046	8847	10594	7225	7870	1562	1707	-1827	4952	0.569

Differences were calculated using ANCOVA (Bonferroni) with baseline values as covariate, comparing Aronia with Control changes from baseline. CFC, changes from Control; SD, standard deviation. P-values (P) obtained following Mann-Whitney non-parametric test. * Mix of two isomers.

Some individual phenolic compounds also tended to increase in the Aronia group compared to baseline. This is the case for 19 PP, including 7 cinnamic acids, 7 phenylpropanoic acids, 3 benzoic acids, 1 phenylacetic acid and 1 benzene diol. None of these compounds were significantly increased with regard to baseline (Figure 4.8).

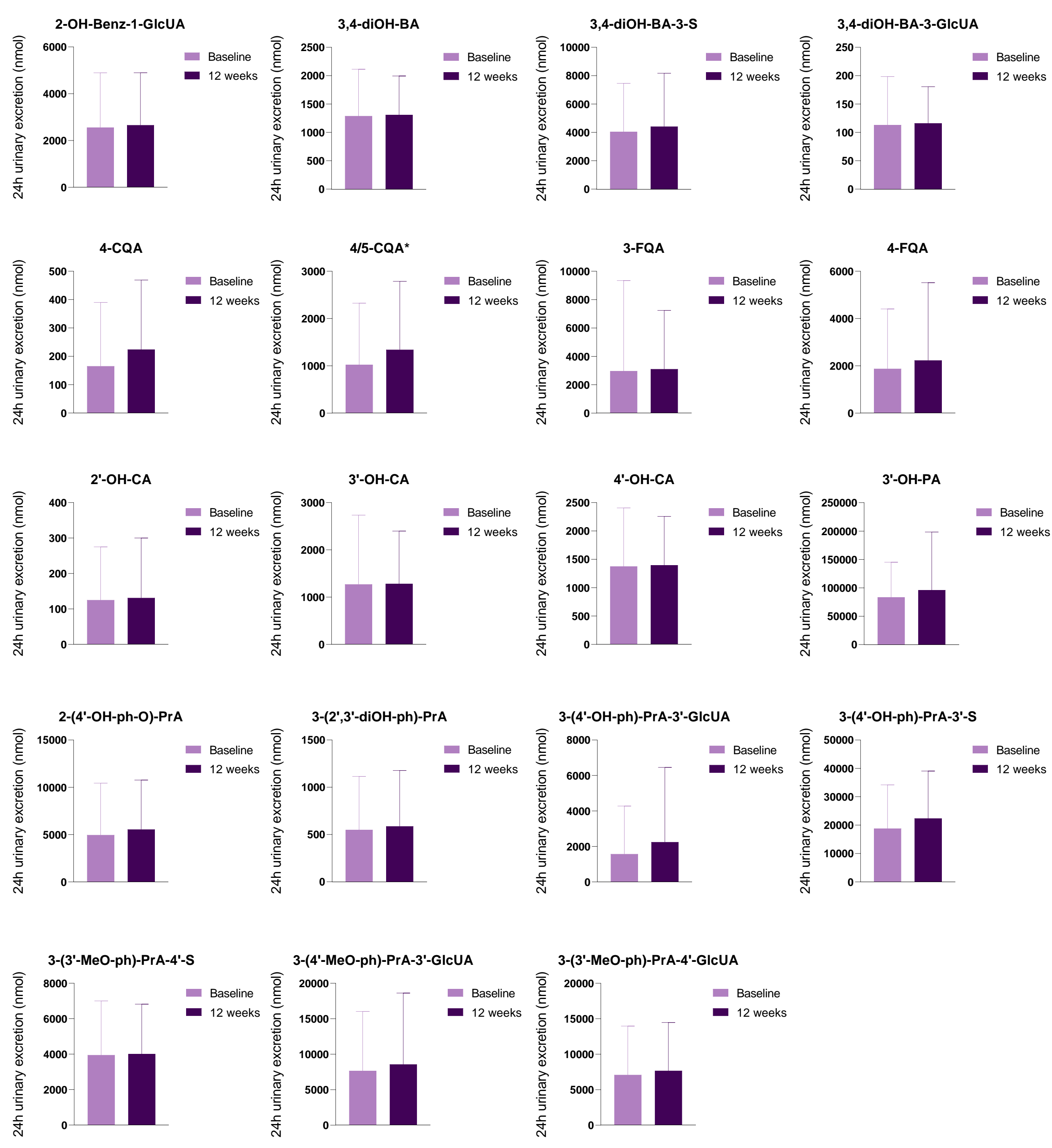


*Figure 4.8: Urinary PP concentrations at baseline and following a 12-week daily consumption of aronia extract, in the Aronia group. * Mix of 2 isomers. Values expressed as mean ± SD.*

4.3.3.2 Changes in plasma PP metabolism after consumption of aronia in comparison with Control

Both Aronia and Control groups showed a tendency for an increase in total plasma PP after 12 weeks of intervention (185 ± 1378 and 291 ± 1853 µM for Aronia and Control, respectively, Table 4.2). However, no significant changes were found after 2 hours or 12 weeks post-consumption of both treatments. No significance was observed between Aronia and Control for any of the calculated changes (Figure 4.9). No significant changes were found between groups or when compared with baseline levels when the main families were compared (Figure 4.10).

Table 4.2: Acute, chronic, and acute on chronic plasma changes (µM) of total PP for both Aronia and Control group.

Plasma changes (µM)	Aronia	Control	*P Aronia vs Control*
Δacute (V1 2h - V1 0h)	-87 ± 932	-81 ± 502	*0.644*
Δchronic (V2 0h - V1 0h)	185 ± 1378	291 ± 1853	*0.674*
Δacute on chronic (V2 2h - V1 2h)	-182 ± 1171	-219 ± 1835	*0.132*

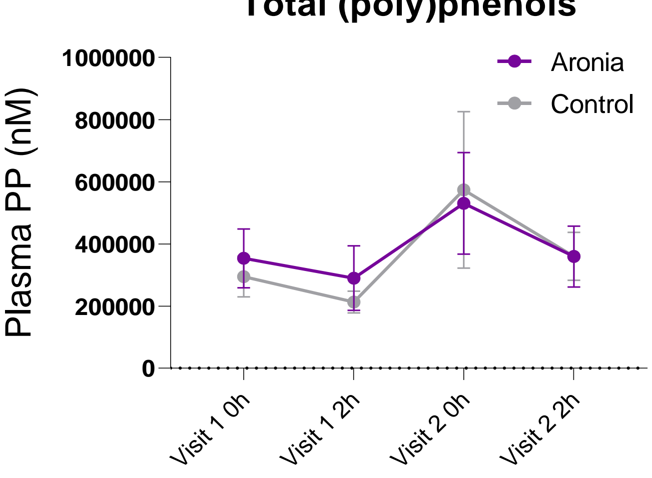
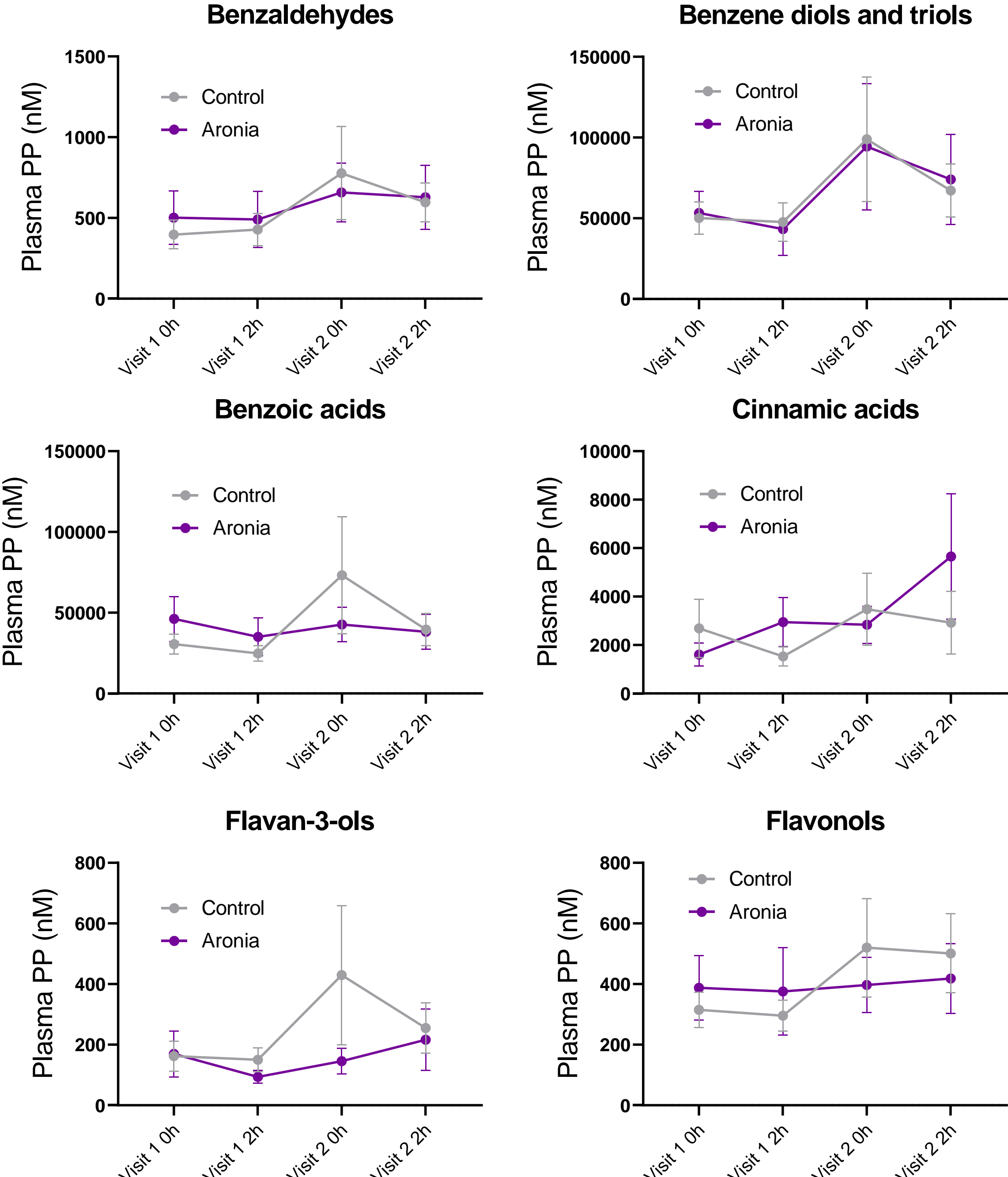


Figure 4.9: Plasma (poly)phenol metabolites after consumption of aronia (poly)phenols or placebo for Aronia and Control groups. Values expressed as mean ± SEM.


Benzaldehydes
Plasma PP (nM)
1500
1000
500
0
Control
Aronia
Visit 1 0h
Visit 1 2h
Visit 2 0h
Visit 2 2h
Benzene diols and triols
150000
100000
50000
0
Benzoic acids
Cinnamic acids
10000
8000
6000
4000
2000
Flavan-3-ols
800
600
400
200
Flavonols

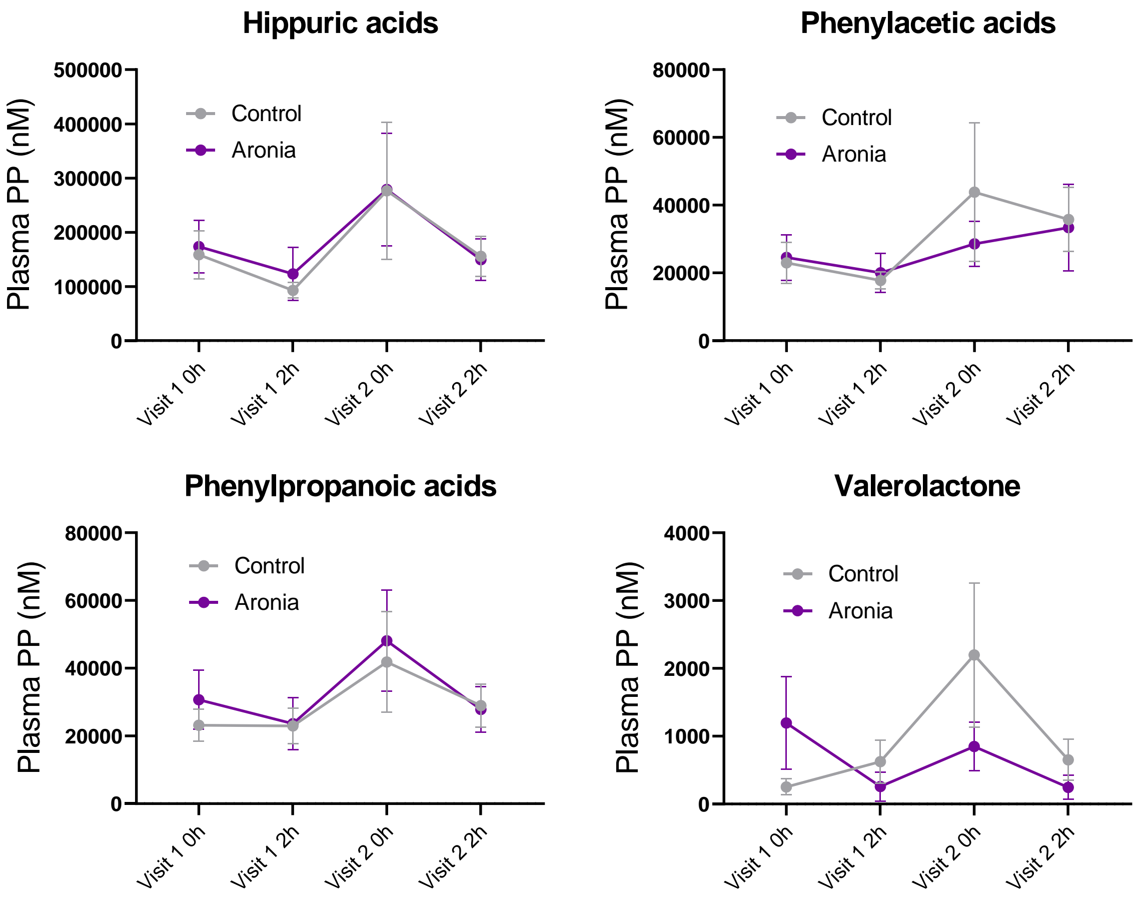


Figure 4.10: Families of plasma PP metabolites after consumption of aronia extract or placebo, for Aronia and Control groups. Values expressed as mean ± SEM.

Regarding individual plasma compounds, a total of 67 plasma PP (20 cinnamic acids, 18 benzoic acids, 10 phenylpropanoic acids, 4 flavonols, 4 benzenes diols and triols, 3 benzaldehydes, 3 hippuric acids, 3 phenylacetic acids and 2 flavan-3-ols) showed a tendency for an increase acutely in the Aronia group 2h after consumption of the first capsule, compared with Control (Supplementary Table S7). Among them, 1 flavanol (quercetin-3-GlcUA), 5 benzoic acids (3,4-diOH-BA-4-S, 3,4-diOH-BA-3-S, 3,4-diOH-BA-3-GlcUA, 4-OH-3-MeO-BA and 3-MeO-BA-4-S) and 12 cinnamic acids (4'-OH-CA-3'-S, 4'-OH-3'-MeO-CA, 3'-MeO-CA-4'-S, 3'-MeO-CA-4'-GlcUA, 3'-OH-4'-MeO-CA, 4'-MeO-CA-3'-S, 4'-MeO-CA-3'-GlcUA, 4-CQA, 4/5-CQA, 3-FQA, 4-FQA and 4'-OH-CA) were significantly increased compared with Control (Table 4.3).

After 12-week daily consumption of the aronia extract or placebo, 32 plasma compounds again tended to increase in the Aronia group with regard to Control group (10 cinnamic acids, 6 benzoic acids, 5 benzene diols and triols, 5 phenylpropanoic acids, 2 hippuric acids, 1 flavan-3-ol, 1 flavonol, 1 phenylacetic acid and 1 valerolactone) (Supplementary Table S7). None of these compounds was found significantly increased in the Aronia group compared with Control.

After 12 weeks, 45 plasma phenolic metabolites increased slightly if not significantly 2 hours following the ingestion of the last capsule in the Aronia group compared to Control (16 cinnamic acids, 11 benzoic acids, 9 phenylpropanoic acids, 3 benzaldehydes, 2 hippuric acids, 1 flavan-3-ol, 1 benzene diol, 1 phenylacetic acid and 1 valerolactone), including 12 that increased significantly (3,4-diOH-BA-4-S, 3,4-diOH-BA-3-S, 3-OH-4-MeO-BA-5-S, 4-OH-3-MeO-BA, 3-MeO-BA-4-S, 4'-OH-CA-3'-S, 4'-OH-3'-MeO-CA, 3'-MeO-CA-4'-S, 4-CQA, 4/5-CQA, 3-FQA, and 3-(3'-OH-ph)-PrA) (Supplementary Table S7 and Table 4.3).

Table 4.3: Significant changes from Control (CFC) for plasma metabolites acutely on the first and the last day, following the intake of aronia berry (poly)phenol extract or placebo.

	Acute CFC (Aronia - Control)				P	Acute on chronic CFC (Aronia - Control)				P
	Mean difference	SEM	95% CI Lower	95% CI Higher		Mean difference	SEM	95% CI Lower	95% CI Higher	
Flavonols										
Quercetin-3-GlcUA	8.5	4.2	0.2	16.8	0.000					
Benzoic acids										
3,4-diOH-BA-4-S	1599	409	787	2412	0.000	5183	2968	-714	11079	0.000
3,4-diOH-BA-3-S	877	182	516	1239	0.000	1829	839	162	3496	0.000
3,4-diOH-BA-3-GlcUA	3.7	2.9	-2.1	9.4	0.037					
3-OH-4-MeO-BA-5-S						84.0	102	-119	287	0.010
4-OH-3-MeO-BA	168	126	-82.4	418	0.025	756	460	-157	1669	0.006
3-MeO-BA-4-S	119	23.2	72.5	165	0.000	334	169	-0.5	669	0.000
Cinnamic acids										
4'-OH-CA-3'-S	421	162	99.6	742	0.000	712	632	-543	1968	0.000
4'-OH-3'-MeO-CA	235	106	24.7	446	0.010	812	655	-488	2113	0.002
3'-MeO-CA-4'-S	545	237	74.8	1016	0.000	950	799	-637	2536	0.001
3'-MeO-CA-4'-GlcUA	264	188	-111	638	0.003					
3'-OH-4'-MeO-CA	161	68.2	25.4	297	0.000					
4'-MeO-CA-3'-S	25.0	14.6	-3.9	54.0	0.042					
4'-MeO-CA-3'-GlcUA	116	56.0	4.7	227	0.002					
4-CQA	12.1	3.6	4.8	19.3	0.000	22.5	11.3	0.0	45.0	0.000
4/5-CQA*	75.4	21.5	32.8	118	0.000	135	67.2	1.4	268	0.000
3-FQA	40.5	8.4	23.9	57.2	0.000	81.3	42.0	-2.1	165	0.000
4-FQA	7.3	4.4	-1.4	16.0	0.004					
4'-OH-CA	57.5	30.3	-2.8	118	0.020					
Phenylpropanoic acids										
3-(3'-OH-ph)-PrA						2534	1994	-1429	6496	0.023

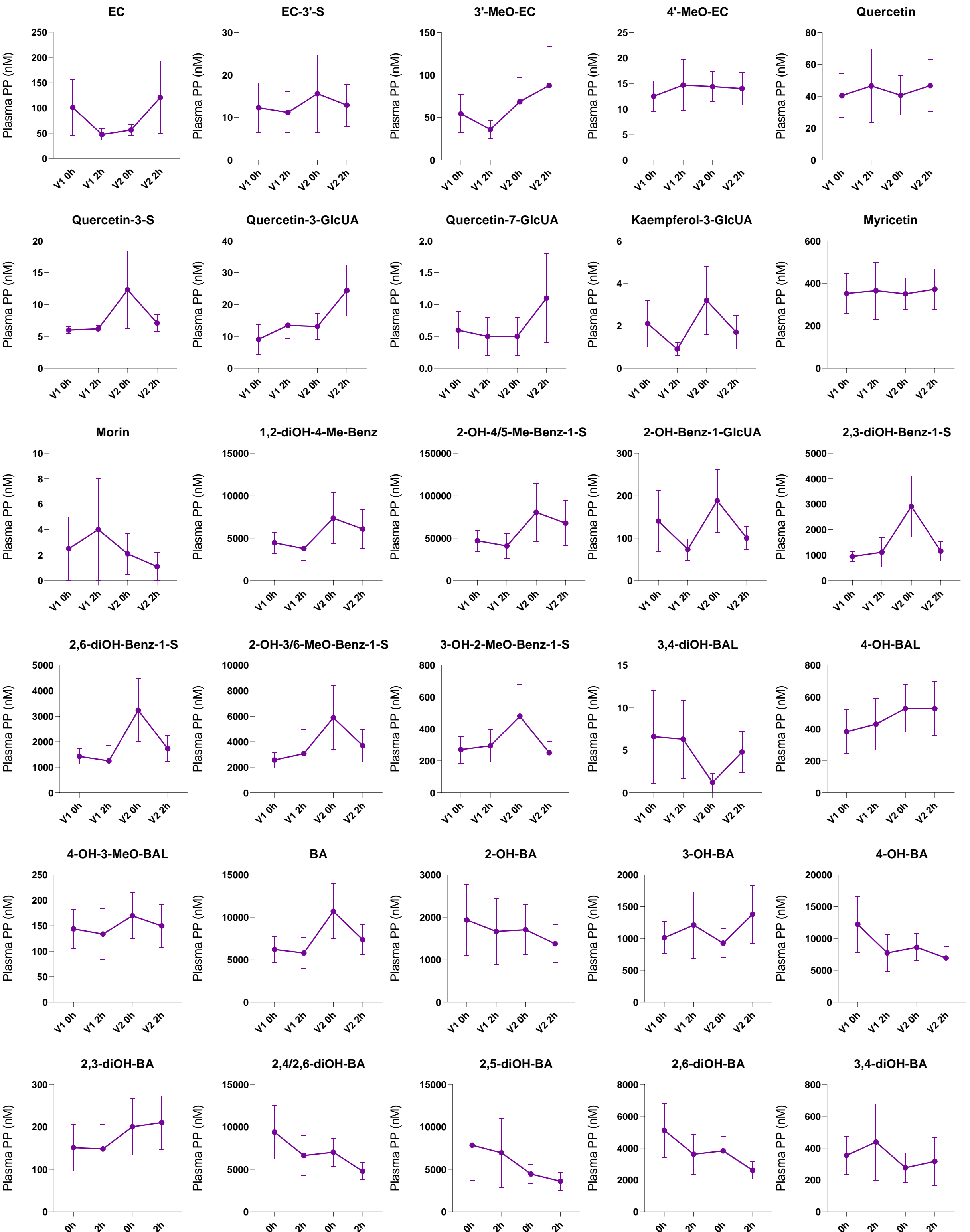
Differences from Control were calculated using ANCOVA (Bonferroni) with baseline values as covariate. 95% CI, 95% confidence interval; CFC, changes from Control; SEM, standard error of mean. P-values (P) obtained following Mann-Whitney non-parametric test. * Mix of two isomers.

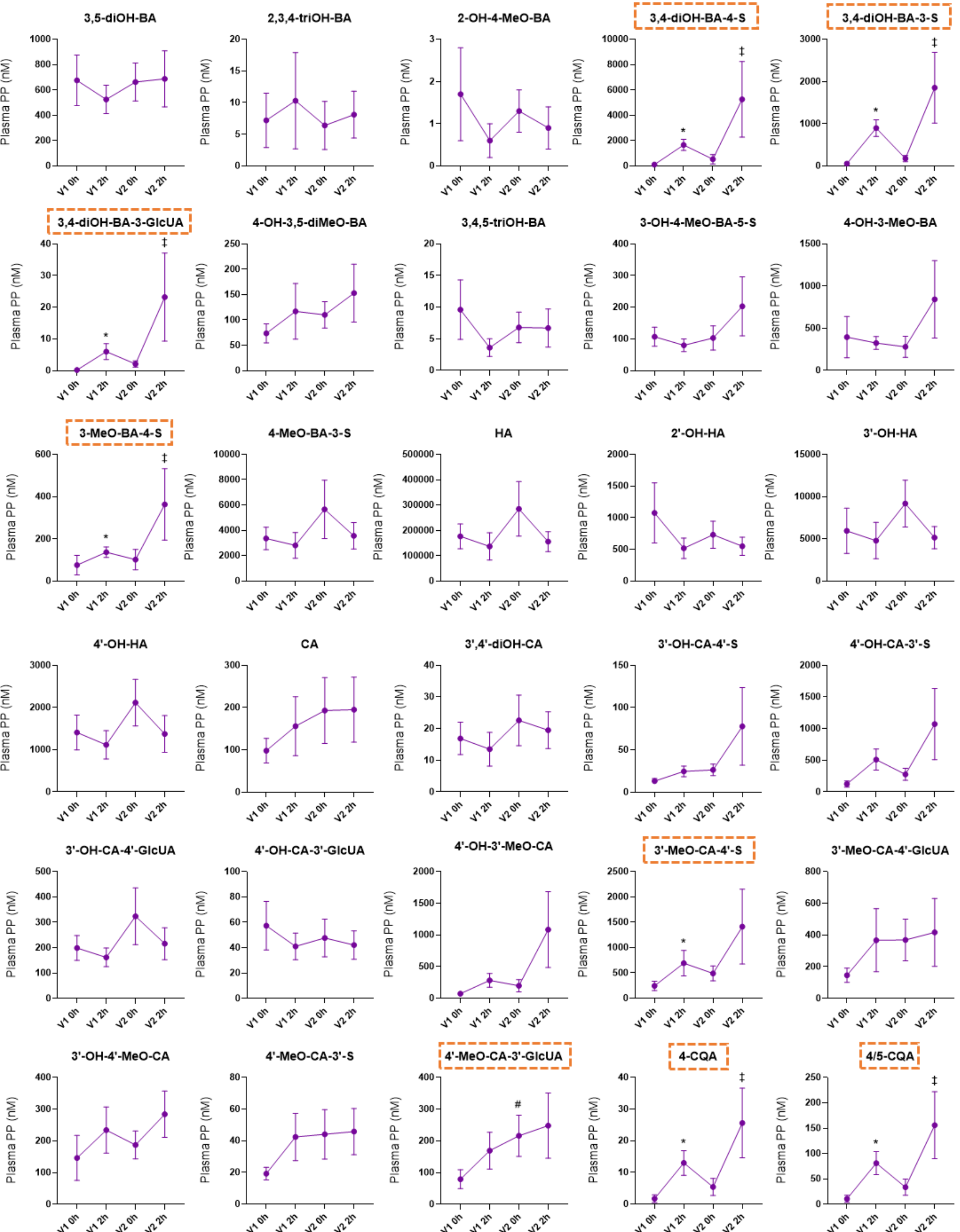
Individual metabolites also increased compared to baseline levels. Acutely, 2 hours post-consumption of the first capsule, a total of 39 compounds had a tendency for an increase in the Aronia group in comparison with baseline (18 cinnamic acids, 8 benzoic acids, 5 flavonols, 3 benzene diols and triols, 3 phenylpropanoic acids, 1 benzaldehyde and 1 flavan-3-ol) (Supplementary Table S6). Among these compounds, 9 were significantly increased with regard to baseline (3,4-diOH-BA-4-S, 3,4-diOH-BA-3-S, 3,4-diOH-BA-3-

GlcUA, 3-MeO-BA-4-S, 4-CQA, 4/5-CQA, 3-FQA, 3'-MeO-CA-4'-S, and 4'-OH-3',5'-diMeO-CA) (Figure 4.11).

After 12-week daily intake of the aronia extract, 64 phenolic compounds similarly tended to increase in the Aronia group (19 cinnamic acids, 14 phenylpropanoic acids, 8 benzoic acids, 7 benzene diols ad triols, 4 flavonols, 3 hippuric acids, 3 flavan-3-ols, 3 phenylacetic acids, 2 benzaldehydes and 1 valerolactone), including 4 significantly increased compared to baseline (3-(3'-OH-ph)-PrA, 3-(3'-MeO-ph)-PrA-4'-GlcUA, 3-(4'-MeO-ph)-PrA-3'-GlcUA and 4'-MeO-CA-3'-GlcUA) (Figure 4.11 and Supplementary Table S6).

At 12-week post-consumption, 43 plasma phenolic metabolites showed a tendency for an increase in the Aronia group 2 hours following the ingestion of the last capsule (18 cinnamic acids, 12 benzoic acids, 4 phenylpropanoic acids, 4 flavonols, 2 phenylacetic acids, 2 flavan-3-ols and 1 benzaldehyde) (Supplementary Table S6). Among them, 8 were significantly increased compared to baseline and were the same metabolites than those significantly raised acutely on the first visit (3,4-diOH-BA-4-S, 3,4-diOH-BA-3-S, 3,4-diOH-BA-3-GlcUA, 3-MeO-BA-4-S, 4-CQA, 4/5-CQA, 3-FQA) (Figure 4.11).


EC
EC-3'-S
3'-MeO-EC
4'-MeO-EC
Quercetin
Quercetin-3-S
Quercetin-3-GlcUA
Quercetin-7-GlcUA
Kaempferol-3-GlcUA
Myricetin
Morin
1,2-diOH-4-Me-Benz
2-OH-4/5-Me-Benz-1-S
2-OH-Benz-1-GlcUA
2,3-diOH-Benz-1-S
2,6-diOH-Benz-1-S
2-OH-3/6-MeO-Benz-1-S
3-OH-2-MeO-Benz-1-S
3,4-diOH-BAL
4-OH-BAL
4-OH-3-MeO-BAL
BA
2-OH-BA
3-OH-BA
4-OH-BA
2,3-diOH-BA
2,4/2,6-diOH-BA
2,5-diOH-BA
2,6-diOH-BA
3,4-diOH-BA
Plasma PP (nM)
V1 0h
V1 2h
V2 0h
V2 2h


3,5-diOH-BA
2,3,4-triOH-BA
2-OH-4-MeO-BA
3,4-diOH-BA-4-S
3,4-diOH-BA-3-S
3,4-diOH-BA-3-GlcUA
4-OH-3,5-diMeO-BA
3,4,5-triOH-BA
3-OH-4-MeO-BA-5-S
4-OH-3-MeO-BA
3-MeO-BA-4-S
4-MeO-BA-3-S
HA
2'-OH-HA
3'-OH-HA
4'-OH-HA
CA
3',4'-diOH-CA
3'-OH-CA-4'-S
4'-OH-CA-3'-S
3'-OH-CA-4'-GlcUA
4'-OH-CA-3'-GlcUA
4'-OH-3'-MeO-CA
3'-MeO-CA-4'-S
3'-MeO-CA-4'-GlcUA
3'-OH-4'-MeO-CA
4'-MeO-CA-3'-S
4'-MeO-CA-3'-GlcUA
4-CQA
4/5-CQA
Plasma PP (nM)
V1 0h
V1 2h
V2 0h
V2 2h

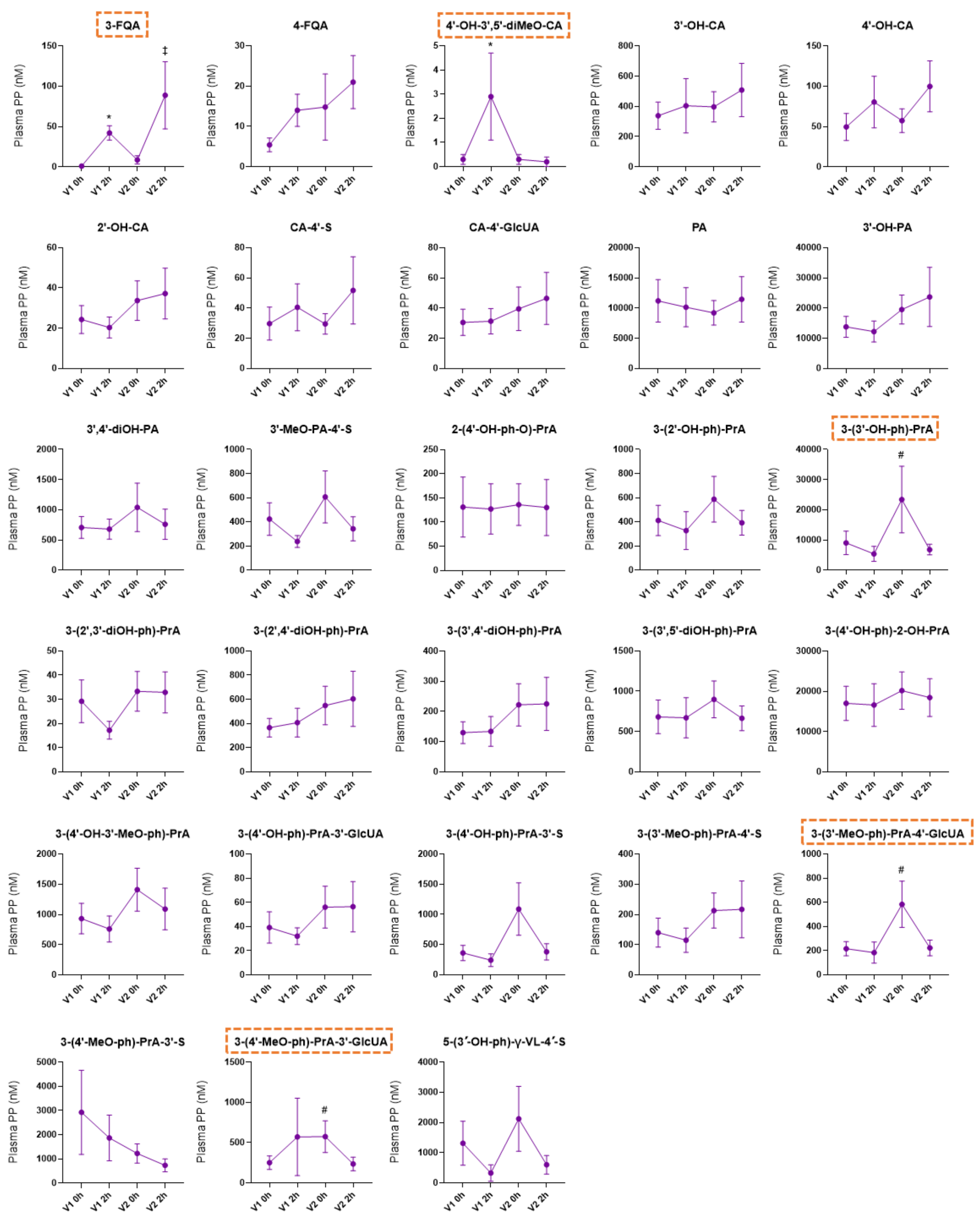


*Figure 4.11: Individual plasma metabolites after consumption of aronia extract in the Aronia group. Values expressed as mean ± SEM. Names framed in orange are compounds that are significantly increased (p< 0.05). * and #: (poly)phenols significantly increased compared to baseline on the first visit (V1 0h) (acute and chronic changes). ‡: (poly)phenols significantly increased with regard to baseline on the second visit (V2 0h) (acute on chronic changes).*

4.3.4 Correlations between PP metabolites and clinical outcomes

Independent Spearman's correlation analysis was run to determine the relationship between changes in urinary and plasma PP and changes in the significant outcomes of the study (Δawake PWV, Δ24h AIx_{ao}, Δawake AIx_{ao}, Δ24h AIx_{br} and awake AIx_{br}) in the Aronia group. Regarding the urinary metabolites (n= 39), 43 compounds were significantly correlated to Δawake PWV, 3 for Δ24h and Δawake AIx_{br}, 3 for Δawake AIx_{ao}, and 1 compound for Δ24h AIx_{ao} (Table 4.4).

Table 4.4: Urinary (poly)phenols correlation with significant outcomes in Aronia group.

Δoutcomes vs Δurinary (poly)phenols in Aronia group	ΔAwake PWV (n=39)	Δ24h AIx_{ao} (n=39)	ΔAwake AIx_{ao} (n=39)	Δ24h AIx_{br} (n=39)	ΔAwake AIx_{br} (n=39)
TOTAL PP	0.009	-	-	-	-
Flavonols					
Quercetin-3-S	0.046	-	-	-	-
Myricetin	0.016	-	-	-	-
Flavan-3-ols					
EC	0.018	-	-	-	-
Hydroxycinnamic acids					
CA	0.003	-	-	-	-
4'-OH-3'-MeO-CA	0.015	0.023	-	0.036	-
3'-MeO-CA-4'-S	0.003	-	-	-	-
3'-MeO-CA-4'-GlcUA	0.005	-	-	-	-
4'-MeO-CA-3'-S	0.025	-	-	-	-
4'-MeO-CA-3'-GlcUA	0.001	-	-	-	-
3'-OH-CA-4'-S	0.001	-	-	-	-
4'-OH-CA-3'-S	0.016	-	-	-	-
3'-OH-CA-4'-GlcUA	0.000	-	-	-	-
4'-OH-CA-3'-GlcUA	0.007	-	-	-	-
4-CQA	0.044	-	-	-	-
3-FQA	0.010	-	-	-	-
Hydroxybenzoic acids					
2,3-diOH-BA	0.037	-	-	-	-
2,5-diOH-BA	0.049	-	-	-	-
3,4-diOH-BA	0.000	-	-	-	-
3,4,5-triOH-BA	0.030	-	-	-	-
3,4,5-triOH-Benz-OEt	0.003	-	-	-	-
3,4-diOH-BA-4-S	0.030	-	-	-	-
3,4-diOH-BA-3-S	0.006	-	-	-	-

3,4-diOH-BA-3-GlcUA	0.003	-	-	-	-
4-MeO-BA-3-S	0.023	-	-	-	-
3-OH-4-MeO-BA-5-S	0.024	-	-	-	-
Phenylacetic acids					
PA	0.033	-	-	-	-
3',4'-diOH-PA	0.037	-	-	-	-
Phenylpropanoic acids					
3-(3'-OH-ph)-PrA	0.006	-	-	-	-
3-(2',3'-diOH-ph)-PrA	0.009	-	-	-	-
3-(3'-MeO-ph)-PrA-4'-S	0.018	-	-	-	-
3-(4'-OH-ph)-PrA-3'-S	0.046	-	-	-	-
3-(3',4'-diOH-ph)-PrA	0.032	-	-	-	-
Benzaldehydes					
4-OH-3-MeO-BAL	0.039	-	-	-	-
Benzene diols and triols					
2,6-diOH-Benz-1-S	0.011	-	0.045	0.041	0.023
2-OH-3/6-MeO-Benz-1-S*	0.021	-	0.033	0.037	0.015
2-OH-Benz-1-GlcUA	0.012	-	0.047	-	-
1,2-diOH-4-Me-Benz	0.015	-	-	-	-
2-OH-4/5-Me-Benz-1-S*	0.008	-	-	-	-
Hippuric acids					
HA	0.035	-	-	-	-
2'-OH-HA	0.017	-	-	-	-
3'-OH-HA	0.009	-	-	-	-
4'-OH-HA	0.027	-	-	-	-
Valerolactones					
5-(3′-OH-ph)-γ-VL-4′-S	0.002	-	-	-	0.049

Values are Spearman ρ. Correlations between changes in urinary metabolite concentrations (with respect to baseline) and significant outcomes changes (with respect to baseline). Spearman ρ was used for correlations of nonparametric data. All data represented had $p < 0.05$. * Mix of two isomers.

The plasma metabolite analysis (n= 34) revealed a significant correlation in 4 compounds after chronic aronia extract consumption (Table 4.5). Indeed, Δawake AIx_{ao} was correlated with 2 benzene diols and triols (pyrogallol-2-O-sulfate and 1-methylpyrogallol-sulfate) as well as 2 hydroxycinnamic acids (chlorogenic acid and cryptochlorogenic acid). Additionally, the only correlation found after 12 weeks of daily consumption of the aronia extract for Δawake AIx_{br} was with chlorogenic acid. No significant correlation was reported for Δawake PWV, Δ24h AIx_{ao} and Δ24h AIx_{br} following the 12-week daily consumption of the aronia extract.

Table 4.5: Plasma (poly)phenols correlation with significant outcomes in Aronia group.

Δoutcomes vs Δplasma (poly)phenols in Aronia group	ΔAwake PWV (n=34)	Δ24h AIx_{ao} (n=34)	ΔAwake AIx_{ao} (n=34)	Δ24h AIx_{br} (n=34)	ΔAwake AIx_{br} (n=34)
CHRONIC					
Benzene diols and triols					
2,6-diOH-Benz-1-S	-	-	0.012	-	-
2-OH-3/6-MeO-Benz-1-S*	-	-	0.029	-	-
Hydroxycinnamic acids					
4-CQA	-	-	0.034	-	-
4/5-CQA*	-	-	0.023	-	0.044

Values are Spearman ρ. Correlations between changes in plasma metabolite concentrations (with respect to baseline) and significant outcomes changes (with respect to baseline). Spearman ρ was used for correlations of nonparametric data. All data represented had $p< 0.05$. AIx, augmentation index; PWV, pulse wave velocity; ao, aortic; br, brachial. * Mix of two isomers.

4.4 Discussion

The study of phenolic compounds bioavailability along with an accurate quantification and identification is the only way to assess the real exposure to these PP (Wiczkowski, Romaszko, and Piskula 2010). The analysis of collected plasma and 24-hour urine samples following the 12-week daily consumption of aronia berry PP or placebo in the present study revealed a significant increase in several compounds, both acutely and after 12-week daily supplementation.

In the present trial, no significant changes were found in total urinary and plasma metabolites following the intervention. However, we saw several compounds increasing following the 12-week daily intake of aronia berry extract. This was the case for 19 and 64 metabolites in urinary and plasma samples, respectively. A similar trend was seen acutely in plasma samples as 39 and 43 compounds were in higher concentration 2 hours after the consumption of the interventional capsule, on the first and last study day, respectively. Among these metabolites, we can cite (–)-epicatechin-3'-sulfate, hippuric acid, 5-O-caffeoylquinic acid or 3',4'-dihydroxyphenylacetic acid, which were part of the most frequent metabolites reported in biofluids following PP-rich supplementation according to a systematic analysis exploring the PP metabolome (Rothwell et al. 2016).

The concentrations found in our trial were in line with other previous papers investigating the bioavailability of PP-rich foods. Indeed, several studies also found a range of plasma metabolites going from nM to µM with hippuric acid accounting for the major part of circulating and excreted metabolites following

the acute and chronic consumption of their investigational product (Feliciano, Boeres, et al. 2016; Vetrani et al. 2016; Hanhineva et al. 2015; Mulder, Rietveld, and van Amelsvoort 2005; Feliciano, Istas, et al. 2016; Loo et al. 2016; Xie et al. 2016). The uptake of metabolites originated from the gut microbiota catabolism may explain the high concentration of hippuric acid in urine (Mecha et al. 2020). However, hippuric acid is also a product of many other endogenous metabolism pathways, such as the tyrosine pathway, so we cannot rule out that a significant amount of the hippuric acid quantified here is coming from other sources, including other PP in the habitual diet of volunteers.

To the best of our knowledge, only 4 studies have investigated the presence of aronia metabolites in 24-hour urine (Loo et al. 2016; Xie et al. 2016; Kay, Mazza, and Holub 2005; Wiczkowski, Romaszko, and Piskula 2010). While 2 of these studies were focusing exclusively on anthocyanin compounds, studies by Loo and Xie also identified some of the compounds of interest of the present study (11 and 2 compounds out of our 92, respectively). However, the units used in these trials to characterize urine concentrations (respectively mg/mg of creatinine and µM, i.e., not reported to the quantity excreted in 24h), does not allow us to compare our findings. Yet, both studies observed a high increase in hippuric acid following aronia supplementation, which is in line with our observations. Moreover, none of the 4 studies listed above and involving 24h collection of urine has identified more than 17 compounds overall, and mainly comprised non-conjugated metabolites. Additionally, study designs are highly divergent between trials, which makes comparisons even harder.

In line with the 24h urine excretion, total PP plasma concentration following 12 weeks daily supplementation of PP-rich aronia berry extract did not significantly increase. This is likely due to the high consumption of PP that our population had in their background diet. A previous study led by our team and exploring the effect of the same aronia extract on a population of young men observed a significant increase in 18 plasma compounds following a 12-week consumption of the berry product, compared to placebo (Istas et al. 2019). While that study required a 24-hour PP-free diet from the volunteers, the usual baseline PP intake was way lower than our participants' (531 ± 357 mg versus 1553 ± 1052 mg in the present study), resulting in a huge difference in terms of ADME and biological effects associated between the two trials, which could account for the chronic differences observed.

Our 12-week intervention led to increases in urine and plasma concentrations compared with Control, mostly for benzoic and cinnamic acids. While no plasma compound was significantly increased in Aronia group with regard to Control following the 12-week intervention, it was the case for 5 urinary compounds (2,3-dihydroxybenzoic acid, 4-O-caffeoylquinic acid, 5-O-caffeoylquinic acid, 3-O-feruloylquinic acid, and 4-O-feruloylquinic acid). Acutely, 18 and 12 compounds were significantly increased acutely in plasma compared with placebo, on the first and last visit respectively, including 10 common compounds: 3-hydroxybenzoic acid-4-sulfate, 4-hydroxybenzoic acid-3-sulfate, 4-hydroxy-3-methoxybenzoic acid, 3-methoxybenzoic acid-4-sulfate, 4'-hydroxycinnamic acid-3'-sulfate, 4'-hydroxy-3'-methoxycinnamic acid, 3'-methoxycinnamic acid-4'-sulfate, 4-O-caffeoylquinic acid, 5-O-caffeoylquinic acid, and 3-O-feruloylquinic acid. One of these compounds,

5-O-caffeoylquinic acid, also known as chlorogenic acid, was, along with its isomer neochlorogenic acid, one of the most represented PP of our aronia extract. As a result, the significant increase of that metabolite in the Aronia group compared to Control confirms the small but significant effect of the investigational product on ADME. Chlorogenic acid was shown to be widely bioavailable and very well absorbed, with recovery rate in 24-hour urine around 30% and derivates mainly related to microbiota catabolism (Czank et al. 2013; Rodriguez-Mateos, Heiss, et al. 2014; Manach et al. 2005). Chlorogenic acid undergoes the early action of esterases located in the upper gastrointestinal tract, resulting in the release of 3',4'-dihydroxycinnamic acid (also known as caffeic acid) which is then conjugated and able to enter the bloodstream (Figures 4.12 and 4.13) (Stalmach, Mullen, Barron, et al. 2009; Lafay et al. 2006).

Metabolites deriving from the extensive metabolism of chlorogenic acid (3',4'-dihydroxycinnamic acid –also known as caffeic acid–, 4'-hydroxy-3'-methoxycinnamic acid –also known as transferulic acid–, 4-hydroxy-3-methoxybenzoic acid –also known as vanillic acid–, and their sulfates and glucuronides forms) were shown to appear and be detected in plasma 1 to 2 hours following the intake of the original food matrix (Rodriguez-Mateos et al. 2013; Rodriguez-Mateos, Vauzour, et al. 2014). This is in line with our findings as 18 chlorogenic-derived compounds increased in plasma 2 hours post-consumption, compared with Control, and 39 compounds increased acutely with regard to baseline. However, metabolic pathways are very complex and sometimes overlapping. Indeed, as seen in Figure 4.13, protocatechuic acid and its derivates can also arise from proanthocyanidins, cyanidin glucosides

and quercetin glucosides, which are also well represented in the aronia extract (Loo et al. 2016; Rios et al. 2003). For example, Gao and colleagues have highlighted that homovanillic acid could be a product of 4 different flavonoids (Gao et al. 2006).

Additionally, no significant difference was found for benzene diols and triols (catechols, pyrogallols) following the 12-week treatment, in both urine and plasma. This result was expected as these compounds are small molecular weight metabolites which are not specific of PP intake (Feliciano et al. 2017).

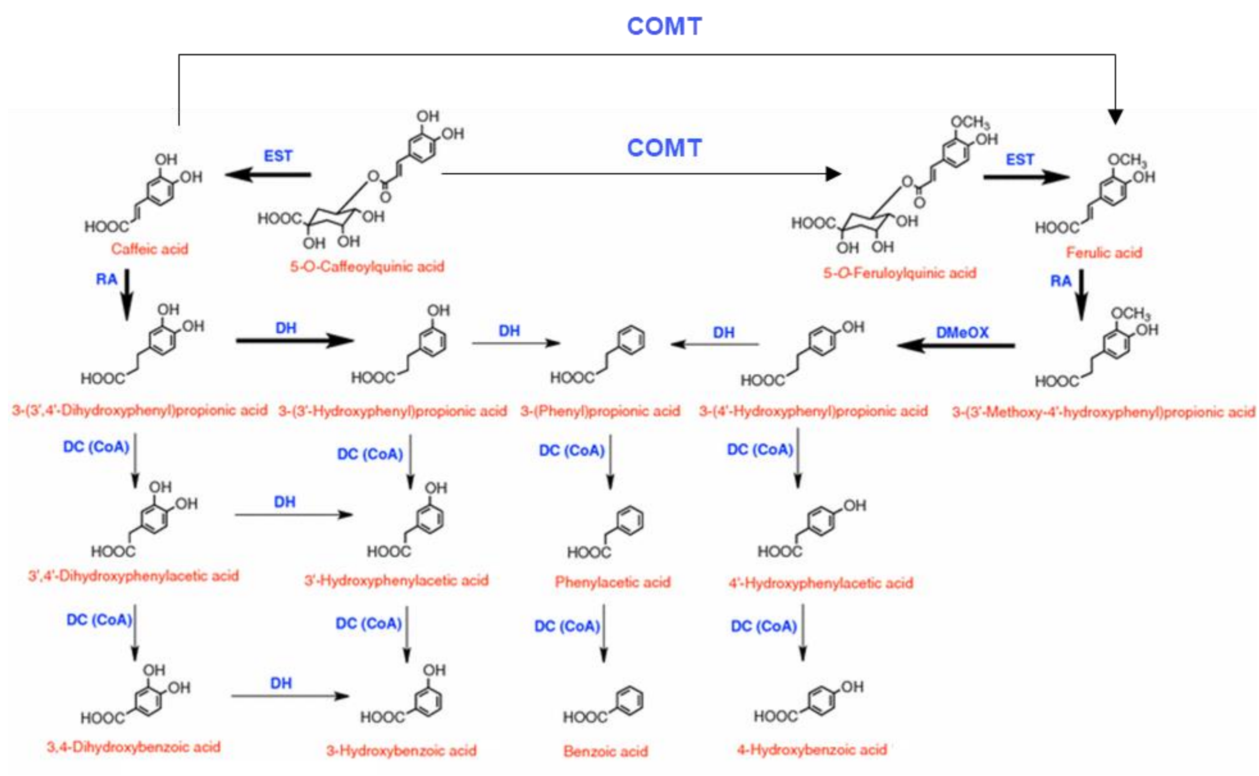


Figure 4.12: Proposed catabolic pathways for 5-CQA and 5-FQA degradation by colonic microbiota. Bold arrows indicate major pathways. EST, esterase; RA, reductase; DH, dehydrogenase; DMeOX, demethoxyesterase; DC (CoA), decarboxylation (co-enzyme A-mediated); COMT, catechol-O-methyltransferase. Adapted from various studies (Rodriguez-Mateos, Vauzour, et al. 2014; Stalmach, Mullen, Barron, et al. 2009).

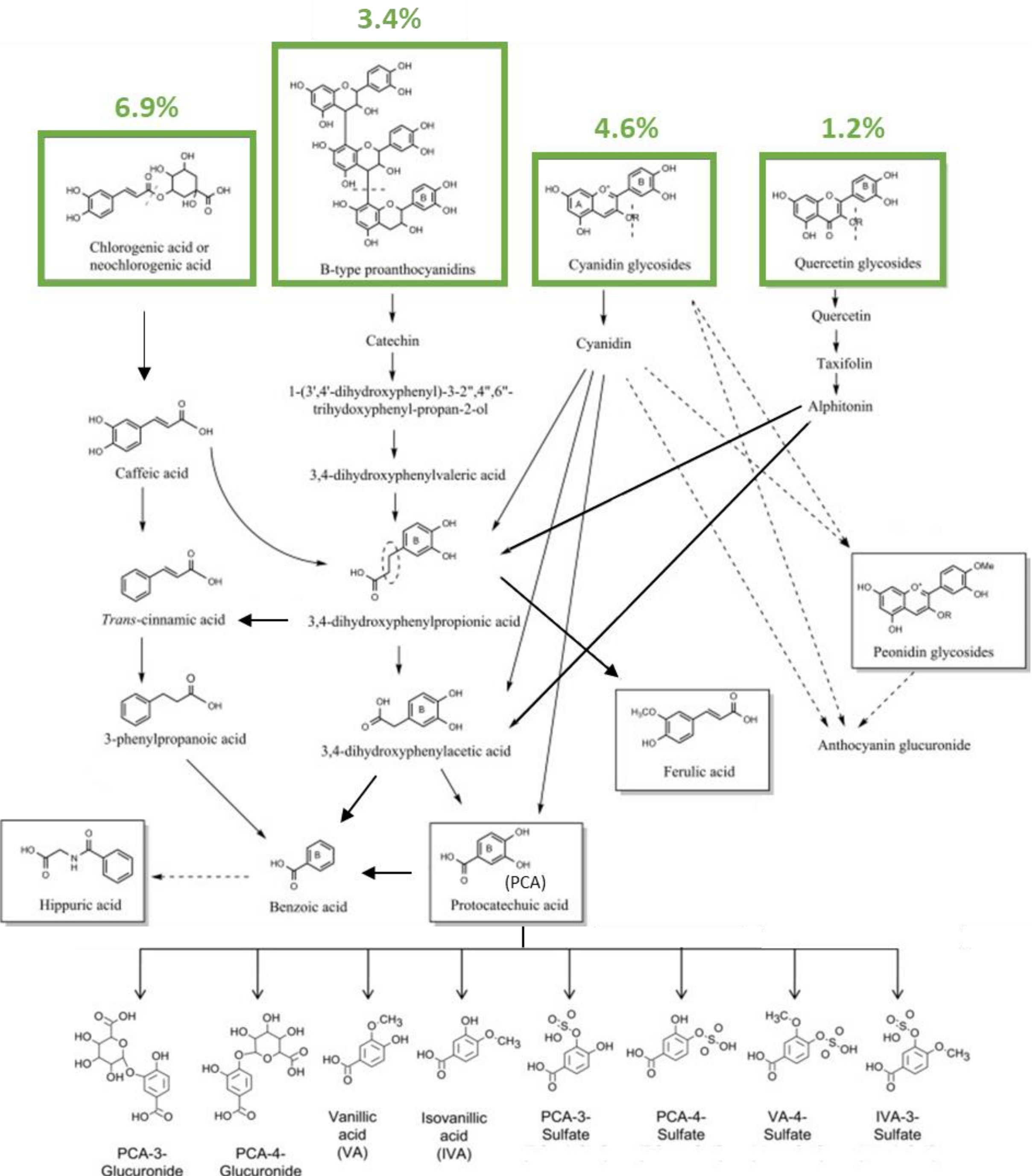


Figure 4.13: Proposed metabolic pathways of aronia (poly)phenols. Solid lines represent microbiota catabolism and dashed lines represent tissue metabolism. Compounds framed in green, and their related percentages correspond to the proportion of these compounds in the berry extract interventional product. Adapted from various studies (Xie et al. 2016; Monagas, Urpi-Sarda, et al. 2010; Wu, Cao, and Prior 2002; Wu et al. 2004; Stalmach et al. 2013; Amin et al. 2015).

Furthermore, a total of 43 and 13 aronia-related metabolites were significantly correlated to significant improvement in awake PWV and awake and 24h central and peripheral AIx in urine and plasma, respectively. This is in line with previous work from our team highlighted the significant association existing between plasma metabolites and enhancement of vascular function (Rodriguez-Mateos et al. 2016; Rodriguez-Mateos et al. 2013). A few papers have found a significant correlation between PWV and a phenolic compound named hydrocinnamate and which belongs to the family of hydroxycinnamic acids (Menni et al. 2015; Li et al. 2018). However, our study to the best of our knowledge is the first double-blind RCT to observe a correlation between augmentation index and PP metabolites.

Our study has notable strengths. The absence of a PP-free diet in the study design offers a more usual diet of participants and is more relevant to personalized nutritional research. The investigational product is an extract of a berry containing a dietarily achievable and relevant dose of phenolic compounds (1 capsule being equivalent to 75g of fresh fruit in terms of PP). This extract is a mix of various individual and pure PP, including that no matrix effect is involved in the ADME of the product. Finally, our study is to date the first to identify so many phenolic compounds in biosamples following intake of aronia berry products. The method used has been validated and includes authentic standards as reference to quantify the metabolites present in the samples, along with short-run times and the use of small volumes of biosamples. Solid phase extraction has also been shown to be a better quantification technique for the analysis of specific metabolites as in our study (González-Domínguez et al. 2020).

However, the study has some limitations. Indeed, participants were intentionally not instructed to follow a PP-free diet in the days preceding the study visits, nor were they asked to avoid the consumption of PP-rich foods in the hours following both visits 1 and 2. As a result, the baseline PP of volunteers was high and extremely variable according to their diet, which caused the analysed biosamples compounds not to be a relevant marker of compliance. Indeed, variations in urinary PP concentrations are known to be high, and largely related to the dietary background of an individual (Zamora-Ros, Achaintre, et al. 2016). Additionally, the duration of the study days and the number of biosamples collected were too few to have a clear vision of the pharmacokinetics of studied phenolic compounds. The absence of segmented urine collection over the 24 hours led to the impossibility to calculate the PP recovery percentage, which could be a greater indicator of the metabolism and excretion range of the metabolites. To get a deeper understanding of the kinetic profile of phenolic compounds with return to baseline level, further studies should be conducted with sample collection extended to 24 or 48h and segmented (Mecha et al. 2020). Indeed, the use of pharmacokinetics is needed to analyse parameters such as urinary clearance, elimination rate or recovery percentage (Wiczkowski, Romaszko, and Piskula 2010).

Moreover, the method used to quantify the phenolic compounds does not allow to identify or quantify any intact anthocyanin while those metabolites represent a large proportion of the PP present in the investigational product. It is thus not possible to have a clear vision of the metabolism of these compounds following their ingestion, although studies have shown that the amount of intact anthocyanins represent less than 0.1% of the total ingested anthocyanins (Kay

et al. 2017; Czank et al. 2013). Finally, the faecal metabolites are not considered in this analysis while they might represent an important part of the overall metabolism of non-absorbed PP, especially for high molecular weight PP such as proanthocyanidins. Indeed, Czank and colleagues showed that 32% of an ingested dose of ^{13}C labelled cyanidin-3-O-glucoside was found in the faeces after 48h (Czank et al. 2013). Similarly, other routes of elimination such as bile or breath are not accounted for, and nor is the pool of metabolites sequestered into body tissues (Crozier, Jaganath, and Clifford 2009).

Overall, the analysis of PP metabolites confirmed that aronia berry compounds are poorly absorbed and widely metabolised. It also highlighted the importance of PP metabolism in the magnitude of biological effects. To conclude, this is the first study investigating the bioavailability of aroqnia berry PP in a population of healthy middle-aged men and women. More information regarding aronia berry PP dose-response relationship and pharmacokinetics needs to be discovered to expand the knowledge related to phenolic compounds bioavailability.

CHAPTER 5

Assessment of interindividual variability following aronia berry (poly)phenol consumption

5.1 Introduction

In the last decade, it has become clear that there is no one-size-fits-all approach when it comes to food, and nutritional studies have demonstrated that a high interindividual variability in response to diets, foods and food components exist (Berry et al. 2020; Gibney et al. 2019; Eagles, Gross, and McLachlan 2020). Those variations in the biological response between subjects could be in part due to differences in the absorption and metabolism of specific food components (Tierney et al. 2011). This is relevant in the context of plant (poly)phenol (PP) consumption, as several studies have shown a high variability in both PP absorption and metabolism and vascular response in several studies (Krga et al. 2016; Manach et al. 2017; Cassidy and Minihane 2017). Another important factor is the gut microbiome, which has an important role in the metabolism of several PP and could as a result be a big contributor to the interindividual variability in responsiveness to the consumption of these plant bioactives (Tomas-Barberan, Selma, and Espin 2016). The impact of the gut microbiota on the variability in the vascular response will be described in Chapter 6.

Along with bioavailability and gut microbiota, several factors related to the environment and lifestyle of individuals can be related to the variability of individual response to PP consumption. This is the case for non-modifiable factors such as genetic background, age, sex, or ethnicity, but also for modifiable factors such as tobacco consumption, medication, physical activity, overall diet and health status (Figure 5.1) (Milenkovic et al. 2017).

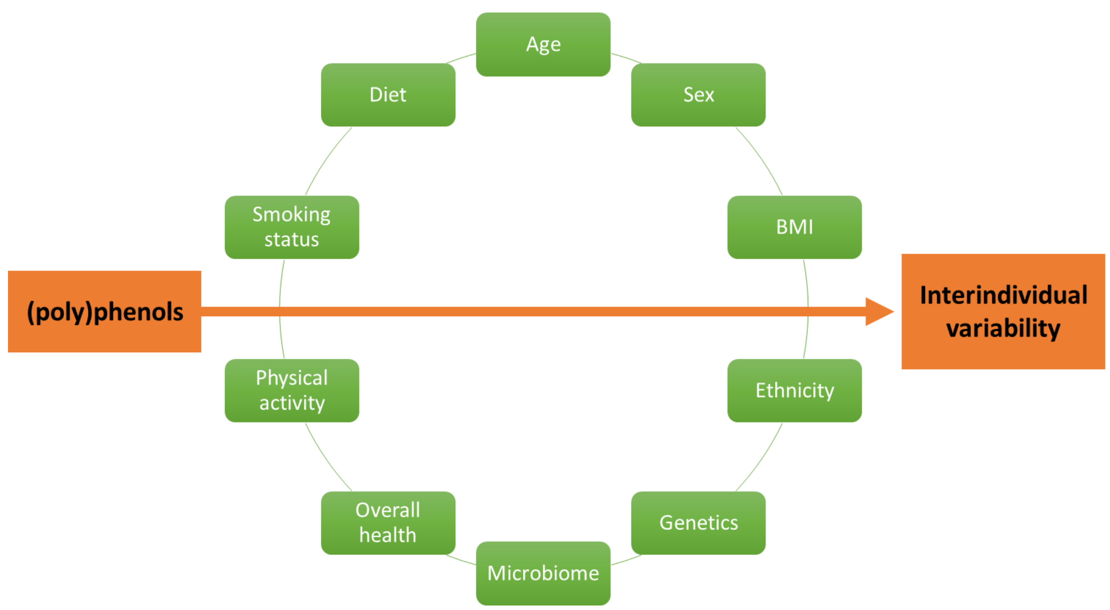


Figure 5.1: Parameters influencing interindividual variability in cardiometabolic response to (poly)phenol consumption. Adapted from Gibney and colleagues (Gibney et al. 2019).

Sex is one of the non-modifiable factors that can be an important contributor to the interindividual variability in metabolism and response to PP. Very few studies focused on investigating sex-related differences in cardiometabolic response to PP intake, with mixed results. Indeed, while some PP-rich interventions reported an amelioration of platelet aggregation (Ostertag et al. 2013) or oxidized LDL (Ibero-Baraibar et al. 2014) in men only, other outcomes such as AIx (West et al. 2014), BP and LDL cholesterol (Shiina et al. 2019) were found significantly improved in female subjects only. Many RCTs investigating the effects of PP on health, particularly cardiovascular outcomes, have included male-only populations because of concern that female subjects could bias the results due to the variations of their hormonal cycle, which affect parameters such as vascular function (Louis et al. 2019). This has unfortunately led to a lack of research on the effects of PP on pre-menopausal women's health, and whether the findings in men can be translated to women.

Age is another non-modifiable risk factor for CVD which influences on cardiometabolic effects mediated by the intake of PP. Although several trials have focused on the age-related effect of phenolic compounds on vascular health, results have been often divergent. Indeed, although SBP and AIx were reported to be significantly improved in the elderly group of a flavanol intervention (Heiss et al. 2015), another study using quercetin supplementation showed that improved SBP was more important for the subgroup of younger individuals. The current evidence suggesting that cardiometabolic response to PP intake is age-dependent is very limited, and no conclusions can be made in this matter.

Furthermore, the influence of BMI on the variability in response to PP interventions has been reported in very few publications, once again with mixed results. A meta-analysis of RCTs on PP-rich dark chocolate concluded that individuals with a BMI higher than 25 kg/m^2 had a higher response to PP compared to those with BMI< 25 kg/m^2 in terms of BP (Almoosawi et al. 2012), while an opposite trend was reported for FMD in another meta-analysis, which improved only in the subgroup of participants with lower BMI (García-Conesa et al. 2018). However, these results need to be taken with caution as only very few publications were included in this analysis, and the RCTs analysed had not been designed to investigate the effect of BMI on the interindividual variability in response.

Very few studies investigated the influence of smoking status on the response to PP intake as most RCTs do not report this parameter or include non-smoker subjects only. A meta-analysis focusing on factors affecting the

cardiometabolic response to ellagitannins (ET) and anthocyanins (ACN) consumption established significant heterogeneity between smokers and non-smokers in the studies analysed, with significant improvements in DBP in non-smokers only, and significant reduction in FMD in smokers only (García-Conesa et al. 2018). As several heterogenous studies were compared in this meta-analysis, that were not designed to investigate the effect of smoking in the variability in response to PP, the findings should be taken with caution. The evidence related to smoking and its impact on responsiveness is scarce and there is a need for a better reporting on the smoking status in nutritional studies.

Interindividual variability in cardiometabolic response to PP consumption related to the physiopathological status has also been described in publications. For instance, chronic intake of PP-rich coffee induced a significant improvement in blood lipids in subjects with hypercholesterolemia, but not in healthy individuals (Martínez-López et al. 2019). Another study reported the beneficial effect of quercetin consumption on SBP in prehypertensive participants while no difference was observed in the normotensive subgroup (Egert et al. 2009). Moreover, a meta-analysis highlighted the beneficial impact of ET-rich foods on blood lipids, SBP and FMD in a subgroup of subjects with CVD risk only (García-Conesa et al. 2018). On the contrary, authors described a significant improvement in FMD for healthy subjects only following supplementation with ACN-rich foods. Additionally, several studies have focused on the impact of aronia berry intake on cardiometabolic response among individuals at risk or with CVD (Kardum et al. 2014; Milutinovic et al. 2019; Naruszewicz et al. 2007; Pokimica et al.

2019; Skoczyńska et al. 2007) but none of them have been comparing their populations to healthy subjects, leading to a lack of information regarding the relevance of aronia berry effect in the context of subgroup analysis.

Subjects' ethnicity is rarely reported in nutritional studies, which highlights the need for a better definition of study populations (Gibney et al. 2019). In one of the few studies investigating this aspect, significant improvement in TG, total and LDL cholesterol in Asian subjects following the consumption of flavonols was described, while no significant difference was observed for the same outcomes in Europe/North America individuals (Menezes et al. 2017). Another meta-analysis investigating the effect of ET and ACN on cardiometabolic outcomes noted a significant improvement in FMD in Asian countries only, while no change was observed for other countries (García-Conesa et al. 2018). Moreover, authors also reported that total cholesterol, LDL and DBP were significantly reduced in the Mediterranean subjects only, whilst FMD was significantly improved in the non-Mediterranean countries only. Based on these results, more research on the influence of ethnicity in nutritional studies is needed to offer valuable new insights for a better grasp of interindividual variability.

Habitual PP consumption was also stated as one of the factors potentially influencing the interindividual cardiometabolic response to PP consumption (Gibney et al. 2019), although no RCT has reported findings stratified based on usual baseline PP composition or intake so far.

To our knowledge, no study has investigated the interindividual variability in cardiometabolic response following PP intake in subgroups based on physical

activity level or alcohol consumption. Which factors are driving the interindividual variability in cardiometabolic response to the consumption of PP are currently unknown, and this area of research is still relatively new. Indeed, the understanding of this variation in responsiveness is paramount to assess the potential of food bioactives on CVD risk and at an individual scale. The few meta-analysis looking at the impact of various factors on the variability in the vascular response to flavonols (Menezes et al. 2017), ellagitannins and anthocyanins (García-Conesa et al. 2018) are highly exploratory, included just few studies, and are not suitable to assess the variability in response, especially as they are based in subgroup analysis of studies reporting the average change in a specific outcome of individual studies, and not on individual data. Very few RCTs have investigated and evaluated the interindividual variability in response following PP intake, and none focused specifically on aronia berry PP consumption. In the present chapter, we aim to conduct an exploratory analysis with individual data from the ABP study to assess the extent of variability in the vascular response and in the bioavailability of aronia berry PP and to identify potential factors that may be important to explain such variability.

5.2 Methods

For the analysis of the variability in responsiveness to treatment, an assessment of interindividual variability was conducted on the primary and secondary outcomes of the trial. This analysis was conducted on subjects from the Aronia group only, to observe how the aronia extract treatment impacted the biological response. Coefficients of variation (CoV) were calculated using the following formula: $CoV = \frac{\sigma}{\mu} \times 100$, with σ representing the standard deviation of the population (i.e., Aronia subjects) and μ characterising the mean of the same population.

Moreover, following the analysis of the various outcomes on the overall population described in Chapters 3 and 4, an exploratory subgroup analysis was also performed to assess the influence of the following parameters: sex, age, BMI, habitual total PP and fruits and vegetables consumption, BP at baseline, as well as habitual alcohol consumption and physical activity level (Table 5.1). These subgroups were chosen for their relevance based on the literature and how well balanced the 2 subgroups were. The data related to sex, age, BMI, and BP was reported from the baseline visit (study day 1). Habitual PP and fruit and vegetable consumption was calculated from the 7-day food diaries completed by participants prior study day 1 (methodology available in Chapter 2, sections 2.7.2 and 2.7.3). Similarly, data used for the subgroup analysis based on physical activity level was processed from the International Physical Activity Questionnaire (IPAQ) filled by volunteers during study day 1 (methodology available in Chapter 2, section 2.7.4). Finally, the information related to the usual intake of alcohol of included subjects was

imported from the associated case report form section completed during the screening visit.

This exploratory subgroup analysis was performed on the overall ITT population and using an ANCOVA with baseline as covariate only (BACO) analysis. The subgroup analysis was done for the chronic results, i.e., changes from baseline after 12 weeks of daily consumption of the interventional product. For more clarity, significant results have been highlighted in green in the tables. This exploratory analysis compares the Aronia subjects of each subgroup with Control, to observe if the response to treatment differs according to some parameters.

No subgroup analysis was performed for smoking status and ethnicity due to the lack of heterogeneity, as only 5 participants were reported as smokers, and more than 80% of participants identified themselves as White. A summary of the different subgroup analyses conducted in the whole population of the ABP study (n= 97, as dropouts were not included) is shown in Table 5.1.

Table 5.1: Subgroup analysis conducted in the ABP study (n=97) for sex, age, BMI, habitual total PP and fruits and vegetables consumption, BP at baseline, habitual alcohol consumption and habitual physical activity level. The number of volunteers in each subgroup is indicated in the last column.

Factors investigated on each subgroup	Subgroup characteristics	Control	Aronia	*Total*
Sex	Men	25	27	*52*
	Women	23	22	*45*
Age	Age < 56.2 years	26	24	*50*
	Age ≥ 56.2 years	22	25	*47*
BMI	BMI < 25 kg/m^2	27	24	*51*
	BMI ≥ 25 kg/m^2	21	25	*46*
Habitual total (poly)phenol (PP) consumption	PP < 700 mg/day	8	9	*17*
	PP ≥ 700 mg/day	40	40	*80*
Habitual fruit and vegetable (FV) consumption	FV < 400 g/day	26	20	*46*
	FV ≥ 400 g/day	22	29	*51*
Blood pressure (BP) at baseline	SBP < 120 mmHg	22	21	*43*
	SBP ≥ 120 mmHg	26	28	*54*
	DBP < 80 mmHg	21	21	*42*
	DBP ≥ 80 mmHg	27	28	*55*
Habitual alcohol consumption	< 3.8 units/week	32	28	*60*
	≥ 3.8 units/week	16	21	*37*
Habitual physical activity	High	32	35	*67*
	Low / Moderate	14	11	*25*

5.3 Results

5.3.1 Analysis of the variability in response to aronia berry PP

An assessment of the interindividual variability in response to aronia berry PP was performed within the Aronia group for the main primary outcomes of the study and for parameters that significantly improved upon its consumption following the overall population analysis, as well as for the total urinary and plasma PP concentrations. We defined responders as individuals presenting changes from baseline (CFB) < 0 for all parameters except FMD and HDL cholesterol, for which they are defined as individuals with CFB> 0.

5.3.1.1 Blood pressure

Plots representing the individual response for the primary outcome (changes in 24h SBP_{br} and DBP_{br}, Figures 5.2 A and B) showed a total of 67 and 73% responders for 24h SBP_{br} and $DBP_{br,}$ respectively. The variability in response was large, ranging from decreases in 24h ambulatory SBP_{br} of -15.8 mmHg to increases of 8.8 mmHg and decreases in 24h ambulatory DBP_{br} of -9.8 mmHg to increases of 5.8 mmHg. Coefficients of variation (CoV) were 245 and 187%, respectively (Table 5.2).

For office SBP_{br} and DBP_{br} (Figures 5.2 C and D), a high variability was also observed, including respectively 69 and 53% of responders to the treatment, with CoV of 286% and 519%, respectively.

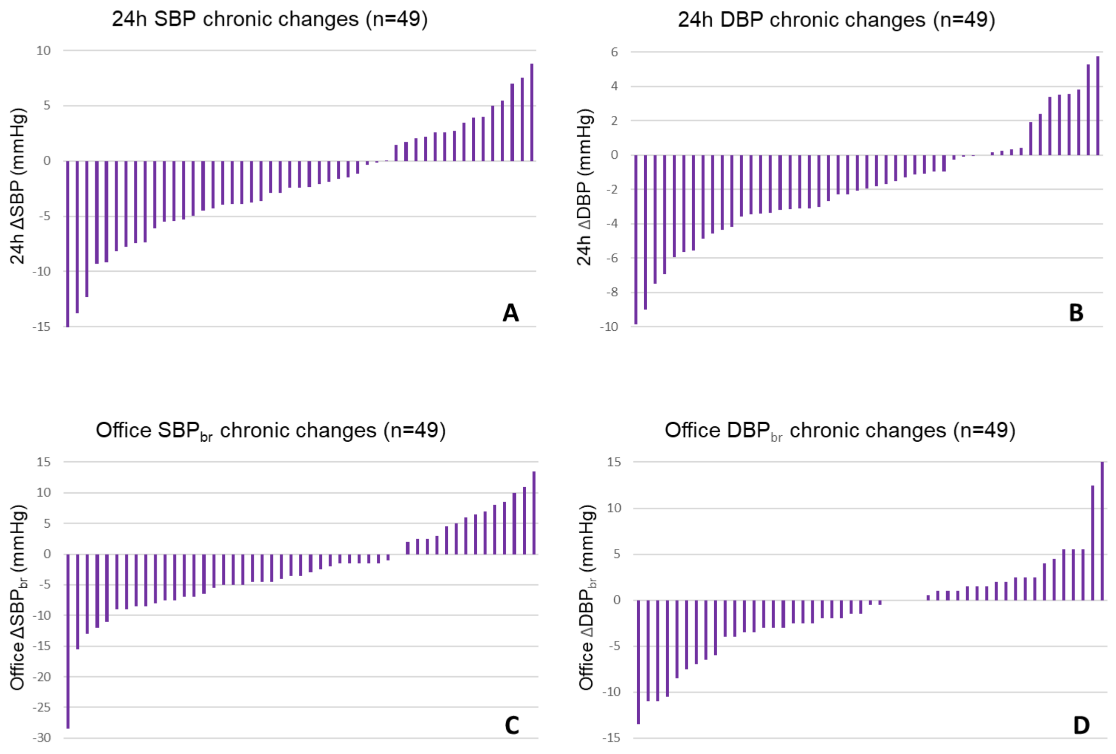


Figure 5.2: Waterfall plots of changes from baseline for chronic changes on ambulatory and office brachial blood pressure. A: 24h ΔSBP; B: 24h ΔDBP; C: office ΔSBP and D: office ΔDBP.

5.3.1.2 Arterial stiffness

The analysis of 24h and awake PWV response measured using Arteriograph24^{TM} following the consumption of the treatment (Figures 5.3 A and B) revealed that more than half of the participants included in the analysis of these parameters were responders (61 and 54% responders for 24h and awake PWV, respectively). The magnitude of responses ranged between -1.3 and 2.2 m/s and between -2.9 and 2.4 m/s for 24h and awake PWV, respectively, with a stronger effect of the intervention observed in the awake PWV, which was significant in the overall population, as indicated in Chapter 3, section 3.4.

The individual PWV response measured with SphygmoCor® was slightly different than the 24h one, with only 40% of participants identified as

responders for this outcome (Figure 5.3 C). The CoV were 1127, 499 and 413% for 24h, awake and office PWV, respectively (Table 5.2).

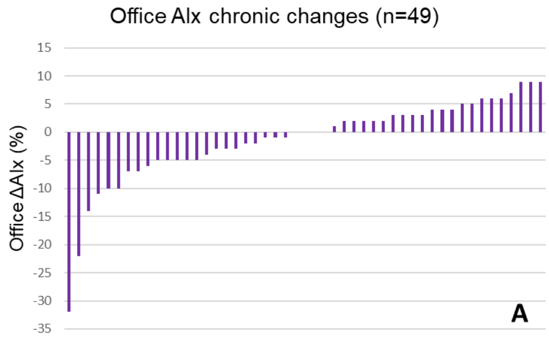
Figure 5.3: Waterfall plots of changes from baseline for chronic changes in ambulatory and office pulse wave velocity. A: 24h ΔPWV; B: awake ΔPWV and C: office ΔPWV.

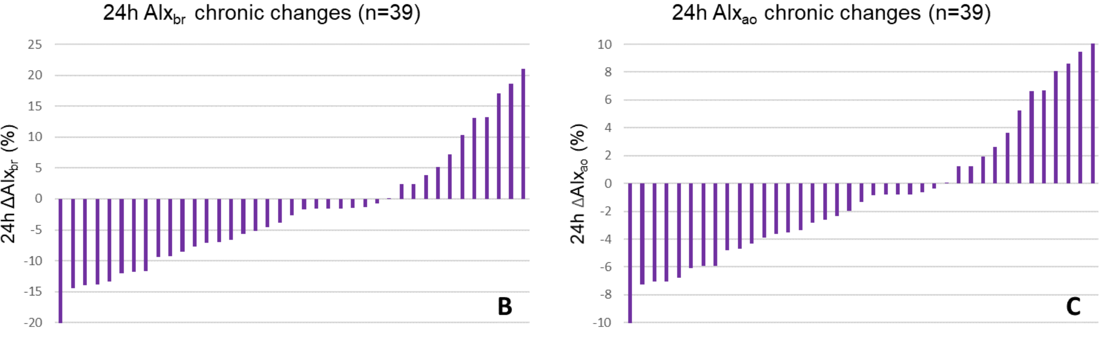
Regarding augmentation index, 2 measures were assessed for ambulatory AIx with the Arteriograph24^{TM}: brachial (AIx_{br}) and central (AIx_{ao}) augmentation index. On the contrary, "office" AIx was measured using the SphygmoCor® device. The individual responses are presented in Figure 5.4. Overall, for the office AIx, the variability in response was high, ranging between -32 and 9% with around half the subjects (47%) being responders in the Aronia group, and a CoV of 559% (Figure 5.4 A and Table 5.2).

The analysis of 24h AIx_{ao} and AIx_{br} exposed some interesting findings regarding the interindividual variability (Figure 5.4 B and C). Indeed, both parameters presented a smooth distribution with only 1 extreme response

behaviour and were both including around the same number of responders (67 and 69% of responders for 24h AIx_{ao} and AIx_{br}, respectively). The range of responses was spread between -10.2 and 10.7% for ambulatory 24h central AIx and between -20.1 and 21.1% for ambulatory 24h brachial AIx, with a CoV of 598 and 454%, respectively (Table 5.2).

Distributions of awake AIx_{ao} and AIx_{br} were very similar in appearance (Figure 5.4 D and E). Both parameters included more responders than non-responders in the Aronia group (64 and 67% for awake AIx_{ao} and AIx_{br}, respectively), with individual responses fluctuating between -12.1 and 29.9% for ambulatory awake central AIx and between -23.9 and 59.0% for ambulatory awake brachial AIx. Coefficients of variations for AIx_{ao} and AIx_{br} were 667 and 522%, respectively (Table 5.2). Both awake AIx_{ao} and AIx_{br} presented significant changes from Control in the overall population (CFC awake AIx_{br}= -6.1%, p= 0.020; CFC awake AIx_{ao}= -2.9%, p= 0.034).





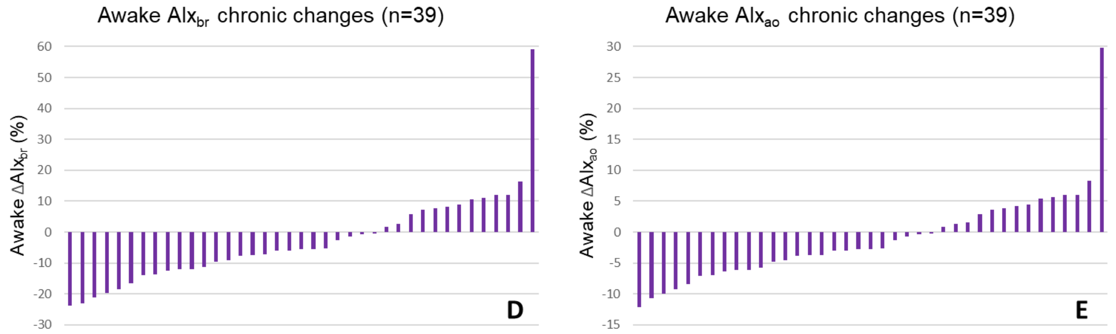


Figure 5.4: Waterfall plots of changes from baseline for chronic changes in AIx 24h brachial and aortic AIx. A: office ΔAIx, B: 24h ambulatory brachial ΔAIx, C: 24h ambulatory aortic ΔAIx, D: awake ambulatory brachial ΔAIx and E: awake ambulatory aortic ΔAIx.

5.3.1.3 FMD

Regarding the interindividual variability in FMD response, acute changes in responses were also investigated as FMD is a parameter which was expected to change acutely. Repartition of responders was different between the 2 types of changes as 64 and 53% of participants were responders for acute and chronic changes in the Aronia group, respectively (Figure 5.5 A and B). Changes from baseline ranged from -2.4 to 2.9% for acute responses, and from -3.5 to 4.1% for chronic responses, with coefficients of variation of 435 and 994%, respectively (Table 5.2).

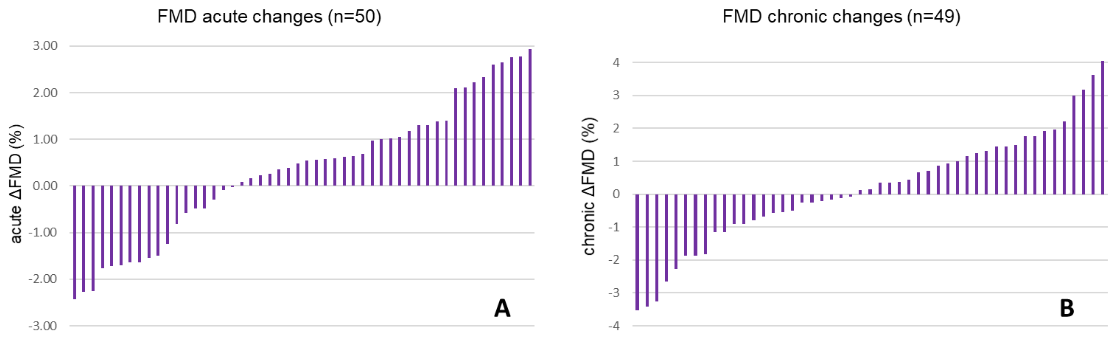


Figure 5.5: Waterfall plots of changes from baseline for acute and chronic changes in FMD. A: acute changes in FMD; B: chronic changes in FMD.

5.3.1.4 Heart rate

Office and ambulatory heart rate (HR) displayed divergent profiles, with changes ranging from -16 to 24.5 bpm for office HR and from -18.9 to 7.3 bpm for ambulatory 24h heart rate (Figures 5.6 A and B). For both parameters, around half (62 and 45%, respectively) the subjects presented an augmentation in HR following the 12-week intervention. Office and 24h ambulatory HR's CoV were high: 1002 and 1518%, respectively (Table 5.2).

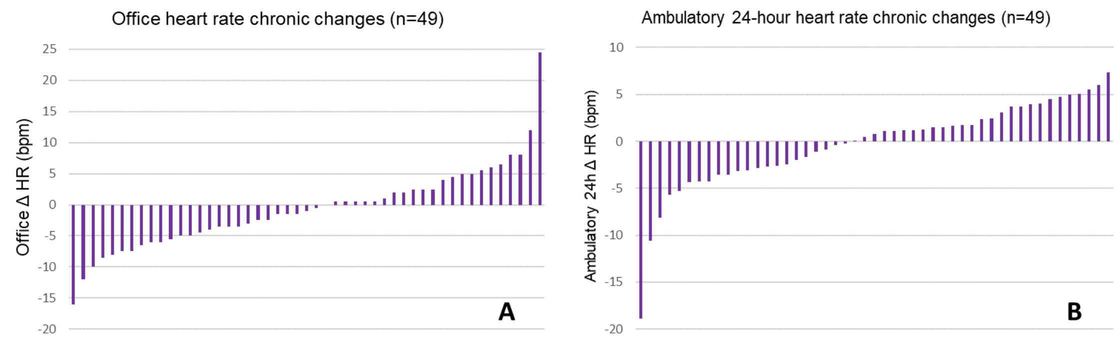


Figure 5.6: Waterfall plots of changes from baseline for chronic changes in office and ambulatory heart rate. A: office heart rate and B: ambulatory 24-hour heart rate.

5.3.1.5 Cortisol levels

In the overall population, the decrease in cortisol levels following the 12-week intervention was substantial although not significant (CFC= -16.8 mmol/L, p= 0.111). In the present analysis of interindividual variability, we observed that almost three quarters (72%) of the subjects from the Aronia group presented a decrease in cortisol level following the 12-week supplementation (Figure 5.7). Changes ranged from -186 to 171 mmol/L with a CoV of 314% (Table 5.2).

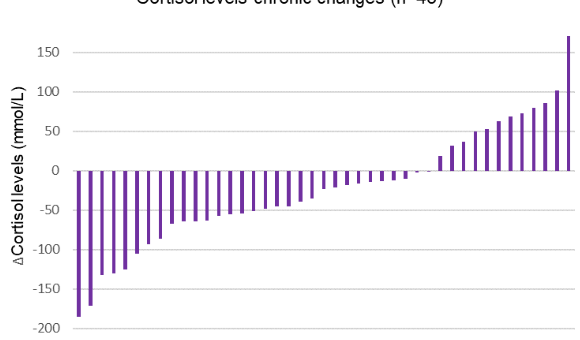
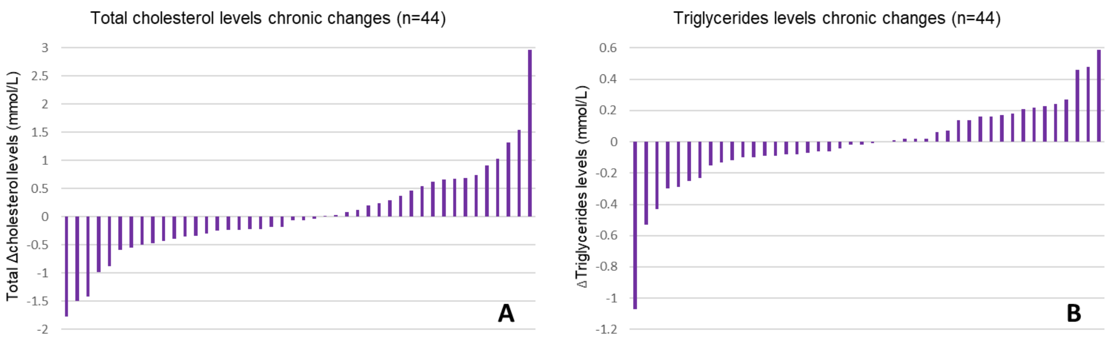


Figure 5.7: Waterfall plots of changes from baseline for chronic changes in cortisol levels.

5.3.1.6 Blood lipid levels

The analysis of the variability in response to treatment for blood lipid levels is presented in Figure 5.8. Total cholesterol, triglycerides, HDL cholesterol and LDL cholesterol had 54, 52, 57, and 43% of responders, respectively. The magnitude of the changes ranged between -1.8 and 3.0 mmol/L for total cholesterol, between -1.1 and 0.6 mmol/L for triglycerides, between -0.4 and 1.1 mmol/L for HDL cholesterol and between -0.9 and 2.0 mmol/L for LDL cholesterol. Coefficients of variations for total cholesterol and triglycerides were the highest among all the parameters analysed (2554 and 2570%, respectively). On the contrary, CoV of HDL and LDL cholesterol were lower: 361 and 389%, respectively (Table 5.2).



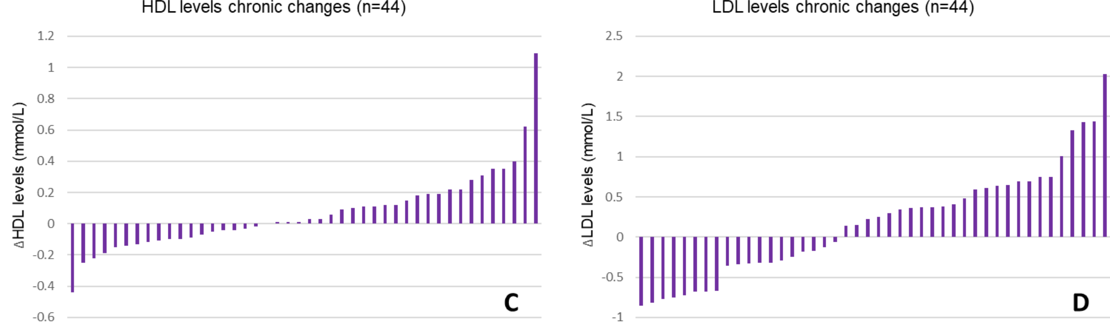


Figure 5.8: Waterfall plots of changes from baseline for chronic changes in blood lipids. A: total cholesterol; B: triglycerides; C: HDL cholesterol and D: LDL cholesterol.

Table 5.2: Parameters analysed in the response analysis and coefficient of variation associated.

Parameter	Unit	n	mean	SD	**CoV (%)**
24h SBP_{br}	mmHg	49	-2.20	5.38	**245**
24h DBP_{br}	mmHg	49	-1.82	3.41	**187**
Office SBP_{br}	mmHg	49	-2.64	7.56	**286**
Office DBP_{br}	mmHg	49	-1.04	5.40	**519**
Office PWV	m/s	48	0.35	1.45	**413**
24h PWV	m/s	38	-0.06	0.73	**1127**
Awake PWV	m/s	39	-0.21	1.06	**499**
Office AIx	%	49	-1.37	7.64	**559**
24h AIx_{ao}	%	39	-0.86	5.16	**598**
24h AIx_{br}	%	39	-2.15	9.77	**454**
Awake AIx_{ao}	%	39	-1.09	7.29	**667**
Awake AIx_{br}	%	39	-2.83	14.78	**522**
FMD	%	49	0.17	1.74	**994**
24h HR	bpm	49	-0.31	4.66	**1518**
Office HR	bpm	49	-0.66	6.65	**1002**
Cortisol	mmol/L	43	-23.70	74.36	**314**
Total cholesterol	mmol/L	44	0.03	0.82	**2554**
Triglycerides	mmol/L	44	-0.01	0.27	**2570**
HDL	mmol/L	44	0.07	0.25	**361**
LDL	mmol/L	44	0.17	0.68	**389**

AIx: augmentation index; ao: aortic; br: brachial; CoV: coefficient of variation (absolute value); DBP: diastolic blood pressure; FMD, flow mediated dilation; HDL: high density lipoprotein; HR: heart rate; LDL: low density lipoprotein; PWV: pulse wave velocity; SBP: systolic blood pressure; SD: standard deviation.

5.3.2 Exploratory subgroup analysis based on biological and lifestyle parameters

5.3.2.1 Sex

The summary of findings resulting from the subgroup analysis based on the sex of participants can be found in Table 5.3 below. No significant differences between men and women were found for the primary outcomes (24h, awake and asleep SBP_{br} and DBP_{br}), FMD, office SBP_{br}, DBP_{br}, SBP_{ao}, DBP_{ao}, PWV, AIx and blood lipids. The only sex-dependent significant differences were found for arterial stiffness:

5.3.2.1.1 **Pulse wave velocity**

When separated in subgroups, a significant reduction in awake PWV (CFC: -0.4 m/s, p= 0.02) and in 24h PWV (CFC: -0.4 m/s, p= 0.025) was found in men, while no significant difference was observed in women.

5.3.2.1.2 **Augmentation index**

Interestingly, a significant difference in AIx was found in women only. Indeed, 24h and awake AIx_{ao} and AIx_{br} were significantly reduced in the women's group (24h AIx_{ao}: CFC: -5.1%, p= 0.003; 24h AIx_{br}: CFC: -10.1%, p= 0.003; Awake AIx_{ao}: CFC: -6.7%, p= 0.000; Awake AIx_{ao}: CFC: -13.2%, p= 0.000) while no significant change was observed in the men's group. No significant difference was found for asleep AIx_{br} and AIx_{ao}.

Table 5.3: Results from the subgroup analysis based on the sex of participants: changes from Control (CFC) and associated p-values calculated using the baseline as covariate only (BACO) analysis.

		Female		Male	
		Control = 25 / Aronia = 27		Control = 23 / Aronia = 22	
ITT population		Chronic - BACO (p-value)	CFC	Chronic - BACO (p-value)	CFC
24h SBP	*mmHg*	0.936	0.117	0.471	-0.874
24h DBP	*mmHg*	0.489	-0.735	0.758	-0.278
Awake SBP	*mmHg*	0.564	0.720	0.374	-1.287
Awake DBP	*mmHg*	0.925	-0.107	0.429	-0.927
Asleep SBP	*mmHg*	0.653	0.889	0.773	-1.108
Asleep DBP	*mmHg*	0.469	-1.054	0.829	-0.379
Office SBP_{br}	*mmHg*	0.103	-3.188	0.588	-1.019
Office DBP_{br}	*mmHg*	0.878	0.205	0.708	0.566
Office SBP_{ao}	*mmHg*	0.497	-1.334	0.491	-1.389
Office DBP_{ao}	*mmHg*	1.000	0.097	0.182	2.227
Office PWV	*m/s*	0.664	-0.131	0.661	0.182
Office AIx	*%*	0.175	-2.789	0.252	0.252
FMD	*%*	0.260	-0.463	0.403	-0.375
Blood flow	*cm/s*	**0.093**	-9.067	0.496	1.987
24h PWV	*m/s*	0.705	0.054	0.025	-0.391
Awake PWV	*%*	0.634	-0.150	0.020	-0.374
Asleep PWV	*%*	0.849	0.095	**0.061**	-0.433
24h AIx_{ao}	*%*	0.003	-5.115	0.520	-0.982
Awake AIx_{ao}	*%*	0.000	-6.665	0.768	0.626
Asleep AIx_{ao}	*%*	0.104	-3.623	0.760	-0.857
24h AIx_{br}	*%*	0.003	-10.10	0.371	-2.678
Awake AIx_{br}	*%*	0.000	-13.161	0.939	0.334
Asleep AIx_{br}	*%*	0.104	-7.166	0.762	-1.679
Cortisol	*mmol/L*	0.376	-21.404	0.894	3.612
Total cholesterol	*mmol/L*	0.801	0.116	0.227	0.229
Triglycerides	*mmol/L*	0.754	-0.020	0.360	0.082
HDL	*mmol/L*	0.451	0.051	0.373	-0.034
LDL	*mmol/L*	0.923	0.016	0.129	0.335

Regarding changes from baseline, a trend for a significant improvement in the Aronia group was observed for the female participants' subgroup, for awake AIx_{ao} and AIx_{br}, cortisol, and HDL plasma levels (CFB awake AIx_{br}= -8.74%, 95% CI= [-13.29; -4.19], CFB awake AIx_{ao}= -4.42%, 95% CI= [-6.73; -2.12], CFB HDL= 0.12 mmol/L, 95% CI= [0.03; 0.22] and CFB cortisol= -33.2 mmol/L, 95% CI= [-60.4; -6.05]) (Table 5.4).

Table 5.4: Significant changes from baseline (CFB) in the different sex subgroups of the Aronia group. SEM: Standard error of mean; 95% CI: 95% confidence interval.

Aronia group	Females			Males		
Parameter	Mean	SEM	95% CI	Mean	SEM	95% CI
CFB Awake AIx_{br}	**-8.74***	**2.25**	**[-13.3; -4.19]**	3.36	3.11	[-2.95; 9.67]
CFB Awake AIx_{ao}	**-4.42***	**1.14**	**[-6.73; -2.12]**	2.28	1.51	[-0.77; 5.34]
CFB HDL cholesterol	**0.12***	**0.05**	**[0.03; 0.22]**	-0.002	0.04	[-0.08; 0.08]
CFB Cortisol levels	**-33.2***	**13.5**	**[-60.4; -6.05]**	-6.02	15.2	[-36.7; 24.7]

5.3.2.2 Age

The average age of the overall population of the ABP study was 56.2 years. This number is close to the median of the age range of the population (55 years) and had thus been chosen as the cut-off age for the subgroup analysis of the population based on the age of participants.

5.3.2.2.1 **Blood pressure**

A summary of the findings can be found in the Table 5.5 below. No significant differences were found for the primary outcomes (24h, awake and asleep SBP_{br} and DBP_{br}), office DBP_{br}, SBP_{ao} and DBP_{ao}. However, a significant reduction in office SBP_{br} was found in the older group (CFC: -4.3 mmHg, p= 0.036), but not in the younger group (CFC: -0.10 mmHg, p= 0.953).

5.3.2.2.2 **Pulse wave velocity and augmentation index**

A significant decrease in awake PWV, awake AIx_{ao} and AIx_{br} was also found for the older group (CFC: -0.5 m/s, p= 0.043; -4.5%, p= 0.008 and -10.1, p= 0.005, respectively), but not for the younger group. No significant difference was found for 24h and asleep PWV. In contrast, peripheral and central AIx, 24h and asleep AIx_{ao} and AIx_{br} were found to be significantly reduced in the younger subgroup (24h AIx_{ao}: CFC: -3.6%, p= 0.027; 24h AIx_{br}: CFC: -7.2%,

p= 0.026; Asleep AIx_{ao}: CFC: -4.9%, p= 0.027; Asleep AIx_{ao}: CFC: -9.6%, p= 0.022).

5.3.2.2.3 Blood flow velocity

A significant drop in blood flow velocity was observed (CFC: -10.4 cm/s, p= 0.031) in the younger group only.

Table 5.5: Results from the subgroup analysis based on the age of participants with a limit of 56.2 years: changes from Control (CFC) and associated p-values calculated using the baseline as covariate only (BACO) analysis.

		Age < 56.2 years		Age ≥ 56.2 years	
		Control = 26 / Aronia = 24		Control = 22 / Aronia = 25	
ITT population		Chronic - BACO (p-value)	CFC	Chronic - BACO (p-value)	CFC
24h SBP	*mmHg*	0.519	0.900	0.260	-1.484
24h DBP	*mmHg*	0.625	-0.524	0.727	-0.340
Awake SBP	*mmHg*	0.207	0.179	0.895	-0.201
Awake DBP	*mmHg*	0.501	-0.865	0.831	0.224
Asleep SBP	*mmHg*	0.287	2.111	0.373	-2.105
Asleep DBP	*mmHg*	0.780	-0.566	0.864	-0.284
Office SBP_{br}	*mmHg*	0.953	-0.101	0.036	-4.319
Office DBP_{br}	*mmHg*	0.321	1.359	0.582	-0.834
Office SBP_{ao}	*mmHg*	0.709	0.690	**0.053**	-3.862
Office DBP_{ao}	*mmHg*	0.122	2.324	0.686	-0.567
Office PWV	*m/s*	0.123	-0.486	0.365	0.456
Office AIx	*%*	0.248	2.351	0.147	-3.245
FMD	*%*	0.648	-0.169	0.158	-0.685
Blood flow	*cm/s*	0.031	-10.443	0.717	-0.010
24h PWV	*m/s*	0.700	-0.071	0.618	-0.124
Awake PWV	*%*	0.809	-0.063	0.043	-0.477
Asleep PWV	*%*	0.370	-0.228	0.462	-0.245
24h AIx_{ao}	*%*	0.027	-3.624	0.137	-2.485
Awake AIx_{ao}	*%*	0.228	-1.384	0.008	-4.513
Asleep AIx_{ao}	*%*	0.027	-4.862	0.930	0.241
24h AIx_{br}	*%*	0.026	-7.186	**0.083**	-5.585
Awake AIx_{br}	*%*	0.228	-2.734	0.005	-10.072
Asleep AIx_{br}	*%*	0.022	-9.595	0.931	0.468
Cortisol	*mmol/L*	0.636	14.116	0.226	-28.036
Total cholesterol	*mmol/L*	0.637	0.084	0.317	0.254
Triglycerides	*mmol/L*	0.403	0.075	0.785	-0.032
HDL	*mmol/L*	0.329	0.095	0.708	-0.024
LDL	*mmol/L*	0.202	0.230	0.665	0.152

Six outcomes revealed some significant changes from baseline in the Aronia group only, all of them in the older subgroup (Table 5.6). This is the case for 24h and awake SBP_{br}, office SBP_{br}, awake AIx_{ao} and AIx_{br}, and office AIx (CFB 24h SBP_{br}= -3.15 mmHg, 95% CI= [-4.94; -1.35], CFB awake SBP_{br}= -2.26 mmHg, 95% CI= [-4.35; -0.16], CFB office SBP_{br}= -3.18 mmHg, 95% CI= [-5.93; -0.43], CFB awake AIx_{br}= -7.73%, 95% CI= [-12.47; -2.99], CFB awake AIx_{ao}= -3.29%, 95% CI= [-5.58; -1.00], and CFB office AIx= -3.22, 95% CI= [-6.19; -0.25]).

Table 5.6: Significant changes from baseline (CFB) in the different age subgroups of the Aronia group. SEM: Standard error of mean; 95% CI: 95% confidence interval.

Aronia group	Age < 56.2 years			Age ≥ 56.2 years		
Parameter	Mean	SEM	95% CI	Mean	SEM	95% CI
CFB 24h SBP_{br}	-0.94	1.00	[-2.94; 1.07]	**-3.15***	**0.89**	**[-4.94; -1.35]**
CFB Awake SBP_{br}	-1.53	1.22	[-3.98; 0.93]	**-2.26***	**1.04**	**[-4.35; -0.16]**
CFB Office SBP_{br}	-1.30	1.23	[-3.78; 1.17]	**-3.18***	**1.36**	**[-5.93; -0.43]**
CFB Awake AIx_{br}	2.27	3.21	[-4.23; 8.77]	**-7.73***	**2.34**	**[-12.5; -2.99]**
CFB Awake AIx_{ao}	1.16	1.62	[-2.13; 4.45]	**-3.29***	**1.13**	**[-5.58; -1.00]**
CFB Office AIx	1.40	1.43	[-1.48; 4.29]	**-3.22***	**1.47**	**[-6.19; -0.25]**

5.3.2.3 Body mass index (BMI)

A subgroup analysis based on the BMI of participants, comparing normoweight (BMI< 25 kg/m^2) and overweight (BMI between 25 and 30 kg/m^2) subjects was carried out and presented in Table 5.7 below.

Twenty-four-hour and awake AIx_{ao} and AIx_{br} were significantly reduced in the normoweight subgroup (24h AIx_{ao}: CFC: -5.0%, p= 0.002; 24h AIx_{br}: CFC: -9.8%, p= 0.002; Awake AIx_{ao}: CFC: -5.9%, p= 0.001; Awake AIx_{br}: CFC: -11.6%, p= 0.001), but not in the overweight subgroup. No significant differences were found for any other parameter of any subgroup.

Table 5.7: Results from the subgroup analysis based on the BMI of participants: changes from Control (CFC) and associated p-values calculated using the baseline as covariate only (BACO) analysis.

		BMI < 25 kg/m^2		BMI ≥ 25 kg/m^2	
		Control = 27 / Aronia = 24		Control = 21 / Aronia = 25	
ITT population		Chronic - BACO (p-value)	CFC	Chronic - BACO (p-value)	CFC
24h SBP	*mmHg*	0.647	-0.640	0.934	-0.113
24h DBP	*mmHg*	0.443	-0.744	0.956	-0.063
Awake SBP	*mmHg*	0.865	-0.685	0.608	-0.791
Awake DBP	*mmHg*	0.637	-0.591	0.569	-0.758
Asleep SBP	*mmHg*	0.866	0.308	0.588	0.167
Asleep DBP	*mmHg*	0.754	-0.485	0.996	-0.008
Office SBP_{br}	*mmHg*	0.108	-2.810	0.400	-1.834
Office DBP_{br}	*mmHg*	0.863	-0.252	0.629	0.679
Office SBP_{ao}	*mmHg*	0.335	-1.905	0.578	-1.147
Office DBP_{ao}	*mmHg*	0.989	-0.024	0.166	2.051
Office PWV	*m/s*	0.865	-0.067	0.633	0.222
Office AIx	*%*	0.770	-0.730	0.964	0.077
FMD	*%*	0.093	-0.604	0.593	-0.266
Blood flow	*cm/s*	0.193	-0.737	0.205	-8.333
24h PWV	*m/s*	0.096	-0.323	0.844	0.05
Awake PWV	*%*	0.262	-0.311	0.701	-0.129
Asleep PWV	*%*	0.271	-0.242	0.742	-0.105
24h AIx_{ao}	*%*	0.002	-4.952	0.484	-1.163
Awake AIx_{ao}	*%*	0.001	-5.856	0.863	0.374
Asleep AIx_{ao}	*%*	0.121	-3.68	0.911	-0.279
24h AIx_{br}	*%*	0.002	-9.847	0.343	-3.111
Awake AIx_{br}	*%*	0.001	-11.634	0.999	-0.007
Asleep AIx_{br}	*%*	0.121	-7.28	0.912	-0.543
Cortisol	*mmol/L*	0.486	-16.728	0.994	0.214
Total cholesterol	*mmol/L*	0.540	0.095	0.575	0.229
Triglycerides	*mmol/L*	0.602	0.03	0.778	0.013
HDL	*mmol/L*	0.984	-0.001	0.469	0.041
LDL	*mmol/L*	0.157	0.215	0.761	0.072

Twenty-four-hour and awake DBP presented a significant change from baseline in the Aronia group, for both subgroups (CFB 24h $DBP_{normoweight}$= -1.86 mmHg, 95% CI= [-3.26; -0.46]; CFB 24h $DBP_{overweight}$= -1.55 mmHg, 95% CI= [-3.06; -0.03]; CFB awake $DBP_{normoweight}$= -1.93 mmHg, 95% CI= [-3.58; -0.29]; CFB awake $DBP_{overweight}$= -1.86 mmHg, 95% CI= [-3.60; -0.12]) (Table 5.8). Office SBP_{br} showed a significant decrease compared to baseline in the

Aronia group among the normoweight subgroup (CFB office SBP_{br}= -3.62 mmHg, 95% CI= [-6.12; -1.11]) while awake SBP_{br} displayed a similar decrease in the overweight subgroup (CFB awake SBP_{br}= -2.48 mmHg, 95% CI= [-4.51; -0.45]).

Interestingly, both 24h and awake AIx_{ao} and AIx_{br} presented some significant CFB in the subgroup of subjects with a BMI< 25kg/m^2 (CFB 24h AIx_{ao}= -2.64%, 95% CI= [-4.86; -0.43], CFB awake AIx_{ao}= -2.86%, 95% CI= [-5.29; -0.44], CFB 24h AIx_{br}= -5.23%, 95% CI= [-9.60; -0.85], CFB awake AIx_{br}= -5.65%, 95% CI= [-10.44; -0.87]).

Table 5.8: Significant changes from baseline (CFB) in the different BMI subgroups of the Aronia group. SEM: Standard error of mean; 95% CI: 95% confidence interval.

Aronia group	BMI < 25 kg/m2			BMI ≥ 25 kg/m2		
Parameter	Mean	SEM	95% CI	Mean	SEM	95% CI
CFB 24h DBP_{br}	**-1.86***	**0.70**	**[-3.26; -0.46]**	**-1.55***	**0.75**	**[-3.06; -0.03]**
CFB Awake SBP_{br}	-2.03	1.21	[-4.45; 0.40]	**-2.48***	**1.01**	**[-4.51; -0.45]**
CFB Awake DBP_{br}	**-1.93***	**0.82**	**[-3.58; -0.29]**	**-1.86***	**0.86**	**[-3.60; -0.12]**
CFB Office SBP_{br}	**-3.62***	**1.25**	**[-6.12; -1.11]**	-1.02	1.42	[-3.89; 1.85]
CFB 24h AIx_{ao}	**-2.64***	**1.09**	**[-4.86; -0.43]**	0.74	1.13	[-1.55; 3.03]
CFB 24h AIx_{br}	**-5.23***	**2.16**	**[-9.60; -0.85]**	0.62	2.23	[-3.89; 5.13]
CFB Awake AIx_{ao}	**-2.86***	**1.20**	**[-5.29; -0.44]**	0.65	1.46	[-2.31; 3.62]
CFB Awake AIx_{br}	**-5.65***	**2.36**	**[-10.4; -0.87]**	0.21	3.03	[-5.94; 6.36]

5.3.2.4 Habitual PP intake

The effect of habitual PP consumption measured using 7-day diaries (Chapter 2, section 2.7) was investigated as a potential factor that could affect the variability in response. Total PP daily intake ranged from 196 mg to 4740 mg, with an average of 1440 mg of total PP per day. A preliminary analysis by tertiles of total PP intake, based on the data sourced from the 7DD, led to the following distribution:

Table 5.9: Distribution of the participants for each tertile of the total daily PP intake.

		Control (n)	Aronia (n)
Tertile 1 (T1)	(Total PP < 1091 mg/day)	14	18
Tertile 2 (T2)	(1091 ≤ Total PP ≤ 1642 mg/day)	15	17
Tertile 3 (T3)	(Total PP > 1642 mg/day)	19	14

Table 5.10 below displays the p-values and changes from Control (CFC) for each parameter of the primary outcome. No significant difference was observed among the different parameters and tertiles.

Table 5.10: Subgroup analysis of the main outcomes based on the distribution by tertile of the total PP intake.

		T1 (PP< 1091 mg/day)		T2 (1091 ≤ PP ≤ 1642 mg/day)		T3 (PP> 1642 mg/day)	
		BACO (p-value)	CFC	BACO (p-value)	CFC	BACO (p-value)	CFC
24h SBP_{br}	*mmHg*	0.550	-1.038	0.774	0.523	0.945	-0.115
24h DBP_{br}	*mmHg*	0.489	-0.952	0.783	-0.362	0.571	-0.622
Awake SBP_{br}	*mmHg*	0.266	-1.759	0.593	1.321	0.957	-0.109
Awake DBP_{br}	*mmHg*	0.366	-1.239	0.791	-0.431	0.648	-0.616
Asleep SBP_{br}	*mmHg*	0.863	0.529	0.548	0.066	0.864	0.046
Asleep DBP_{br}	*mmHg*	0.652	-0.936	0.708	0.680	0.566	-0.998

As the lowest tertile investigated above was already very high (< 1091 mg total PP/day) a subgroup analysis with a lower cut-off of 700 mg/day of PP was chosen to achieve the largest possible difference in PP intake but at the same time include at least 20% of the study population in the subgroup with lower PP intake. Despite this, the groups are very uneven, with n= 17 versus n= 80 in lower versus higher PP intake, respectively (Table 5.11).

5.3.2.4.1 Blood pressure

A tendency for a stronger effect on ambulatory BP was seen in the lower PP intake group (Table 5.11), with 24h ambulatory and asleep SBP significantly reduced in the subgroup with an intake of less than 700 mg of total PP per day

(24h SBP_{br}: CFC: -5.2 mmHg, p= 0.023; Asleep SBP_{br}: CFC: -6.4 mmHg, p= 0.02). The higher PP group had no significant effect.

5.3.2.4.2 Augmentation index

Moreover, awake AIx_{br} also significantly decreased in the group consuming lower PP only (CFC: -9.7%, p= 0.049). However, 24h AIx_{ao} and 24h AIx_{br} significantly decreased in the subgroup with the higher PP intake after the 12-week intervention (CFC: -3.2%, p= 0.017 and -6.7%, p= 0.01, respectively). No significant change was observed for the other outcomes. Those findings need to be taken with caution as the subgroup of participants having less than 700 mg/day is only composed of 17 volunteers.

Table 5.11: Results from the subgroup analysis based on the baseline (poly)phenol (PP) intake of volunteers, with a cut-off limit of 700 mg/day, calculated based only on the data collected 7DD: changes from Control (CFC) and associated p-values calculated using the baseline as covariate only (BACO) analysis.

		PP < 700 mg/day		PP ≥ 700 mg/day	
		Control = 8 / Aronia = 9		Control = 40 / Aronia = 40	
ITT population		Chronic - BACO (p-value)	CFC	Chronic - BACO (p-value)	CFC
24h SBP	*mmHg*	0.023	-5.217	0.431	0.822
24h DBP	*mmHg*	0.291	-2.246	0.970	0.028
Awake SBP	*mmHg*	0.266	-3.039	0.285	0.505
Awake DBP	*mmHg*	0.615	-1.237	0.794	-0.235
Asleep SBP	*mmHg*	0.020	-6.400	0.469	-1.711
Asleep DBP	*mmHg*	0.120	-3.538	0.773	0.357
Office SBP_{br}	*mmHg*	0.921	0.315	0.069	-2.726
Office DBP_{br}	*mmHg*	0.987	-0.034	0.558	0.674
Office SBP_{ao}	*mmHg*	0.886	0.456	0.247	-1.783
Office DBP_{ao}	*mmHg*	0.926	-0.187	0.281	1.414
Office PWV	*m/s*	0.287	-0.587	0.519	0.155
Office AIx	*%*	0.421	3.294	0.896	-1.222
FMD	*%*	0.476	0.385	0.065	-0.6
Blood flow	*cm/s*	0.606	-8.014	0.152	-3.468
24h PWV	*m/s*	0.661	-0.132	0.410	-0.146
Awake PWV	*%*	0.383	-0.412	0.196	-0.209
Asleep PWV	*%*	0.542	0.198	0.189	-0.28
24h AIx_{ao}	*%*	0.267	-2.448	0.017	-3.214
Awake AIx_{ao}	*%*	**0.060**	-4.712	0.135	-2.488
Asleep AIx_{ao}	*%*	0.697	1.304	0.128	-3.195

24h AIx_{br}	%	0.241	-5.241	0.010	-6.74
Awake AIx_{br}	%	0.049	-9.774	0.111	-5.40
Asleep AIx_{br}	%	0.696	2.582	0.128	-6.308
Cortisol	*mmol/L*	0.489	-25.674	0.475	-14.74
Total cholesterol	*mmol/L*	0.890	0.100	0.251	0.138
Triglycerides	*mmol/L*	0.222	0.316	0.225	-0.029
HDL	*mmol/L*	0.555	0.084	0.843	-0.009
LDL	*mmol/L*	0.801	0.118	0.215	0.169

When compared with baseline, the lower PP intake group presented significant decreases in 24h SBP_{br} and asleep SBP_{br} and DBP_{br} (CFB 24h SBP_{br}= -5.18 mmHg, 95% CI= [-8.19; -2.17], CFB asleep SBP_{br}= -5.62 mmHg, 95% CI= [-9.22; -2.02], CFB asleep DBP_{br}= -3.15 mmHg, 95% CI= [-6.29; -0.01]) following the intervention in the Aronia group (Table 5.12). On the contrary, office SBP_{br} significantly decreased in the subgroup with the higher daily intake of PP (CFB office SBP_{br}= -2.26 mmHg, 95% CI= [-4.34; -0.19]).

Table 5.12: Significant changes from baseline (CFB) in the different baseline PP intake subgroups of the Aronia group. SEM: Standard error of mean; 95% CI: 95% confidence interval.

Aronia group	PP < 700 mg/day			PP ≥ 700 mg/day		
Parameter	Mean	SEM	95% CI	Mean	SEM	95% CI
CFB 24h SBP_{br}	**-5.18***	**1.40**	**[-8.19; -2.17]**	-1.33	0.73	[-2.79; 0.13]
CFB Asleep SBP_{br}	**-5.62***	**1.68**	**[-9.22; -2.02]**	-0.17	1.23	[-2.62; 2.29]
CFB Asleep DBP_{br}	**-3.15***	**1.47**	**[-6.29; -0.01]**	-0.42	0.88	[-2.17; 1.33]
CFB Office SBP_{br}	-2.50	2.13	[-7.06; 2.06]	**-2.26***	**1.04**	**[-4.34; -0.19]**

5.3.2.5 Habitual fruits and vegetable intake

UK dietary guidelines recommends the intake of 5 portions of fruits and vegetables (FV) per day, representing a total daily quantity of 400g of FV (Public Health England 2016; World Health Organization 1990), which is the cut-off limit chosen for the subgroup analysis based on the baseline FV intake of the ABP study participants, calculated from the data collected from their baseline 7-day food diaries.

5.3.2.5.1 Blood pressure

As presented in Table 5.13 below, awake SBP_{br} was significantly reduced in the subgroup with lower FV intake (CFC: -2.1 mmHg, p= 0.039) following the 12-week intervention. No other significant change was observed for the other parameters.

5.3.2.5.2 Augmentation index

Twenty-four-hour and asleep AIx_{ao} and AIx_{br} were found significantly decreased in the subgroup of volunteers having a higher intake of FV (24h AIx_{ao}: CFC: -5.3%, p= 0.001; Asleep AIx_{ao}: CFC: -5.5%, p= 0.042; 24h AIx_{br}: CFC: -10.5%, p= 0.001; Asleep AIx_{br}: CFC: -10.8%, p= 0.042). No significant changes were observed for awake AIx_{ao} and AIx_{br}, cortisol and blood lipids levels.

Table 5.13: Results from the subgroup analysis based on the baseline intake of fruits and vegetables, with a cut-off limit of 400 g of fruits and vegetables (FV) per day: changes from Control (CFC) and associated p-values calculated using the baseline as covariate only (BACO) analysis.

		FV < 400 g/day		FV ≥ 400 g/day	
		Control = 26 / Aronia = 20		Control = 22 / Aronia = 29	
ITT population		Chronic - BACO (p-value)	CFC	Chronic - BACO (p-value)	CFC
24h SBP	*mmHg*	0.058	-2.586	0.356	1.270
24h DBP	*mmHg*	0.125	-1.728	0.668	0.410
Awake SBP	*mmHg*	0.039	-2.099	0.482	1.091
Awake DBP	*mmHg*	0.148	-1.772	0.791	0.292
Asleep SBP	*mmHg*	0.804	-1.108	0.257	1.940
Asleep DBP	*mmHg*	0.810	-0.391	0.884	0.219
Office SBP_{br}	*mmHg*	0.186	-2.398	0.267	-2.330
Office DBP_{br}	*mmHg*	0.921	0.156	0.733	0.471
Office SBP_{ao}	*mmHg*	0.321	-1.892	0.342	-2.016
Office DBP_{ao}	*mmHg*	0.390	1.467	0.324	0.747
Office PWV	*m/s*	0.605	0.203	0.888	-0.061
Office AIx	*%*	0.448	1.461	0.094	-3.506
FMD	*%*	0.993	-0.004	0.048	-0.81
Blood flow	*cm/s*	0.200	-10.087	0.342	-0.617
24h PWV	*m/s*	0.178	-0.255	0.752	-0.068
Awake PWV	*%*	0.104	-0.35	0.426	-0.209
Asleep PWV	*%*	0.125	-0.458	0.494	0.057

24h AIx_{ao}	%	0.655	-0.711	0.001	-5.338
Awake AIx_{ao}	%	0.147	-2.733	0.083	-3.715
Asleep AIx_{ao}	%	0.708	0.925	0.042	-5.455
24h AIx_{br}	%	0.446	-2.362	0.001	-10.545
Awake AIx_{br}	%	0.092	-6.627	0.083	-7.335
Asleep AIx_{br}	%	0.707	1.833	0.042	-10.777
Cortisol	*mmol/L*	0.845	5.074	0.350	-24.435
Total cholesterol	*mmol/L*	0.336	0.377	0.840	0.029
Triglycerides	*mmol/L*	0.348	0.077	0.409	-0.067
HDL	*mmol/L*	0.147	0.119	0.376	-0.049
LDL	*mmol/L*	0.063	0.497	0.902	-0.02

Both subgroups presented some significant decreases from baseline in 24h and awake DBP (CFB 24h $DBP_{FV<400g/d}$= -2.26 mmHg, 95%CI= [-3.93; -0.60], CFB 24h $DBP_{FV\geq400g/d}$= -1.50 mmHg [-2.75; -0.24], CFB awake $DBP_{FV<400g/d}$= -2.12 mmHg [-3.92; -0.31], CFB awake $DBP_{FV\geq400g/d}$= -1.85 mmHg [-3.30; -0.40]) (Table 5.14). However, only the lower FV intake subgroup showed significant drops when compared to baseline in 24h and awake SBP_{br} (CFB 24h SBP_{br}= -3.41 mmHg [-5.40; -1.42], CFB awake SBP_{br}= -3.11 mmHg [-5.67; -0.54]), as well as office SBP_{ao} (CFB= -3.09 mmHg [-5.89; -0.29]).

Table 5.14: Significant changes from baseline (CFB) in the different baseline fruits and vegetables intake subgroups of the Aronia group. SEM: Standard error of mean; 95% CI: 95% confidence interval.

Aronia group	F&V < 400 g/day			F&V ≥ 400 g/day		
Parameter	Mean	SEM	95% CI	Mean	SEM	95% CI
CFB 24h SBP_{br}	**-3.41***	**0.99**	**[-5.40; -1.42]**	-1.33	0.89	[-3.13; 0.46]
CFB 24h DBP_{br}	**-2.26***	**0.83**	**[-3.93; -0.60]**	**-1.50***	**0.62**	**[-2.75; -0.24]**
CFB Awake SBP_{br}	**-3.11***	**1.27**	**[-5.67; -0.54]**	-1.38	1.01	[-3.41; 0.66]
CFB Awake DBP_{br}	**-2.12***	**0.90**	**[-3.92; -0.31]**	**-1.85***	**0.72**	**[-3.30; -0.40]**
CFB Office SBP_{ao}	**-3.09***	**1.39**	**[-5.89; -0.29]**	-0.91	1.36	[-3.64; 1.82]

5.3.2.6 *Systolic blood pressure at baseline*

Normal SBP is defined as below 120 mmHg. As a result, this cut-off limit was selected for the present subgroup analysis, which summary can be found in Table 5.15 below.

5.3.2.6.1 **Blood pressure**

No significant differences were found for the primary outcomes (24h, awake and asleep SBP_{br} and DBP_{br}) or office peripheral and central BP, office PWV and AIx, and cortisol levels.

5.3.2.6.2 **Pulse wave velocity and augmentation index**

Significant decreases in 24h, awake and asleep AIx_{br} and AIx_{ao} were observed in the subgroup with lower baseline office SBP (24h AIx_{ao}: CFC: -5.3%, p= 0.001; 24h AIx_{br}: CFC: -10.4%, p= 0.001; Awake AIx_{ao}: CFC: -5.6%, p= 0.001; Awake AIx_{br}: CFC: -11.0%, p= 0.002; Asleep AIx_{ao}: CFC: -5.5%, p= 0.04; Asleep AIx_{br}: CFC: -11.0%, p= 0.4). On the contrary, awake PWV was significantly reduced in the subgroup with higher baseline SBP (CFC: -0.5 m/s, p= 0.008).

Table 5.15: Results from the subgroup analysis based on the baseline SBP of volunteers, with a cut-off limit of 120 mmHg: changes from Control (CFC) and associated p-values calculated using the baseline as covariate only (BACO) analysis.

		Baseline SBP < 120 mmHg		Baseline SBP ≥ 120 mmHg	
		Control = 22 / Aronia = 21		Control = 26 / Aronia = 28	
ITT population		Chronic - BACO (p-value)	CFC	Chronic - BACO (p-value)	CFC
24h SBP	*mmHg*	0.972	0.058	0.663	-0.466
24h DBP	*mmHg*	0.450	-0.847	0.821	-0.214
Awake SBP	*mmHg*	0.496	1.354	0.267	-1.432
Awake DBP	*mmHg*	0.885	0.187	0.327	-1.059
Asleep SBP	*mmHg*	0.199	-2.051	0.346	1.857
Asleep DBP	*mmHg*	0.082	-2.819	0.386	1.241
Office SBP_{br}	*mmHg*	0.168	-2.782	0.454	-1.349
Office DBP_{br}	*mmHg*	0.531	0.876	0.961	-0.072

Office SBP_{ao}	*mmHg*	0.324	-2.255	0.740	-0.635
Office DBP_{ao}	*mmHg*	0.392	0.615	0.374	1.395
Office PWV	*m/s*	0.699	0.141	0.797	-0.186
Office AIx	*%*	0.556	-1.235	0.416	0.244
FMD	*%*	0.008	-0.986	0.949	0.029
Blood flow	*cm/s*	0.419	-4.911	0.138	-3.135
24h PWV	*m/s*	0.927	-0.02	0.241	-0.262
Awake PWV	*%*	0.870	0.042	0.008	-0.482
Asleep PWV	*%*	0.253	-0.314	0.682	-0.106
24h AIx_{ao}	*%*	0.001	-5.319	0.448	-1.221
Awake AIx_{ao}	*%*	0.001	-5.633	0.670	-0.904
Asleep AIx_{ao}	*%*	0.040	-5.531	0.910	0.274
24h AIx_{br}	*%*	0.001	-10.431	0.307	-3.205
Awake AIx_{br}	*%*	0.002	-10.992	0.526	-2.757
Asleep AIx_{br}	*%*	0.040	-10.927	0.909	0.546
Cortisol	*mmol/L*	0.742	-11.034	0.626	-9.742
Total cholesterol	*mmol/L*	0.494	-0.14	0.032	0.373
Triglycerides	*mmol/L*	0.208	0.006	0.685	0.045
HDL	*mmol/L*	0.389	0.061	0.197	-0.035
LDL	*mmol/L*	0.889	-0.028	0.069	0.327

Additionally, several parameters presented some significant changes from baseline in the Aronia group (Table 5.16). For instance, awake SBP and DBP, as well as office SBP_{ao} were significantly reduced from baseline in the subgroup with higher baseline SBP only (CFB awake SBP_{br}= -2.95 mmHg, 95% CI= [-4.71; -1.18]; CFB awake DBP_{br}= -1.94 mmHg, 95% CI= [-3.43; -0.46]; CFB office SBP_{ao}= -3.11 mmHg, 95% CI= [-5.73; -0.49]). On the contrary, asleep DBP_{br} presented a significant decrease in CFB for the lower baseline SBP subgroup of the Aronia group (CFB asleep DBP_{br}= -2.43 mmHg, 95% CI= [-4.75; -0.12]). Both subgroups showed significant CFB in 24h DBP_{br} (CFB 24h $DBP_{SBP<120mmHg}$= -1.94 mmHg, 95% CI= [-3.54; -0.34], CFB 24h $DBP_{SBP\geq120mmHg}$= -1.58 mmHg, 95% CI= [-2.89; -0.27]).

Table 5.16: Significant changes from baseline (CFB) in the different baseline SBP subgroups of the Aronia group. SEM: Standard error of mean; 95% CI: 95% confidence interval.

Aronia group	Baseline SBP < 120 mmHg			Baseline SBP ≥ 120 mmHg		
Parameter	Mean	SEM	95% CI	Mean	SEM	95% CI
CFB 24h DBP_{br}	**-1.94***	**0.79**	**[-3.54; -0.34]**	**-1.58***	**0.65**	**[-2.89; -0.27]**
CFB Awake SBP_{br}	-0.72	1.41	[-3.57; 2.13]	**-2.95***	**0.88**	**[-4.71; -1.18]**
CFB Awake DBP_{br}	-1.64	0.92	[-3.50; 0.22]	**-1.94***	**0.74**	**[-3.43; -0.46]**
CFB Asleep DBP_{br}	**-2.43***	**1.14**	**[-4.75; -0.12]**	0.00	0.98	[-1.98; 1.98]
CFB Office SBP_{ao}	0.08	1.47	[-2.90; 3.05]	**-3.11***	**1.31**	**[-5.73; -0.49]**

5.3.2.7 Diastolic blood pressure at baseline

Normal DBP is defined as under 80 mmHg. The summary of findings resulting of the subgroup analysis based on the baseline office DBP of participants can be found in Table 5.17 below.

5.3.2.7.1 **Blood pressure**

No significant differences were found in the subgroups based on baseline DBP for the primary outcomes (24h, awake and asleep SBP_{br} and DBP_{br}) or office peripheral and central BP.

5.3.2.7.2 **Augmentation index**

The group with higher baseline DBP had significant decreases in 24h AIx (24h AIx_{ao}: CFC: -4.0%, p= 0.012; 24h AIx_{br}: CFC: -7.8%, p= 0.013), while awake AIx was decreased in the group with lower baseline DBP (Awake AIx_{ao}: CFC: -4.8%, p= 0.008; Awake AIx_{br}: CFC: -11.0%, p= 0.004). No significant differences in other parameters were found.

Table 5.17: Results from the subgroup analysis based on the baseline office DBP of volunteers, with a cut-off limit of 80 mmHg: changes from Control (CFC) and associated p-values calculated using the baseline as covariate only (BACO) analysis.

		Baseline DBP < 80 mmHg		Baseline DBP ≥ 80 mmHg	
		Control = 21 / Aronia = 21		Control = 27 / Aronia = 28	
ITT population		Chronic - BACO (p-value)	CFC	Chronic - BACO (p-value)	CFC
24h SBP	*mmHg*	0.377	-1.386	0.725	0.431
24h DBP	*mmHg*	0.295	-1.275	0.808	-0.222
Awake SBP	*mmHg*	**0.054**	-1.639	0.647	0.660
Awake DBP	*mmHg*	0.242	-1.549	0.700	-0.401
Asleep SBP	*mmHg*	0.404	-2.225	0.268	2.132
Asleep DBP	*mmHg*	0.203	-2.266	0.468	1.038
Office SBP_{br}	*mmHg*	0.106	-3.159	0.387	-1.495
Office DBP_{br}	*mmHg*	0.705	0.635	0.979	0.034
Office SBP_{ao}	*mmHg*	0.310	-2.256	0.655	-0.800
Office DBP_{ao}	*mmHg*	0.822	0.576	0.435	1.051
Office PWV	*m/s*	0.408	0.362	0.505	-0.352
Office AIx	*%*	0.804	-0.449	0.786	-0.579
FMD	*%*	0.346	-0.441	0.346	-0.379
Blood flow	*cm/s*	0.166	-8.877	0.054	-1.401
24h PWV	*m/s*	0.219	-0.292	0.481	-0.045
Awake PWV	*%*	0.161	-0.396	0.136	-0.181
Asleep PWV	*%*	0.346	-0.31	0.628	-0.109
24h AIx_{ao}	*%*	0.250	-1.968	0.012	-4.022
Awake AIx_{ao}	*%*	0.008	-4.849	0.474	-1.513
Asleep AIx_{ao}	*%*	0.835	0.514	0.177	-4.064
24h AIx_{br}	*%*	0.141	-4.885	0.013	-7.823
Awake AIx_{br}	*%*	0.004	-11.03	0.502	-2.804
Asleep AIx_{br}	*%*	0.835	1.018	0.117	-8.024
Cortisol	*mmol/L*	0.184	-39.38	0.714	8.018
Total cholesterol	*mmol/L*	0.106	0.295	0.950	0.059
Triglycerides	*mmol/L*	**0.086**	0.086	0.403	-0.042
HDL	*mmol/L*	0.393	0.069	0.282	-0.013
LDL	*mmol/L*	0.209	0.23	0.908	0.12

Five parameters showed some significant changes from baseline in the Aronia group (Table 5.18). This is the case for awake SBP_{br}, DBP_{br}, AIx_{ao}, AIx_{br} and cortisol levels, all for the subgroup presenting a baseline office DBP< 80 mmHg (CFB awake SBP_{br}= -2.83 mmHg, 95% CI= [-5.41; -0.25]; CFB awake DBP_{br}= -2.24 mmHg, 95% CI= [-4.02; -0.45]; CFB awake AIx_{ao}= -3.13%, 95% CI= [-5.70; -0.57]; CFB awake AIx_{br}= -7.594%, 95% CI= [-12.9; -2.26]; CFB

cortisol= -40.5 mmol/L, 95% CI= [-66.4; -14.6]). Moreover, a significant change from baseline for awake DBP_{br} was also seen in the subgroup with the higher baseline office DBP (CFB awake DBP_{br}= -1.84 mmHg, 95% CI= [-3.31; -0.38]).

Table 5.18: Significant changes from baseline (CFB) in the different baseline DBP subgroups of the Aronia group. SEM: Standard error of mean; 95% CI: 95% confidence interval.

Aronia group	Baseline DBP < 80 mmHg			Baseline DBP ≥ 80 mmHg		
Parameter	Mean	SEM	95% CI	Mean	SEM	95% CI
CFB Awake SBP_{br}	**-2.83***	**1.28**	**[-5.41; -0.25]**	-1.46	1.00	[-3.47; 0.55]
CFB Awake DBP_{br}	**-2.24***	**0.88**	**[-4.02; -0.45]**	**-1.84***	**0.73**	**[-3.31; -0.38]**
CFB Awake AIx_{ao}	**-3.13***	**1.26**	**[-5.70; -0.57]**	0.43	1.44	[-2.49; 3.34]
CFB Awake AIx_{br}	**-7.60***	**2.62**	**[-12.9; -2.26]**	0.81	2.86	[-4.96; 6.57]
CFB Cortisol levels	**-40.5***	**12.80**	**[-66.5; -14.6]**	-2.36	14.01	[-30.5; 25.8]

5.3.2.8 Habitual alcohol consumption

The average consumption of alcohol in the overall ABP study population was 3.8 units per week, which represents an intake of 1.6 pint of lager at 4% alc/vol, or 280 mL of wine at 13% alc/vol. This is the cut-off limit chosen for the subgroup analysis. Main findings are presented in Table 5.19 below.

5.3.2.8.1 Blood pressure

The subgroup consuming a higher quantity of alcohol showed significant decreases in 24h SBP_{br} (CFC: -3.8 mmHg, p= 0.012), office SBP_{br} (CFC: -4.3 mmHg, p= 0.019), office SBP_{ao} (CFC: -4.0 mmHg, p= 0.032), awake SBP_{br} (CFC: -4.6 mmHg, p= 0.002) and awake DBP_{br} (CFC: -2.3 mmHg, p= 0.03).

5.3.2.8.2 Pulse wave velocity

This is also the case for 24h, awake and asleep and office PWV, which were significantly decreased in the subgroup consuming more alcohol (24h PWV: CFC: -0.5 m/s, p= 0.03; Awake PWV: CFC: -0.5 m/s, p= 0.017; Asleep PWV: CFC: -0.8 m/s, p= 0.032; Office PWV: CFC: -1.0 m/s, p= 0.025).

5.3.2.8.3 Augmentation index

In contrast, significant changes in AIx were found for volunteers consuming a lower amount of alcohol. In fact, this pattern is observed for 24h central and brachial AIx, as well as asleep central and brachial AIx (24h AIx_{ao}: CFC: -4.1%, p= 0.005; 24h AIx_{br}: CFC: -8.1%, p= 0.005; Asleep AIx_{ao}: CFC: -5.0%, p= 0.016; Asleep AIx_{br}: CFC: -9.9%, p= 0.016).

Table 5.19: Results from the subgroup analysis based on weekly alcohol consumption of participants: changes from Control (CFC) and associated p-values calculated using the baseline as covariate only (BACO) analysis.

		Alcohol < 3.8 units/week		Alcohol ≥ 3.8 units/week	
		Control = 32 / Aronia = 28		Control = 16 / Aronia = 21	
ITT population		Chronic - BACO (p-value)	CFC	Chronic - BACO (p-value)	CFC
24h SBP	*mmHg*	0.121	1.918	0.012	-3.767
24h DBP	*mmHg*	0.948	0.062	0.160	-1.589
Awake SBP	*mmHg*	0.882	2.757	0.002	-4.621
Awake DBP	*mmHg*	0.677	0.493	0.030	-2.289
Asleep SBP	*mmHg*	0.951	1.417	0.714	-2.771
Asleep DBP	*mmHg*	0.556	-0.758	0.758	-0.657
Office SBP_{br}	*mmHg*	0.918	-0.188	0.019	-4.258
Office DBP_{br}	*mmHg*	0.360	1.250	0.316	-1.460
Office SBP_{ao}	*mmHg*	0.764	0.571	0.032	-3.986
Office DBP_{ao}	*mmHg*	0.106	2.562	0.306	-1.548
Office PWV	*m/s*	**0.052**	0.701	0.025	-1.041
Office AIx	*%*	0.779	0.617	0.231	-1.779
FMD	*%*	0.253	-0.422	0.521	-0.358
Blood flow	*cm/s*	**0.075**	-0.084	0.391	-0.021
24h PWV	*m/s*	0.862	0.034	0.03	-0.5
Awake PWV	*%*	0.543	-0.171	0.017	-0.495
Asleep PWV	*%*	0.563	0.12	0.032	-0.769
24h AIx_{ao}	*%*	0.005	-4.13	0.558	-1.194
Awake AIx_{ao}	*%*	**0.093**	-3.308	0.355	-1.636
Asleep AIx_{ao}	*%*	0.016	-5.019	0.378	3.203
24h AIx_{br}	*%*	0.005	-8.079	0.404	-3.279
Awake AIx_{br}	*%*	**0.100**	-6.408	0.204	-5.007
Asleep AIx_{br}	*%*	0.016	-9.916	0.377	6.336
Cortisol	*mmol/L*	0.809	5.472	0.426	-35.286
Total cholesterol	*mmol/L*	0.833	0.049	0.258	0.229
Triglycerides	*mmol/L*	0.667	-0.037	0.154	0.11
HDL	*mmol/L*	0.726	0.037	0.832	0.007
LDL	*mmol/L*	0.466	0.128	0.367	0.172

Regarding changes from baseline after aronia consumption, 24h and awake SBP_{br} and DBP_{br}, as well as office SBP_{br} and office SBP_{ao} significantly decreased in the group consuming a higher quantity of alcohol (CFB 24h SBP_{br}= -4.39 mmHg, 95% CI= [-6.27; -2.51]; CFB 24h DBP_{br}= -2.24 mmHg [-3.71; -0.76]; CFB awake SBP_{br}= -4.41 mmHg, 95% CI= [-6.23; -2.59]; CFB awake DBP_{br}= -2.26 mmHg, 95% CI= [-3.61; -0.91]; CFB office SBP_{br}= -4.41 mmHg, 95% CI= [-6.72; -2.09]; CFB office SBP_{ao}= -3.16 mmHg, 95% CI= [-5.54; -0.77]). A significant decrease in 24h DBP_{br} was also observed in the subgroup consuming less than 3.8 units of alcohol per week (CFB 24h DBP= -1.42 mmHg, 95% CI= [-2.79; -0.05]) (Table 5.20).

Table 5.20: Significant changes from baseline (CFB) in the different alcohol consumption subgroups of the Aronia group. SEM: Standard error of mean; 95% CI: 95% confidence interval.

Aronia group	Alcohol < 3.8 units/weeks			Alcohol ≥ 3.8 units/week		
Parameter	Mean	SEM	95% CI	Mean	SEM	95% CI
CFB 24h SBP_{br}	-0.37	0.89	[-2.14; 1.41]	**-4.39***	**0.93**	**[-6.27; -2.51]**
CFB 24h DBP_{br}	**-1.42***	**0.69**	**[-2.79; -0.05]**	**-2.24***	**0.73**	**[-3.71; -0.76]**
CFB Awake SBP_{br}	-0.08	1.13	[-2.35; 2.19]	**-4.41***	**0.90**	**[-6.23; -2.59]**
CFB Awake DBP_{br}	-1.49	0.86	[-3.20; 0.22]	**-2.26***	**0.66**	**[-3.61; -0.91]**
CFB Office SBP_{br}	-0.47	1.32	[-3.12; 2.18]	**-4.41***	**1.14**	**[-6.72; -2.09]**
CFB Office SBP_{ao}	-0.43	1.37	[-3.17; 2.32]	**-3.16***	**1.17**	**[-5.54; -0.77]**

5.3.2.9 Habitual physical activity

Physical activity level was estimated using the International Physical Activity Questionnaire (IPAQ) (Craig et al. 2003). The completion of this questionnaire led to the calculation of a score equivalent to the MET minutes/week, with MET minutes defined by the amount of energy expended carrying out physical activity. One MET represents 1 kcal/kg/hour and can be related to the energy cost of being seated. This score was then able to discriminate the participants into 3 categories of physical activity: low, moderate, and high. As the ABP

population was very active, only 1 volunteer was allocated in the low category, and only 24 in the moderate, with the rest of the participants presenting high physical activity level. For the purpose of the subgroup analysis, low and moderate categories were grouped together. The summary of findings resulting of the subgroup analysis based on the physical activity level of participants can be found in Table 5.21 below.

5.3.2.9.1 Blood pressure

Regarding the primary outcomes, 24h and awake SBP_{br} and DBP_{br} were found significantly reduced in the moderate/low physical activity group after 12-week consumption of the aronia extract (24h SBP_{br}: CFC: -3.5 mmHg, p= 0.02; 24h DBP_{br}: CFC: -3.6 mmHg, p= 0.005; Awake SBP_{br}: CFC: -3.9 mmHg, p= 0.028; Awake DBP_{br}: CFC: -3.8 mmHg, p= 0.007).

5.3.2.9.2 Augmentation index

Furthermore, although a reduction in AIx was observed in the overall population, only the subgroup of participants with high physical activity level presented some significant improvement in 24h and awake AIx_{ao} and AIx_{br} (24h AIx_{ao}: CFC: -2.9%, p=0.026; 24h AIx_{br}: CFC: -6.3%, p= 0.013; Awake AIx_{ao}: CFC: -2.8%, p= 0.028; Asleep AIx_{br}: CFC: -6.2%, p= 0.011). No significant difference was found for asleep AIx.

Table 5.21: Results from the subgroup analysis based on the physical activity level of participants: changes from Control (CFC) and associated p-values calculated using the baseline as covariate only (BACO) analysis.

		Physical activity level = High		Physical activity level = Moderate/Low	
		Control = 32 / Aronia = 35		Control = 14 / Aronia = 11	
ITT population		Chronic - BACO (p-value)	CFC	Chronic - BACO (p-value)	CFC
24h SBP	*mmHg*	0.364	1.104	0.020	-3.526
24h DBP	*mmHg*	0.451	0.656	0.005	-3.582
Awake SBP	*mmHg*	0.522	1.100	0.028	-3.909
Awake DBP	*mmHg*	0.551	0.573	0.007	-3.845
Asleep SBP	*mmHg*	0.674	1.853	0.525	-1.549
Asleep DBP	*mmHg*	0.479	0.981	0.141	-2.794
Office SBP_{br}	*mmHg*	0.293	-1.698	0.715	-0.985
Office DBP_{br}	*mmHg*	0.611	0.623	0.236	2.325
Office SBP_{ao}	*mmHg*	0.438	-1.306	0.870	0.468
Office DBP_{ao}	*mmHg*	0.190	1.722	0.193	2.055
Office PWV	*m/s*	0.621	0.182	0.175	-0.696
Office AIx	*%*	0.478	-1.372	0.822	0.599
FMD	*%*	0.573	-0.209	0.007	-1.343
Blood flow	*cm/s*	0.179	-3.532	0.860	-1.613
24h PWV	*m/s*	0.674	-0.084	0.214	-0.304
Awake PWV	*%*	**0.090**	-0.271	0.260	-0.419
Asleep PWV	*%*	0.722	-0.084	0.405	-0.222
24h AIx_{ao}	*%*	0.026	-2.906	0.300	-2.735
Awake AIx_{ao}	*%*	0.028	-2.774	**0.054**	-4.66
Asleep AIx_{ao}	*%*	0.706	-0.792	0.154	-6.023
24h AIx_{br}	*%*	0.013	-6.29	0.307	-5.298
Awake AIx_{br}	*%*	0.011	-6.243	**0.056**	-9.05
Asleep AIx_{br}	*%*	0.706	-1.565	0.154	-11.90
Cortisol	*mmol/L*	0.547	-10.761	0.666	-21.017
Total cholesterol	*mmol/L*	0.682	0.169	0.700	-0.101
Triglycerides	*mmol/L*	0.295	0.061	0.328	-0.148
HDL	*mmol/L*	0.537	-0.032	0.754	0.754
LDL	*mmol/L*	0.214	0.212	0.789	0.789

Several parameters presented some significant changes from baseline in the Aronia group (Table 5.22). This is the case for 24h and awake SBP_{br} which presented significantly decreases CFB for the moderate/low physical activity subgroup (CFB 24h SBP_{br}= -3.99 mmHg, 95% CI= [-6.17; -1.82]; CFB awake SBP_{br}= -4.04 mmHg, 95% CI= [-6.61; -1.47]). Twenty-four and awake DBP were significantly reduced compared to baseline in both subgroups (CFB 24h

DBP_{High}= -1.38 mmHg, 95% CI= [-2.57; -0.19], CFB 24h $DBP_{Mod./Low}$= -3.35 mmHg, 95% CI= [-5.14; -1.56], CFB awake DBP_{High}= -1.71 mmHg, 95% CI= [-3.03; -0.39], CFB awake $DBP_{Mod./Low}$= -3.45 mmHg, 95% CI= [-5.46; -1.44]). Office SBP_{br} was significantly decreased with regard to baseline in the subgroup with high physical activity only (CFB office SBP_{br}= -2.32 mmHg, 95% CI= [-4.53; -0.11]).

Table 5.22: Significant changes from baseline (CFB) in the different physical activity level subgroups of the Aronia group. SEM: Standard error of mean; 95% CI: 95% confidence interval.

Aronia group	Physical activity level = High			Physical activity level = Low / Moderate		
Parameter	Mean	SEM	95% CI	Mean	SEM	95% CI
CFB 24h SBP_{br}	-1.35	0.83	[-3.01; 0.32]	**-3.99***	**1.05**	**[-6.17; -1.82]**
CFB 24h DBP_{br}	**-1.38***	**0.60**	**[-2.57; -0.19]**	**-3.35***	**0.86**	**[-5.14; -1.56]**
CFB Awake SBP_{br}	-1.50	1.00	[-3.50; 0.50]	**-4.04***	**1.24**	**[-6.61; -1.47]**
CFB Awake DBP_{br}	**-1.71***	**0.66**	**[-3.03; -0.39]**	**-3.45***	**0.97**	**[-5.46; -1.44]**
CFB Office SBP_{br}	**-2.32***	**1.11**	**[-4.53; -0.11]**	-0.67	1.98	[-4.79; 3.44]

5.4 Discussion

Understanding the individual variability in response to PP intake is paramount to be able to understand the potential beneficial effects of plant bioactives on CVD and to give better dietary recommendations to the general public. In this analysis, we assessed the extent of the variability in response to treatment in subjects consuming the aronia extract (section 5.3.1). We also explored the influence of several modifiable and non-modifiable factors such as sex, age, BMI, diet, cardiometabolic health status, alcohol intake and physical activity on the overall population compared to placebo to observe in which subgroups the effect of the treatment was the more important (section 5.3.2).

The variability in response for our main outcome 24-hour ambulatory BP was high, with a CoV of 245 and 187% for 24-hour SBP_{br} and DBP_{br}, respectively, but was overall one of the lowest variabilities among the outcomes analysed. Total cholesterol and triglycerides showed the highest variability in response overall (more than 2500%), indicating that aronia berry extract does not present the same magnitude of effect depending on the individuals for these factors.

Gender seems to be the factor influencing the overall population response the most. In the subgroup analysis, a significant reduction in awake PWV was found in men, while no significant difference is observed for women. On the contrary, 24h and awake AIx_{ao} and AIx_{br} are significantly lower in female subjects following the 12-week intervention. This agrees with a RCT involving 30 middle-aged adults which highlighted a significant decrease (-12.9%, p= 0.01) in AIx_{br} in women only, following a flavanol-rich chocolate intake daily

for 4 weeks (West et al. 2014). These observations could be explained by two hypotheses. First, AIx is related to the reflection of the arterial wave originating from the various branching of the aorta and arterial conduits. Moreover, as the length of the aorta matches with the overall body height, wave reflections appears earlier in shorter individuals (Smulyan et al. 1998). As men from our study were in average 12.4 cm higher than women, height of volunteers is thus one possible explanation regarding the discrepancies in arterial function results between genders. However, AIx measurements from the present study are adjusted for height through an algorithm so this hypothesis is not validated. Another explanation of the divergence between female and male participants could be related to the prevalence for women to present a higher arterial stiffness compared with men with similar height (Gatzka et al. 2001). Indeed, baseline AIx_{ao} for women and men were 36.1 ± 10.1% and 23.2 ± 8.0%, respectively. This may be linked to the smaller arterial diameter reported in women, which would cause the reflection waves to travel quicker through the arterial system (Costa-Hong et al. 2018). A higher baseline arterial stiffness could thus lead to the ability for the interventional product to exert a larger magnitude of effect on this parameter.

Physiologically, increasing age is related to elevated arterial stiffness, decreased endothelial function and hypertension (Kaess et al. 2012). These phenomena are mainly related to the loss of elasticity of large arteries related to the diminution of elastin production towards collagen, which induces a reduction of the buffer function of the arteries and leads to an increase in PWV and BP (Sun 2015). Dietary interventions involving PP supplementations were shown to counterbalance the effect of age on CVD (Estruch et al. 2018).

It was observed that although SBP constantly increased with age, DBP reaches a maximum peak around 55 years which then diminishes (Kannel 1999; Avolio et al. 2018). In a similar manner, aortic stiffening occurs gradually with age, independently of gender (van der Heijden-Spek et al. 2000; AlGhatrif et al. 2013). Although aortic PWV was observed to increase by around 0.1 m/s per year of age (Asmar et al. 1995), it also has been indicated that this increase does not follow a linear evolution but is more noticeable after 55 years of age (Lajemi et al. 2001), which supports the findings of the Framingham study which noted an increased prevalence for hypertension from that same age (Franklin et al. 1997).

In the present interindividual variability study, we observed a significant decrease in office SBP_{br}, awake PWV, awake AIx_{ao} and AIx_{br} in subjects older than 56.2 years in the Aronia group compared with Control. In contrast, blood flow velocity, 24h and asleep AIx_{ao} and AIx_{br} were significantly reduced in the subgroup younger than 56.2 years compared with Control. Our results are partially in line with findings from Heiss and colleagues, who have, to date, led the only RCT investigating the influence of age on the impact of PP supplementation on vascular parameters (Heiss et al. 2015). Indeed, considering 2 subgroups of subjects of different ages (younger, < 35 years versus older, > 80 years), they observed a significant decrease in SBP (-6 mmHg) and AIx_{ao} (-7%) in the group of older participants only, following daily intake of cocoa flavanols for 2 weeks. In the present study, a decrease in office SBP_{br} compared to Control was observed only in participants older than 56.2 years, which could be related to higher BP and stiffer arteries usually described for this older category of the population and leading to the

phenomenon of systolic hypertension (Chaudhry, Krumholz, and Foody 2004). A similar explanation could be used to depict the significant decrease of awake arterial stiffness in subjects aged 56.2 and above. However, with the lack of study specifically designed to investigate the age-dependent effects of PP consumption on vascular function after ACN intake, it is difficult to establish clear and reliable suggestions explaining the discrepancies in vascular responses related to age.

Although the relationship between BMI and CVD risk is more intricate for subjects diagnosed with diabetes or other chronic cardiometabolic diseases, it is well understood that higher BMI is associated with an elevated risk of CVD events and mortality (Dwivedi et al. 2020). In the present study, we have observed some significant improvement for 24h and awake AIx_{ao} and AIx_{br} compared to Control in the subgroup of subjects with a BMI inferior to 25 kg/m^2. No significant difference was observed for any other cardiovascular parameter. Our results are not in line with the findings from the meta-analysis by Garcia-Conesa and colleagues who investigated the interindividual response to ACN consumption on various vascular outcomes (García-Conesa et al. 2018). Indeed, while they did not report any significant change for BP, FMD and blood lipids in the subgroup of normoweight subjects, they observed a significant reduction in total cholesterol (-0.23 mmol/L, p= 0.009), SBP (-1.54 mmHg, p= 0.000) and DBP (-1.62 mmHg, p= 0.000) in the subgroup of overweight participants. However, similarly to our study, the meta-analysis by Menezes and colleagues focusing on the difference in responsiveness to flavanol intake did not show any difference for triglycerides, HDL, LDL, and total cholesterol for either normoweight or overweight subjects (Menezes et al.

2017). However, both meta-analyses from Garcia-Conesa and Menezes were highly exploratory and used averages from studies presenting different outcomes, interventional products, and study design and direct comparison of our findings with their results may not be relevant. Additionally, a previous study led by our team and investigating the effect of aronia berry on vascular function in healthy young men with a normal BMI (mean BMI= 23 kg/m^2) did not report any change in arterial stiffness following 12-week daily intake of the aronia extract (Istas et al. 2019). The significant results of the present study could thus be explained by other inherent parameters such as physical activity level or health status. Unfortunately, the dearth of similar RCT involving the measurement of arterial stiffness and the inclusion of different ranges of BMI does not allow any comparison with the present trial.

Nutrition studies investigating the effects of specific PP supplementation do not usually consider the effect of the background diet of the participants, which can be significant. As shown in Chapter 3, individuals participating in the ABP study had a high consumption of PP in their habitual diet (1537 ± 989 mg per day). This is higher than the total daily PP intake of 1035 ± 545 mg reported for participants aged 50 to 64 years in the UK National Diet and Nutrition Survey Rolling Programme (Ziauddeen et al. 2019). As the aronia extract used in this work had a relatively modest PP content (105.9 mg per day) in comparison with other similar trials, it is possible that the habitual PP consumption of participants may affect the response to the intervention. To investigate this, we performed a subgroup analysis based on the total PP consumption and separated participants in 2 groups based on a cut-off of 700 mg of PP per day. When comparing the subgroups with low versus high

habitual PP intake, a trend for stronger BP lowering effects following aronia extract consumption was seen in the low PP intake group, with significant changes from Control of -5.2 and -6.4 mmHg for 24h and asleep ABP, respectively, versus changes of 0.8 and -1.7 mmHg in the high PP intake group. This was in line with the exploratory analysis comparing subgroups based on fruits and vegetables' intake, which reported a similar tendency. Indeed, following 12-week daily aronia extract consumption, we observed that awake SBP was significantly improved for participants with an intake of FV lower than 400g per day only. Twenty-four-hour and asleep SBP were also greatly reduced in Aronia participants with a daily PP intake of less than 700 mg, compared to Control. This is in line with the hypothesis that the impact of PP supplementation on BP is higher in participants presenting a low baseline PP intake presented in a recent review (Vendrame and Klimis-Zacas 2019). Besides, a meta-analysis looking at the influence of specific dietary patterns (DASH, Nordic, and Mediterranean diet) on BP reported a significantly reduced SBP and DBP in populations following these patterns (Ndanuko et al. 2016). To sum up, a lower PP intake and lower FV consumption seemed to lead to a higher vascular response to the aronia intervention on BP.

However, for arterial stiffness, that trend for an improvement was observed in the subgroup with the higher intake. In fact, greater BP lowering effects of the aronia extract were seen in the high FV intake group, with significant changes from Control of -5.3 and -10.6% for 24h AIx_{ao} and AIx_{br}, respectively, versus changes of -0.7 and -2.4% in the low FV intake group. To date, only 1 trial investigating the effect of berry consumption on arterial stiffness has shown an improvement of AIx (-2.24%, p= 0.04) following a 6-month daily intake of

150g of fresh blueberries in middle-aged men and women (Curtis et al. 2019). However, this RCT was conducted on overweight and obese participants with metabolic syndrome and the baseline anthocyanin intake of participants was 18.6 mg/day, which is almost three times less than the baseline anthocyanin intake of the present study population (68.0 ± 65.9 mg/day). All these differences in study design and subjects included cannot allow us to extrapolate the observed results to our population. Unfortunately, most studies investigating the effect of PP-rich dietary patterns on arterial stiffness only considered PWV as a marker of vascular dysfunction, and not AIx (Jennings et al. 2012; Curtis et al. 2013). Additionally, most studies are not considering arterial stiffness as a primary outcome, which implies that trials might be underpowered for the assessment of AIx. Augmentation index improvement has mostly been observed following medium- and long-term investigations, suggesting that time of exposure as well as study design can influence the modulation in AIx (Martini et al. 2020).

In most trials, the variability of participant's baseline PP intake is often unreported, which is an obstacle to the accurate estimation of the interindividual variability in response to phenolic compounds supplementation (Cassidy and Minihane 2017). A better reporting of dietary patterns and the standardization of interventions may lessen the potential bias involved in the identification of biological elements impacting interindividual variability (Gibney et al. 2019).

The primary inclusion criteria of this RCT were a SBP and/or DBP in the prehypertensive range of BP, defined as SBP comprised between 120 and

139 mmHg and/or DBP between 80 and 89 mmHg. Through an exploratory analysis, we evaluated the impact of baseline SBP and DBP on the interindividual variability in response to our treatment. We divided the volunteers into subgroups of normotensive (SBP and DBP< 120 and 80 mmHg, respectively) versus prehypertensive, for both BP parameters (SBP and DBP comprised between 120-139 and 80-89 mmHg, respectively). Results were highly inconsistent, as we observed a significant reduction in 24h, awake and asleep AIx_{br} and AIx_{ao} in the subgroup with SBP< 120 mmHg, compared to Control, while awake PWV was driven by the subgroup with a prehypertensive SBP at baseline. Moreover, awake AIx was significantly decreased in the subgroup of normotensive DBP participants while 24h AIx was significant in the subgroup of subjects with DBP> 80 mmHg. In addition, we did not report any significant change or trend for ambulatory or office BP following this subgroups analysis based on baseline SBP and DBP. Several reviews and meta-analysis investigating the factors influencing the effects of ACN and cocoa flavanols on BP highlighted that (pre)hypertensive subjects presented a greater BP-reducing effect of the intervention compared to normotensive participants (Vendrame and Klimis-Zacas 2019; Ried, Fakler, and Stocks 2017). One explanation to this discrepancy in results between our study and the existing literature –on top of the low dose of PP coming from the aronia extract which was discussed earlier– could be the low BP average of the general population. Indeed, mean baseline SBP and DBP were 121.7 ± 9.3 and 80.4 ± 5.8 mmHg, respectively, which, although being in the prehypertensive range, was low and could almost be considered as normotensive.

Along with age, alcohol consumption is one of the factors which has not been taken into account in the only exploratory meta-analysis focusing of interindividual variability in response to ACN intake because of a lack of existing data (García-Conesa et al. 2018). For the present study, a cut-off of 3.8 units of alcohol (equivalent to 2 small glasses of 12% wine or 2 bottles of 5% beer) per week has been chosen for the subgroup analysis as it represented the average alcohol intake of our population. We observed some significant decreases in BP and PWV in the subgroup of subjects with the higher intake of alcohol (> 3.8 units) compared to Control, but not in the other subgroup with the lowest intake of alcohol. In contrast, we reported significant reductions in AIx in the subgroup with the lowest intake of alcohol per week. While several epidemiological studies have reported the beneficial impact of light alcohol consumption on BP (Jaubert et al. 2014; Vallée et al. 2019; Fisher, Orav, and Chang 2018) and arterial stiffness (Gonzalez-Sanchez et al. 2020; Nishiwaki, Kora, and Matsumoto 2017; Sasaki et al. 2013; Mattace-Raso et al. 2005), –with most studies showing the presence of a J-shaped relationship between alcohol intake and these vascular parameters–, there is a real lack of evidence for the influence of alcohol consumption on interindividual variability in response following PP-rich foods coming from the RCTs. Moreover, the cut-off of 3.8 units per week used in the analysis is still far below the threshold established by a combined analysis including around 600 000 drinkers without pre-existing CVD which defined a limit of 10 to 12 units per week (equivalent to 100g of alcohol) to be associated to the lowest risk for all-cause mortality (Wood et al. 2018). In the UK, moderate drinking pattern is defined by the National Health Service as an intake of maximum 14 units of alcohol per week.

Based on this guideline, only 4 participants of the present study are considered as heavy drinkers. It is thus impossible to perform a subgroup analysis segregating moderate and heavy drinkers. Moreover, a third of our volunteers (35 out of 102) were teetotallers, who were reported to present significantly higher CVD risk compared to low/moderate drinkers (Fillmore et al. 2007). Our study participants mainly consumed beer, followed by wine and spirits (Table 5.23). Interestingly, subjects from the Aronia group consumed more wine than the Control group (5.2 ± 3.7 versus 3.4 ± 4.0 units/ week), and this trend persists when looking at the participants with an intake ≥ 3.8 units per week in both interventional groups (7.4 ± 3.3 versus 5.9 ± 4.8 units/ week). Due to the high concentration of PP found in wine, this inter-group divergence could explain some of the positive effects on vascular function reported in this exploratory analysis.

Table 5.23: Alcohol intake of the ABP population.

	Total alcohol	Wine	Beer	Spirits
Overall population	3.8 ± 4.8	4.3 ± 3.9	5.7 ± 5.5	2.1 ± 1.4
Aronia	4.0 ± 4.8	5.2 ± 3.7	5.4 ± 5.5	2.7 ± 1.2
Control	3.6 ± 4.9	3.4 ± 4.0	6.0 ± 5.8	1.9 ± 1.6
Aronia < 3.8	0.8 ± 1.1	2.0 ± 0.7	1.0 ± 0.0	-
Aronia ≥ 3.8	8.5 ± 4.3	7.4 ± 3.3	6.8 ± 5.6	3.0 ± 1.4
Control < 3.8	0.8 ± 1.0	1.3 ± 0.9	1.0 ± 0.7	1.0 ± 0.7
Control ≥ 3.8	9.6 ± 4.4	5.9 ± 4.8	8.2 ± 5.7	3.0 ± 1.7

Values expressed in units per week (mean ± SD)

To summarize, no clear explanation can explain the variations in response to aronia extract intake following the exploratory subgroup analysis based on alcohol consumption. There is a real need for better reporting of alcohol consumption in interventional studies to determine the real impact of drinking patterns on biological response to plant bioactive supplements.

In our present study, we observed a significant reduction of 24h and awake SBP and DBP in the subgroup of participants presenting low/moderate physical activity level only. Along with the significant improvement of BP, we have reported some significant decreases in 24h and awake AIx_{ao} and AIx_{br} in the subgroup of subjects with a high physical activity. The impact of physical activity on cardiometabolic health has been investigated in the past decades. For example, six weeks of high or medium intensity training on sedentary young men presenting a BMI> 25 kg/m^2 led to some significant improvement in blood lipids and cardiorespiratory fitness (Fisher et al. 2015). It is now well established that a low cardiorespiratory fitness, measured by maximal oxygen consumption VO_{2max}, is recognized as a CVD risk factor (Gupta et al. 2011; Kodama et al. 2009). The main physiological process behind cardioprotective effects of exercise is based on the secretion of musclin myokine from exercising muscle which improves cardiorespiratory fitness by increasing the expression of mitochondrial respiratory chain complex proteins (Subbotina et al. 2015). A meta-analysis including 2001 participants showed the beneficial and significant influence of walking interventions on 7 different CVD risk factors including SBP and DBP (Oja et al. 2018). However, the effect of exercise on the cardiometabolic response to PP intake has, to our knowledge, never been investigated. Baseline physical activity is rarely investigated in nutritional RCTs, which prevents us to compare the findings of the present study with other similar trials, as it appears that physical activity levels vary highly between specific populations. For example, a RCT testing the impact of quercetin supplementation on inflammation and BP in women with rheumatoid arthritis reported a baseline physical activity level of the participants to be 848

MET/min/week, which is in the lower part of the moderate activity level range (Javadi et al. 2014). On the contrary, another study investigating the relationship between cocoa consumption and physical activity and health status in 270 young students (71 males and 199 females) described the average physical activity level of the overall population to be 2692 MET/min/week, which is close to the cut-off moderate/high physical activity level (Rodríguez-Lagunas et al. 2019). No previously conducted RCT looking at the impact of aronia berry consumption on BP has recorded the exercise status of their populations. Recently, a study investigated the association between physical activity and PWV and AIx in 84 middle-aged men and women (Haapala, Lee, and Laukkanen 2020). Authors determined that there was no relationship between the different parameters and that reduction in AIx following minimal physical exercise was greater in subjects who were prone to regular resistance training. This could imply the significant difference observed in our study could be influence by an external factor.

To summarize, an exploratory analysis investigating factors which could be responsible for the interindividual variability in responsiveness following aronia extract daily consumption was conducted. Tables 5.24 represent the summary of the main subgroup analyses performed in this study. Following the study of various factors, from diet and health status to alcohol intake and physical activity level, we observed a pattern among the different outcomes analysed towards a bigger improvement of ambulatory arterial stiffness (measured as PWV and AIx) mainly in volunteers we classified as “very healthy profile” (i.e. female subjects with a total PP intake ≥ 700 mg/day, total FV ≥ 400 g/day, age < 56.2 years, BMI < 25 kg/m^2, alcohol intake < 3.8 units per week, and a high

physical activity level) (Table 5.24 A). On the contrary, BP was mostly improved in “less healthy profile” volunteers, especially for subjects with a low or moderate physical activity level, a total PP intake lesser than 700 mg/day and an alcohol consumption of more than 3.8 units per week (Table 5.24 B). Several subgroups presented some significant decreases in BP, including participants from the Aronia group with a total PP intake of less than 700 mg per day who showed a significant drop of 5.2 mmHg compared to Control. This is of clinical relevance, as a reduction of 2 mmHg in SBP has been shown to be associated to a 6% drop in the risk of coronary heart disease (Cook et al. 1995).

Tables 5.24: Summary of the changes from Control observed in subjects for the according parameters used in the subgroup analysis. A: subgroup parameters classified as “very healthy profile” and B: subgroups parameters classified as “less healthy profile”.

A	Female		Age < 56.2 years		BMI < 25		Total PP ≥ 700 mg/day		FV ≥ 400 g/day		Alcohol < 3.8 units/wk		Physical activity level = High	
	C = 25 / A = 27		C = 26 / A = 24		C = 27 / A = 24		C = 40 / A = 40		C = 22 / A = 29		C = 32 / A = 28		C = 32 / A = 35	
Parameter	p-value	CFC	p-value	CFC	p-value	CFC	p-value	CFC	p-value	CFC	p-value	CFC	p-value	CFC
24h SBP	0.94	0.12	0.52	0.90	0.65	-0.64	0.43	0.82	0.36	1.27	0.12	1.92	0.36	1.10
24h DBP	0.49	-0.74	0.63	-0.52	0.44	-0.74	0.97	0.03	0.67	0.41	0.95	0.06	0.45	0.66
Awake SBP	0.56	0.72	0.21	0.18	0.87	-0.69	0.29	0.51	0.48	1.09	0.88	2.76	0.52	1.10
Awake DBP	0.93	-0.11	0.50	-0.87	0.64	-0.59	0.79	-0.24	0.79	0.29	0.68	0.49	0.55	0.57
Office SBP_{br}	0.10	-3.19	0.95	-0.10	0.11	-2.81	**0.07**	-2.73	0.27	-2.33	0.92	-0.19	0.29	-1.70
Office DBP_{br}	0.88	0.21	0.32	1.36	0.86	-0.25	0.56	0.67	0.73	0.47	0.36	1.25	0.61	0.62
Office PWV	0.66	-0.13	0.12	-0.49	0.87	-0.07	0.52	0.16	0.89	-0.06	**0.05**	0.70	0.62	0.18
Office AIx	0.18	-2.79	0.25	2.35	0.77	-0.73	0.90	-1.22	**0.09**	-3.51	0.78	0.62	0.48	-1.37
24h PWV	0.71	0.05	0.70	-0.07	0.10	-0.32	0.41	-0.15	0.75	-0.07	0.86	0.03	0.67	-0.08
Awake PWV	0.63	-0.15	0.81	-0.06	0.26	-0.31	0.20	-0.21	0.43	-0.21	0.54	-0.17	**0.09**	-0.27
24h AIx_{ao}	**0.003**	-5.12	**0.027**	-3.62	**0.002**	-4.95	**0.017**	-3.21	**0.001**	-5.34	**0.005**	-4.13	**0.026**	-2.91
Awake AIx_{ao}	**0.000**	-6.67	0.23	-1.38	**0.001**	-5.86	0.14	-2.49	**0.08**	-3.72	**0.09**	-3.31	**0.028**	-2.77
24h AIx_{br}	**0.003**	-10.10	**0.026**	-7.19	**0.002**	-9.85	**0.010**	-6.74	**0.001**	-10.55	**0.005**	-8.08	**0.013**	-6.29
Awake AIx_{br}	**0.000**	-13.16	0.23	-2.73	**0.001**	-11.63	0.11	-5.40	**0.08**	-7.34	**0.10**	-6.41	**0.011**	-6.24

B	Male		Age ≥ 56.2 years		BMI ≥ 25		Total PP < 700 mg/day		FV < 400 g/day		Alcohol ≥ 3.8 units/wk		Physical activity = Moderate/Low	
	C = 23 / A = 22		C = 22 / A = 25		C = 21 / A = 25		C = 8 / A = 9		C = 26 / A = 20		C = 16 / A = 21		C = 14 / A = 11	
Parameter	p-value	CFC	p-value	CFC	p-value	CFC	p-value	CFC	p-value	CFC	p-value	CFC	p-value	CFC
24h SBP	0.47	-0.87	0.26	-1.48	0.93	-0.11	**0.023**	-5.22	**0.06**	-2.59	**0.012**	-3.77	**0.020**	-3.53
24h DBP	0.76	-0.28	0.73	-0.34	0.96	-0.06	0.29	-2.25	0.13	-1.73	0.16	-1.59	**0.005**	-3.58
Awake SBP	0.37	-1.29	0.90	-0.20	0.61	-0.79	0.27	-3.04	**0.039**	-2.10	**0.002**	-4.62	**0.028**	-3.91
Awake DBP	0.43	-0.93	0.83	0.22	0.57	-0.76	0.62	-1.24	0.15	-1.77	**0.030**	-2.29	**0.007**	-3.85
Office SBP_{br}	0.59	-1.02	**0.036**	-4.32	0.40	-1.83	0.92	0.32	0.19	-2.40	**0.019**	-4.26	0.72	-0.99
Office DBP_{br}	0.71	0.57	0.58	-0.83	0.63	0.68	0.99	-0.03	0.92	0.16	0.32	-1.46	0.24	2.33
Office PWV	0.66	0.18	0.37	0.46	0.63	0.22	0.29	-0.59	0.61	0.20	**0.025**	-1.04	0.18	-0.70
Office AIx	0.25	0.25	0.15	-3.25	0.96	0.08	0.42	3.29	0.45	1.46	0.23	-1.78	0.82	0.60
24h PWV	**0.025**	-0.39	0.62	-0.12	0.84	0.05	0.66	-0.13	0.18	-0.26	**0.030**	-0.50	0.21	-0.30
Awake PWV	**0.020**	-0.37	**0.043**	-0.48	0.70	-0.13	0.38	-0.41	0.10	-0.35	**0.017**	-0.50	0.26	-0.42
24h AIx_{ao}	0.52	-0.98	0.14	-2.49	0.48	-1.16	0.27	-2.45	0.66	-0.71	0.56	-1.19	0.30	-2.74
Awake AIx_{ao}	0.77	0.63	**0.008**	-4.51	0.86	0.37	**0.06**	-4.71	0.15	-2.73	0.36	-1.64	**0.05**	-4.66
24h AIx_{br}	0.37	-2.68	**0.08**	-5.59	0.34	-3.11	0.24	-5.24	0.45	-2.36	0.40	-3.28	0.31	-5.30
Awake AIx_{br}	0.94	0.33	**0.005**	-10.07	1.00	-0.01	**0.049**	-9.77	**0.09**	-6.63	0.20	-5.01	**0.06**	-9.05

A: Aronia; AIx: augmentation index; BMI: body mass index; br: brachial; C: Control; CFC: changes from Control; DBP: diastolic blood pressure; FV: fruits and vegetables; PP: (poly)phenols; PWV: pulse wave velocity; SBP: systolic blood pressure. P-value and CFC obtained using ANCOVA analysis (Bonferroni post-hoc) with baseline as covariate and treatment as fixed factor. Blood pressure values in mmHg, PWV values in m/s and AIx values in %. Significant changes from Control have been highlighted in grey and a gradient of colour, from green to red, represents the highest (in green) and lowest (in red) decreases for each of the parameters, following the 12-week daily intake of aronia extract.

The present analysis of interindividual variability presents some positive points. First, it is to our knowledge the first study to perform an interindividual analysis of the main parameters studied in out RCT as well as an exploratory analysis to look at variations in responsiveness between two groups of intervention in more than 100 participants regarding alcohol consumption, fruits and vegetable intake, and physical activity. Very few studies have been recorded with such precision these parameters, which often led to the impossibility of analysing the influence of these factors on the subjects' responsiveness. We used robust validated 7-day food diaries and physical activity questionnaires to record our data in the most standardised way possible. Besides, our population subjects were healthy, middle-aged, and constituted of men and women, which allows us to extrapolate the results of this analysis on a large portion of the general public, rather than on specific

segments of the population including only young men or volunteers with CVD risk factors.

However, there are a few weaknesses in the present analysis. First, the main limitation of this exploratory analysis is the oversimplification of our approach, using arbitrary subgroups that were statistically underpowered and considering the assessed factors independently rather than as a continuum of interactions able to influence the biological response to PP intake. In addition, the overall population of the trial was mainly constituted of non-smokers (only 5 smokers in total) and was very homogenous in terms of ethnicity which made the subgroup analysis based on smoking status and ethnicity impossible to perform. These parameters were taken into consideration by Garcia-Conesa and colleagues in their meta-analysis looking at the interindividual variability of response following anthocyanin consumption. They indeed highlighted that only non-smokers were showing a significant decrease in SBP and DBP (-1.99 and -1.28 mmHg, respectively). They also investigated the influence of geographical location of studies and notably the variation in response between Mediterranean and non-Mediterranean countries and did not observe a significant decrease in BP in non-Mediterranean countries. The population of the present study being in majority British could explain the absence of significant result when looking at the overall population. Moreover, participants were extremely healthy and conscious about their diet. This caused the subgroup analysis based on baseline PP intake to be very imbalanced, which might lead to some statistical irrelevance and bias. Additionally, although it was possible to segregate the population intro normo- and overweight subjects for the subgroup analysis based on BMI, no obese subjects were included in

the trial, as per the inclusion criteria, which made the comparison of normal weight individual response and obese subjects' response to treatment impossible. Finally, some assessments performed in the present study regarding the exploratory subgroup analysis (physical activity, background diet and smoking status) were based on participants' self-reporting. These methods are limited by a potential lack of accuracy which can lead to a risk of bias in the results.

To conclude, variations occurring in the interindividual response to PP consumption may lead to ambiguous relationships between plant bioactive intake and biological response. This can result in some hidden health benefits within an overall cohort of heterogenous subjects and thus prevent to expand our knowledge of phenolic compounds impact on cardiometabolic health. Therefore, well-designed trials and epidemiological studies involving multi-omics approaches will be a key to understand the influence of various factors on the interindividual variability of response following plant bioactive consumption and ensure their integration in future tailored nutrition approaches.

CHAPTER 6

Impact of aronia berry (poly)phenols on gut microbiota

6.1. Introduction

The term microbiome stands for the combined genomes of all microorganisms (bacteria, fungi, viruses) living in a specific environment (Hills et al. 2019). As a result, gut microbiota refers to the overall population of microorganisms living in the gastrointestinal tract. Recent metagenomic techniques have highlighted the presence of more than 2500 bacteria species in the human gut, for a total population of trillions of entities (Almeida et al. 2019). The interest in gut microbiota and its impact for the host has increased notably in the last decades, particularly due to recent advances showing its close relationship with a large number of diseases including cardiometabolic diseases and mental disorders (Valdes et al. 2018). Recent studies have also highlighted the importance of diet as a strong modulator of the gut microbiome (Leeming et al. 2019; Leeming et al. 2021). (Poly)phenols (PP) were shown to be able to influence the gut microbiota, and increasing evidence suggests that their mechanisms of action regarding their beneficial effects on human health may be strongly related to the bidirectional relationship they establish with gut bacteria.

6.1.1. Interactions between gut microbiota and PP

6.1.1.1. Gut microbial metabolism of PP

Following ingestion, the majority of PP are not absorbed in the upper gastrointestinal tract and reach the large intestine where enzymes of the gut microbiota will cleave and catabolize the native compounds into a large variety of smaller molecules easily absorbed (Cardona et al. 2013; Bowey,

Adlercreutz, and Rowland 2003; Santhakumar, Battino, and Alvarez-Suarez 2018). These enzymes include hydrolases, reductases, decarboxylases, and demethylases, all acting to decrease the complexity and size of phenolic metabolites (Zanotti et al. 2015; Selma, Espín, and Tomás-Barberán 2009). Some potential cleavage sites for berry PP are shown in Figure 6.1 below.

As a result of these scissions, a large number of low molecular weight compounds are formed, such as benzoic acids, catechols, phenylpropanoic acids and hydroxycinnamic acids (Aura et al. 2005). These catabolites will be able to reach the systemic circulation where they can be distributed to various peripheral organs and tissues before being eliminated by the kidneys via the urine (Rodriguez-Mateos et al. 2014). The gut microbial metabolism of PP plays an essential role in the bioavailability of these compounds due to their overall low absorption in the small intestine, in particular large molecular weight PP such as proanthocyanidins, which favour the interaction of PP with colonic bacteria (Marchesi et al. 2016). This relationship between PP and gut microbiota is bidirectional, and not only gut bacteria can metabolize PP, but phenolic compounds can also modulate the composition and diversity of the microbiome through their “prebiotic effect” (Espin, Gonzalez-Sarrias, and Tomas-Barberan 2017).

Figure 6.1: Potential ring cleavage sites for berry flavonoids and chlorogenic acid by gut microbiota. Adapted from Lavefve et al. 2019.

As mentioned earlier, the main reactions related to PP metabolism by the gut microbiota occurring in the large intestine are decarboxylations, demethylations, reductions, and hydrolysis (Espin, Gonzalez-Sarrias, and Tomas-Barberan 2017). Many metabolites has been identified to be products of microbial metabolism, such as urolithins (derived from ellagitannins), enterolactones and enterodiols (produced from lignans), equol (coming from isoflavones catabolism) or γ-valerolactones (derived from catechin and proanthocyanidins) (Tomas-Barberan, Selma, and Espin 2016). These compounds are typically present in higher concentrations than the intact or phase II conjugated PP absorbed in the small intestine, appear later in circulation, and have a longer half-life (Liu and Hu 2007).

In recent years, a high interindividual variability in PP gut microbial metabolism has been demonstrated (Cortés-Martín et al. 2020). In this context, the notion of metabotype has been developed, referring to a "*metabolic phenotype with*

specific gut microbiome-derived metabolites that characterize the metabolism of the parent compound" (Espin, Gonzalez-Sarrias, and Tomas-Barberan 2017). This concept is an important discovery in the field of personalised nutrition and could potentially explain some of the mixed effects shown in clinical trials investigating the health benefits of PP (Palmnäs et al. 2020; Espin, Gonzalez-Sarrias, and Tomas-Barberan 2017). Interindividual variability in gut microbiota function and composition is therefore likely responsible for the variability observed in bioavailability and biological efficacy of PP (Bolca et al. 2010; van Dorsten et al. 2010). The interindividual variations observed in gut microbiota are related to environmental factors (diet, lifestyle, exercise, physio pathological status), but also to genetic factors (Arumugam et al. 2011).

6.1.1.2. Prebiotic effect of PP

Due to their nature of compounds modulating the composition and activity of the colonic microbiota, PP can be defined as prebiotics (Bindels et al. 2015; Kennedy 2014; Fogliano et al. 2011; Oteiza et al. 2018; Tomas-Barberan, Selma, and Espin 2016). For example, tea PP were shown to inhibit the development of detrimental bacteria such as *Staphylococcus aureus*, *Salmonella typhimurium*, *Helicobacter pylori* or *Escherichia coli in vitro* (Duda-Chodak et al. 2015). PP can also promote the development of some beneficial bacteria such as *Lactobacillus* spp., *Bifidobacterium* spp., *Akkermansia muciniphila* or *Faecalibacterium prausnitzii* which is responsible for the production of the short chain fatty acid (SCFA) butyrate (Gwiazdowska et al.

2015; Jin et al. 2012; Koutsos et al. 2017; Derrien, Belzer, and de Vos 2017; Eppinga et al. 2016).

There is some clinical evidence of the modulation of microbiome by PP. For instance, a 4-week trial investigating the influence of red wine PP consumption on gut microbiota showed a significant increase in several beneficial genus, such as *Bifidobacterium*, *Bacteroides* and *Enterococcus* (Queipo-Ortuno et al. 2012). More specifically, phenolic anthocyanin metabolites were associated with greater abundance in *Bifidobacterium*, a genus associated with positive effects on health (Boto-Ordonez et al. 2014; Saulnier, Kolida, and Gibson 2009). This was confirmed by another trial showing significant increases in *Bifidobacterium* following anthocyanin-rich blueberry intervention (Vendrame et al. 2011). Recent reviews and meta-analysis of human and animal trials and mechanistic studies have corroborated the potential of anthocyanins (3 *in vitro*, 2 animal and 1 RCT), tea PP (6 human trials and 18 *in vitro* studies) and grape and red wine PP (7 human trials) consumption to induce a significant proliferative effect on *Bifidobacterium* spp., –a widely used probiotic–, the inhibition of pathogenic *Clostridium* species, and a modulation of gut microbial diversity (Igwe et al. 2019; Bond and Derbyshire 2019; Nash et al. 2018).

The *Firmicutes*/*Bacteroidetes* balance has also been shown to be modulated by specific PP such as rutin, anthocyanins and proanthocyanidins (Parkar, Trower, and Stevenson 2013; Guo et al. 2018; Wu, Hu, et al. 2018; Jin et al. 2018). However, most studies were performed in rodents and very limited evidence from human trials is available. Nonetheless, this ratio is thought to be an indicator of health conditions such as type 2 diabetes and obesity, for which a higher ratio has been associated (Cani and Everard 2016). However,

it was recently demonstrated that the interindividual variability in the relative abundance of *Bacteroidetes* and *Firmicutes* is high and influenced by lifestyle factors such as physical activity or diet, which could explain the contradictory results observed in normoweight and obese participants (Magne et al. 2020). Vaiserman and colleagues also observed that the *Firmicute/Bacteroidetes* balance increases with age independently from gender (Vaiserman et al. 2020).

6.1.2. Factors influencing gut microbial composition

Gut microbial composition changes significantly with age and physiopathological status, as they both affect diversity and composition of the colonic microbiome (Lakshminarayanan et al. 2014; Konturek et al. 2015). At the age of 3, gut microbiota is close to adults' in terms of diversity and composition, and is already dominated by the 3 main phyla, i.e. *Firmicutes*, *Bacteroidetes* and *Actinobacteria* (Yatsunenko et al. 2012). Reported changes in composition with regard to age included a decrease in diversity, reduced abundance of dominant species and improved abundance of subdominant species, increased *Proteobacteria*, decreased *Bifidobacteria* counts, and reduction of the *Firmicutes/Bacteroidetes* ratio (Bischoff 2016; Biagi et al. 2016; Rondanelli et al. 2015; Pérez Martínez, Bäuerl, and Collado 2014).

Similarly, sex was shown to be a factor influencing gut microbiota. Indeed, while no difference in diversity was found between men and women (Haro, Rangel-Zúñiga, et al. 2016), richness appeared to be substantially different between sexes in young adults (de la Cuesta-Zuluaga et al. 2019). Women seem to reach adulthood with a more diverse gut microbiome compared to

men, although both sexes attain a plateau around the age of 40 years. One hypothesis related to these sex-dependant variations could be linked to the presence of specific enzyme expression profiles which could be modulated by sex hormones (Manach et al. 2017)

Moreover, Haro and colleagues revealed that in a population of individuals matched for diet and sex, and stratified by BMI, men with a BMI below 33 kg/m^2 presented a higher proportion of *Firmicutes* compared to individuals with BMI > 33 kg/m^2 (Haro, Rangel-Zúñiga, et al. 2016). The impact of BMI on gut microbiota composition has been mostly investigated in children and adolescents, and it was observed that over- and normoweight subjects presented a higher bacterial diversity compared with underweight individuals (Bai, Hu, and Bruner 2019; Yun et al. 2017).

Due to the strong link between microbiome and diet, the latter has been extensively investigated in the context of abundance and diversity modulation. For example, a study comparing children fed with Western diet with children having a diet from Burkina Faso (rich in grains, vegetables, with low lipid intake) revealed that African children presented a significantly higher abundance for beneficial *Prevotella* and *Xylanibacter,* while pathogens *Shigella* and *Escherichia* were under-represented (De Filippo et al. 2010). Another study compared the impact of a plant-based diet with an animal-based diet on gut microbiota quality and quantity in healthy adults (David et al. 2014). Authors demonstrated a that the diet low in fibres and high in fats led to an augmentation in the abundance of *Alistipes*, *Bilophila*, and *Bacteroides* –which are bile-tolerant bacteria–, while *Firmicutes* such as *Roseburia*, *Eubacterium*,

and *Ruminococcus* (which are known as plant polysaccharides fermenters) increased within individuals consuming the diet rich in fibres and low in fats.

Finally, the beneficial impact of physical activity on gut microbiota diversity has been demonstrated in a cohort of 40 male athletes, in comparison with their low- and high-BMI controls (Clarke et al. 2014). A similar trend was observed in children and adolescents for whom daily physical activity increased the abundance of *Clostridiales*, *Roseburia*, *Erysipelotrichaceae*, and *Lachnospiraceae,* associated with an improvement of the production of SCFA (Bai, Hu, and Bruner 2019).

6.1.3. Short-chain fatty acids and their biological role

SCFA, such as acetate, propionate, and butyrate, are metabolites coming from the gut bacterial catabolism of indigestible carbohydrates which can modulate the immune response through the regulation of pathways (Kawabata, Yoshioka, and Terao 2019). SCFA have also been shown to exert a role in the maintenance of a healthy cardiometabolic function (Anselmi et al. 2020). In fact, the homeostasis of the microbiome is maintained by peroxisome proliferator-activated receptor gamma (PPAR-γ), a nuclear receptor which becomes activated in contact with butyrate for example. In the case of dysbiosis such as colitis, PPAR-γ synthesis decreases, the abundance in *Proteobacteria* increases, while the *Firmicutes* proportion diminishes (Hills et al. 2019).

Various PP, such as rutin, hesperidin, epicatechin, procyanidins, chlorogenic acid or ellagitannins were shown to enhance the production of SCFA following the consumption of various plant-based foods in animal models (Sadeghi

Ekbatan et al. 2016; Zduńczyk, Juśkiewicz, and Estrella 2006; Aprikian et al. 2003). Among the bacteria producing SCFA and enhanced by the intake of phenolic acids, *Akkermansia muciniphila*, *Lactobacillus* and *Bifidobacterium spp.* have been often reported (Kawabata, Yoshioka, and Terao 2019). Specifically, the main acetate-producing species are *Streptococcus spp., Prevotella spp., Bifidobacterium spp., Clostridium spp.,* and *A. muciniphila,* while propionate is metabolised mostly by *Bacteroides spp., Salmonella spp., Dialister spp., Veillonella spp., Roseburia inulinivorans, Coprococcus catus,* and *Blautia obeum.* Additionally, *Lachnospiraceae, Ruminococcaceae,* and *Acidaminococcaceae* families are the main butyrate producers among gut microbiome (Duttaroy 2021). Although the PP-induced impact on SCFA was demonstrated in the rodent model, very few studies have investigated these effects in humans, and results have mostly been unconclusive and limited (Mosele et al. 2015; Jacobs et al. 2008).

6.1.4. Characterisation of the gut microbiome

Different techniques are used to characterise the gut microbiome. The oldest and most accessible technique is the 16S rRNA sequencing, which uses polymerase chain reaction (PCR) to amplify a specific region of bacterial 16S gene. The other technique, –more recent and expensive–, is called shotgun metagenomic sequencing and is considered as the gold standard for microbiome characterization. This method is based on the sequencing of the whole bacterial DNA present in a sample, which delivers more thorough data on microbial gene content (Liu, Qin, et al. 2021). Both techniques present strengths and weaknesses. Indeed, while 16S is widely used and represents

a cheaper and easier way to map the gut microbiome, it also lacks in precision and does not allow the direct profiling of bacterial genes but is an efficient tool to identify gut bacteria at a species level (Durazzi et al. 2021). On the other hand, shotgun analysis is more precise and can deliver a strain-level resolution. However, as the whole DNA is analysed, some non-microbial genetic information (e.g., host DNA) may bias the results.

Once sequenced, several statistical tools are available to characterize the composition and abundance of gut microbiome. Among them, alpha and beta diversity indices are the most used. Alpha (α) diversity assesses the intra-sample diversity, and a higher α diversity indicates a higher evenness and/or richness in a sample and is correlated with healthy bacterial communities. Main indices for α diversity are Shannon and Simpson indices. On the contrary, beta (β) diversity compares the differences or similarities between 2 samples or 2 bacterial communities. A lower β diversity stands for a low similarity between the 2 entities, indicating these 2 are highly different. The Bray-Curtis dissimilarity index, one of the most used for β diversity, is based on the variability of abundances between 2 samples and presents values ranging from 0 to 1, with 0 implicating samples have the exact same abundances and 1 standing for samples with completely different abundances (Ricotta and Podani 2017). Statistical representation of β diversity is often presented through a Principal Coordinates Analysis (PCoA), which is a 3D graphic highlighting the (dis)similarity of a data set.

Besides these indices assessing the diversity of microbial species have been developed some parameters focusing more on the magnitude of effect of an

intervention on gut microbiome profile. Indeed, gene richness (also called gene count) was shown to be a potent predictive factor for the efficacy of a treatment, as gut microbiota low richness was reported in patients with inflammation or intestinal disorders (Cotillard et al. 2013; Le Chatelier et al. 2013). The concept of bacterial gene richness relies on the idea of the gut composition to stay stable throughout the adulthood, and thus uses richness as a characteristic feature of a subject (Costello et al. 2009).

6.1.5. Aronia berry PP and gut microbiota

To date, only 4 studies have investigated the effect of aronia berry PP on microbiome composition, on three different models: human (n= 1), animal (n= 2), and *in vitro* (n= 1).

To our knowledge, only one RCT has investigated the impact of aronia berry consumption on human gut microbiota composition and diversity in healthy young men after consumption of aronia extracts for 12 weeks (Istas et al. 2019). While no change in microbiome diversity was reported, a significant increase in the concentration of *Anaerostipes* and *Bacteroides* was observed. *Anaerostipes* are known to be butyrate producers, a SCFA associated with positive effects in cardiometabolic diseases such as stroke or hypercholesterolaemia (Muñoz-Tamayo et al. 2011). Additionally, *Bacteroides* were described as polysaccharide A producers, a metabolite reported to prevent inflammatory bowel disease in mice (Mazmanian, Round, and Kasper 2008). Additionally, a study investigating the impact of red wine consumption for 1 month also showed a significant increase in *Bacteroides* abundance in healthy subjects (Queipo-Ortuno et al. 2012). A limitation of the study by Istas

and colleagues was the use of 16S rRNA sequencing, which presents a lower resolution and does not allow a direct profiling of microbial genes compared with shotgun metagenomic sequencing. Another limitation of this trial is that only young men were recruited in the study, which does not reflect the complexity of metabolic profiles found in the general population.

Two other studies investigating the effects of aronia berry PP on gut microbiota used animal models. A study using a model of high-fat fed rats reported a decrease of the *Firmicute*/*Bacteroidetes* ratio after consumption of aronia berry PP (1g / kg of body weight / day) for 40 days, as well as an increase in the abundance of *Bacteroides*, *Prevotella* and *Akkermansia* (Zhu et al. 2020). Another study involving wild type mice (n= 6) and administrating aronia extract (1.6g / kilo of body weight / day) for one week showed no significance in terms of α diversity measured with Shannon index compared with the control group (Liu, Martin, et al. 2021). Authors also reported that the concentration in propionate in faecal samples was twice as high in the aronia extract group compared with control. Finally, authors revealed that 11 microbial pathways were significantly enriched in the aronia extract group in comparison with control. Among them, pathways related to the tricarboxylic acid cycle, sulfur oxidation, L-lysine fermentation to acetate and butanoate, and biosynthesis of various compounds (thiamine diphosphate, phosphopantothenate, queuosine) were reported.

Finally, a study using an *in vitro* simulation of the human intestinal microbial ecosystem (SHIME) tested the effect of aronia juice (6.5 g/L) on gut microbiome composition and SCFA production. The aronia juice led to a change in the composition of SCFA with reduction of the concentration of

acetate in favour of an augmentation of the propionate and butyrate concentrations. (Wu, Grootaert, et al. 2018). Besides, a significant increase in the concentration of *Firmicutes* and *Akkermansia* bacteria following the treatment with aronia juice was found, which agrees with the results from the *in vivo* study on rats. Of interest, the main bacteria involved in the catabolism of flavonols and flavan-3-ols present in aronia berries belong to the *Firmicutes* phylum (Cueva et al. 2016). Moreover, *Akkermansia* was noted as a beneficial genus implicated in the regulation of cardiometabolic diseases by contributing to the metabolism homeostasis and producing SCFA (Lyu et al. 2017).

6.1.6. Studies evaluating the effect of other berries on gut microbiota

6.1.6.1. Animal studies

Several studies have investigated the effect of different berry PP on rodents (Table 6.1). Animal model provides an insight of the response to PP interventions, through long term experimentations (4 to 21 weeks) and the use of doses which would be dietarily achievable if applied to the human model.

Across many of the studies, the most consistent effect was the enrichment in beneficial *Bifidobacterium* and *Akkermansia*, independently from the diet or the health status of the animal (Anhê et al. 2015; Pan et al. 2017; Molan, Liu, and Kruger 2010; Lacombe et al. 2013; Petersen et al. 2019). A reduction in the abundance of *Clostridiales* was also reported in several studies, following blackcurrant, black raspberry, and blueberry PP supplementation, in healthy rodents but also in mice with inflammatory bowel disease (Molan, Liu, and Kruger 2010; Gu et al. 2019; Paturi et al. 2012). Moreover, the enrichment in

the butyrate-producing species *Oscillibacter* and *Pseudoflavonifractor* was noted for both high-fed rats and rats with normal diet following the 17-week intake of blackberry PP (Marques et al. 2018).

Furthermore, a study by Overall and colleagues comparing the effects of 6 berries (blackberry, blackcurrant, black raspberry, blueberry, Concord grape and maqui berry) presenting structurally different anthocyanin profiles on the gut microbiota in high-fed mice, showed different results in microbiota abundance, implicating a PP- or metabolite-specific action of the berry on colonic bacteria based on the structure (Overall et al. 2017).

Table 6.1: Summary of animal intervention investigating the impact of berry PP on gut microbiome.

Species / health status	Intervention (/day)	Duration (w)	Results	Reference
HFD mice	400 µg ACN from **blackberry** and **black raspberry**	12	No effect on microbiota	(Overall, Bonney et al. 2017)
HFD mice	400 µg ACN from **blueberry**	12	↑ *Bacteroidetes* and *Actinobacteria*	
HFD and normal diet rats	24 mg/kg/day of **blackberry** PP	17	HFD: ↑ *Pseudoflavonifractor, Oscillibacter*; normal diet: ↑ *Oscillibacter* and ↓ *Rumminococcus*	(Marques, Fernandes et al. 2018)
Healthy rats	2 mL **blackcurrant** extract	4	↑ *Bifidobacteria, Lactobacilli* ↓ *Bacteroides, Clostridia*	(Molan, Liu et al. 2010)
Healthy rats	5% **black raspberry** powder	6	↑ *Akkermansia, Desulfovibrio, Anaerostipes*	(Pan, Lam et al. 2017)
Healthy mice	10% **black raspberry** powder	6	↑ *Barnesiella*, ↓ *Clostridium*, *Lactobacillus*	(Gu, Thomas-Ahner et al. 2019)
Healthy rats	8% **blueberry** powder	6	↓ *Lactobacillus*, *Enterococcus*, ↑ *Bifidobacteriales*	(Lacombe, Li et al. 2013)
Mice with IBD	10% **blueberry** powder	21	↓ *Clostridium perfringens*, *Enterococcus* spp., *Lactobacillus* spp., *Escherichia coli*	(Paturi, Mandimika et al. 2012)
Diabetic mice	2.5% **strawberry** powder	10	↑ *Bifidobacterium*, ↓ *Verrucomicrobia*	(Petersen, Wankhade et al. 2019)
HFD mice	200 mg/kg/day **cranberry** extract	9	↑ *Akkermansia*	(Anhê, Roy et al. 2015)

ACN, anthocyanins; HFD, high fat diet; IBD, inflammatory bowel disease; PP, (poly)phenols.

6.1.6.2. Human studies

Very few human trials have investigated the impact of berries and berry PP on the gut microbiota, and the existing ones involve short- to medium-term supplementation (3 to 8 weeks) within a small number of individuals (n< 46) (Table 6.2).

A first study involving 46 middle-aged obese men and women and the consumption of 215 mg of anthocyanins from blueberry and blackcurrant daily for 8 weeks reported an increase in the abundance of *Bacteroidetes* and decrease in *Firmicutes*, *Actinobacteria*, leading to a reduction of the *Firmicute/Bacteroidetes* ratio (Hester et al. 2018). While 16S rRNA sequencing was used and the administered dose was dietarily relevant, findings cannot apply to a healthy population of subjects with BMI< 25 kg/m^2.

In contrast, 2 studies including healthy adults found rather contrasted results. A first one recruiting only men and studying the effect of a blueberry PP supplementation for 6 weeks observed an increase in the abundance of the beneficial *Bifidobacterium* spp. and *Lactobacillus acidophilus* (Vendrame et al. 2011). Quite the opposite, a trial composed of 10 healthy women instructed to consume a red grape pomace extract for 3 weeks did not report any change in the gut microbiome abundance (Gil-Sánchez et al. 2018). These 2 studies were led on a short period of time (≤ 6 weeks) and included only a very small sample size (n≤ 20). Moreover, a limitation of these trials was the use of qPCR sequencing technique, which was proven to show a reduced resolution compared to shotgun metagenomic sequencing (Durazzi et al. 2021).

Table 6.2: Summary of human intervention investigating the impact of berry PP on gut microbiome.

Population	Health status	Intervention (/day)	Duration (w)	Sequencing method	Results	Reference
34 ♀ 12 ♂ MA: 43y	Obese	215 mg ACN from **blueberry** and **blackcurrant**	8	16S rRNA	↑ *Bacteroidetes*, ↓ *Firmicutes*, *Actinobacteria*, F/B ratio	(Hester, Mastaloudis et al. 2018)
20 ♂ MA: 46y	Healthy	10% **blueberry** powder in water	6	qPCR	↑ *Bifidobacterium* spp., *Lactobacillus acidophilus*	(Vendrame, Guglielmetti et al. 2011)
10 ♀ Age: 25-65y	Healthy	1.4g **red grape pomace** extract	3	qPCR	No significant change in gut microbiota	(Gil-Sánchez, Esteban-Fernández et al. 2018)

ACN, anthocyanins; F/B, *Firmicutes/Bacteroidetes*; MA, mean age; PP, (poly)phenols; SG, shotgun.

Data from clinical trials investigating the role of gut microbiota function and composition on the cardiovascular health benefits of PP is scarce and lacking (Cassidy and Minihane 2017). The objective of this chapter is to investigate the impact of aronia berry PP consumption on gut microbiome abundance and composition, and to explore associations between gut microbiome, aronia PP metabolites and vascular outcomes, in the ABP population.

6.2. Methods

Faecal samples were collected at baseline and after 12 weeks of daily consumption of the aronia extract or the placebo and immediately stored at -80°C. Detailed information regarding the collection and processing of faecal samples can be found in Chapter 2, section 2.6.3.

As stated in Chapter 2, section 2.9, the Per Protocol (PerP) population included the Intention To Treat (ITT) participants without any major protocol deviations and who have taken at least 80% of the study treatment. Sixteen samples were not analysed in this chapter due to drop-outs (n= 5), consumption of antibiotics within 3 months (n= 4), history of aortic stenosis (n= 1), abnormal rise of cholesterol level associated with thyroid deficiency (n= 1), suspicion of flu at visit 2 (n= 1), low compliance (n= 1), abnormal increase in GGT, ALT and TG at visit 2 along with a high alcohol consumption at screening visit (n= 1), or missing faecal samples (n= 2). As a result, the remaining PerP population assessed in this chapter consisted of 86 subjects evenly split into Aronia group (n= 43) and Control group (n= 43).

6.3. Results

6.3.1. Quality check of homogeneity at different timepoints

A quality check for data homogeneity in read counts was conducted across all timepoints and treatments (baseline and 12 weeks, in Aronia and Control groups). No significant difference was found (Figure 6.2), indicating a homogeneous quality of sequencing between groups.

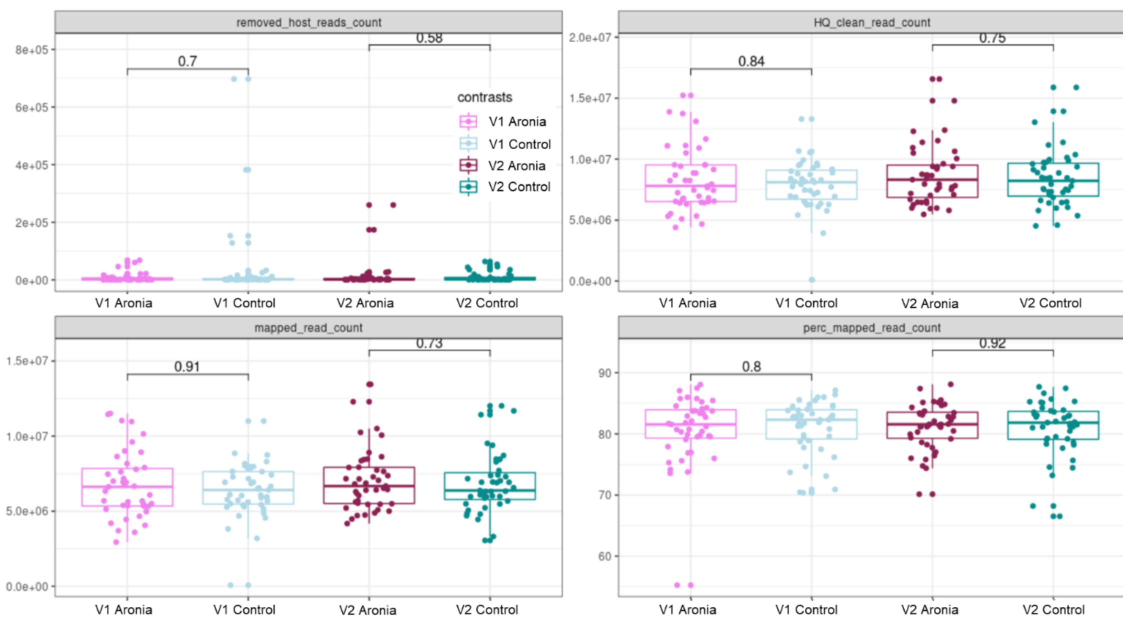


Figure 6.2: Boxplot of read counts between Aronia and Control groups at baseline (V1) and at the end of trial (V2). The significance was tested by applying unpaired Wilcoxon rank-sum tests. Subjects were coloured according to treatment and time points: Aronia subjects at baseline (pink), after 12 weeks (burgundy). Control subjects at baseline (light blue) and after 12 weeks (green).

6.3.2. Overall similarity and hierarchical clustering

A Spearman correlation between samples was performed using the MSP abundance profile matrix quantified in each subgroup (Figure 6.3, Aronia; Figure 6.4, Control). The hierarchical clustering of the samples was performed

using the Ward's method and the colours of rows and columns defined the treatments and timepoints. All individual samples were correctly clustered, indicating that each volunteer's microbiome was consistent between visit 1 and visit 2. However, we observed an unpaired clustering for subject ABP042, with a microbiome similarity (baseline versus 12 weeks) of ρ= 0.15, which was considered too low. To avoid any bias in the analysis which may be caused by this participant's microbiome, this subject was excluded from further analysis. Therefore, the PerP population consisted of 85 subjects, distributed between Aronia group (n= 42) and Control group (n= 43).

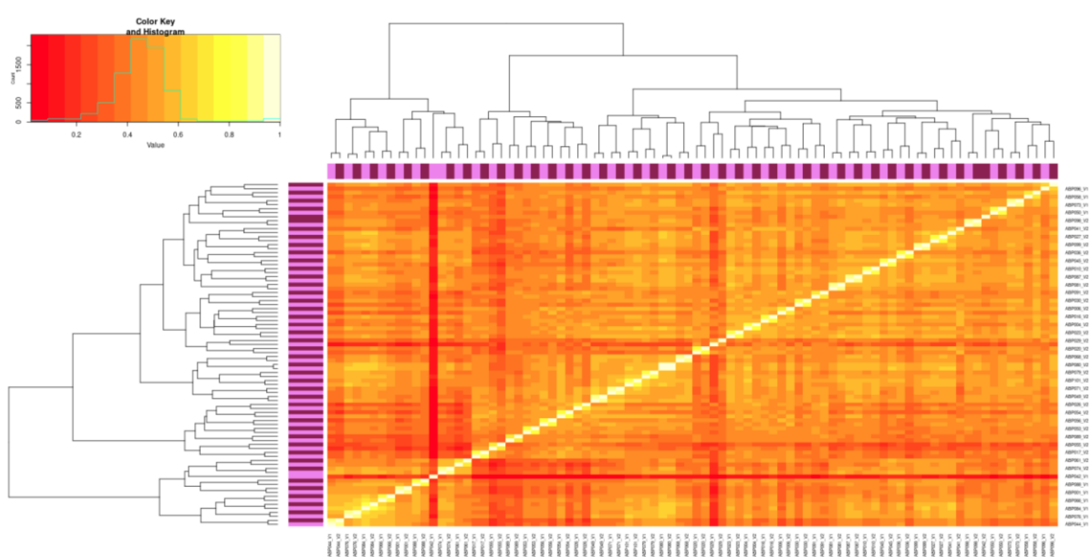


Figure 6.3: Spearman correlation between all Aronia samples at species level. Aronia group subjects at baseline (pink) and after 12 weeks (dark violet).

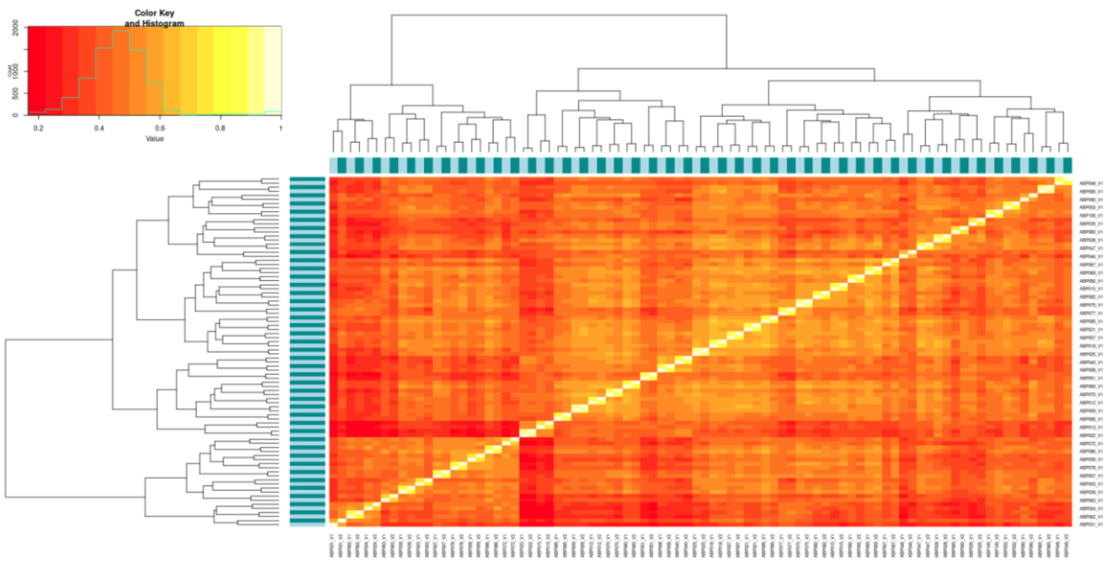


Figure 6.4: Spearman correlation between all Control samples at species level. Control subjects at baseline (light blue) and after 12 weeks (green).

6.3.3. Species abundance and gene richness

The richness of the samples was evaluated at gene and MSP levels. The number of genes seen in a sample defined the gene count while the number of observed species in a sample determined the MSP count. At baseline, no differences were observed between Aronia and Control groups either for MSP count (Figure 6.5 A) or gene count (Figure 6.5 B). Significant differences were found for gene count when considering the changes from baseline between Aronia and Control groups (p= 0.021) (Figure 6.6). These results indicate that consumption of Aronia extract for 12 weeks led to an increase in the gene count, suggesting a favourable increase in the gut microbiota richness after aronia consumption.

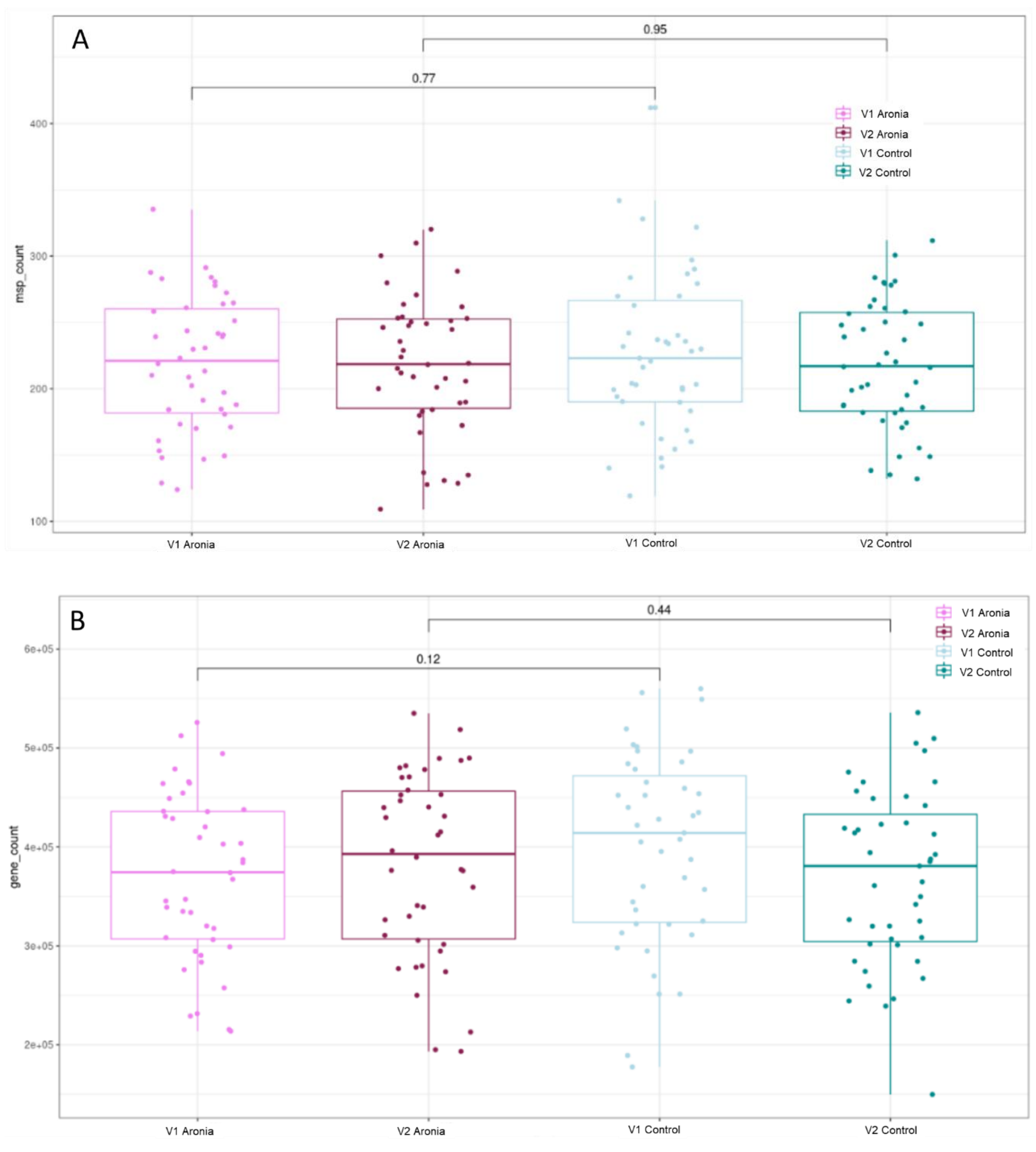


Figure 6.5: Boxplot showing differences in species abundance and gene richness. A) MSP count (number of observed species in a sample); B) gene count. P-values refer to unpaired two-samples Wilcoxon test. Subjects belonging to different categories were coloured according to treatment and time points: Aronia subjects at baseline (pink) and after 12 weeks (burgundy). Control subjects at baseline (light blue) and after 12 weeks (green).

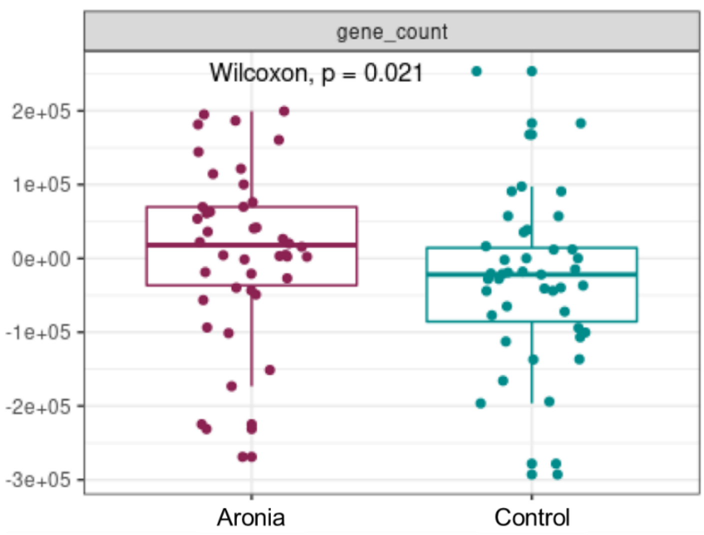


Figure 6.6: Boxplot showing changes from baseline in gene count after 12-week intervention. The significance was tested by applying unpaired Wilcoxon rank-sum tests for variation at 12 weeks compared with baseline in Aronia versus Control groups.

In addition, a non-parametric analysis for longitudinal data was applied to investigate the effect of the two treatments, time, and influence of other factors, respectively. Time was reported as the main parameter significantly influencing gene count for both Aronia and Control groups (Table 6.3). Moreover, age, gender, physical activity, diet, and smoking habits did not influence the gene count.

Table 6.3: Summary of the relative treatment effect upon gene count.

Call	Relative treatment effect	P-value
Treatment	0.17	0.67
Time	0.96	0.32
Treatment * Time	**5.25**	**0.02**
Treatment * Time * Age	0.5	0.87
Treatment * Time * Gender	0.29	0.58
Treatment * Time * Physical activity	0.42	0.58
Treatment * Time * Diet	0.3	0.6
Treatment * Time * Smoking habits	0.2	0.6

6.3.4. Alpha diversity

Alpha diversity translates the richness and evenness within a sample or an environment. Here, Shannon and Simpson indices were calculated for both Aronia and Control group at the different timepoints (Table 6.4). No significant differences were found at baseline between Aronia and Control groups for the 2 alpha diversity indices.

Table 6.4: Characteristics of Shannon-Wiener and Simpson indices at baseline and after 12-week daily supplementation, in Aronia and Control groups.

	Aronia			Control		
Index	V1 (n = 42)	V2 (n = 42)	ΔV2 - V1	V1 (n = 43)	V2 (n = 43)	ΔV2 - V1
Shannon	3.99 ± 0.41	3.97 ± 0.61	-0.03 ± 0.68	4.11 ± 0.46	3.98 ± 0.55	-0.12 ± 0.53
Simpson	0.95 ± 0.03	0.94 ± 0.09	-0.01 ± 0.09	0.96 ± 0.04	0.95 ± 0.05	-0.01 ± 0.05

V1: Visit 1; V2: Visit 2

No significant changes in alpha diversity were found following the 12-week consumption of aronia extract when compared with baseline and with the control group (Table 6.5).

Table 6.5: Changes from baseline in Aronia group and changes from Control of Shannon-Wiener and Simpson indices following the 12-week intervention.

Index	CFB Aronia	CFC	Sig.
Shannon	-0.06 [-0.23; 0.11]	0.033	NS
Simpson	-0.01 [-0.034; 0.01]	-0.006	NS

CFB: Changes from baseline; CFC: Changes from Control; NS: Non significant. Differences were calculated using ANCOVA (Bonferroni) with baseline values as covariate, comparing Aronia versus Control changes from baseline.

6.3.5. Beta diversity

Variations in microbial communities between samples belonging to different groups (Aronia versus Control groups) and timepoints (visit 1 versus visit 2)

were evaluated through a Bray-Curtis dissimilarity metric and PCoA plot (Figure 6.7).

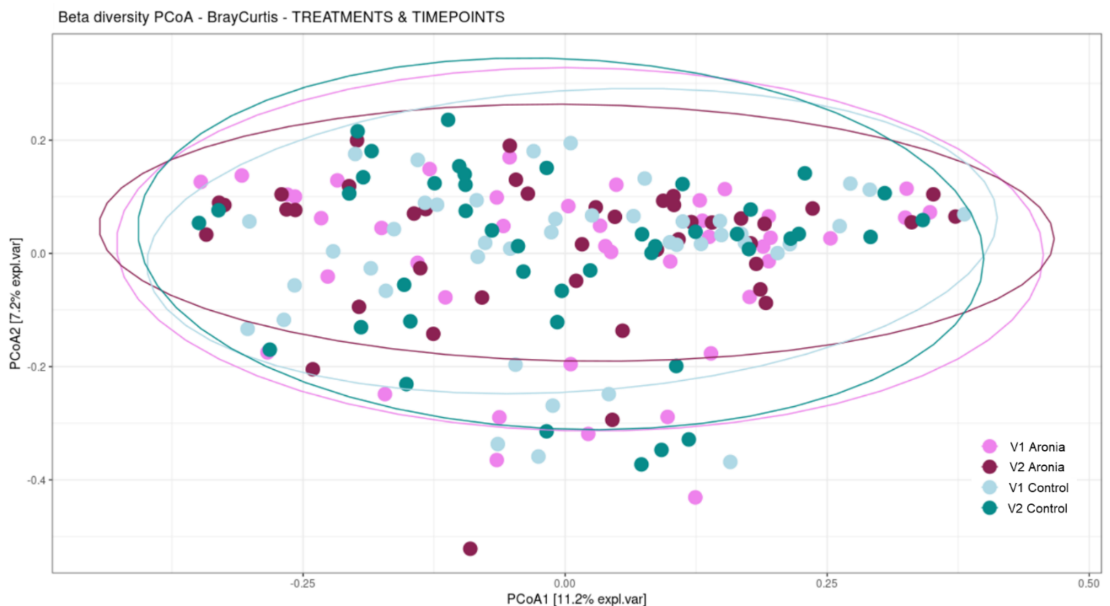


Figure 6.7: PCoA plots of gut microbiome.
Subjects belonging to different categories were coloured according to treatment and timepoints: Aronia subjects at baseline (pink) and after 12 weeks (burgundy). Control subjects at baseline (light blue) and after 12 weeks (green). Ellipsoids represent a 95% confidence interval surrounding each group.

The Bray-Curtis test was not significant, indicating that there were no differences between treatments groups and timepoints considering the overall gut microbiome composition.

6.3.6. Baseline gut microbiome abundance and composition

To be able to compare the changes in gut microbiome profile after 12 weeks, the analysis of the abundance and composition of bacterial species at baseline was investigated. The bar plot in Figure 6.8 shows the cliff delta value (a nonparametric effect-size measure which quantifies how often one value in distribution A is higher than the values in distribution B) of all species significantly and relevantly contrasted (relevance defined as absolute value of

cliff delta > 0.2) between Aronia and Control groups. Differences in abundance of bacteria were found between Aronia and Control groups at baseline. At baseline, a total of 18 bacteria were significantly more abundant in Control group (in red) while 4 species were more abundant in Aronia group (in blue) (Figure 6.8 and Table 6.6).

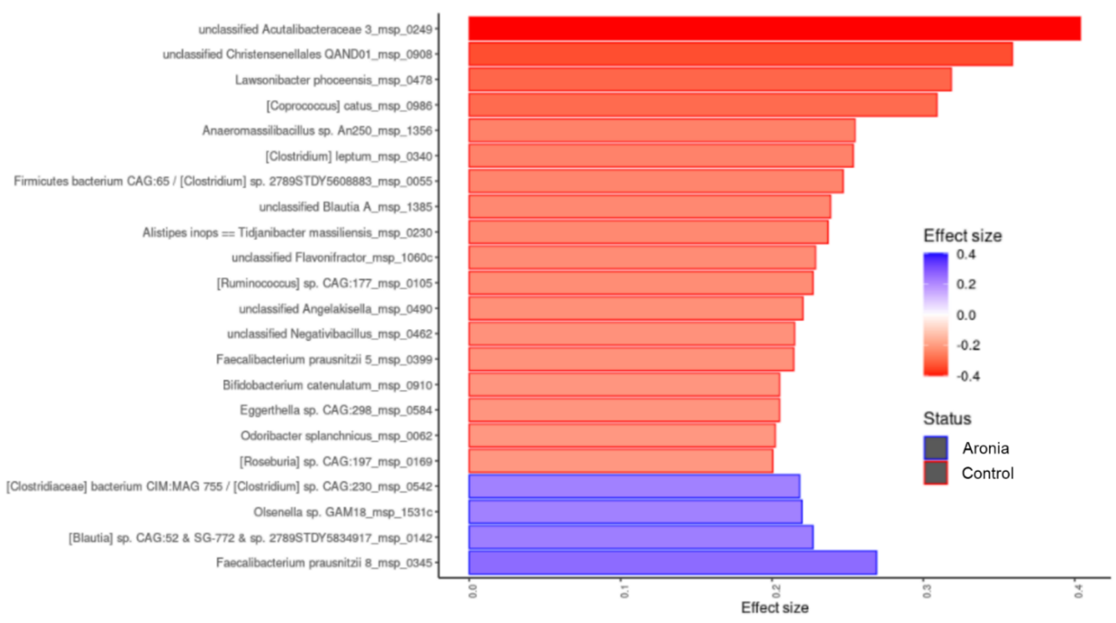


Figure 6.8: Bar plot of the species significantly different between Aronia and Control groups at baseline. In blue, Aronia-related species and in red, Control-related species.

Table 6.6: Species significantly different between Aronia and Control groups at baseline. In blue, Aronia-related species and in red, Control-related species.

Control	Aronia
Unclassified *Acutalibacteraceae* 3	*Blautia* sp.
Unclassified *Christensenellales*	*Olsenella* sp.
Lawsonibacter phoceensis	*Clostridiaceae* bacterium / *Clostridium* sp.
Coprococcus catus	*Faecalibacterium prausnitzii* 8
Anaeromassilibacillus sp.	
Clostridium leptum	
Firmicutes bacterium / *Clostridium* sp.	
Unclassified *Blautia* A	
Tidjanibacter massiliensis	
Unclassified *Flavonifractor*	
Ruminococcus sp.	
Unclassified *Angelakisella*	
Unclassified *Negativibacillus*	
Faecalibacterium prausnitzii 5	
Bifidobacterium catenulatum	
Eggerthella sp.	
Odoribacter splanchnicus	
Roseburia sp.	

6.3.7. Effects of Aronia supplementation in gut microbiota abundance and composition

A similar analysis of the bacterial abundance and composition was conducted for the changes from baseline to depict the differences in species abundance between the 2 treatment groups following 12-week daily intake of aronia extract or placebo (Figure 6.9). A total of 18 and 4 species were significantly more abundant in Aronia (in blue) and Control (in red) groups, respectively. Among them, *Intestinimonas butyriciproducens* was the most abundant bacteria in the Aronia group compared to Control, following the 12-week intervention. Other bacteria significantly more abundant in Aronia group were

Clostridiales bacterium, *Oscillibacter* sp. *Firmicutes* bacterium, *Lawsonibacter phoceensis*, *Oscillospirales* 5, *Clostridium* sp., *Butyricimonas faecihominis*, *Turicibacter sanguinis*, *Bacteroides dorei*, *Oscillospiraceae*, *Bacteroides xylanisolvens, Ruminococcus* sp. /*Blautia* sp., *Dialister invisus*, *Flavonifractor* sp. /*Clostridium* sp., *Faecalibacterium prausnitzii* 2, *Christensenellales*, and *Blautia* A (Figure 6.9 and Table 6.7).

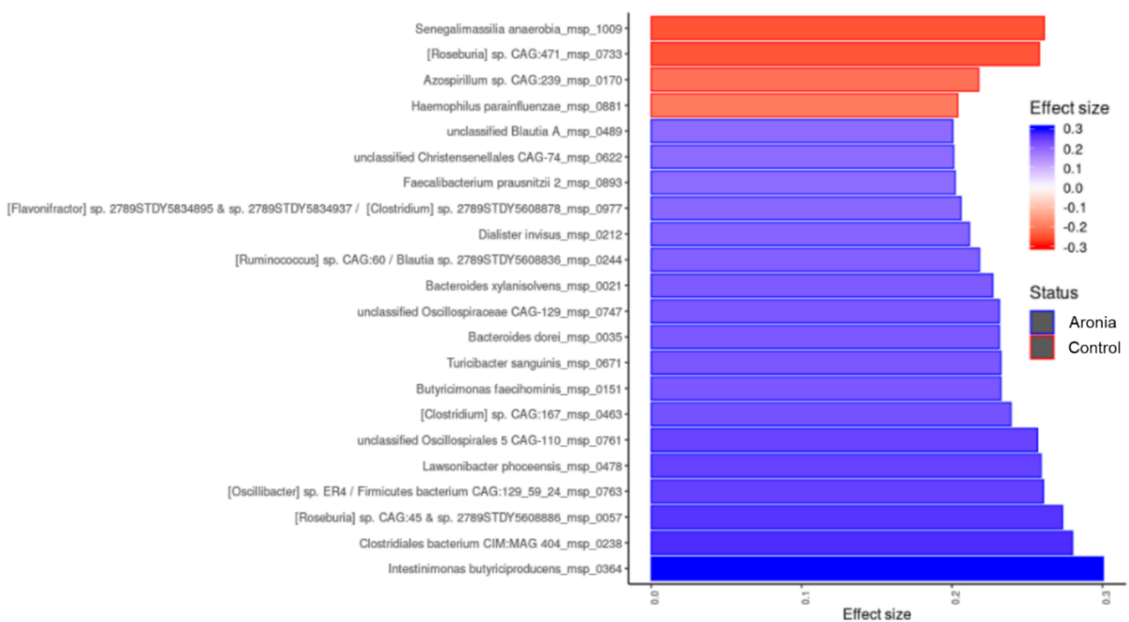


Figure 6.9: Bar plot of the species significantly different between Aronia and Control groups after considering the changes from baseline. In blue, Aronia-related species and in red, Control-related species.

Table 6.7: Species significantly different between Aronia and Control groups after considering the changes from baseline. In blue, Aronia-related species and in red, Control-related species.

Control	Aronia
Senegalimassilia anaerobia	Unclassified *Blautia* A
Roseburia sp.	Unclassified *Christensenellales*
Azospririllum sp.	*Faecalibacterium prausnitzii* 2
Haemophilus parainfluenzae	*Flavonifractor* sp. / *Clostridium* sp.
	Dialister invisus
	Ruminococcus sp. / *Blautia* sp.
	Bacteroides xylanisolvens
	Unclassified *Oscillospiraceae*
	Bacteroides dorei
	Turicibacter sanguinis
	Butyricimonas faecihominis
	Clostridium sp.
	Unclassified *Oscillospirales* 5
	Lawsonibacter phoceensis
	Oscillobacter sp. / *Firmicutes* bacterium
	Roseburia sp.
	Clostridiales bacterium
	Intestimonas butyriciproducens

Furthermore, to understand the effect of aronia berry PP consumption on gut microbiota abundance and composition, further analysis was done comparing baseline versus 12-week timepoints in the intervention group. Abundance of *Intestinimonas butyriciproducens* ($p < 0.001$) (Figure 6.10 A) significantly increased in the Aronia cohort following 12-week intervention, while an inverse trend for *Ruminococcus* ($p < 0.01$) (Figure 6.10 B) was observed.

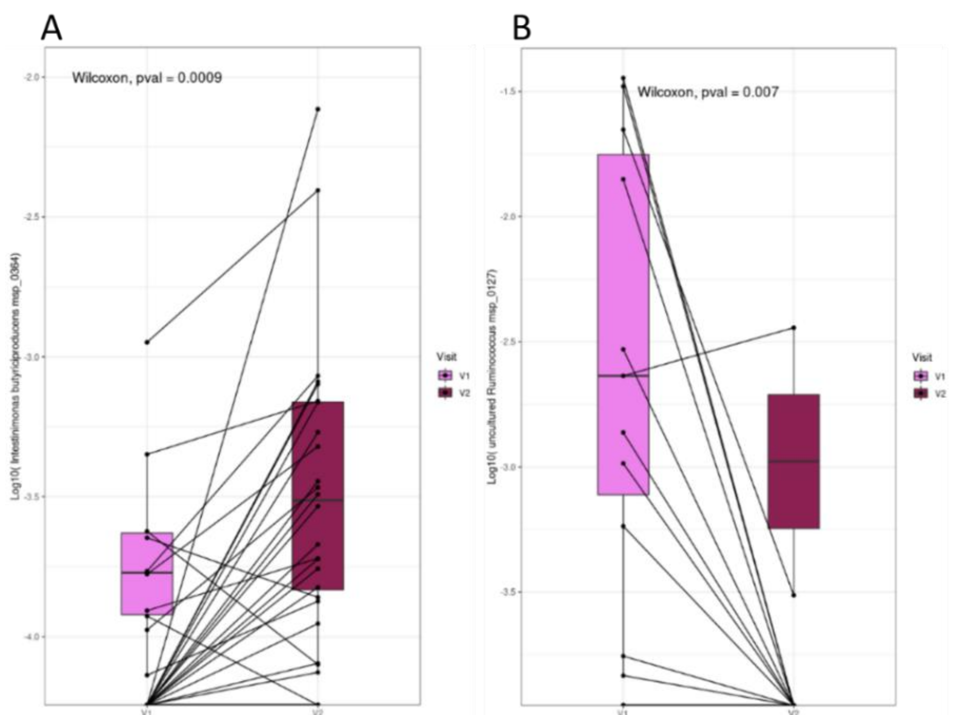


Figure 6.10: Boxplot showing differently abundant species in the Aronia group. P-values refer to paired two-samples Wilcoxon test. Subjects belonging to different timepoints were coloured accordingly. Aronia subjects at baseline (pink), after 12 weeks (burgundy). A: Intestinimonas butyriciproducens; B: uncultured Ruminococcus.

6.3.8. Function pathways related to gut microbiome composition

The beneficial effect of treatment on gut microbiome and its impact on several functional relevant pathways (|cliff delta| > 0.2) was investigated (Table 6.8). Thirteen pathways were identified as relevant and associated with Aronia group including 7 statistically significant. Among them, γ-aminobutyric acid (GABA) production, a neurotransmitter produced by bacteria such as *Lactobacillus* to decrease the intracellular pH (Strandwitz 2018); histidine degradation and pyruvate to ferredoxin reduction, both implicated in the formation of the SCFA acetate (Andreu et al. 2021; Li, Yin, et al. 2020); γ-hydroxybutyrate degradation, related to the pathway resulting in the production of butyrate (Louis and Flint 2017); superoxide dismutase, an

antioxidant enzyme involved in the oxidative stress response; polysaccharide A, a capsular carbohydrate found in *Bacteroides fragilis* and presenting anti-inflammatory properties (Alvarez et al. 2020), and pathways involved in the production of the SCFA propionate. Three pathways were related with Control group, including 2 statistically significant.

Table 6.8: Functional pathways related to Aronia and Control groups when considering changes from baseline.

Pathways	Group	Cliff delta	p-value
Lipopolysaccharide transport system	Aronia	0.24	0.060
Phosphotransferase system	Aronia	0.28	**0.028**
MtrB-MtrA (osmotic stress response) regulatory system	Aronia	0.25	**0.047**
Propionate synthesis II	Aronia	0.22	0.080
ϒ-Hydroxybutyrate degradation	Aronia	0.24	0.053
Polysaccharide A	Aronia	0.28	**0.022**
Superoxide dismutase	Aronia	0.21	0.091
Propionate production	Aronia	0.22	0.080
Pyruvate:ferredoxin oxidoreductase	Aronia	0.27	**0.035**
Histidine degradation	Aronia	0.30	**0.018**
GABA biosynthesis	Aronia	0.22	**0.037**
Siroheme biosynthesis	Aronia	0.23	0.068
Nitrogen fixation	Aronia	0.26	**0.028**
Osmoprotectant transport system	Control	-0.27	**0.035**
LiaS-LiaR (cell wall stress response) regulatory system	Control	-0.24	0.058
Pyrimidine deoxyribonucleotide biosynthesis	Control	-0.24	**0.042**

6.3.9. Multi-omics analysis to investigate associations between gut microbiome, cardiometabolic outcomes and circulating PP metabolites

Our objective in this section was to look for the potential interactions between gut microbiome species, clinical outcomes and circulating PP metabolites. More precisely, we focused on the 18 microbial taxa enriched in the Aronia group following the 12-week intervention (as seen in section 6.3.7) and their association with of clinical parameters improvement and the abundance of

metabolites derived from aronia-PP related compounds. For each analysis, changes from baseline (for urinary and plasma metabolites, as well as clinical outcomes) were correlated with the changes from baseline of the 18 bacteria's abundance.

6.3.9.1. Correlations between microbial species and plasma PP metabolites after aronia consumption

In the group of volunteers receiving the aronia extract, a total of 43 plasma metabolites correlated positively with 8 different microbial species (Table 6.9). Among the most significant, plasma 4-methylgallic acid-3-sulfate and 1-methylpyrogallol-sulfate correlated with *Bacteroides xylanisolvens* ($p < 0.01$), while 2-hydroxybenzoic acid and dihydroisoferulic acid-3'-sulfate correlated significantly with *Flavonifractor* and *Clostridium* species ($p < 0.01$ and $p < 0.001$, respectively).

Table 6.9: Summary of positive and significant correlations between Aronia PP-related plasma metabolites and gut microbiome (, p< 0.05, **, p< 0.01, and ***, p< 0.001).*

Metabolite	Full / usual name	Bacterial species	p-value
Kaempferol-3-GlcUA	Kaempferol-3-glucuronide		*
3'-MeO-CA-4'-S	Ferulic acid-4'-sulfate		*
3-(4'-OH-ph)-PrA-3'-S	Dihydrocaffeic acid-3'-sulfate	*Clostridium* sp.	*
3-(3',5'-diOH-ph)-PrA	3-(3',5'-Dihydroxyphenyl)propanoic acid		*
4-CQA	Cryptochlorogenic acid		*
3-(3',4'-diOH-ph)-PrA	Dihydrocaffeic acid		*
2-OH-BA	2-Hydroxybenzoic acid		**
3-(4'-MeO-ph)-PrA-3'-S	Dihydroisoferulic acid-3'-sulfate		***
3-OH-BA	3-Hydroxybenzoic acid		*
3-(2',4'-diOH-ph)-PrA	3-(2',4'-Dihydroxyphenyl)propanoic acid		*
4-OH-BAL	4-Hydroxybenzaldehyde	*Flavonifractor* sp. / *Clostridium* sp.	*
BA	Benzoic acid		*
4'-OH-CA	p-Coumaric acid		*
3'-MeO-PA-4'-S	Homovanillic acid-sulfate		*
Myricetin	Myricetin		*
3-(4'-OH-ph)-2-OH-PrA	2-Hydroxy-3-(4'-hydroxyphenyl)propanoic acid		*
Quercetin	Quercetin		*

4'-OH-HA	4'-Hydroxyhippuric acid	*Flavonifractor sp. / Clostridium sp.*	*
3-OH-2-MeO-Benz-1-S	2-Methylpyrogallol-sulfate		*
CA-4'-S	p-Coumaric acid-4'-sulfate		*
4-OH-3-MeO-BA	Vanillic acid		*
4-OH-BA	4-Hydroxybenzoic acid		*
2'-OH-HA	2'-Hydroxyhippuric acid		*
PA	Phenylacetic acid		*
4-OH-3,5-diMeO-BA	Syringic acid		*
3-OH-BA	3-Hydroxybenzoic acid	*Roseburia* sp.	*
4'-OH-3'-MeO-CA	trans-Ferulic acid	*Ruminococcus* sp. / *Blautia* sp.	*
Quercetin-3-S	Quercetin-3-sulfate	*Bacteroides xylanisolvens*	*
BA	Benzoic acid		*
3'-MeO-PA-4'-S	Homovanillic acid-sulfate		*
CA	Cinnamic acid		*
3-(3'-MeO-ph)-PrA-4'-S	Dihydroferulic acid-4'-sulfate		*
3,4-diOH-BA-3-S	Protocatechuic acid-3-sulfate		*
HA	Hippuric acid		*
3-(2',3'-diOH-ph)-PrA	3-(2',3'-Dihydroxyphenyl)propanoic acid		*
3-OH-4-MeO-BA-5-S	4-Methylgallic acid-3- sulfate		*
2-OH-3/6-MeO-Benz-1-S*	1-Methylpyrogallol-sulfate		**
2,6-diOH-Benz-1-S	Pyrogallol-2-O-sulfate		**
3-(4'-OH-ph)-PrA-3'-S	Dihydrocaffeic acid-3'-sulfate		*
Quercetin-3-GlcUA	Quercetin-3-glucuronide		*
4'-MeO-CA-3'-S	Isoferulic acid-3'-sulfate		*
3'-OH-HA	3'-Hydroxyhippuric acid		*
3'-MeO-CA-4'-GlcUA	Ferulic acid-4'-glucuronide		*
3-MeO-BA-4-S	Vanillic acid-4-sulfate		*
4/5-CQA*	Chlorogenic acid		*
3'-OH-CA-4'-S	Caffeic acid-4'-sulfate	*Clostridiales* bacterium	*
3-(4'-OH-ph)-PrA-3'-S	Dihydrocaffeic acid-3'-sulfate		*
2-(4'-OH-ph-O)-PrA	2-(4'-Hydroxyphenoxy)propanoic acid		*
3,4-diOH-BA-3-GlcUA	Protocatechuic acid-3-glucuronide	*Dialister invisus*	*
3,4-diOH-BAL	3,4-Dihydroxybenzaldehyde	Unclassified *Blautia* A	*

*: p< 0.05; **: p< 0.01; ***: p< 0.001

A total of 7 plasma metabolites were significantly and negatively correlated with 3 microbial species in the Aronia group: unclassified *Blautia* A, *Turicibacter sanguinis*, and *Oscillibacter* sp./ *Firmicutes* bacterium (Table 6.10).

Table 6.10: Summary of negative and significant correlations between Aronia PP-related plasma metabolites and gut microbiome (, p< 0.05, **, p< 0.01).*

Metabolite	Full / usual name	Bacterial species	p-value
3'-MeO-CA-4'-S	Ferulic acid-4'-sulfate	*Oscillibacter* sp. / *Firmicutes* bacterium	*
3,4-diOH-BAL	3,4-Dihydroxybenzaldehyde		*
4'-OH-3',5'-diMeO-CA	Sinapic acid		**
3,4,5-triOH-Benz-OEt	Gallic acid ethyl ester	*Turicibacter sanguinis*	**
4'-OH-3'-MeO-CA	trans-Ferulic acid		*
PA	Phenylacetic acid	Unclassified *Blautia* A	*
4-OH-3,5-diMeO-BA	Syringic acid		*

*: p< 0.05; **: p< 0.01

6.3.9.2. Correlations between microbial species and urinary PP metabolites after aronia consumption

In the Aronia group, 35 urinary compounds were positively correlated to the changes from baseline of some bacterial species (Table 6.11). Notably, 3'-hydroxyphenylacetic acid and protocatechuic acid-4-sulfate were strongly correlated (p< 0.001) with *Intestinimonas butyriciproducens* and *Roseburia* sp., respectively. Moreover, quercetin and dihydroisoferulic acid-3'-sulfate were correlated (p< 0.01) with *Christensenellales* and *Lawsonibacter phoceensis*, respectively.

Table 6.11: Summary of positive and significant correlations between targeted Aronia-PP related urinary compounds and gut microbiome (, p< 0.05, **, p< 0.01, and ***, p< 0.001).*

Metabolite	Full / usual name	Bacterial species	p-value
2,3-diOH-Benz-1-S	Pyrogallol-1-O-sulfate	Unclassified *Blautia* A	*
Quercetin	Quercetin	Unclassified *Christensenellales*	**
2,4/2,6-diOH-BA*	2,4-Dihydroxybenzoic acid		*
3,4-diOH-BA	Protocatechuic acid		*
4-OH-BA	4-Hydroxybenzoic acid		*
3'-OH-PA	3'-Hydroxyphenylacetic acid	*Intestinimonas butyriciproducens*	***

3-(2',4'-diOH-ph)-PrA	3-(2',4'-Dihydroxyphenyl)propanoic acid	*Lawsonibacter phoceensis*	*
2-OH-Benz-1-GlcUA	Catechol-1-glucuronide		*
3-(4'-OH-ph)-PrA-3'-GlcUA	Dihydrocaffeic acid-3'-glucuronide		*
3,4-diOH-BA	Protocatechuic acid		*
3,4-diOH-BA-3-GlcUA	Protocatechuic acid-3-glucuronide		*
CA-4'-GlcUA	p-Coumaric acid-4'-glucuronide		*
3'-MeO-CA-4'-S	Ferulic acid-4'-sulfate		*
4'-OH-3'-MeO-CA	trans-Ferulic acid		*
4'-OH-CA	p-Coumaric acid		*
3-(4'-MeO-ph)-PrA-3'-S	Dihydroisoferulic acid-3'-sulfate		**
4-OH-BA	4-Hydroxybenzoic acid		*
Myricetin	Myricetin	*Faecalibacterium prausnitzii 2*	*
3,4-diOH-BA	Protocatechuic acid		*
3,4-diOH-BA-3-S	Protocatechuic acid-3-sulfate		*
3'-OH-CA-4'-GlcUA	Caffeic acid-4'-glucuronide		*
4'-OH-CA-3'-S	Caffeic acid-3'-sulfate		*
3'-OH-CA	m-Coumaric acid		*
3-(4'-MeO-ph)-PrA-3'-S	Dihydroisoferulic acid-3'-sulfate		*
4-OH-BAL	4-Hydroxybenzaldehyde	*Bacteroides dorei*	*
CA	Cinnamic acid	*Butyricimonas faecihominis*	*
3-(2',4'-diOH-ph)-PrA	3-(2',4'-Dihydroxyphenyl)propanoic acid	*Clostridium* sp.	*
4'-MeO-EC	4'-Methoxy-(–)-Epicatechin		*
3,4-diOH-BA	Protocatechuic acid		*
4/5-CQA*	Chlorogenic acid	*Flavonifractor* sp. / *Clostridium* sp.	*
4-CQA	Cryptochlorogenic acid		*
4-FQA	4-O-Feruloylquinic acid		*
3-(4'-MeO-ph)-PrA-3'-S	Dihydroisoferulic acid-3'-sulfate		*
HA	Hippuric acid		*
3'-MeO-EC	3'-Methoxy-(–)-epicatechin		*
2,5-diOH-BA	2,5-Dihydroxybenzoic acid	*Oscillibacter* sp. / *Firmicutes*	*
2,3-diOH-BA	2,3-Dihydroxybenzoic acid		*
CA	Cinnamic acid	*Roseburia* sp.	*
2,6-diOH-Benz-1-S	Pyrogallol-2-O-sulfate		*
2-OH-Benz-1-GlcUA	Catechol-1-glucuronide		*
EC	(–)-Epicatechin		*
3,4-diOH-BA	Protocatechuic acid		*
3,4-diOH-BA-3-S	Protocatechuic acid-3-sulfate		*
3-MeO-BA-4-S	Vanillic acid-4-sulfate		*
3,4-diOH-BA-4-S	Protocatechuic acid-4-sulfate		***

*: p< 0.05; **: p< 0.01; ***: p< 0.001

On the contrary, 12 urinary metabolites were significantly and negatively correlated to some of the microbial species analysed in the Aronia group (Table 6.12). This is the case for example for 4-methylcatechol and 4-methylcatechol-1-sulfate, which were negatively correlated ($p < 0.01$) with

Turicibacter sanguinis, or (–)-epicatechin-3'-sulfate which was correlated with unclassified *Oscillospiraceae*.

Table 6.12: Summary of negative and significant correlations between targeted Aronia-PP related urinary compounds and in gut microbiome (, p< 0.05, **, p< 0.01).*

Metabolite	Full / usual name	Bacterial species	p-value
Kaempferol-3-GlcUA	Kaempferol-3-glucuronide	*Turicibacter sanguinis*	*
2-OH-4/5-Me-Benz-1-S	4-Methylcatechol-1-sulfate		**
1,2-diOH-4-Me-Benz	4-Methylcatechol		**
EC-3'-S	(–)-Epicatechin-3'-sulfate	Unclassified *Oscillospiraceae*	**
CA-4'-GlcUA	p-Coumaric acid-4'-glucuronide		*
CA-4'-S	p-Coumaric acid-4'-sulfate		*
2,3,4-triOH-BA	2,3,4-Trihydroxybenzoic acid	*Ruminococcus* sp. / *Blautia* sp.	*
3-OH-2-MeO-Benz-1-S	2-Methylpyrogallol-sulfate	*Clostridiales* bacterium	*
2-OH-4/5-Me-Benz-1-S	4-Methylcatechol-1-sulfate		*
4-OH-BA	4-Hydroxybenzoic acid		*
2'-OH-HA	2'-Hydroxyhippuric acid		*
3,5-diOH-BA	3,5-Dihydroxybenzoic acid	*Dialister invisus*	*
3-MeO-BA-4-S	Vanillic acid-4-sulfate	*Butyricimonas faecihominis*	*

*: p< 0.05; **: p< 0.01

6.3.9.3. Correlations between microbial species and clinical outcomes after aronia consumption

In the group of volunteers of the Aronia group, 27 clinical outcomes out of the 36 included in the analysis significantly correlated negatively with 11 bacterial species (Figure 6.11). The strongest negative correlations were between changes from baseline in 24h and awake SBP_{ao} and SBP_{br} and changes in *Roseburia* sp. (p< 0.001), as well as between Asleep AIx_{ao} and AIx_{br} and changes in *Oscillospiraceae* (p< 0.01) (Table 6.13), implicating that lower blood pressure correlated with higher bacterial abundance.

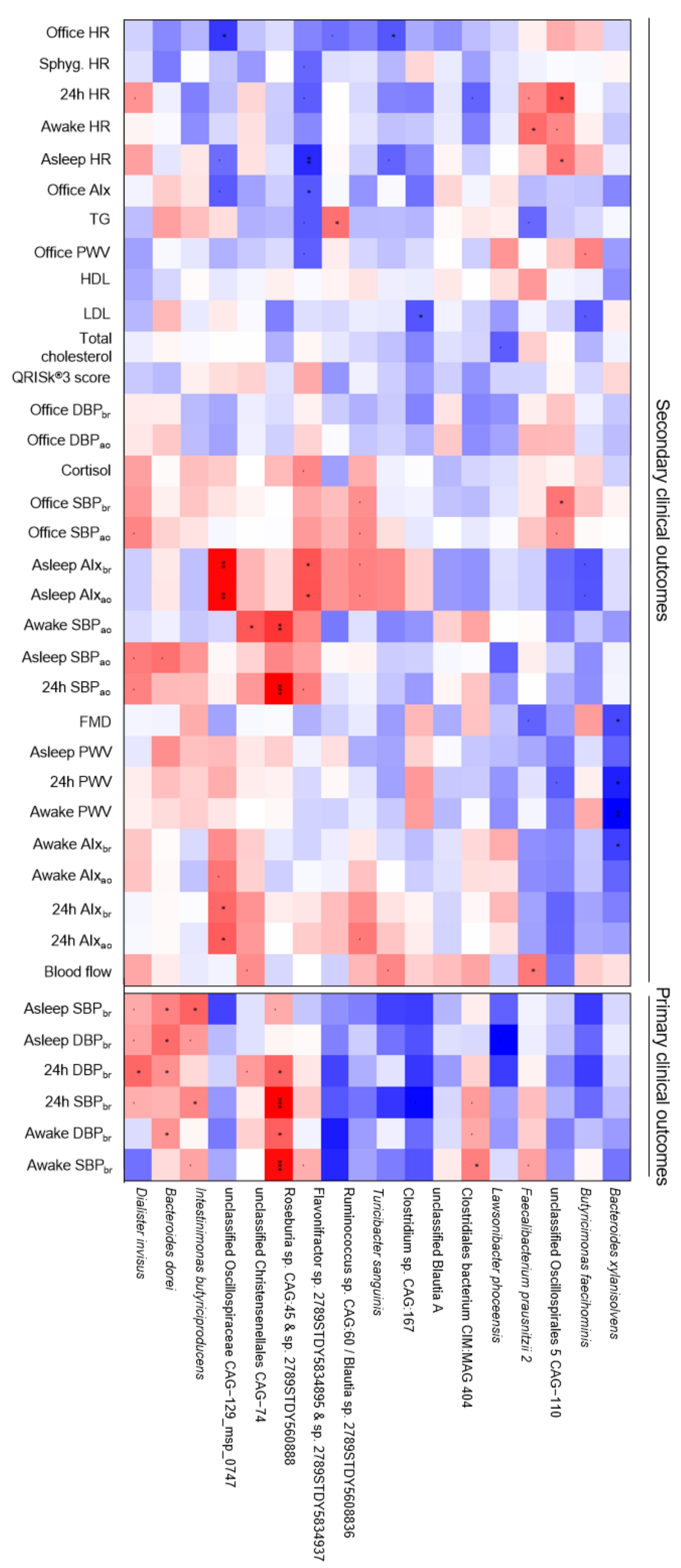


Figure 6.11: Heatmap showing hierarchical Ward-linkage clustering of correlations between clinical metadata and gut microbiome (, p<0.05, **, p<0.01 and ***, p<0.001).*

Table 6.13: Summary of negative and significant correlations between clinical outcomes and significantly different species in gut microbiome (, p<0.05, **, p<0.01 and ***, p<0.001).*

Clinical outcome	Bacterial species	p-value
24h HR	unclassified *Oscillospirales* 5	*
Asleep HR		*
Office SBP_{br}		*
Awake HR	*Faecalibacterium prausnitzii 2*	*
Blood flow		*
Awake SBP_{br}	*Clostridiales* bacterium	*
TG	*Flavonifractor* sp. / *Clostridium* sp.	*
Asleep AIx_{br}	*Oscillibacter* sp. / *Firmicutes* bacterium	*
Asleep AIx_{ao}		*
Awake DBP_{br}	*Roseburia* sp.	*
24h DBP_{br}		*
Awake SBP_{ao}		**
24h SBP_{ao}		***
Awake SBP_{br}		***
24h SBP_{br}		***
Awake SBP_{ao}	unclassified *Christensenellales*	*
24h AIx_{br}	unclassified *Oscillospiraceae*	*
24h AIx_{ao}		*
Asleep AIx_{br}		**
Asleep AIx_{ao}		**
24h SBP_{br}	*Intestinimonas butyriciproducens*	*
Asleep SBP_{br}		*
Asleep SBP_{br}	*Bacteroides dorei*	*
24h DBP_{br}		*
Awake DBP_{br}		*
Asleep DBP_{br}		*
24h DBP_{br}	*Dialister invisus*	*

*: p< 0.05; **: p< 0.01; ***: p< 0.001.

On the contrary, 9 outcomes correlated positively with 5 bacterial species, indicating that the increase in abundance for these bacteria was correlated with an increase for the clinical outcome compared with baseline. The most significant were awake PWV and asleep HR, which were significantly (p< 0.01) correlated *Bacteroides xylanisolvens* and *Oscillibacter* sp., respectively (Table 6.14).

Table 6.14: Summary of positive and significant correlations between clinical outcomes and significantly different species in gut microbiome (, p<0.05, **, p<0.01 and ***, p<0.001).*

Clinical outcome	Bacterial species	p-value
FMD	*Bacteroides xylanisolvens*	*
24h PWV		*
Awake PWV		**
Awake AIx_{br}		*
LDL	*Clostridium* sp.	*
Office HR	*Turicibacter sanguinis*	*
Asleep HR	*Oscillibacter* sp. / *Firmicutes* bacterium	**
Office AIx		*
Office HR	unclassified *Oscillospiraceae*	*

*: p< 0.05; **: p< 0.01.

6.3.10. Role of gut microbiota on the interindividual variability in vascular response to aronia berry PP

6.3.10.1. Cluster analysis to identify Responders and Non-Responders

As shown in Chapter 5, a high variability in the vascular response to Aronia consumption was found among the study population. We hypothesised that this interindividual variability in response could be related to differences in the gut microbiome. Responders (R) versus Non-Responders (NR) were categorized among participants from the Aronia group (n= 42) based on an unsupervised method of clustering k-means. The following variables were included in the model as relevant clinical parameters based on the results of the RCT presented in Chapter 3. All clinical parameters were primary and secondary outcomes of the RCT and included ambulatory blood pressure (BP) (primary outcome), office SBP, ambulatory AIx and PWV, FMD and cortisol levels (Table 6.15). The cut-off decision for each parameter was made based

on clinical relevance, overall magnitude of effects and range of responses in our study, as well as the ability to perform statistical analysis on big enough groups.

Table 6.15: Summary of the parameters included in the cluster analysis, with detail of their cut-off limit.

	Primary / Secondary outcome	Parameters	Cut-off
Ambulatory BP	Primary	Δ 24h SBP_{br} Δ 24h DBP_{br}	≤ -2 vs > -2 mmHg
Office BP	Secondary	Δ SBP_{br}	≤ -2 vs > -2 mmHg
Ambulatory AIx	Secondary	Δ 24h AIx_{ao} Δ 24h AIx_{br} Δ Awake AIx_{ao} Δ Awake AIx_{br}	< 0 vs ≥ 0%
Ambulatory PWV	Secondary	Δ Awake PWV	< 0 vs ≥ 0 m/s
FMD	Secondary	Δ FMD	< 0.7 vs ≥ 0.7%
Cortisol levels	Secondary	Δ Cortisol	< 0 vs ≥ 0 mmol/L

ao, aortic; br, brachial; BP, blood pressure; CVD, cardiovascular disease; DBP, diastolic BP; FMD, flow-mediated dilation; PWV, pulse wave velocity; SBP, systolic BP.

The best number of clusters (k= 2) was defined using the NbClust() function (NbClust R package) on a matrix containing the changes of the given variables. This analysis was repeated 200 times to ensure the robustness of the observations, and 37 out of the 42 volunteers were included in 2 clusters. The other 5 participants were classified as “jumping subjects” as they oscillated from one cluster to another during the repetitions (Figure 6.12).

Therefore, the R group consisted of 23 volunteers (labelled aronia_2 in Figure 6.12) who responded to the aronia berry treatment, while the NR group consisted of 14 volunteers who did not respond to the treatment (labelled aronia_1 in Figure 6.12).

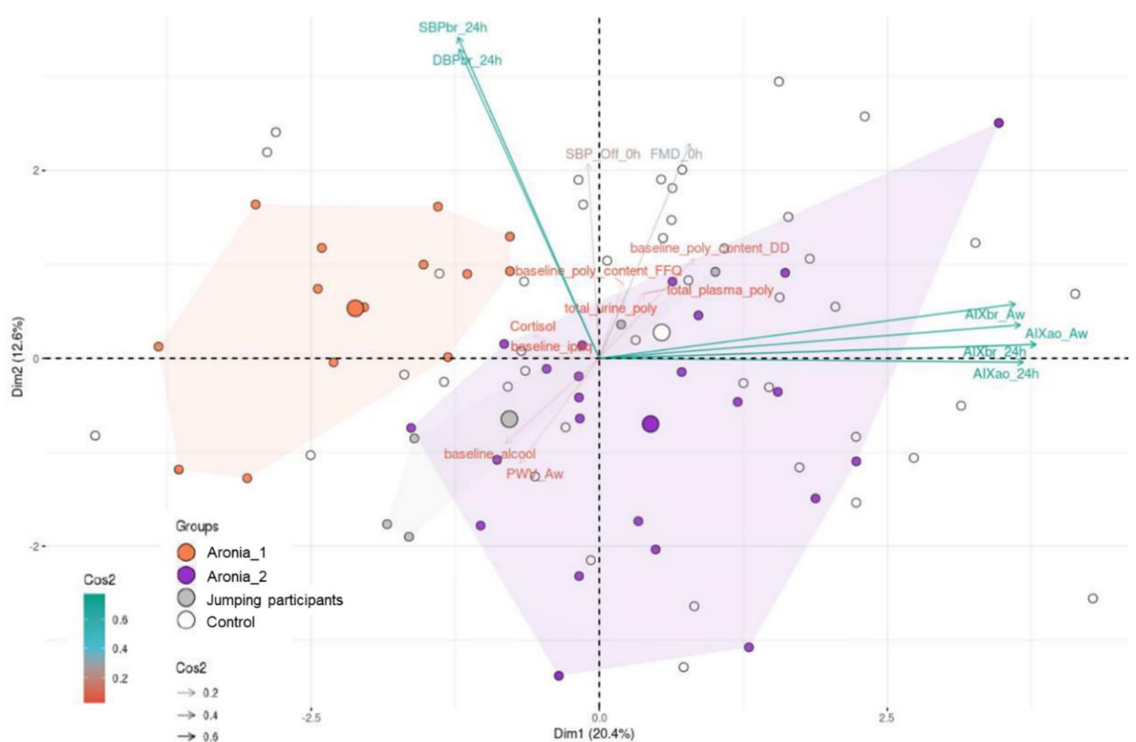


Figure 6.12: Principal component analysis from k-means analysis based on relevant clinical parameters highlighted by the partner after k-means algorithm. Participants in the Aronia group were separated in the aronia_1 (n=14, orange), aronia_2 (n=23, violet) and jumping subjects (n=5, grey).

Table 6.16 below describes the background baseline characteristics of the R and NR groups. No significant differences were observed between R and NR except for our primary outcome 24h SBPbr and DBPbr, which was included in the cluster analysis.

Table 6.16: Background baseline parameters for R and NR.

	Subgroup	N	Mean ± SD
Sex	NR	14	1.5 ± 0.5
	R	23	1.6 ± 0.5
Age (years)	NR	14	59.8 ± 8.3
	R	23	56.1 ± 9.9
Alcohol consumption (unit/week)	NR	14	3.9 ± 5.3
	R	23	4.5 ± 4
Weight (kg)	NR	14	69.9 ± 10
	R	23	71.3 ± 10.4
IPAQ score (MET/min/week)	NR	13	4995 ± 2948
	R	22	5000 ± 3405
BMI (kg/m^2)	NR	14	24.4 ± 3.3
	R	23	24.9 ± 2.9
24h SBP_{br} (mmHg)*	NR	14	115 ± 6.6*
	R	23	124 ± 11*
24h DBP_{br} (mmHg)*	NR	14	66.5 ± 4.5*
	R	23	71.3 ± 5.7*
24h HR (bpm)	NR	14	68.6 ± 8.3
	R	23	70.2 ± 7.8
24h SBP_{ao} (mmHg)	NR	14	112 ± 10
	R	18	120 ± 16.4
24h AIx_{ao} (%)	NR	14	31.7 ± 11
	R	18	28.2 ± 13.2
24h AIx_{br} (%)	NR	14	-11.7 ± 21.8
	R	18	-17.6 ± 24.8
24h PWV (m/s)	NR	14	9.5 ± 1.1
	R	18	9.3 ± 0.9
Office SBP (mmHg)	NR	14	118 ± 7.8
	R	23	125 ± 11.6
Office DBP (mmHg)	NR	14	80.4 ± 6.1
	R	23	80.1 ± 5.9
Office peripheral heart rate (bpm)	NR	14	69.2 ± 13.4
	R	23	65.3 ± 5.7
Office AIx (%)	NR	14	25.3 ± 11.4
	R	23	21.1 ± 9.3
Office PWV (m/s)	NR	14	7.5 ± 1.4
	R	23	7.4 ± 1.6
Office central SBP (mmHg)	NR	14	112 ± 8.6
	R	23	117 ± 11.5
Office central DBP (mmHg)	NR	14	81.7 ± 6.5
	R	23	81.2 ± 6
Office central heart rate (bpm)	NR	14	63.3 ± 9
	R	23	60.2 ± 6.7
Flow mediated dilation (%)	NR	14	5 ± 1.5
	R	23	4.9 ± 1.3
10-year QRISK®3 score (%)	NR	13	6.5 ± 4
	R	22	5.5 ± 3.7
Cortisol (mmol/L)	NR	13	263 ± 66.7
	R	21	252 ± 86.7
hsCRP (mg/L)	NR	13	1.3 ± 1.6
	R	22	0.9 ± 0.8

Total cholesterol (mmol/L)	NR	13	5.7 ± 0.8
	R	22	5.3 ± 1
Triglycerides (mmol/L)	NR	13	0.9 ± 0.5
	R	22	0.9 ± 0.5
HDL cholesterol (mmol/L)	NR	13	1.9 ± 0.5
	R	22	1.6 ± 0.4
LDL cholesterol (mmol/L)	NR	13	3.7 ± 1.1
	R	22	3.5 ± 1.2
Total PP 7DD (mg)	NR	14	1148 ± 504
	R	23	1371 ± 530
Fibre 7DD (g)	NR	14	25.1 ± 12.6
	R	23	26.8 ± 11.2

7DD, 7-day food diary; AIx, augmentation index; ao, aortic; br, brachial; DBP, diastolic blood pressure; hsCRP, high-sensitivity C-reactive protein; IPAQ, international physical activity questionnaire; NR, non-responders; PWV, pulse wave velocity; R, responders; SBP, systolic blood pressure; SD, standard deviation. *Parameters with significance at $p< 0.05$ between R and NR groups. In green, parameters included in the cluster analysis to define R and NR.

Differences among clinical parameters were investigated between R and NR groups to confirm that the cluster classification could explain the variability in clinical efficacy to aronia extract supplementation. The R group had a significant reduction in the primary outcome, 24h SBP_{br} of -6.6 and -3.6 mmHg compared with the NR group, and the Control group (n= 43), respectively ($p< 0.01$). Similar observations were found regarding 24h DBP_{br} (-4.6 and -2.8 mmHg decrease in the R group compared with NR group and Control, respectively, $p< 0.01$). These observations suggest that subjects in the R group (aronia_2) responded to aronia extract consumption, while those in the NR (aronia_1) cluster were non-responders (NR) (Figure 6.13 A). Moreover, significant reductions in the R group in Awake SBP_{br} and DBP_{br} were found when compared with NR and control groups (-5.3 and -3.8 mmHg for awake SBP_{br} and -4.6 and -3.3 mmHg for awake DBP_{br} compared with NR and Control, respectively) (Figure 6.13 B)

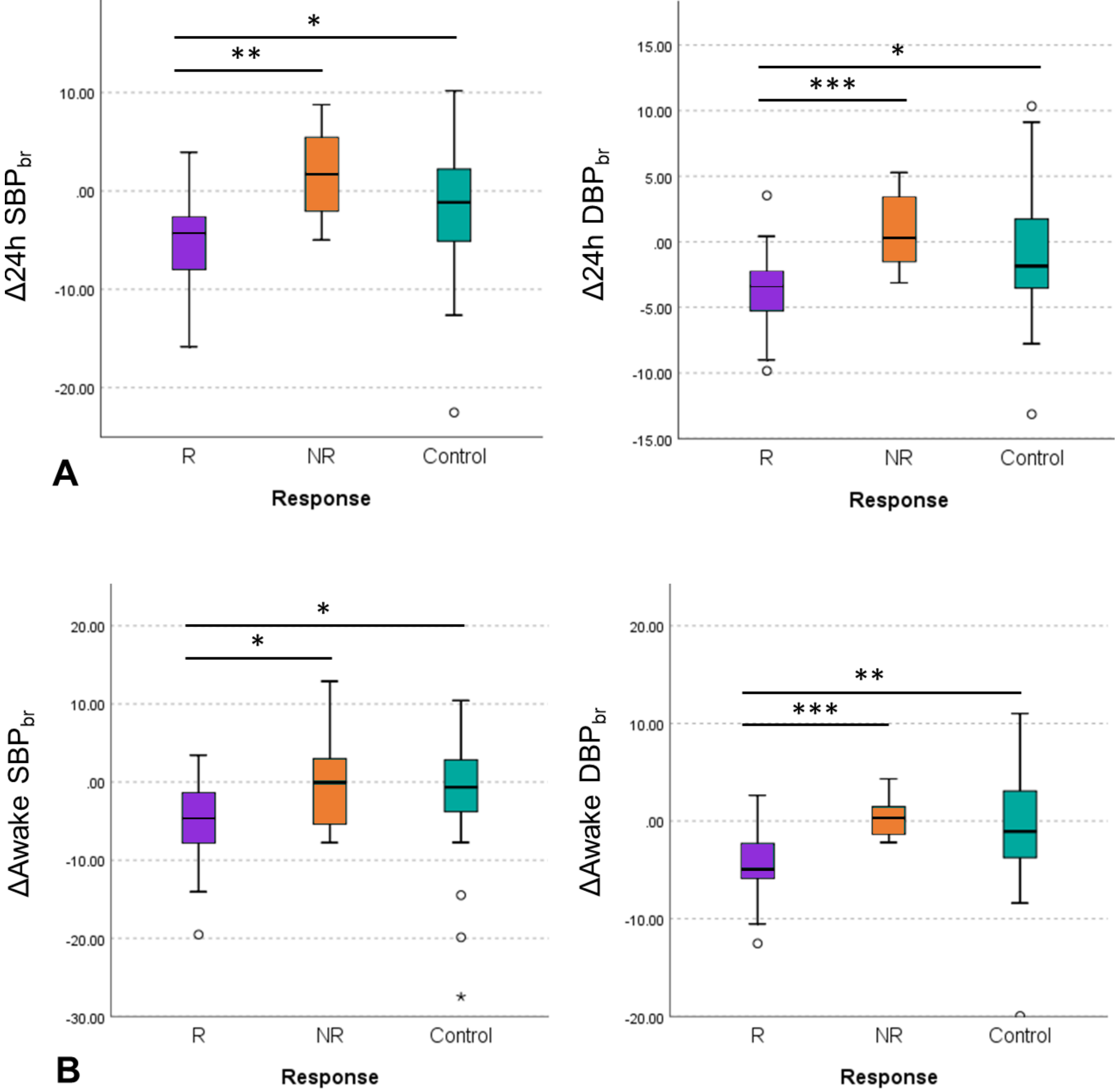
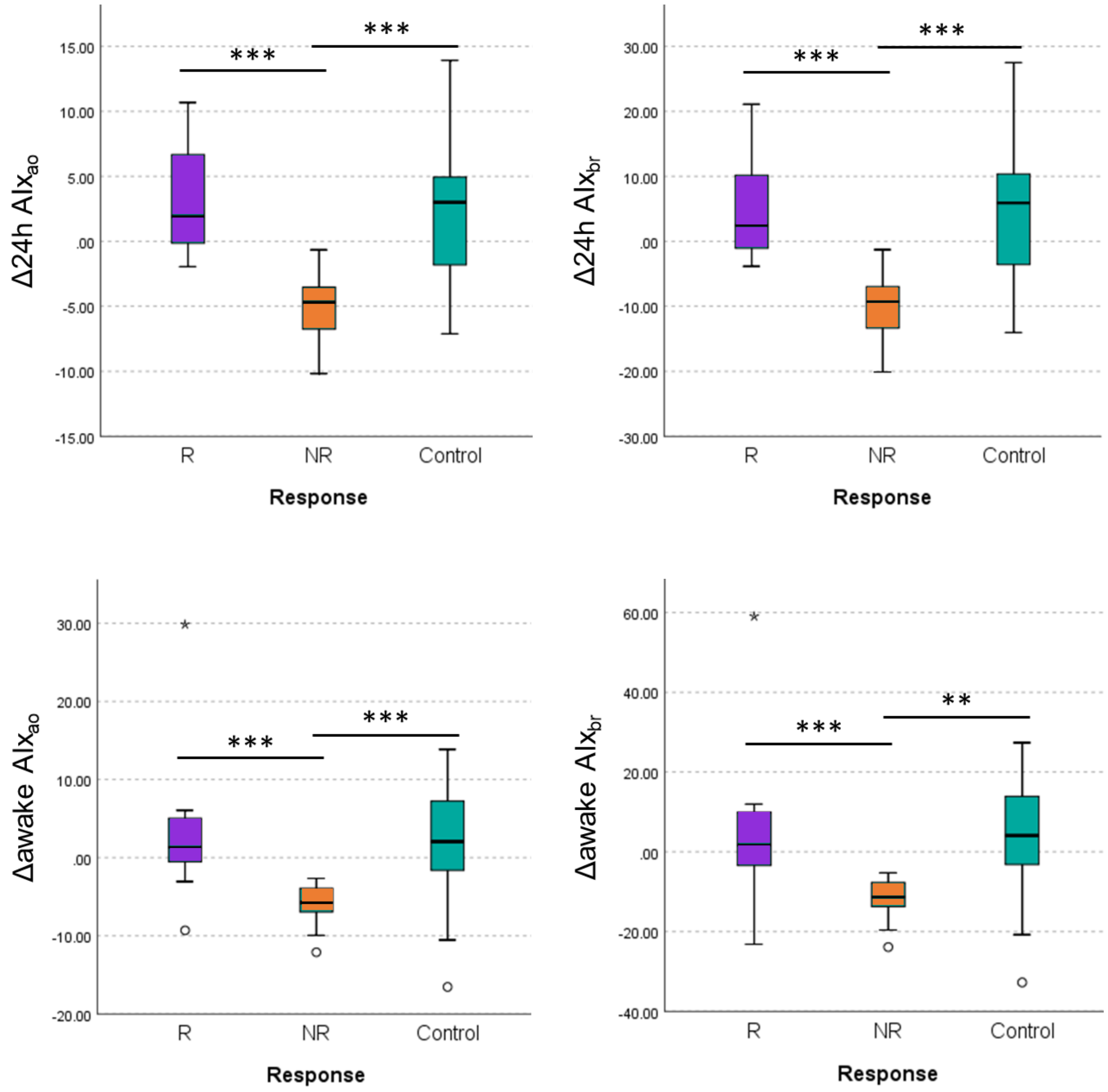


Figure 6.13: Boxplot showing statistical differences between Aronia R, NR, and Control groups for changes in 24h and awake SBP_{br} and 24h and awake DBP_{br} following 12-week consumption. For each comparison, significance was tested by applying a Kruskal Wallis test with Bonferroni adjustment. Subjects belonging to different categories were coloured according to treatment and response: Aronia subjects belonging to Responders (violet), to Non-Responders (orange), and Control subjects (green).

Regarding changes in augmentation index, significant changes were found between R and NR, however the changes were in the opposite direction than the one expected, with R presenting a significant increase in AIx (central and peripheral 24h, awake and asleep AIx) in comparison with NR and control groups, instead of decreasing AIx and therefore improving arterial stiffness. Instead, NR present significant decreases in AIx compared to both R and

Control subgroups (Figure 6.14). A summary of these findings is found in Table 6.14.



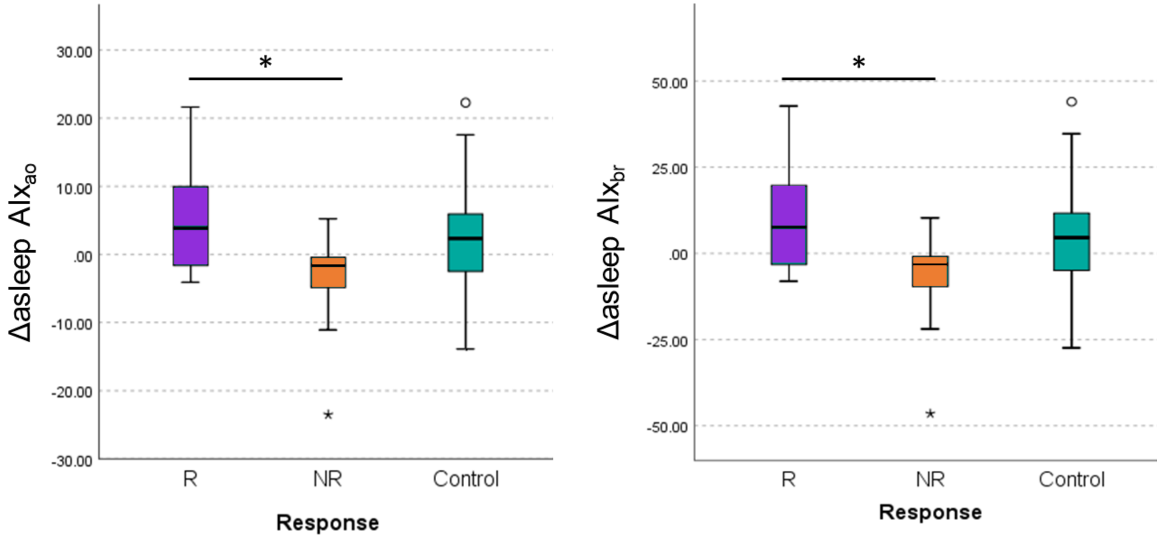


Figure 6.14: Boxplot showing statistical differences between Aronia R, NR, and Control groups for changes in outcomes presenting significant differences between categories of response following 12-week consumption. For each comparison, significance was tested by applying a Kruskal Wallis test with Bonferroni adjustment. Subjects belonging to different categories were coloured according to treatment and response: Aronia subjects belonging to Responders (violet), to Non-Responders (orange), and Control subjects (green).

Table 6.17: Statistical differences between Aronia R, NR, and Control groups for chronic changes in primary and secondary outcomes. For each comparison, significance was tested by applying a Kruskal Wallis test with Bonferroni adjustment.

			R versus C		**NR versus R**		**NR versus C**	
Parameter	**N**	**p-value KWT**	**Δ**	**Sig.**	**Δ**	**Sig.**	**Δ**	**Sig.**
24h SBP_{br}	80	**0.002**	**-3.6**	0.038	**-6.6**	0.002	3.0	0.258
24h DBP_{br}	80	**0.000**	**-2.8**	0.018	**-4.6**	0.000	1.8	0.155
Awake SBP_{br}	80	**0.008**	**-3.8**	0.012	**-5.3**	0.046	1.4	1.000
Awake DBP_{br}	80	**0.001**	**-3.3**	0.006	**-4.6**	0.001	1.3	0.595
24h AIx_{ao}	64	**0.000**	0.8	1.000	**-8.1**	0.000	**-7.3**	0.000
24h AIx_{br}	64	**0.000**	0.5	1.000	**-15**	0.000	**-14**	0.000
Awake AIx_{ao}	64	**0.000**	0.6	1.000	**-8.8**	0.001	**-8.2**	0.000
Awake AIx_{br}	64	**0.000**	-0.5	1.000	**-16**	0.006	**-16**	0.000
Asleep AIx_{ao}	63	**0.022**	3.5	0.974	-9.1	0.081	**-5.6**	0.022
Asleep AIx_{br}	63	**0.022**	7.0	0.974	-18	0.081	**-11**	0.022

C, control; KWT, Kruskal-Wallis test; NR, non-responders; R, responders.

6.3.10.2. Differences in baseline gut microbiome richness and composition between Responders and Non-Responders

As shown in section 6.3.3, consumption of Aronia extract for 12 weeks led to an increase in the gene count, implicating a favourable increase in the richness of the gut microbiota after aronia consumption. Here, we investigated and compared the gene richness in R versus NR subjects.

Responders had significant lower gene count at baseline compared to both NR individuals and placebo (Figure 6.15, p< 0.05) and the same conclusion was observed for species richness, although not significant after Bonferroni adjustment for multiple comparisons (Kruskal-Wallis p= 0.18; Dunn *post-hoc* test p-value (R vs NR) = 0.03, without correction).

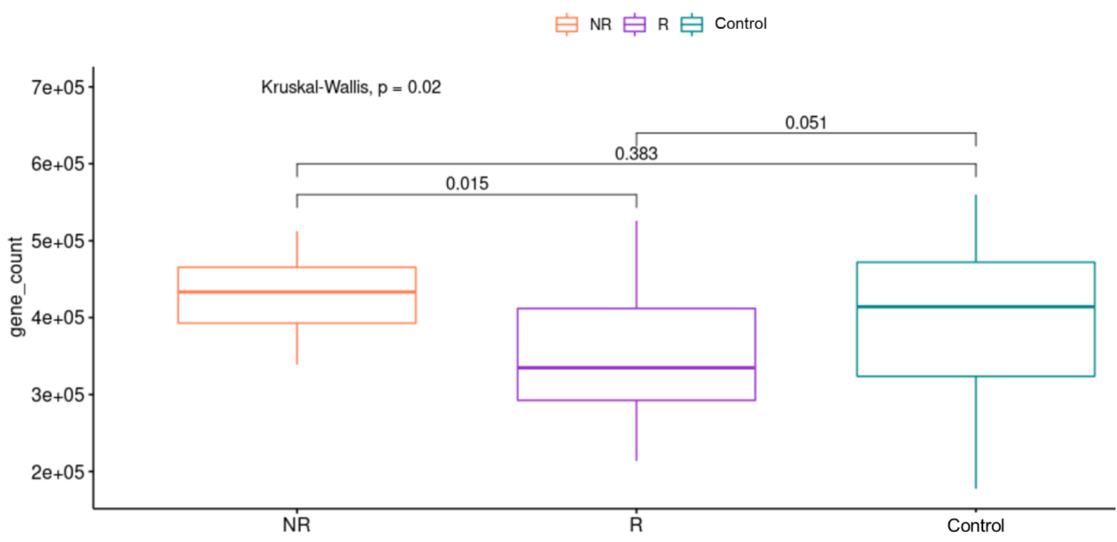


Figure 6.15: Boxplot showing statistical differences between Aronia R, NR, and Control groups for Δ gene count. The significance was tested by applying a Dunn post-hoc test after Kruskal Wallis test and Bonferroni adjustment.

Baseline gut microbiome composition was investigated for both R and NR subgroups of the Aronia subjects. Following a Kruskal Wallis test with Dunn *post-hoc* test, the abundances of *Faecalibacterium prausnitzii*, *Acutalibacteraceae* 3, *Firmicutes* bacterium and *Bifidobacterium adolescentis* taxa appeared significantly enriched the R group compared to NR at baseline. *Bifidobacterium adolescentis* was also enriched in R compared with Control group (Figure 6.16).

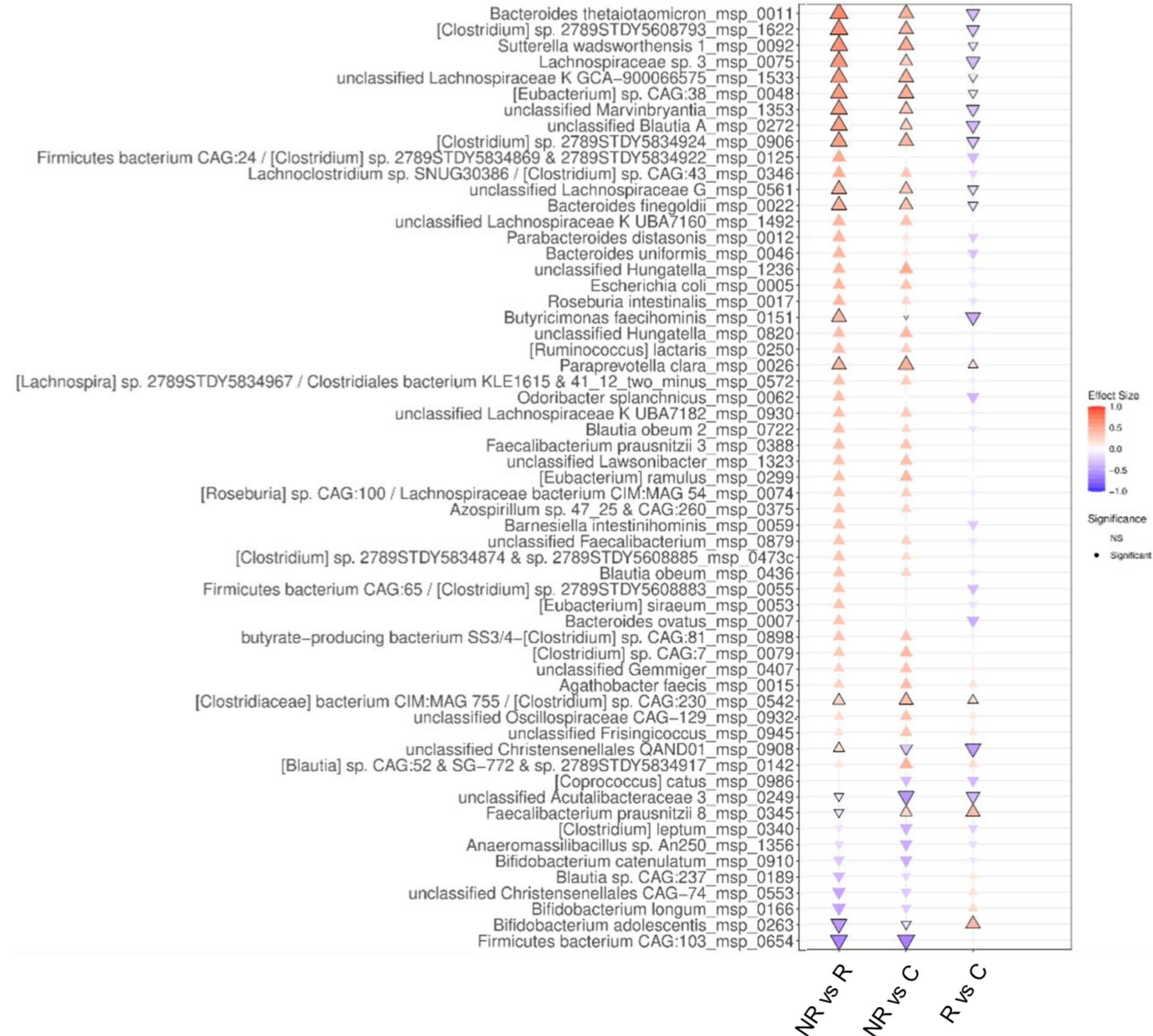


Figure 6.16: Cuneiform plot showing bacterial taxa significantly enriched in R, NR, or Control at baseline. For each comparison, a triangle pointing up indicates that species are enriched in the NR group. Signed effect size are shown through marker direction and colour, hue and size represent absolute effect size. Solid borders indicate significance. Markers not shown had no differences in statistical analysis. C, Control; R, responders; NR: non responders

In order to predict the response to aronia extract supplementation according to the variation in gut microbiota composition at baseline, an analysis focusing exclusively on the 4 species enriched in the R cluster (listed above) was performed. We observed that baseline abundances in *Bifidobacterium adolescentis* showed a significant and negative correlation with the increase in 24h DBP for the Aronia group (Spearman's ρ= -0.32, p= 0.05) (Figure 6.17).

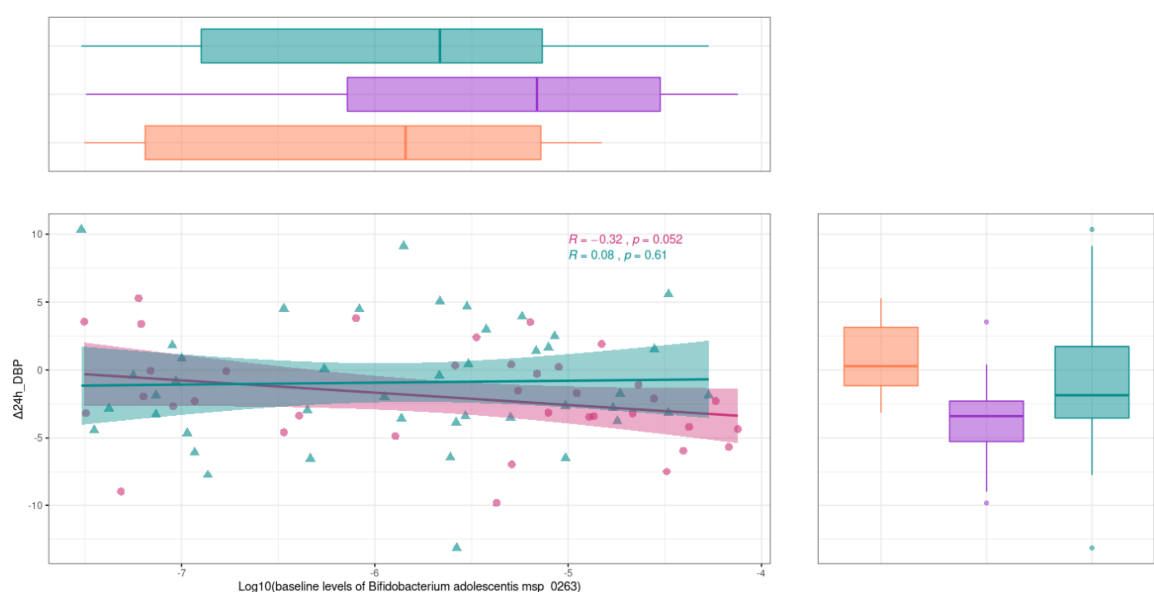


Figure 6.17: Spearman's correlation plot between baseline levels of B. adolescentis and chronic changes in 24h DBP. On the right, boxplots reporting the delta changes in 24h DBP. On top, boxplots for log_{10} baseline levels of B. adolescentis. In the boxplots, Aronia subjects belonging to NR are coloured in orange, the R are coloured in violet and Control subjects are coloured in green.

6.3.10.3. Effects of aronia berry PP on gut microbiome composition in Responders versus Non-Responders

Finally, the impact of the aronia extract supplementation in R and NR subgroups was assessed by comparing the gut microbiome composition of both subgroups following the 12-week intervention (Figure 6.18 and Table 6.17). A total of 26 and 10 species were significantly more abundant following the intervention in the R and NR groups, respectively. Among the species

enriched in the R group were found *Gemmiger*, *Lachnospiraceae* G, *Intestimonas butyriciproducens*, *Eggerthella lenta*, *Lawsonibacter*, *Faecalibacterium prausnitzii* (1, 9, 2, 6), *Oscillospirales* (3, 5), *Faecalibacterium*, *Roseburia intestinalis*, *Ruthenibacterium lactatiformans*, *Lachnoclostridium* sp. /*Clostridium* sp., *Acutalibacteraceae* 3, *Clostridium* sp., *Collinsella bouchesdurhonensis*, *Firmicutes* bacterium / *Clostridium* sp., *Dysomobacter welbionis, Ruminococcus* sp., *Clostridiales* bacterium, and *Clostridia* bacterium. On the contrary, the species significantly enriched in the NR individuals were *Streptococcus salivarius*, *Oscillospirales* 4, *Streptococcus australis*, *Eubacterium* sp., unclassified *Lachnospiraceae* C, *Firmicutes* bacterium, *Bacteroides vulgatus*, *Ruminococcus bicirculans*, *Lachnospira pectinoschiza*, and *Intestinibacter.*

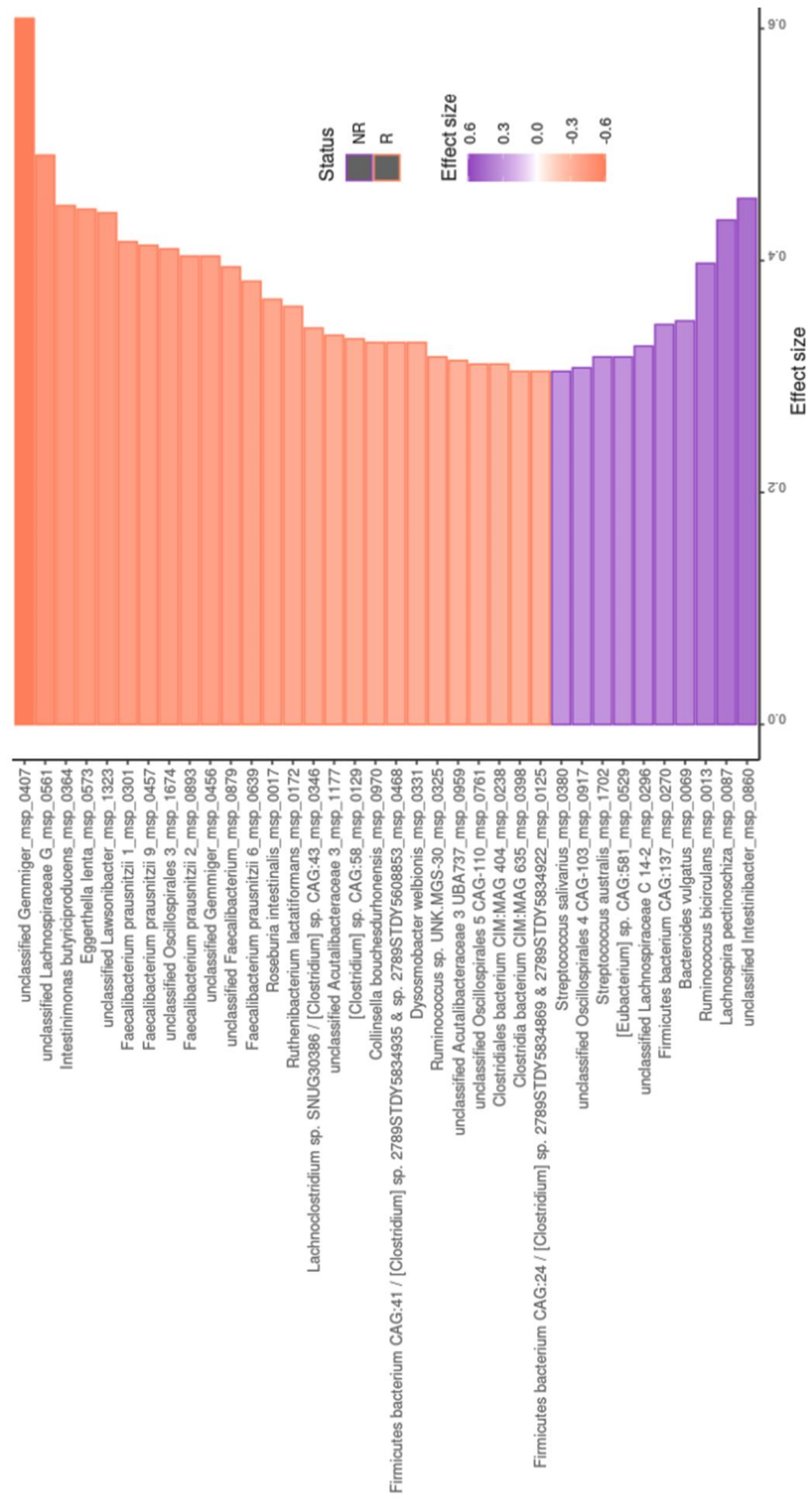


Figure 6.18: Species significantly different between R and NR after 12 weeks of intervention. The bar plot shows the Cliff delta value of all species significantly and relevantly contrasted between R and NR. In red: species significantly more abundant in the R. In blue: species significantly more abundant in the NR group when considering the chronic changes in abundance for the species of interest.

Table 6.18: Species significantly different between R and NR after 12 weeks of intervention. In red: species significantly more abundant in the R. In blue: species significantly more abundant in the NR group when considering the chronic changes in abundance for the species of interest.

Responders	Non-responders
Unclassified *Gemmiger*	*Streptococcus salivarius*
Unclassified *Lachnospiraceae* G	Unclassified *Oscillospirales* 4
Intestimonas butyriciproducens	*Streptococcus australis*
Eggerthella lenta	*Eubacterium* sp.
Unclassified *Lawsonibacter*	Unclassified *Lachnospiraceae* C
Faecalibacterium prausnitzii 1, 9, 2, 6	*Firmicutes* bacterium
Unclassified *Oscillospirales* 3, 5	*Bacteroides vulgatus*
Unclassified *Faecalibacterium*	*Ruminococcus bicirculans*
Roseburia intestinalis	*Lachnospira pectinoschiza*
Ruthenibacterium lactatiformans	Unclassified *Intestinibacter*
Lachnoclostridium sp. / *Clostridium* sp.	
Unclassified *Acutalibacteraceae* 3	
Clostridium sp.	
Collinsella bouchesdurhonensis	
Firmicutes bacterium / *Clostridium* sp.	
Dysomobacter welbionis	
Ruminococcus sp.	
Clostridiales bacterium	
Clostridia bacterium	

6.4. Discussion

6.4.1. Effect of aronia berry PP on gut microbiota abundance and composition

In the present trial, we first investigated the effects of aronia berry PP on gut microbiota composition and richness. In the Aronia group, we noted a significant increase in *Intestinimonas butyriciproducens* as well as a significant decrease in *Ruminococcus* following the 12-week intervention. *Intestimonas butyriciproducens* is known to be a beneficial bacteria thanks to its ability to convert lysine into butyrate and to release ammonium which acts as an acidity buffer in the process of SCFA production (Bui et al. 2016). On the contrary, increases in *Ruminococcus* were observed in Crohn's disease subjects as well as in obese and diabetic mice models compared with healthy individuals (Joossens et al. 2011; Geurts et al. 2011). These suggests that aronia PP may have a positive favourable modulation of the gut microbiome.

Several species were found to be enriched in Aronia group compared with Control following the 12-week supplementation. For instance, this is the case for *Turicibacter sanguinis*, belonging to the *Firmicutes* phylum and able to interact with host-derived compounds (Maki, Nielsen, and Looft 2020), *Butyricimonas faecihominis*, a newly identified butyrate-producing species (Nguyen et al. 2019), *Bacteroides dorei*, shown to inhibit atherosclerosis (Yoshida et al. 2018), *Bacteroides xylanivsolvens*, an acetate-, succinate-, and propionate-producer and probiotic (Chassard et al. 2008; Chen, Liu, et al. 2020), *Clostridium* sp., beneficial for the gut homeostasis and reported to reduce allergic diseases and inflammation (Guo et al. 2020), *Blautia*,

described as a regulator of host health and able to reduce metabolic syndrome (Liu, Mao, et al. 2021), and *Christensenellaceae*, a bacterial family shown to be a potential therapeutic probiotic (Waters and Ley 2019) and reported enriched in normoweight individuals compared with obese subjects. Among the significantly enriched bacteria observed in Aronia group were also reported *Dialister invisus*, *Roseburia* sp. and *Faecalibacterium prausnitzii*, which were related with unbalanced microbiome, inflammatory bowel disease, obesity and type 2 diabetes when reduced in abundance (Morelli, Callegari, and Patrone 2018; Reygner and Kapel 2019; Tamanai-Shacoori et al. 2017; Karlsson et al. 2013; Miquel et al. 2014). Among all the species significantly increased in Aronia group after the intervention, the increase in *Bacteroides* sp., *Clostridium* sp., and *Oscillibacter* sp. were in line with a previous trial studying the impact of aronia on gut microbiome (Istas et al. 2019).

Our analysis also showed the impact of aronia consumption over gut microbiome following the 12-week intervention, especially in terms of species and gene richness, compared with Control. Gene richness has previously been shown to improve significantly following dietary intervention (energy-restricted high-fibre diet) and an increased gene count was associated with a diminution of total cholesterol and inflammation (measured as C-reactive protein) in a population of overweight individuals (Cotillard et al. 2013). Authors hypothesised that a lower gene count could be a potent tool for health risk detection as individuals with a less rich gut microbiome are the less healthy. Another study investigating the correlation between gut microbiome richness and metabolic markers observed that subjects with high gene count were more likely to present an increased production of SCFA, which is in line with the

pathways significantly enriched in Aronia (Le Chatelier et al. 2013). In addition, gene count was also significantly lower in prehypertensive and hypertensive subjects compared with control in a cohort of 196 individuals (Li et al. 2017). Low gene count was also related with obesity and insulin resistance (Molinaro et al. 2020). In rats, significantly lower gene count was reported for rodents which underwent induced heart failure, compared with healthy rats, indicating that gene dysbiosis could influence the predisposition to heart failure (Zheng et al. 2019).

Furthermore, the analysis of microbiome α diversity did not show any difference following the 12-week intervention in both Shannon and Simpson indices, which is in line with the results from another trial led by our team and exploring the effect of the same aronia extract on vascular function and gut microbiome in healthy young men (Istas et al. 2019). To our knowledge, this is the first study to investigate the impact of aronia berry PP consumption on gene count. The discrepancy in results between α diversity and gene count has previously been observed in several non-interventional studies. For example, it was reported that gene count was significantly lower in individuals with asthma and attention-deficit/hyperactivity disorder (ADHD) compared with healthy controls, while no change for α diversity (assessed by Shannon index) was found (Wang et al. 2018; Yan et al. 2021). While α diversity translated the number of individual bacteria in a pool of samples, gene count is the number of unique genes. Since each bacterium presents a large number of genes, we can hypothesise several reasons explaining our results. In fact, aronia PP consumption could have introduced new bacteria to the gut or, the intervention benefited to strains that were present in very low

amount (i.e., not enough to be detected) and these strains grew in larger numbers following 12-week intake of aronia berry PP. These 2 hypotheses could explain an increase in gene count but no significant change in α diversity.

6.4.2. Associations between gut microbiome, vascular outcomes, and PP metabolites

Increasing evidence suggests that modifications in gut microbiome composition and abundance could be related to protective effects against cardiometabolic diseases (Tomas-Barberan, Selma, and Espin 2016). The multi-omic analysis performed in this study explored the influence of plasma and urine PP metabolites on the changes in microbial species, by correlating the 12-week changes in phenolic metabolites with changes in the bacteria of interest. Indeed, a large portion of the phenolic metabolites analysed and quantified in this trial were identified as microbial breakdown products (Cerdá, Tomás-Barberán, and Espín 2005; Gross et al. 2010) which are believed to influence gut microbiome (Williamson and Clifford 2010). A total of 88% and 78% of species versus metabolites' correlations were positive, for plasma and urine, respectively. This confirms the strong and bidirectional link between PP and gut microbiome. Among bacteria positively correlated with plasma metabolites was observed *Ruminococcus* sp., a genus associated with long-term plant-based diets and able to degrade complex carbohydrates (i.e., starch or cellulose) (David et al. 2014). Moreover, *Bacteroides xylanisolvens*, a species able to degrade complex polysaccharides from fruits and vegetables (i.e., xylans or pectin), was also reported among the positive correlations in the plasma metabolites (Henning

et al. 2017). *Ruminococcus* sp. and *B. xylanisolvens* were correlated positively with 20 and 18 plasma metabolites, respectively. When looking at the urinary metabolites, we observed the presence of health-related species such as butyrate-producer *Lawsonibacter phoceensis*, correlated with 11 metabolites, and whose genus was found to be associated to coffee consumption (Asnicar et al. 2021). Similarly, *Faecalibacterium prausnitzii*, one of the most important species regarding SCFA production, was also correlated with 7 urinary metabolites (Lopez-Siles et al. 2017). Interestingly, the rises in these taxa were positively correlated to the presence of 3,4-diOH-BA (also known as protocatechuic acid), which has been documented with anti-inflammatory and antioxidant effects in humans (Wang et al. 2015). This corroborates our hypothesis for the aronia extract supplementation to exert positive effects on gut microbial composition and clinical outcomes. Three species were significantly and positively correlated with both urinary and plasma metabolites: *Clostridium* sp., *Flavonifractor* sp., and *Roseburia* sp., although the metabolites were different for each category. While PP were shown to increase the abundance of the butyrate-producer *Roseburia* sp. (Moreno-Indias et al. 2016), species from *Clostridium* and *Flavonifractor* genus were described as active players in the metabolism and degradation of flavan-3-ols and proanthocyanidins, two of the most abundant PP present in the aronia extract used in the intervention (Rodriguez-Castaño et al. 2020; Moco, Martin, and Rezzi 2012; Luca et al. 2020). This shows once again the robust relationship between phenolic metabolites and gut microbiome composition.

Moreover, we also analysed the potential interactions between gut microbiome species and clinical outcomes, and found a total of 36 significant correlations,

including 75% of them being negative, indicating that a higher abundance in species would correlate to a greater improvement of the clinical outcome. Correlations were mainly with BP (44% of the correlations). Among them, the genus *Oscillospira*, previously found to be negatively correlated with SBP (Chen, Zheng, et al. 2020). This was also the case for the butyrate-producers *Roseburia* sp., *I. butyriciproducens*, and unclassified *Christensenellales* which were found enriched in normotensive participants or negatively correlated with BP (Dan et al. 2019; Li et al. 2017; Calderón-Pérez et al. 2020; Silveira-Nunes et al. 2020; Yan et al. 2020; Yan et al. 2017; Verhaar et al. 2020). Increases in BP were related with a poor gut microbiota diversity and a decrease in SCFA production (Li et al. 2017; Yang et al. 2015). Indeed, butyrate was associated with benefits for health, and in particular with stroke, hypercholesterolemia or cancer (Canani et al. 2011; Vitale et al. 2021). Furthermore, abundances in *Oscillospira* and *B. dorei*, two of the bacterial species negatively correlating BP, were reported to be reduced in subjects with cardiometabolic or inflammatory diseases (Yoshida et al. 2018; Gophna, Konikoff, and Nielsen 2017). This multi-omic analysis also highlighted that the changes in abundance in unclassified *Oscillospiraceae* and *Oscillibacter* sp. were significantly and negatively correlated with AIx. To our knowledge, this is the first time these species are shown to have a beneficial impact on arterial stiffness. These correlations are not in line with the main study focusing on this aspect and which described that arterial stiffness was negatively correlated with the *Ruminococcaceae* family, in a British cohort of 617 middle-aged women (Menni et al. 2018). However, this trial only considered PWV in the analysis and not AIx. The results of the present study are thus novel and of

interest in the investigation of understanding the impact of gut microbiota in the onset of CVD.

6.4.3. Role of gut microbiome in explaining variation in response to aronia consumption

It is becoming more and more clear based on recent evidence that gut microbiota has a strong impact on cardiometabolic diseases (Asnicar et al. 2021). Therefore, gut microbiota may have an important role in explaining the variability in cardiometabolic response of aronia berry PP discussed in previous chapters of this thesis. However, very few studies have investigated this, in particular in clinical trials.

The analysis of gut microbial composition based on the variation in response to aronia extract treatment (Responders versus Non-Responders) revealed that the consumption of the aronia extract could reduce BP (ambulatory 24h and awake SBP_{br} and $DBP_{br)}$ in subjects with low gene count at baseline compared to Control group. Among the 4 taxa significantly enriched at baseline in the R group compared with NR, a higher abundance in *Bifidobacterium adolescentis* was related with a greater decrease in 24h DBP. This bacterium was reported to undergo a stimulatory effect of PP in *in vitro* essays (Gwiazdowska et al. 2015; Kawabata et al. 2018). Furthermore, along with *Faecalibacterium prausnitzii* which was also part of the significantly increased bacteria in R group at baseline, *Bifidobacterium adolescentis* was reported to be restored following a Mediterranean diet in subjects with metabolic syndrome (Haro, Garcia-Carpintero, et al. 2016).

When looking at the microbiome composition and abundance after the 12-week intervention, 26 species were significantly enriched in R versus NR. This was for instance the case for many beneficial SCFA-producing bacteria such as *Intestimonas butyriciproducens*, *Lachnospiraceae G, Flavonifractor* sp., *Faecalibacterium prausnitzii*, *Dysomobacter welbionis*, and *Clostridiales* bacterium (Rinninella et al. 2019; Le Roy et al. 2020; Rodriguez-Castaño et al. 2020). This was also the case for *Roseburia intestinalis*, another butyrate-producing species able to impact atherosclerosis in *in vivo* models (La Rosa et al. 2019; Kasahara et al. 2018) and for *Eggerthella lenta* species, a human colon taxa metabolizing ellagitannins, lignans and catechin, and shown to improve intestinal barrier function as well as being associated with lower cardiometabolic risk (Selma et al. 2018; Meslier et al. 2020; Struijs, Vincken, and Gruppen 2009; Kutschera et al. 2011).

On the contrary, 10 species were significantly enriched in the NR group after the intervention. Among them, entities from *Ruminococcaceae* and *Lachnospiraceae* families, which were reported to be significantly and negatively correlated with arterial stiffness (Menni et al. 2018; Huang et al. 2020), which is in line with our analysis of R versus NR response to treatment showing an improvement in AIx_{ao} and AIx_{br} only in NR individuals.

6.4.4. Strengths and weaknesses

Our investigation of the impact of 12-week daily intake of aronia berry PP intervention on gut microbiome composition and abundance presented several strengths. First, faecal samples were collected only hours before the study days, which allow us to correlate the clinical outcomes with the gut composition

of the volunteers. Samples were analysed through a shotgun metagenomic analysis which involves a sequencing of all the DNA present, which represents a more thorough method compared with 16S sequencing. Moreover, our mathematical approach was robust and used novel algorithms and models to assess the variation in response to treatment. Besides, this study is one of the first to focus on such a multi-omic methodology, including metabolomics, microbiomics and clinical outcomes. We also proved that a 12-week intervention was long enough to detect effects on microbiome, even with a low-dose treatment. Future work could involve the quantification of SCFA in faecal and blood samples to confirm whether the increase seen in butyrate-producing species influences the concentration in SCFA in biosamples.

However, this assessment also presents some limitations. Indeed, as our sample size was calculated based on our primary outcome 24h BP, this analysis might be underpowered to perform some gut microbiome exploration. Another limitation of our analysis is related to the specific population of prehypertensive individuals targeted in the trial, involving that our results cannot be extrapolated to a more general population.

In conclusion, we have shown that consumption of aronia PP led to a beneficial impact on gut microbiome composition and an in bacterial gene richness. Moreover, correlations between metabolites, microbiome, and health outcomes highlighted the bidirectional modulation between PP and gut microbiota. Finally, this overall analysis showed the importance of gut microbiota in explaining variability in response to aronia berry PP consumption.

CHAPTER 7

Discussion

This chapter reviews the main outcomes of the experiments and analysis conducted through this thesis and discusses them according to the current state of literature. Additionally, an outline of the main strengths and limitations related to the present work is given, which need to be considered when interpreting the results. Finally, recommendations for future work needed in the field of (poly)phenol (PP) research associated to cardiovascular diseases is presented.

7.1. Summary of main findings

The main purpose of this thesis was to investigate whether the consumption of an aronia berry extract could improve cardiometabolic health in healthy middle-aged subjects. A randomised controlled human intervention trial was conducted to investigate this, as described in Chapter 3. To understand the potential mechanisms of action and the role of aronia berry PP on the improvements in vascular function found in the clinical trial, the bioavailability of aronia berry PP was investigated with the plasma and urine of the volunteers participating in the study (Chapter 4). As a high variability in vascular response to aronia berry PP was found among participants, a comprehensive assessment of potential factors affecting the interindividual variability in response to the intervention was conducted (Chapter 5). Finally, the impact of aronia berry PP on gut microbiota and its role in the interindividual variability in response was investigated in Chapter 6.

A summary of the main discoveries for each chapter can be found hereunder:

- **Main findings Chapter 3 (*Effects of aronia berry (poly)phenols on cardiometabolic health*):**
  - Twelve-week daily consumption of an aronia berry extract did not improve 24-hour ambulatory blood pressure (BP) in a group of 102 healthy middle-aged men and women (primary outcome).
  - No changes in FMD, blood flow velocity, office BP, arterial stiffness, heart rate, blood lipids, and blood cortisol levels (secondary outcomes) were observed after Aronia berry consumption. Significant improvements in SBP were found in the aronia group when compared with baseline (-2.4 ± 0.9 mmHg), but not when compared with the control group.
  - Daily aronia berry PP consumption for 12 weeks significantly improved 24h ambulatory central and peripheral arterial stiffness measured as augmentation index when compared with the control group (Δ24h AIx_{br}= -6.8%, Δ24h AIx_{ao}= -3.3%). The same trend was observed for awake AIx_{br}, awake AIx_{ao} and awake PWV (Δ= -6.1%, -2.9%, and -0.24 m/s, respectively).

- **Main findings Chapter 4 (*Bioavailability and metabolism of aronia berry (poly)phenols*):**
  - Aronia berry PP were found to be bioavailable in plasma and urine with 92 PP metabolites found in plasma and urine of

volunteers after consumption of aronia PP for 12 weeks. The main compounds found were predominantly hippuric acids, followed by cinnamic acids and benzoic acids for urinary samples and benzene diols and triols and benzoic acids for plasma samples.

- Five individual urinary metabolites significantly increased compared to Control in the Aronia group following 12-week supplementation: 2,3-dihydroxybenzoic acid, 4-O-caffeoylquinic acid, 5-O-caffeoylquinic acid, 3-O-feruloylquinic acid, and 4-O-feruloylquinic acid. A total of 18 and 12 plasma compounds were significantly increased compared to Control 2h post-consumption, on the first and last study visit, respectively. Some compounds, such as 5-O-caffeoylquinic acid (i.e., chlorogenic acid), were also increased compared to baseline in both urine and plasma samples after 12 weeks.
- Correlations were investigated between plasma and urinary PP metabolites and significant vascular outcomes. Plasma and urinary metabolites of aronia PP (mainly hydroxybenzoic acids, phenylacetic acids, hydroxycinnamic acids, and benzene diols and triols) correlated significantly with improvements in awake PWV, as well as 24h and awake central and peripheral AIx.

- **Main findings Chapter 5 (*Assessment of interindividual variability following aronia berry (poly)phenol consumption*):**
    - A high variability in response to aronia berry PP was found with coefficients of variation ranging from ≈200% for 24h SBP and DBP to ≈2560% for total cholesterol and triglycerides.

    - Subgroup analysis showed that the beneficial effects of aronia berry on arterial stiffness measured as AIx were found in women and individuals with high fruit and vegetable intake, presenting a high physical activity level, normoweight, normotensive, younger than 56 years and consuming less than 3.8 units of alcohol per week.

    - On the contrary, beneficial effect of the aronia berry supplementation on blood pressure was observed in subjects presenting a low or moderate physical activity level, a total PP intake lesser than 700 mg/day and an alcohol consumption of more than 3.8 units per week.

- **Main findings Chapter 6 (*Impact of aronia berry (poly)phenols on gut microbiota*):**
    - Twelve-week aronia berry supplementation impacted positively and significantly bacterial gene count compared to placebo, suggesting that the intervention improved microbial richness.

    - In Aronia group, abundance in beneficial SCFA-producer bacteria *Intestinimonas butyriciproducens* increased 12 weeks

after daily consumption, and 18 species were significantly enriched in Aronia compared with Control following the intervention, including beneficial bacteria such as *Bacteroides dorei*, *Bacteroides xylanivsolvens*, *Dialister invisus*, *Roseburia* sp. and *Faecalibacterium prausnitzii*.

- A multi-omic approach revealed significant correlations between urinary and plasma PP metabolites, clinical outcomes, and gut bacterial abundances following 12-week intervention.

- An interindividual variability analysis based on k-means clustering showed that responders presented a lower gene count at baseline compared with non-responders, and baseline abundance of the beneficial bacterium *B. adolescentis* was significantly correlated with improvement in 24h DBP.

- This analysis of responsiveness also highlighted that responders presented significant decreases in Δ24h and Δawake SBP_{br} and DBP_{br} compared with Control and non-responders. On the contrary, non-responders showed significant reductions in Δ24h and Δawake AIx_{ao} and AIx_{br} compared with responders and Control.

- Gut microbiome composition was found to be an important factor in explaining variability in vascular response, confirming the strong and bidirectional link between PP and gut microbiome.

7.2. Strengths of this work

One of the strengths of the work presented here is the high quality of the clinical trial which was central to this PhD thesis. The study design consisted of a double blind randomised controlled trial which included 102 participants, making this study the largest trial ever performed investigating the effects of aronia PP on cardiovascular health. Subjects were randomly allocated into Aronia and Control groups, to prevent any “pre-analytical” variations which may induce bias. The randomization was carried out using a computerized random number generator by an external researcher. Each subject entering the study was assigned a participant’s number corresponding to one of the 2 intervention groups. The relationship between the participant’s number and the treatment group was unknown to both investigators and participants. Moreover, a stratification according to gender was also performed.

Both investigators and participants were blinded to the allocated treatments, as the interventional aronia extract product tested in the RCT was indistinguishable from the control product. In addition, the interventional product was well characterised in terms of nutrients and PP content and represented a relevant and dietarily achievable amount of PP that can be taken as part of a normal diet. Subjects followed their normal diet without any restrictions, which constitutes a better representation of the habitual life and diet of volunteers than other clinical trials conducted under more artificial conditions, which are harder to reproduce in real life.

The population involved was a well-defined and relatively homogenous group composed of middle-aged men and women (n= 47 and 55, respectively), who were prehypertensive but otherwise healthy.

Furthermore, the techniques and methodology applied during the trial were gold standard procedures such as flow-mediated dilation measurement, 24-hour recordings of vascular outcomes, or the use of robust and validated questionnaires to assess the baseline diet and physical activity level of participants. This includes the use of 7-day food diary to assess the diet of participants, which is a gold standard in nutritional studies. All this allowed us to collect the data in a pertinent and standard manner. Regarding the analytical work on biosamples, our trial used one of the most comprehensive methods existing so far, which is validated for more than 120 PP metabolites including phase II and gut microbial metabolites. Gut microbiome analysis was conducted using shotgun metagenomics sequencing which is also a gold standard technique for microbiome analysis.

7.3. Limitations

Several limitations need to be considered when interpreting the main results emanating from the present thesis.

First, the investigation of bioactivity and bioavailability of aronia berry PP was conducted in a population of middle-aged participants, implying that extrapolation of the findings to other segments of the general population such as younger or older individuals cannot be undertaken. Indeed, metabolism and cardiovascular function are widely dependant on age, and the improvement of arterial stiffness observed in our study may not be seen on a younger population with more flexible blood vessels.

Similarly, most men and women included in the trial were healthy, i.e., not at risk of CVD, with a diet generally rich in PP, and presenting a high level of physical activity. The amount of PP given through the treatment was small compared with the habitual intake. This high baseline intake might be the reason why we did not see many or stronger effect of the treatment on the various outcomes. Therefore, we cannot estimate the effect of the intervention on subjects at higher risk or presenting CVD, who may be a better target to observe the impact of aronia PP on hard clinical endpoints rather than surrogate biomarkers of CVD risk.

Another weakness of our trial concerns the timepoints used to measure the vascular outcomes. Indeed, we only measured baseline and 12-week timepoints, without intermediary point such as 4, 6 and 8 weeks. As some parameters such as blood pressure can vary for a number of reasons, having

more timepoints in this trial could have improved the robustness of the findings. Moreover, the overall duration of the trial may not be long enough, and it could have been interesting to assess the effects of the treatment on the longer term (6 month or 1 year).

Compliance based on capsule count was high (> 99%) but it was not assessed during the course of the study. The implementation of additional study visits in between endpoints over the 12 weeks could improve the assessment of this parameter.

Another limitation is related to the fact we did not measure the socioeconomic status of the participants, or other factors such as pollution and environment, which could be important factors for cardiovascular health.

Moreover, as previously stated, our RCT did not restrict the consumption of PP prior to the study visits or during the study. This led to a baseline concentration of phenolic compounds to be high, potentially masking some of the effects of the PP supplementation. This aspect of our protocol also implies that results may be extensively correlated to the food intake of subjects taking place in the last days preceding the study visits. However, significant improvements in arterial stiffness were observed despite this absence of strictly controlled diet, suggesting that even a small dose of PP can induce beneficial health effects among a population with high PP intake. Likewise, background diet was assessed in this trial using a 7-day food diary at baseline and following the 12-week intervention and we did not see any significant change in the diet. However, we cannot rule out that other bioactives present in the diet of volunteers could potentially exert beneficial health effects.

7.4. Conclusions

This work demonstrated that dietarily achievable amounts of aronia berry PP consumed daily over 12 weeks significantly decreased components of arterial stiffness in middle-aged healthy men and women. The magnitude of the effect was -0.24 m/s for awake PWV, and between -2.9 to -6.8% for AIx. Although no cut-off has been defined to relate a decrease in AIx to an improvement of cardiovascular health, the clinical relevance of arterial stiffness improvement allows us to think our findings could be associated with a decrease in the risk of CVD.

Moreover, we were able to significantly correlate the improvement in arterial stiffness to increases in specific plasma and urinary metabolites, suggesting that such phenolic metabolites may be related to the improvements observed, although more work is needed to confirm these findings and the causality of these relationships.

Through the subgroup analysis findings, we found a high variability in response to aronia berry PP, with some factors appearing relevant such as gender, age, or physical activity. These findings could be valuable to widen our understanding of the interindividual variation in response to plant-based interventions, and to set some basis for future approaches to personalised nutrition.

Finally, the metagenomic approach used in our trial showed the impact of the aronia supplementation on gut microbiota, with improvements in gene count and beneficial bacterial species. Importantly, gut microbiota seems to have an important role in explaining the variability in response, as we demonstrated for

the first time the strong correlation between some beneficial butyrate-producing bacterial species and blood pressure as well as arterial stiffness following chronic intake of aronia berry extract. This is one of the very few existing RCTs designed with a multi-omic approach to link gut microbiome composition, PP metabolites concentration, and vascular outcomes.

7.5. Future work

Cardiovascular diseases account for more than 25% of the overall deaths in the UK each year, which represents a huge social and economic burden for the population (British Heart Foundation). As a result, it is necessary to raise awareness in the population and implement accessible nutritional guidelines to promote healthy eating behaviour and prevent these age-related disorders.

The health benefits of fruits, vegetables, and plant-based diets are increasingly being recognized, in particular for the prevention of cardiometabolic diseases. While the beneficial role of PP consumption on health has been demonstrated in preclinical studies and short-term small scale clinical trials, there is a need for larger cohorts investigating the clinical efficacy of PP consumption on the general public. There is also a need to investigate in more details the absorption, distribution, metabolism, and excretion of PP, to predict the interindividual response to dietary intakes.

It is with this aim that tailored nutrition comes into play with a promising approach targeting both management and prevention of chronic CVD. Although personalized nutrition is at its early stages of development, the key aspects of this tactic are well defined and include the monitoring of physical activity and dietary habits, the development of “deep phenotyping” techniques and the exploration of omic techniques such as nutrigenomics and metabolomics (de Toro-Martín et al. 2017).

The present study, with the use of gold standard dietary assessment techniques, advanced targeted metabolomics, physical activity monitoring and gut microbiome sequencing, is in line with the mind set of tailored nutrition.

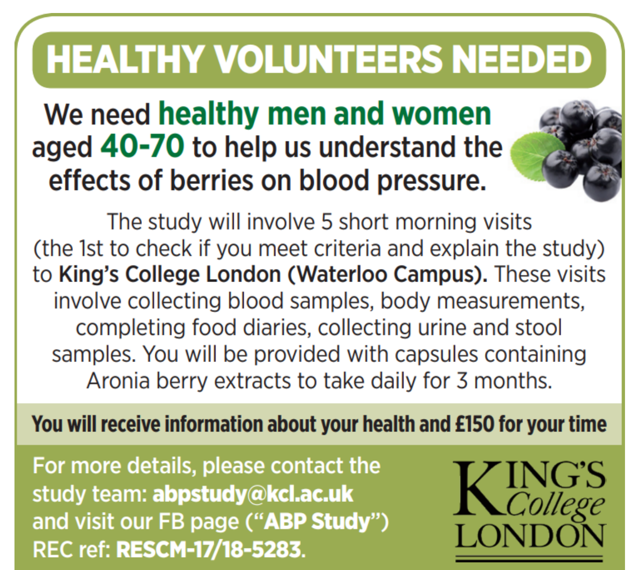
Based on the high interindividual variability in response observed in this work, further studies need to be conducted to assess the reason for such unpredictability. It is crucial to implement study designs accounting for genetics, ethnicity, dietary background, physical activity, age, or socioeconomic status to pursue the work related to factors associated to interindividual variability in response to phenolic compounds intake and, as a result, build tailored nutritional programs based on these parameters. Indeed, the current national recommendations, e.g., the “5 a day” campaigns, offer a “one-size fits all” tactic, which would need to be reviewed to fulfil the requirements of the population on a segmented basis, according to everyone’s need. Based on this statement, the future of nutrition research will probably involve the creation of cooperative databases and longitudinal clinical trials to take into consideration the factors related to variations in responsiveness.

Moreover, results from nutritional RCTs are still divergent in terms of results and magnitude of effects, and there is a necessity for the development of trials involving standardized and relevant protocols, larger cohorts, gold standard assessments, long-term intervention, and dietarily achievable doses of supplementation, to be able to move forward in the field of nutrition, improve our knowledge of variability in response to PP interventions, and compare results within trials. All these improvements and future directives will provide some essential information to make headway into the field of personalized nutrition and contribute to the promotion of healthy cardiovascular ageing.

APPENDICES

Appendix A: Poster displayed in Clapham Junction station (A0 poster).

Appendix B: Advertisement published in Evening Standard and Metro newspapers.



Appendix C: Participant information sheet of the ABP study.

Version Number 03
BDM ref: DBS-05ARN

INFORMATION SHEET FOR PARTICIPANTS:

Randomized, double-blind, placebo controlled, parallel study to evaluate the effect of chokeberry (*Aronia melanocarpa*) polyphenols on blood pressure in prehypertensive healthy men and women: the ABP study

BDM Protocol Number DBS-05ARN - RESCM-17/18-5283
YOU WILL BE GIVEN A COPY OF THIS INFORMATION SHEET

We would like to invite you to participate in this nutritional research project undertaken as part of a PhD program. You should only participate if you want to. Choosing not to take part will not disadvantage you in any way. Before you decide whether you want to take part, it is important for you to understand why the research is being done and what your participation will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information.

What is the purpose of the study?

This study primarily aims to investigate the effect of aronia berries (chokeberries) on vascular function. Ambulatory blood pressure will be measured for 24 hours using a non-invasive 24-hour monitor. This dietary intervention is a randomized placebo-controlled trial (RCT) where consumption of 2 different capsules, containing aronia berries extract or a placebo, will be investigated. We will also measure arterial elasticity through a non-invasive ultrasound-based technique called endothelium-dependent flow-mediated dilation (FMD).

In order to assess the long-term effects of aronia berries, volunteers will be asked to take one capsule filled with aronia powder or placebo every morning for 84 days. The blood of the volunteers will be analyzed on day 1 and day 84. In addition, fecal samples will be collected (self-collection) on day 0 and day 83 to analyze changes in gut bacterial populations. 24-hour urine samples will also be collected prior both study day 1 and 2. The results that will be obtained from this study will help to understand whether consumption of berries can prevent the development of cardiovascular diseases.

Why have I been invited to take part?

You have been invited as you have expressed an interest in our research. We would like to study 100 men and women who can answer 'Yes' to all of the following items:

- I am aged between **40** and **70** years
- My BMI is **below 30**
- I **do not suffer** from peripheral artery disease, cerebrovascular disease, hypertension, obesity, acute inflammation, abnormal heartbeat or terminal renal failure
- I **have never had** a heart attack, stroke, diabetes, allergies to berries or other significant food allergy
- I **do not take** medication or vitamin/dietary supplements
- I **do not follow** a diet, I **did not lose** more than **10%** of my body weight in the previous 6 months and I am willing to maintain my drinking/eating/exercise habits for the duration of the study
- I am a **non-smoker** or a **smoker** who is smoking a **regular** amount of cigarettes per day

Do I have to take part?

No. It is up to you to decide whether to take part. If you do decide to take part, we will go through this information sheet with you and answer any questions you may have. You will be given this information sheet to keep and asked to sign 2 copies of a consent form. You can withdraw from the study at any time by informing one of the researchers and you are not obliged to give a reason. If you decide to withdraw once the study has started, the data may be used in the final report unless you request withdrawal of the data. Data cannot be withdrawn once the study has been submitted as a study report, which will be on the 06/01/2020. If you decide to take part, please let us know if you have been involved in any other study in the previous year.

You should also be aware that unexpected results could be discovered. If so, we may contact your GP to be informed of these results. Consequently, you should not take part in this study if you do not want your GP to be contacted in that context.

What will happen to me if I take part?

If you answer 'yes' to all the above questions and remain interested in participating, we will ask some further questions about your health and eating habits via telephone or email (approx. 10 mins). You will be invited to a screening visit to confirm eligibility, and then you will be enrolled on the study. The study will last 3 months and you will attend King's College London for cardiovascular measurements, give blood samples and collect fecal and urine samples on 2 occasions. Moreover, you will have to wear an ambulatory blood pressure monitor 24 hours prior to each study day, without changing your daily routine. Finally, you will also be asked to record information about your diet and physical activity.

Screening visit:

If we think you are potentially suitable for the study you will be invited to attend a clinic screening appointment (approx. 30 mins) in the **Metabolic Research Unit on 4th Floor, Corridor A, Franklin-Wilkins Building, 150 Stamford Street, London, SE1 9NH** (close to Waterloo Station). On arrival, the study will be explained in detail and you will have the opportunity to ask any questions to ensure you will be giving fully informed consent. Following screening, if your results comply with the study inclusion criteria you will be invited to attend the Metabolic Research Unit on 2 further occasions. Prior to your second visit you will be asked to complete a food diary where you will record your activity level and everything that you eat and drink for a 7 day period. We will also ask you to collect a fecal sample and 24-hour urine sample on the day before the second visit.

Study visits (2st and 3rd visits):

On the days before each visit, we will ask you to avoid eating or drinking (except water) for 12h overnight until the arranged time of your morning appointment. You will have to make sure you drink some water on the morning of the study to avoid dehydration. You will also need to wear a 24-hour ambulatory blood pressure monitor that we will give you prior to both visit 2 and 3. Upon arrival on both study visits you will return the 7-day food diary and the collected fecal and 24-hour urine sample. Visits last approximately 3 h.

- **Visit 2**:
  - Baseline measurements: We will start with the non-invasive cardiovascular measurements like blood pressure, pulse wave velocity (PWV) and augmentation index (AIx) in order to assess arterial stiffness (≈ 30 min). Next we perform the FMD technique (≈ 10 min). Then we will take fasting blood samples (44mL, ≈ 3 tablespoons) to assess your blood glucose and cholesterol, blood cell count, liver and kidney enzymes, plasma polyphenol concentration and

gene activity. We will also collect the data from the 24-hour blood pressure monitor. In the next step you will take your first capsule containing either the placebo or the aronia extract.

- Questionnaires: The polyphenols present in the capsules need about 2h the time to be absorbed, metabolized and transported into your blood. During this waiting time you will be asked to fill in some questionnaires related to your physical activity and diet.

- Acute measurements: After 90 minutes we will repeat the analysis exactly as we did for the baseline measurements. Blood samples will be taken again, this time 22mL to assess the amount of polyphenols in your plasma.

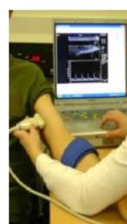
➢ **Visit 3**: Exactly the same as visit 2.

About the cardiovascular measurements:

All measurements taken during the study are non-invasive (except for the blood taking).

- PWV and AIx: A pressure-sensitive probe is applied on the skin will measure the pulse from the carotid (neck), femoral (thigh) and brachial (wrist) arteries. Together with information about the ECG, blood pressure, the distance between the carotid and the femoral artery the pulse wave velocity and augmentation index will be calculated. These parameters give an indication of arterial stiffness.

- FMD: Elasticity of the brachial artery will be measured through echography. With an ultrasound probe we will measure the diameter of the brachial artery in the upper arm. To test the flexibility of the artery, a cuff will be placed on the forearm and will be inflated during 5 min. After deflation an increased blood flow will expand the artery by 5-8%. This expansion is called flow-mediated dilation (see picture).



Dietary strategy:

We will ask you to consume one capsule daily for a 3-month period with breakfast (except on the days of the visits). We ask you to take the capsules with water, not with milk or any other juice. You will be given enough capsules for 84 days.

Incentives

Participants will be compensated for their time and travel expenses with a payment of **£75 per visit** (a total of £150 upon completion of the 2 study days).

What are the possible benefits and risks of taking part?

Benefits: You will receive information about your health and will contribute to nutrition science knowledge.

Risks: Venipuncture (taking a blood sample) may cause brief discomfort and there is a risk of bruising. The cuff used to measure blood pressure and flow-mediated dilation may cause a tingling sensation in the arm. In the unlikely event we discover that you have a medical condition (high blood pressure or elevated blood cholesterol) that you did not know about before, we will tell you about it and discuss how this should be dealt with. Aronia berries can cause vomiting, diarrhea or nausea when a person is allergic or when taken in high amounts. We like to remind you that the extracts in the capsules represent only 10g of aronia berries per day.

Study flowchart

Screening visit	• Lasts approximately **30 min** • Will give you a copy of this information sheet • Will explain the details of the study and answer your questions • You will sign a consent form • Will ask you questions about your medical history, food habits • Will measure your height, weight and blood pressure
24-hour before Visit 2 *Day -1*	• Lasts approximately **30 min** • Place ambulatory blood pressure monitor on your arm and give you the instructions • Give you the bottles and cooling bags in order to collect the 24-hour urine samples • Will give you the self-collection kit to collect the fecal sample
Visit 2 (Study day number 1) *Day 0*	• Lasts approximately **3h30** • Will collect the monitors, as well as the urine and fecal samples • After resting for 10 minutes, we will perform blood pressure, PWV, AIx and FMD measurements (30 min in total) • We will take a 44mL blood sample at the baseline • You will take the first capsule • During waiting time (90 min), you will have to fill in questionnaires • 2 hours after the intake of the capsule, we will perform the same measurements (30 min) • A final blood sample (22mL) will be taken
24-hour before Visit 3 *Day 83*	• Lasts approximately **30 min** • Place ambulatory blood pressure monitor on your arm and give you the instructions • Give you the bottles and cooling bags in order to collect the 24-hour urine samples • Will give you the self-collection kit to collect the fecal sample
Visit 3 (study day number 2) *Day 84*	• Exactly the same as Visit 2, but you will take this time the last capsule • The study is then completed

Data handling and confidentiality

All personal information collected during the study will be kept strictly confidential, in accordance with the General Data Protection Regulation (GDPR). Subject confidentiality and anonymity will be maintained throughout the study by use of subject codes in place of names and the storage of subject details in a secure place. Only the investigators have access to this data and only anonymized data will be shared with other researchers. Anonymized data will be stored for 3 years from the point that collection commence. Should you wish to find out your personal results or the results of this study you are welcome to contact the ABP study team (details below) for a copy of the final report once the study is finished. Questions, comments and requests about your personal data can be sent to the King's College London Data Protection Officer Mr Albert Chan: info-compliance@kcl.ac.uk. If you wish to lodge a complaint with the Information Commissioner's Office, please visit www.ico.org.uk

How is the project being funded?

The study is organized by the Diabetes and Nutritional Sciences Division, King's College London and funded by Naturex-DBS, US.

What will happen to the results of the study?

The study results will be presented in a report and will be published in a scientific journal. You will not be identified in the results of the study or any publication that might arise from this study. We will be happy to discuss the results with you when the study is completed, and will let you know how you can get a copy of the published results if you wish. The results of the ABP study will be also presented during scientific conferences and workshops, and they may be published on the university website or in other events aiming to disseminate the results to the general public.

Who should I contact for further information?

If you have any questions or require more information about this study, please contact Dr Ana Rodriguez-Mateos or the ABP study team using the following contact details:

Dr Ana Rodriguez-Mateos: +44 (0)20 7848 4349 - ana.rodriguez-mateos@kcl.ac.uk

ABP study team: +44 (0)20 7848 4162 - abpstudy@kcl.ac.uk

What if something goes wrong?

If this study has harmed you in any way or if you wish to make a complaint about the conduct of the study you can contact King's College London using the details below for further advice and information: **The Chair, BDM Research Ethics Subcommittee (RESC),** rec@kcl.ac.uk

Thank you for reading this document and for considering taking part in this research

Name of Participant	Date	Signature
	/ /20	
Name of Researcher	**Date**	**Signature**
	/ /20	

Appendix D: Case report form of the ABP study.

CASE REPORT FORM

Randomized, double-blind, placebo controlled, parallel study to evaluate the effect of Aronox® on blood pressure in prehypertensive healthy men and women for 3 months: the ABP study

Protocol Number: **DBS-05ARN**

Principal Investigator: Ana Rodriguez-Mateos, PhD.
Sponsor: NATUREX-DBS LLC
Name of site: Kings College London
CRF Version Number: 2.0

Participant Initials	☐☐☐	Subject screening No.	S☐☐☐
Randomised No.	☐☐☐☐	Subject study No.	☐☐☐☐

CRF Completion Instructions

General

Complete the CRF using a **black ballpoint pen** and ensure that all entries are complete and legible.

Avoid the use of abbreviations and acronyms.

The CRF should be completed as soon as possible after the scheduled visit.

Do not use subject identifiers anywhere on the CRF, such as name, hospital number etc., in order to maintain the confidentiality of the subject. Ensure that the header information (i.e. subject's initials and ID number) is completed consistently throughout the CRF. Missing initials should be recorded with a dash (i.e. D-L).

Each CRF page should be signed and dated by the person completing the form.
The 'completed by' Name in the footer of each page must be legible and **CRFs should only be completed by individuals delegated to complete CRFs on the Site Delegation log (and signed by the PI).**

Ensure that all fields are completed on each page:

- If a test was Not Done record **ND** in the relevant box(es)
- Where information is Not Known write **NK** in relevant box(es)
- Where information is not applicable write **NA** in the relevant box(es)

Corrections to entries

If an error is made, draw a single line through the item, then write the correct entry on an appropriate blank space near the original data point on the CRF and initial and date the change.

Do NOT

- Obscure the original entry by scribbling it out
- Try to correct/ modify the original entry
- Use Tippex or correction fluid

Medications taken by the subject during the trial should be recorded on the "Concomitant Medications Log" using the generic name whenever possible, except combination products which will be recorded using the established trade name. All non-IMPs mentioned in the protocol should also be recorded on the "Concomitant medication Log" for the duration of the trial.

Verbatim Adverse Event terms (initial medical term) should be recorded as the final diagnosis whenever possible.

Complete all **dates** as day, month, year i.e. 13/11/2008. Partial dates should be recorded as NK/11/2008.

All **times** are to be recorded in 24 hour format without punctuation and always use 4-digits; i.e. 0200 or 2130. Midnight is recorded as 0000.

Weights should be recorded to the nearest 0.1 kg.

Source documents such as lab reports, ECG reports etc. should be filed separately from the CRF (if not in the medical notes) for each subject and be signed and dated by a delegated Investigator as proof of review of the assessment during the trial. Questionnaire should be considered as the CRF appendices (except standard approved questionnaire e.g. EQ-5D)

If a subject prematurely withdraws from the trial a single line must be drawn across each uncompleted page to correspond with the last visit of the subject as mentioned on the "Trial Completion" page.

The protocol deviation/violation/serious breach log should be used to record comments relating to each CRF visit that cannot be captured on the page itself. This includes reason for delayed or missed protocol visits or trial assessments, unscheduled visits etc.

The Chief Investigator (for lead site)/Principal Investigator is responsible for the accuracy of the data reported on the CRF. The CI/PI must sign and date the Principal Investigator's Sign Off page to certify accuracy, completeness and legibility of the data reported in the CRF.

Serious Adverse Events (SAEs)

SAEs should be faxed **within 24 hours** of the site being aware of the event using the trial specific SAE report form to **020 3108 2312 or preferably emailed to sae@ucl.ac.uk**

Storage

CRF documents should be stored in a locked, secure area when not in use where confidentiality can be maintained. Ensure that they are stored separately to any other documents that might reveal the identity of the subject.

Participant Initials ☐☐☐ Study No. ☐☐☐

Screening No. S☐☐☐ Randomised No. ☐☐☐

VISIT 1

(SCREENING)

Date of Assessment: __ __ / __ __ / __ __ __ __

(DD / MM / YYYY)

ABP study

Screening No.	S				Study No.				Site	KCL - FWB

VISIT 1 (SCREENING) **DEMOGRAPHIC DATA**

Informed Consent:			
Date participant signed written consent form:	__/__/____ (DD / MM / YYYY)	**Date of first trial-related procedure:**	__/__/____ (DD / MM / YYYY)
Name of person taking informed consent: ________________________________			

Demographic Data:				
Date of Birth:	__/__/____ (DD / MM / YYYY)			
Ethnicity:				
White	White British ☐	White Irish ☐	White Other ☐	
Mixed race	White & Black Caribbean ☐	White & Black African ☐	White & Asian ☐	Other mixed background ☐
Asian or Asian British	Indian ☐	Bangladeshi ☐	Pakistani ☐	Other Asian background ☐
Black or Black British	Caribbean ☐	African ☐	Black Other ☐	
Chinese or other ethnicity	Chinese ☐	Other ☐ (please specify)		
Sex:	☐ Male ☐ Female			
Profession: ________________________________				

	Name	Signature	Date
Completed by			

ABP study

Screening No.	S				Study No.				Site	KCL - FWB

VISIT 1 (SCREENING) **MEDICAL HISTORY**

Has the patient had any relevant medical history?	☐ **No** ☐ **Yes,** Complete below

Condition / illness /surgical procedure	**Start date** (DD/MM/YYYY)	**Stop date** (DD/MM/YYYY)	**Or tick if ongoing at Screening Visit?**
Current medications and medications consumed within the previous 6 months (including "over-the-counter")	___/____/____	___/____/____	☐
Known allergies and reactions to medications	___/____/____	___/____/____	☐
Medical/psychiatric illnesses	___/____/____	___/____/____	☐
Past surgeries/injuries/hospitalizations	___/____/____	___/____/____	☐
Immunizations	___/____/____	___/____/____	☐
Current and past tobacco/alcohol/drug use	___/____/____	___/____/____	☐

	Name	Signature	Date
Completed by			

ABP study

Screening No. | S | | | | Study No. | | | | Site | KCL - FWB

VISIT 1 (SCREENING) VITAL SIGNS & ANTHROPOMETRY

	Systolic Blood Pressure (SBP, mmHg, seated)	Diastolic Blood Pressure (DBP, mmHg, seated)	Pulse (beats/min)
Replicate 1	___ ___ ___ . ___	___ ___ ___ . ___	___ ___ ___ . ___
Replicate 2	___ ___ ___ . ___	___ ___ ___ . ___	___ ___ ___ . ___
Replicate 3	___ ___ ___ . ___	___ ___ ___ . ___	___ ___ ___ . ___
Average R2 + R3	___ ___ ___ . ___	___ ___ ___ . ___	___ ___ ___ . ___

Distance carotid – heart:	___ ___ ___ ___ mm
Distance heart – femoral:	___ ___ ___ ___ mm
Height:	___ . ___ ___ m
Weight:	___ ___ ___ . ___ kg

	Name	Signature	Date
Completed by			

ABP study

Screening No. | S | | | | Study No. | | | | Site | KCL - FWB |

VISIT 1 (SCREENING) SCREENING CONCOMITANT MEDICATIONS

Is the participant taken any concomitant medications at screening or visits 2 and 3					☐ No ☐ Yes, Complete below		
Medication (Record Generic or trade name)	**Reason for use** (Medical History diagnosis or other reason, e.g. Prophylaxis)	Dose and units	**Frequency**	**Route**	**Start Date** (DD/MM/YYYY)	**Stop Date** (DD//MM/YYY)	**Or tick if ongoing at Screening Visit**
1.					___/___/___	___/___/___	☐
2.					___/___/___	___/___/___	☐
3.					___/___/___	___/___/___	☐
4.					___/___/___	___/___/___	☐
5.					___/___/___	___/___/___	☐
6.					___/___/___	___/___/___	☐
7.					___/___/___	___/___/___	☐
8.					___/___/___	___/___/___	☐
9.					___/___/___	___/___/___	☐
10.					___/___/___	___/___/___	☐

	Name	Signature	Date
Completed by			

ABP study

Screening No. | S | | | | Study No. | | | | Site | KCL - FWB |

VISIT 1 (SCREENING) SMOKING / ALCOHOL / DIETARY SUPPLEMENT STATUS

Has the participant ever smoked? ☐ **No** ☐ **Yes,** Complete below

☐ **Current Smoker**	**Participant's average daily use:** - Number of cigarettes : ___ ___ - Number of cigars : ____ ___ - Number of pipes : ___ ___ - Number of shishas: ___ ___ **Smoked for ___ ___ months/years**	☐ "Social" smoker
☐ **Former smoker**	**Smoked for ___ ___ months/years** **Date when smoking ceased:** __ __/___ /__ __ __ __ (DD / MM / YYYY) **When smoking, participant's average daily use:** - Number of cigarettes : ___ ___ - Number of cigars : ____ ___ - Number of pipes : ___ ___ - Number of shishas: ___ ___	☐ "Social" smoker

Participant's alcohol consumption (see protocol for definition of units)
Participant's average consumption per week:
- Number of units of wine : ___ ___
- Number of units of beer : ____ ___
- Number of units of spirits : ___ ___

Has the participant taken vitamins/minerals/dietary supplements in the last year? ☐ **No** ☐ **Yes**

Name and brand:

Amount and frequency:

For how long and until when:

Willing to stop taking them before and during the study (At least 1 month before): ☐ **No** ☐ **Yes**

	Name	Signature	Date
Completed by			

ABP study

Screening No. | S | | | | Study No. | | | | Site | KCL - FWB |

VISIT 1 (SCREENING) INCLUSION CRITERIA

The following criteria MUST be answered YES for participant to be included in the trial (except where NA is appropriate):		Yes	No	N/A
1.	Healthy men or women aged 40-70 years old	☐	☐	☐
2.	Blood pressure comprised between: SBP 120/139 mmHg or DBP 80/89 mmHg	☐	☐	☐
3.	Willing to maintain their normal eating/drinking habits and exercise habits to avoid changes in body weight over the duration of the study	☐	☐	☐
4.	Able to understand the nature of the study	☐	☐	☐
5.	Able to give signed written informed consent	☐	☐	☐
6.	Signed informed consent form	☐	☐	☐
7.	Comply with all study protocol procedures	☐	☐	☐
If any of the above criteria is answered NO, the participant is NOT eligible for the trial and must not be included in the study. Please list reason(s) for ineligibility for screen failure on Participant Eligibility Review page.				

Comments about inclusion and/or exclusion criteria:

	Name	Signature	Date
Completed by			

Screening No.	S				Study No.				Site	KCL - FWB

VISIT 1 (SCREENING) **EXCLUSION CRITERIA**

	The following criteria MUST be answered NO for the participant to be included in the trial:	Yes	No
1.	Have required treatment for hypertension at any time (e.g. statins, aspirin, blood pressure lowering drugs)	☐	☐
2.	Hypertensive, as defined as SBP superior or equal to 140 mmHg or DBP superior or equal to 90 mmHg	☐	☐
3.	Manifest cardiovascular disease including coronary artery disease, cerebrovascular disease and peripheral artery disease	☐	☐
4.	Currently treated with a diet	☐	☐
5.	Chronic-acute disease	☐	☐
6.	Unstable psychological condition	☐	☐
7.	Obese participants, defined as BMI superior or equal to 30	☐	☐
8.	Diabetes mellitus, metabolic syndrome, acute inflammation, terminal renal failure, malignancies or abnormal heart rhythm (lower or higher than 60-100 bpm)	☐	☐
9.	History of cancer, myocardial infarction, cerebrovascular incident or kidney abnormality	☐	☐
10.	Allergies to berries or other significant food allergy	☐	☐
11.	Requiring chronic antimicrobial or antiviral treatment	☐	☐
12.	Reported having taken food supplements, dietary supplement or herbal remedies within 1 month of study start	☐	☐
13.	Weight loss of more than 10% body weight in the previous 6 months	☐	☐
14.	Reported participant in another study within one month before the study start	☐	☐
15.	Smoke an irregular amount of cigarettes per day or planning to quit smoking in the next 3 months	☐	☐
16.	Unable to swallow capsules	☐	☐
18.	Any reason or condition that in the judgment of the clinical investigator(s) may put the subject at unacceptable risk or that may preclude the subject from understanding or complying with the study's requirements	☐	☐
19.	Pregnant woman/ lactating woman/ woman planning to become pregnant/ premenopausal women who do not have a regular menstrual cycle/ premenopausal women who do not have adequate method of contraception[1]	☐	☐
	If any of the above criteria is answered YES, the participant is NOT eligible for the trial and must not be included in the study. Please list reason(s) for ineligibility for screen failure on Participant Eligibility Review page.		

[1] Highly effective methods of contraception are defined as one of the following: Complete abstinence from intercourse that could result in pregnancy from 14 days prior to Day 1 until 28 days after completion of study treatment, Levonorgestrel Implant, Injectable progesterone, Intrauterine device (IUD), Oral contraceptives (either combined or progesterone only), Double barrier method: condom, cervical cap or diaphragm with spermicidal agent, Transdermal contraceptive patch, Male partner who is sterile prior to the female participant's entry into the study and is the sole sexual partner of the female participant

	Name	Signature	Date
Completed by			

ABP study

Screening No. S _ _ _ Study No. _ _ _ Site KCL - FWB

VISIT 1 (SCREENING) **PARTICIPANT ELIGIBILITY REVIEW**

End of Screening Visit Checklist:			
		Yes	**No**
1.	Have all Screening Visit procedures been completed?	☐	☐
2.	Have the Medical History and Concomitant Medication pages been completed?	☐	☐
3.	Is the participant still willing to proceed in the trial?	☐	☐
4.	Does the participant satisfy the inclusion and exclusion criteria to date?	☐	☐
If no, please give the reason for the screen failure below			
Reason(s) for screen failure:			
1.			
2.			

7-day food diary dispensed?	☐ Yes ☐ No
Fecal sample kit dispensed?	☐ Yes ☐ No
Is the volunteer invited for a second blood pressure checking?	☐ Yes ☐ No

PRE-VISIT 2 **BLOOD PRESSURE CHECKING** (1)

Double check if the blood pressure measured at the screening visit is reproducible	
Systolic Blood Pressure (SBP, seated):	___ ___ ___ . ___ mmHg
Diastolic Blood Pressure (DBP, seated):	___ ___ ___ . ___ mmHg
Pulse:	___ ___ ___ . ___ beats/min

	Name	Signature	Date
Completed by			

Screening No.	S				Study No.				Site	KCL - FWB

PRE-VISIT 2 **BLOOD PRESSURE CHECKING** (2)

	Yes	No
Is the blood pressure still in the range required for the study?	☐	☐
Is the participant eligible to take part in the Clinical Trial?	☐	☐
If no, please give the reason for the failure below		
Reason(s) for failure:		
1.		
2.		

Participant's eligibility Investigator Sign-Off:
I confirm that the volunteer number: S __ __ __ **is elegible for the ABP study** ☐
Investigator's Name: ______________________________
Investigator's Signature: ______________________________
Date : __ __/ __ __/ __ __ __ __ (DD/MM/YYYY)

	Name	Signature	Date
Completed by			

Participant Initials ☐☐☐ Subject No. ☐☐☐☐

Screening No. S☐☐☐ Randomised No. ☐☐☐☐

VISIT 2

Date of Assessment: __ __ / __ __ / __ __ __ __

(DD / MM / YYYY)

ABP study

Subject No.	☐☐☐☐	Site	KCL - FWB

VISIT 2 RANDOMISATION/ENROLMENT

Participant Randomisation/Enrolment	
Participant study Number allocated:	ABP __ __ __ __
Date of Randomisation/Enrolment:	__ __/ __ __ / __ __ __ __ (DD / MM / YYYY)

Date & Time of first treatment consumption:	__ __/__ __/__ __ __ __ (DD / MM / YYYY) ____:____ HH:MM
Number of capsules dispensed	

Room temperature	___ ___ . ___ °C

Urine volume	___ ___ ___ ___ mL

VISIT 2 AMBULATORY BLOOD PRESSURE MONITOR

ABPM placed by:	
ABPM functioning ?	☐ Yes ☐ No
Date & Time:	__ __/__ __/__ __ __ __ (DD / MM / YYYY) ____:____ HH:MM

	Name	Signature	Date
Completed by			

ABP study

Subject No. | | | | | Site **KCL - FWB**

ABPM collected and checked by:	
Date & Time:	__ __ / __ __ / __ __ __ __ ____:____ (DD / MM / YYYY) HH:MM
24-hour record ok?	☐ Yes ☐ No (Comment below)
Comment*:	

VISIT 2 **BLOOD PRESSURE & BODY FAT**

Peripheral blood pressure and heart rate performed?	☐ No (Comment Below) ☐ Yes, Complete below Comment*: ____________________
Date: __ __ / __ __ / __ __ __ __ **(DD / MM / YYYY)**	Time of measurement 0h: ____:____ HH:MM Time of measurement 2h: ____:____ HH:MM
Position: Seated	Device: Automatic Sphygmomanometer

BLOOD Pressure	SBP (mmHg)	DBP (mmHg)	HR (bpm)
0h replicate 1			
0h replicate 2			
0h replicate 3			
Average R2 + R3 0h			
2h replicate 1			
2h replicate 2			
2h replicate 3			
Average R2 + R3 2h			

	Name	Signature	Date
Completed by			

ABP study

Subject No. | | | | | Site **KCL - FWB**

BODY FAT - Tanita	
Weight	___ ___ ___ . ___ kg
BMI	___ ___ ___ . ___ kg/m²
Body fat	___ ___ ___ ___ %
BMR	___ ___ ___ ___ Kcal

VISIT 2 **PWV, AIX AND CBP**

PWV, AIx and CBP performed?	☐ No (Comment Below) ☐ Yes, Complete below Comment*: ______________________________
Date: __ __ / __ __ / __ __ __ __ **(DD / MM / YYYY)**	Time of measurement 0h: ________:_______ HH:MM Time of measurement 2h: ________:_______ HH:MM
Position: Supine **Device (if different than usual): Sphygmocor**	

CBP, PWV, AIx	CSBP (mmHg)	CDBP (mmHg)	HR (bpm)	PWV (cm/s)	AIx (%)
Time point 0h					
Time point 2h					

Side of the carotid measurement	☐ Left	☐ Right
Side of the femoral measurement	☐ Left	☐ Right

	Name	Signature	Date
Completed by			

Subject No.					Site	KCL - FWB

VISIT 2 **FMD**

FMD performed?	☐ No (Comment Below) ☐ Yes, Complete below Comment*: ____________________
Date: __ __ / __ __ / __ __ __ __ **(DD / MM / YYYY)**	Time of measurement 0h: ____:____ HH:MM Time of measurement 2h: ____:____ HH:MM
Position: Supine **Device/probe (if different than usual): Vivid i**	
Comments	

FMD	Baseline diameter (mm)	%FMD	Blood flow velocity (m/s)
0h	FMD results can be found at: External hard drive MLS01 (silver) In folder “ABP Study” → “FMD_results” → “ABP_FMD results.xcl”		
2h			

	Name	Signature	Date
Completed by			

ABP study

Subject No. | | | | | Site **KCL - FWB**

VISIT 2 **BLOOD COLLECTION**

Blood collection performed?	☐ No (Comment Below) ☐ Yes, Complete below Comment*: ______________________________
Date: __ __ / __ __ / __ __ __ __ **(DD / MM / YYYY)**	Time of measurement 0h: ________:________ HH:MM Time of measurement 2h: ________:________ HH:MM

BLOOD COLLECTION (68.5 mL)	ANALYSIS	0h	2h
10 ml EDTA (purple large)	(Poly)phenols	☐ No ☐ Yes	☐ No ☐ Yes
12 ml heparin (green)	(Poly)phenols	☐ No ☐ Yes	☐ No ☐ Yes
4 mL EDTA (purple small)	FBC	☐ No ☐ Yes	☐ No ☐ Yes
7 mL Serum (gold)	Lipids, Liver fct, Urate	☐ No ☐ Yes	☐ No ☐ Yes
3 mL Na/F K/oxalate (grey)	Glucose	☐ No ☐ Yes	☐ No ☐ Yes

BLOOD COLLECTION Laboratory Parameter	0h	2h	Number of Eppendorf tubes 0h	Number of Eppendorf tubes 2h	Stored in box number (-80°C)
0.6 ml plasma EDTA with 2% FA	☐ No ☐ Yes	☐ No ☐ Yes			
0.6 ml plasma EDTA no FA	☐ No ☐ Yes	☐ No ☐ Yes			
0.6 ml Plasma heparin with 2% FA	☐ No ☐ Yes	☐ No ☐ Yes			
0.6 ml Plasma heparin no FA	☐ No ☐ Yes	☐ No ☐ Yes			
Buffy coat	☐ No ☐ Yes	☐ No ☐ Yes			
1 ml full blood	☐ No ☐ Yes	☐ No ☐ Yes			
PBMC	☐ No ☐ Yes	☐ No ☐ Yes			
Paxgene	☐ No ☐ Yes	☐ No ☐ Yes			

	Name	Signature	Date
Completed by			

ABP study

Subject No.	☐☐☐☐	Site	KCL - FWB

VISIT 2 **HAEMATOLOGY**

Clinical Haematology Laboratory tests performed?	☐ **No** (comment below) ☐ **Yes** Comment*: ______________________
Date of Sample	__ __/ __ __/ __ __ __ __ (DD / MM / YYYY)
Time of Sample	_____:_____ HH:MM
Was laboratory sample taken at KCL hospital	☐ No ☐ Yes

HAEMATOLOGY Laboratory Parameter	Unit	If out of normal range on report, please check if clinically significant:		
WBC	10^9/L	☐ No	☐ Yes	Blood results can be found at: External hard drive MLS01 (silver) in folder "ABP Study" → "Blood_results" → "ABP_blood results.xcl"
RBC	10^{12}/L	☐ No	☐ Yes	
Hb	g/L	☐ No	☐ Yes	
HCT (PCV)	L/L	☐ No	☐ Yes	
MCV	fL	☐ No	☐ Yes	
MCH	pG	☐ No	☐ Yes	
MCHC	g/dL	☐ No	☐ Yes	
RDW	%	☐ No	☐ Yes	
PLT	10^9/L	☐ No	☐ Yes	
MTV (MPV)	fL	☐ No	☐ Yes	
NEUTROPHILS	10^9/L	☐ No	☐ Yes	
LYMPHOCYTES	10^9/L	☐ No	☐ Yes	
MONOCYTES	10^9/L	☐ No	☐ Yes	
EOSINOPHILS	10^9/L	☐ No	☐ Yes	
BASOPHILS	10^9/L	☐ No	☐ Yes	
RETICULOCYTES (NURBC)	%	☐ No	☐ Yes	
LARGE UNSTAINED CELLS	10^9/L	☐ No	☐ Yes	
THR	10^9/L	☐ No	☐ Yes	
WCPC	10^9/L	☐ No	☐ Yes	
%HYPO	10^9/L	☐ No	☐ Yes	

	Name	Signature	Date
Completed by			

Subject No. | | | |

Site **KCL - FWB**

VISIT 2 BIOCHEMISTRY

Clinical Biochemistry Laboratory tests performed?	☐ **No (comment below)** ☐ **Yes** Comment*: ______________________
Date of Sample	__ __ / __ __ / __ __ __ __ (DD / MM / YYYY)
Time of Sample	______:______ HH:MM
Were laboratory samples taken at different hospital other than taken at KCL hospital to Tracy Neal?	☐ No ☐ Yes

BIOCHEMISTRY Laboratory Parameter	Unit	If out of normal range on report, please check if clinically significant:	
UREA	mmol/L	☐ No ☐ Yes	Blood results can be found at: External hard drive MLS01 (silver) in folder “ABP Study” → “Blood_results” → “ABP_blood results.xcl”
CREATININE	mmol/L	☐ No ☐ Yes	
TOTAL BILIRUBIN	µmol/L	☐ No ☐ Yes	
TOTAL CHOLESTEROL	mmol/L	☐ No ☐ Yes	
HDL-CHOLESTEROL	mmol/L	☐ No ☐ Yes	
LDL-CHOLESTEROL	mmol/L	☐ No ☐ Yes	
TRIGLYCERIDES	mmol/L	☐ No ☐ Yes	
LDH	IU/L	☐ No ☐ Yes	
AST	IU/L	☐ No ☐ Yes	
GGT	IU/L	☐ No ☐ Yes	
ALKALINE PHOSPHATASE	IU/L	☐ No ☐ Yes	
GLUCOSE	mmol/L	☐ No ☐ Yes	
Na	mmol/L	☐ No ☐ Yes	
K	mmol/L	☐ No ☐ Yes	
EGFR	mL/min	☐ No ☐ Yes	
TOTAL PROTEIN	g/L	☐ No ☐ Yes	
ALBUMIN	g/L	☐ No ☐ Yes	
NON-HDL CHOLESTEROL	mmol/L	☐ No ☐ Yes	
GLOBULIN	g/L	☐ No ☐ Yes	

	Name	Signature	Date
Completed by			

ABP study

Subject No. | | | | | Site **KCL - FWB**

VISIT 2 **QUESTIONNAIRES & FECAL COLLECTION**

Epic FFQ filled?	☐ No (Comment Below) ☐ Yes Comment*: ______________
Reading FFQ filled?	☐ No (Comment Below) ☐ Yes Comment*: ______________
24 h pre-study diet diary filled?	☐ No (Comment Below) ☐ Yes Comment*: ______________
Exercise questionnaire filled?	☐ No (Comment Below) ☐ Yes Comment*: ______________
7 day diary filled?	☐ No (Comment Below) ☐ Yes Comment*: ______________
New 7 day diary dispensed?	☐ No (Comment Below) ☐ Yes Comment*: ______________
Willing to be contacted 3 times during the study to check compliance, adverse events etc ?	☐ No (Comment Below) ☐ Yes Comment*: ______________
Willing to be contacted by SMS every week during the study to check compliance?	☐ No (Comment Below) ☐ Yes Comment*: ______________
Preference of times to be called:	

Fecal sample collected?	☐ No (Comment Below) ☐ Yes, Complete below Day & time collected by subject: __ __ / __ __ / __ __ __ __ (DD / MM / YYYY) ____:____ HH:MM Comment*: ______________
New fecal collection kit dispensed?	☐ No ☐ Yes

	Name	Signature	Date
Completed by			

Participant Initials ☐☐☐ Subject No. ☐☐☐☐

Randomised No. ☐☐☐☐

VISIT 3

Date of Assessment: __ __/__ __/__ __ __ __

(DD / MM / YYYY)

ABP study

Subject No. | | | | | Site **KCL - FWB**

VISIT 2 AMBULATORY BLOOD PRESSURE MONITOR

ABPM placed by:	
ABPM functioning ?	☐ Yes ☐ No
Date & Time:	__ __/__ __/__ __ __ __ (DD / MM / YYYY) ____:____ HH:MM

VISIT 3 CHECKLIST

Date of Visit: __ __/__ __/__ __ __ __ (DD / MM / YYYY)

Visit Checklist:			
		Yes	**No**
1.	**Have there been any new Adverse Events?** (If yes, please record in Adverse Events page)	☐	☐
2.	**Have there been any changes in Concomitant Medications?** (If yes, please record in Concomitant Medications Log)	☐	☐
3.		☐	☐

Date & Time of last treatment consumption:	__ __/__ __/__ __ __ __ (DD / MM / YYYY) ____:____ HH:MM
Room temperature	___ ___ . ___ °C
Urine volume	___ ___ ___ ___ mL

	Name	Signature	Date
Completed by			

ABP study

Subject No. | | | | | Site **KCL - FWB**

ABPM collected and checked by:	
Date & Time:	__ __/__ __/__ __ __ __ ______:______ (DD / MM / YYYY) HH:MM
24-hour record ok?	☐ Yes ☐ No (Comment below)
Comment*:	

VISIT 3 **BLOOD PRESSURE & BODY FAT**

Peripheral blood pressure and heart rate performed?	☐ No (Comment Below) ☐ Yes, Complete below Comment*: ______________________
Date: __ __/__ __/__ __ __ __ **(DD / MM / YYYY)**	Time of measurement 0h: ______:______ HH:MM Time of measurement 2h: ______:______ HH:MM
Position: Seated	Device: **Automatic Sphygmomanometer**

BLOOD Pressure	SBP (mmHg)	DBP (mmHg)	HR (bpm)
0h replicate 1			
0h replicate 2			
0h replicate 3			
Average R2 + R3 0h			
2h replicate 1			
2h replicate 2			
2h replicate 3			
Average R2 + R3 2h			

	Name	Signature	Date
Completed by			

Subject No. | | | | | **Site** KCL - FWB

BODY FAT - Tanita	
Weight	___ ___ ___ . ___ kg
BMI	___ ___ ___ . ___ kg/m²
Body fat	___ ___ ___ . ___ %
BMR	___ ___ ___ ___ Kcal

VISIT 3 **PWV, AIX AND CBP**

PWV, AIx and CBP performed?	☐ No (Comment Below) ☐ Yes, Complete below Comment*: ______________________________
Date: __ __/ __ __/ __ __ __ __ **(DD / MM / YYYY)**	Time of measurement 0h: ________:_______ HH:MM Time of measurement 2h: ________:_______ HH:MM
Position: Supine **Device (if different than usual): Sphygmocor**	

CBP, PWV, AIx	CSBP (mmHg)	CDBP (mmHg)	HR (bpm)	PWV (cm/s)	AIx (%)
Time point 0h					
Time point 2h					

Side of the carotid measurement	☐ Left	☐ Right
Side of the femoral measurement	☐ Left	☐ Right

	Name	Signature	Date
Completed by			

ABP study

Subject No.	||||	Site	KCL - FWB

VISIT 3 **FMD**

FMD performed?	☐ No (Comment Below) ☐ Yes, Complete below Comment*: ______________________
Date: __ __/__ __/__ __ __ __ **(DD / MM / YYYY)**	Time of measurement 0h: ____:____ HH:MM Time of measurement 2h: ____:____ HH:MM
Position: Supine **Device/probe (if different than usual): Vivid i**	
Comments	

FMD	Baseline diameter (mm)	%FMD	Blood flow velocity (m/s)
0h	FMD results can be found at: External hard drive MLS01 (silver) in folder "ABP Study" → "FMD_results" → "ABP_FMD results.xcl"		
2h			

	Name	Signature	Date
Completed by			

ABP study

Subject No. ☐☐☐☐ Site **KCL - FWB**

VISIT 3 BLOOD COLLECTION

Blood collection performed?	☐ No (Comment Below) ☐ Yes, Complete below Comment*: ____________________
Date: __ __/__ __/__ __ __ __ **(DD / MM / YYYY)**	Time of measurement 0h: ____:____ HH:MM Time of measurement 2h: ____:____ HH:MM

BLOOD COLLECTION (68.5 mL)	ANALYSIS	0h	2h
10 ml EDTA (purple large)	(Poly)phenols	☐ No ☐ Yes	☐ No ☐ Yes
12 ml heparin (green)	(Poly)phenols	☐ No ☐ Yes	☐ No ☐ Yes
4 mL EDTA (purple small)	FBC	☐ No ☐ Yes	☐ No ☐ Yes
7 mL Serum (gold)	Lipids, Liver fct, Urate	☐ No ☐ Yes	☐ No ☐ Yes
3 mL Na/F K/oxalate (grey)	Glucose	☐ No ☐ Yes	☐ No ☐ Yes

BLOOD COLLECTION Laboratory Parameter	0h	2h	Number of Eppendorf tubes 0h	Number of Eppendorf tubes 2h	Stored in box number (-80°C)
0.6 ml plasma EDTA with 2% FA	☐ No ☐ Yes	☐ No ☐ Yes			
0.6 ml plasma EDTA no FA	☐ No ☐ Yes	☐ No ☐ Yes			
0.6 ml Plasma heparin with 2% FA	☐ No ☐ Yes	☐ No ☐ Yes			
0.6 ml Plasma heparin no FA	☐ No ☐ Yes	☐ No ☐ Yes			
Buffy coat	☐ No ☐ Yes	☐ No ☐ Yes			
1 ml full blood	☐ No ☐ Yes	☐ No ☐ Yes			
PBMC	☐ No ☐ Yes	☐ No ☐ Yes			
Paxgene	☐ No ☐ Yes	☐ No ☐ Yes			

	Name	Signature	Date
Completed by			

ABP study

Subject No. | | | | | Site **KCL - FWB**

VISIT 3 **HAEMATOLOGY**

Clinical Haematology Laboratory tests performed?	☐ **No** (comment below) ☐ **Yes** Comment*: ___________________________
Date of Sample	__ __ / __ __ / __ __ __ __ (DD / MM / YYYY)
Time of Sample	______:______ HH:MM
Was laboratory sample taken at KCL hospital	☐ No ☐ Yes

HAEMATOLOGY Laboratory Parameter	Unit	If out of normal range on report, please check if clinically significant:	
WBC	10^9/L	☐ No ☐ Yes	Blood results can be found at: External hard drive MLS01 (silver) in folder "ABP Study" → "Blood_results" → "ABP_blood results.xcl"
RBC	10^{12}/L	☐ No ☐ Yes	
Hb	g/L	☐ No ☐ Yes	
HCT (PCV)	L/L	☐ No ☐ Yes	
MCV	fL	☐ No ☐ Yes	
MCH	pG	☐ No ☐ Yes	
MCHC	g/dL	☐ No ☐ Yes	
RDW	%	☐ No ☐ Yes	
PLT	10^9/L	☐ No ☐ Yes	
MTV (MPV)	fL	☐ No ☐ Yes	
NEUTROPHILS	10^9/L	☐ No ☐ Yes	
LYMPHOCYTES	10^9/L	☐ No ☐ Yes	
MONOCYTES	10^9/L	☐ No ☐ Yes	
EOSINOPHILS	10^9/L	☐ No ☐ Yes	
BASOPHILS	10^9/L	☐ No ☐ Yes	
RETICULOCYTES (NURBC)	%	☐ No ☐ Yes	
LARGE UNSTAINED CELLS	10^9/L	☐ No ☐ Yes	
THR	10^9/L	☐ No ☐ Yes	
WCPC	10^9/L	☐ No ☐ Yes	
%HYPO	10^9/L	☐ No ☐ Yes	

	Name	Signature	Date
Completed by			

Subject No. | | | | |

Site **KCL - FWB**

VISIT 3 **BIOCHEMISTRY**

Clinical Biochemistry Laboratory tests performed?	☐ **No (comment below)** ☐ **Yes** Comment*: ___________________________
Date of Sample	__ __/__ __/__ __ __ __ (DD / MM / YYYY)
Time of Sample	_______:______ HH:MM
Were laboratory samples taken at different hospital other than taken at KCL hospital to Tracy Neal?	☐ No ☐ Yes

BIOCHEMISTRY Laboratory Parameter	Unit	If out of normal range on report, please check if clinically significant:	
UREA	mmol/L	☐ No ☐ Yes	Blood results can be found at: External hard drive MLS01 (silver) in folder “ABP Study” → “Blood_results” → “ABP_blood results.xcl”
CREATININE	mmol/L	☐ No ☐ Yes	
TOTAL BILIRUBIN	µmol/L	☐ No ☐ Yes	
TOTAL CHOLESTEROL	mmol/L	☐ No ☐ Yes	
HDL-CHOLESTEROL	mmol/L	☐ No ☐ Yes	
LDL-CHOLESTEROL	mmol/L	☐ No ☐ Yes	
TRIGLYCERIDES	mmol/L	☐ No ☐ Yes	
LDH	IU/L	☐ No ☐ Yes	
AST	IU/L	☐ No ☐ Yes	
GGT	IU/L	☐ No ☐ Yes	
ALKALINE PHOSPHATASE	IU/L	☐ No ☐ Yes	
GLUCOSE	mmol/L	☐ No ☐ Yes	
Na	mmol/L	☐ No ☐ Yes	
K	mmol/L	☐ No ☐ Yes	
EGFR	mL/min	☐ No ☐ Yes	
TOTAL PROTEIN	g/L	☐ No ☐ Yes	
ALBUMIN	g/L	☐ No ☐ Yes	
NON-HDL CHOLESTEROL	mmol/L	☐ No ☐ Yes	
GLOBULIN	g/L	☐ No ☐ Yes	

	Name	Signature	Date
Completed by			

ABP study

Subject No. ☐☐☐☐ Site **KCL - FWB**

VISIT 3 **QUESTIONNAIRES & FECAL COLLECTION**

24 h pre-study diet diary filled?	☐ No (Comment Below) ☐ Yes Comment*: ____________________
7 day diary filled?	☐ No (Comment Below) ☐ Yes Comment*: ____________________

Fecal sample collected?	☐ No (Comment Below) ☐ Yes, Complete below Day & time collected by subject: __ __ / __ __ / __ __ __ __ (DD / MM / YYYY) ____:____ HH:MM Comment*: ____________________

VISIT 3 **COMPLIANCE**

Number of capsules (investigational product) left:	
Volunteer had one capsule per day during the 12 weeks?	☐ **No (Comment Below)** ☐ **Yes** **Comment*:** ____________________
Exact number of days taking capsules:	

	Name	Signature	Date
Completed by			

ABP study

Subject No.					Site	KCL - FWB

TRIAL COMPLETION

Did participant complete the trial?	☐ **Yes,** Please provide **date of last visit**: __ __ / __ __ / 2 0 __ __ (DD / MM / YYYY) ☐ **No,** Please provide **date of withdrawal** and complete below: __ __ / __ __ / 2 0 __ __ (DD / MM / YYYY)

Early Withdrawal: **please tick most appropriate reason for participant not completing the trial:**

☐ **Adverse Events related:** please state related AE: ________________________________ (add details to AE page)

☐ **Participant's decision, specify:** ___________________________

☐ **Investigator's decision, specify:** ___________________________

☐ **Sponsor's decision**

☐ **Lost to follow up**

☐ **Patient deceased**

☐ **Other, specify:** ___________________________

	Name	Signature	Date
Completed by			

ABP study Subject No. [ ][ ][ ][ ] Site **KCL - FWB**

ADVERSE EVENTS PAGE

AE Nº	Event Name (Please give Diagnosis if known)	Start date (DD/MM/YYYY)	Stop date (DD/MM/YYYY)	Serious? If serious, please complete a JRO SAE form	Concomitant Medication given	Severity 0 - Mild 1 - Moderate 2 - Severe	Study Drug Action 0 - None 1 - Temporarily Interrupted 2 - permanently withdrawn	Outcome 0 – Resolved 1- Resolved with sequelea 2 - Not resolved	Relationship to Study Drug 0 - Definitely 1 - Probably 2 - Possibly 3 - Unlikely 4 - Not related 5 - Not assessable
1		___/___/___	___/___/___	☐ No ☐ Yes	☐ No ☐ Yes				
2		___/___/___	___/___/___	☐ No ☐ Yes	☐ No ☐ Yes				
3		___/___/___	___/___/___	☐ No ☐ Yes	☐ No ☐ Yes				
4		___/___/___	___/___/___	☐ No ☐ Yes	☐ No ☐ Yes				
5		___/___/___	___/___/___	☐ No ☐ Yes	☐ No ☐ Yes				
6		___/___/___	___/___/___	☐ No ☐ Yes	☐ No ☐ Yes				

I have reviewed the AEs on this page and have assessed them for seriousness, causality, severity and outcome and confirm that, to the best of my knowledge, it accurately reflects the study information obtained for this participant

PI signature ______________________________ **Date:**________________________ ☐ **Please check box if this is the last page used**

	Name	Signature	Date
Completed by			

ABP study Subject No. [ ][ ][ ][ ] Site **KCL - FWB**

ADVERSE EVENTS PAGE (CONTINUATION PAGE)

AE Nº	Event Name (Please give Diagnosis if known)	Start date (DD/MM/YYYY)	Stop date (DD/MM/YYYY)	Serious? If serious, please complete a JRO SAE form	Concomitant Medication given	Severity 0 - Mild 1 - Moderate 2 - Severe	Study Drug Action 0 - None 1 - Temporarily Interrupted 2 - Permanently withdrawn	Outcome 0 - Resolved 1- Resolved with sequelea 2 - Not resolved	Relationship to Study Drug 0 - Definitely 1 - Probably 2 - Possibly 3 - Unlikely 4 - Not related 5 - Not assessable
__		___/___/___	___/___/___	☐ No ☐ Yes	☐ No ☐ Yes				
__		___/___/___	___/___/___	☐ No ☐ Yes	☐ No ☐ Yes				
__		___/___/___	___/___/___	☐ No ☐ Yes	☐ No ☐ Yes				
__		___/___/___	___/___/___	☐ No ☐ Yes	☐ No ☐ Yes				
__		___/___/___	___/___/___	☐ No ☐ Yes	☐ No ☐ Yes				
__		___/___/___	___/___/___	☐ No ☐ Yes	☐ No ☐ Yes				

I have reviewed the AEs on this page and have assessed them for seriousness, causality, severity and outcome and confirm that, to the best of my knowledge, it accurately reflects the study information obtained for this participant

PI signature______________________________ **Date:**________________________ ☐ **Please check box if this is the last page used**

	Name	Signature	Date
Completed by			

ABP study Subject No. | | | | | Site **KCL - FWB**

CONCOMITANT MEDICATIONS LOG

Has the participant used any Concomitant Medications? ☐ **No** ☐ **Yes,** Complete below

CM Nº	Medication name (Record <specify Generic or Brand> name)	Start date (DD/MM/YYYY)	Stop date (DD/MM/YYYY)	Or tick if ongoing at end of study	Reason for use (Enter related AE diagnosis, or other reasons for use, e.g. Prophylaxis)	Dose (Units)	Route	Frequency
1.		___/___/____	___/___/____	☐				
2.		___/___/____	___/___/____	☐				
3.		___/___/____	___/___/____	☐				
4.		___/___/____	___/___/____	☐				
5.		___/___/____	___/___/____	☐				
6.		___/___/____	___/___/____	☐				
7.		___/___/____	___/___/____	☐				

☐ **Please check box if this is the last page used**

Note: Use the Concomittent log to record Non-IMPs

	Name	Signature	Date
Completed by			

ABP study Subject No. | | | | | Site **KCL - FWB**

CONCOMITANT MEDICATIONS LOG (CONTINUATION PAGE)

CM Nº	Medication name (Record <specify Generic or Brand> name)	Start date (DD/MM/YYYY)	Stop date (DD/MM/YYYY)	Or tick if ongoing at end of study?	Reason for use (Enter related AE diagnosis, or other reasons for use, e.g. Prophylaxis)	Dose (Units)	Route	Frequency
__.		___/___/____	___/___/____	☐				
__.		___/___/____	___/___/____	☐				
__.		___/___/____	___/___/____	☐				
__.		___/___/____	___/___/____	☐				
__.		___/___/____	___/___/____	☐				
__.		___/___/____	___/___/____	☐				
__.		___/___/____	___/___/____	☐				
__.		___/___/____	___/___/____	☐				

☐ **Please check box if this is the last page used**

	Name	Signature	Date
Completed by			

PRINICIPAL INVESTIGATOR'S SIGN OFF

Principal Investigator's Signature Statement:	
I have reviewed this CRF and confirm that, to the best of my knowledge, it accurately reflects the study information obtained for this participant. All entries were made either by me or by a person under my supervision who has signed the Delegation and Signature Log.	
Principal Investigator's Signature: ____________________________ **Principal Investigator's Name:** ____________________________	**Date of Signature:** __ __/ __ __/ __ __ __ __ (DD / MM / YYYY)

ONCE SIGNED, NO FURTHER CHANGES CAN BE MADE TO THIS CRF WITHOUT A SIGNED DATA QUERY FORM.

Appendix E: Consent form of the ABP study.

Consent form V3 (18/06/18)
ABP study DBS-05ARN

CONSENT FORM FOR PARTICIPANTS IN RESEARCH STUDIES

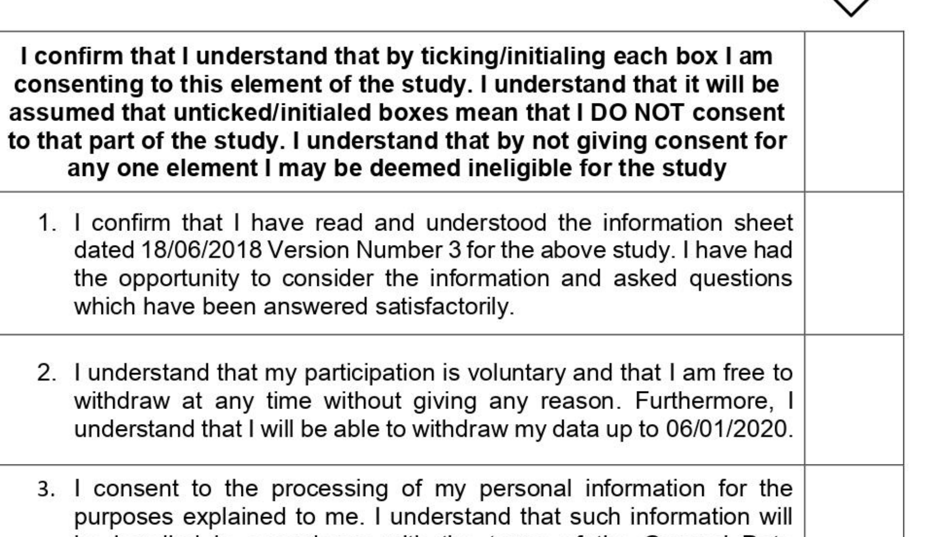
Please complete this form after you have read the Information Sheet and/or listened to an explanation about the research.

Title of Study: Randomized, double-blind, placebo controlled, parallel study to evaluate the effect of chokeberry (*Aronia melanocarpa*) polyphenols on blood pressure in prehypertensive healthy men and women: the ABP Study

King's College Research Ethics Committee Ref: RESCM-17/18-5283

Thank you for considering taking part in this research. The person organising the research must explain the project to you before you agree to take part. If you have any questions arising from the Information Sheet or explanation already given to you, please ask the researcher before you decide whether to join in. You will be given a copy of this Consent Form to keep and refer to at any time.

Please tick or initial



I confirm that I understand that by ticking/initialing each box I am consenting to this element of the study. I understand that it will be assumed that unticked/initialed boxes mean that I DO NOT consent to that part of the study. I understand that by not giving consent for any one element I may be deemed ineligible for the study	
1. I confirm that I have read and understood the information sheet dated 18/06/2018 Version Number 3 for the above study. I have had the opportunity to consider the information and asked questions which have been answered satisfactorily.	
2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason. Furthermore, I understand that I will be able to withdraw my data up to 06/01/2020.	
3. I consent to the processing of my personal information for the purposes explained to me. I understand that such information will be handled in accordance with the terms of the General Data Protection Regulation (GDPR).	

4. I understand that my information may be subject to review by responsible individuals from the College for monitoring and audit purposes.	
5. I understand that confidentiality and anonymity will be maintained and it will not be possible to identify me in any publications.	
6. I agree to be contacted in the future by King's College London researchers who would like to invite me to participate in follow up studies to this project, or in future studies of a similar nature.	
7. I agree that the research team may use my data for future research and understand that any such use of identifiable data would be reviewed and approved by a research ethics committee. (In such cases, as with this project, data would/would not be identifiable in any report).	
8. I understand that the information I have submitted will be published as a report and I will be able to receive a copy of it by contacting the ABP study team whose contact details can be found on the patient information sheet.	
9. I understand that I must not take part if I fall under the exclusion criteria as detailed in the information sheet and explained to me by the researcher.	
10. I agree that my GP may be contacted if any unexpected results are found in relation to my health.	
11. I have informed the researcher of any other research in which I am currently involved or have been involved in during the past 12 months.	

Name of Participant	**Date**	**Signature**
	/ /20	
Name of Researcher	**Date**	**Signature**
	/ /20	

Appendix F: Example of food-frequency questionnaire section (EPIC-Study).

PLEASE PUT A TICK (✓) ON EVERY LINE

FOODS AND AMOUNTS	AVERAGE USE LAST YEAR								
DRINKS	Never or less than once/month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day
Tea (cup)								✓	
Coffee, instant or ground (cup)						✓			
Coffee, decaffeinated (cup)	✓								
Coffee whitener, eg. Coffee-mate (teaspoon)	✓								
Cocoa, hot chocolate (cup)						✓			
Horlicks, Ovaltine (cup)	✓								
Wine (glass)	✓								
Beer, lager or cider (half pint)	✓								
Port, sherry, vermouth, liqueurs (glass)	✓								
Spirits, eg. gin, brandy, whisky, vodka (single)	✓								
Low calorie or diet fizzy soft drinks (glass)	✓								
Fizzy soft drinks, eg. Coca cola, lemonade (glass)						✓			
Pure fruit juice (100%) eg. orange, apple juice (glass)	✓								
Fruit squash or cordial (glass)							✓		
FRUIT (1 fruit or medium serving) **For very seasonal fruits such as strawberries, please estimate your average use when the fruit is in season**									
Apples				✓					
Pears				✓					
Oranges, satsumas, mandarins		✓							
Grapefruit	✓								
Bananas			✓						
Grapes			✓						
Melon	✓								
Peaches, plums, apricots				✓					
Strawberries, raspberries, kiwi fruit						✓			
Tinned fruit		✓							
Dried fruit, eg. raisins, prunes	✓								
	Never or less than once/month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day

Please check that you have a tick (✓) on EVERY line

7

Appendix G: Example of 7-day food diary section (EPIC-Study).

DATE 23 10 1993 DAY OF WEEK SATURDAY.

BEFORE BREAKFAST

Food/Drink	Description and Preparation	Amount
Orange Squash	Robinsons whole Orange - Sweetened	1 Glass

BREAKFAST

Food/Drink	Description and Preparation	Amount
Beef Patty with onion	Homebaked cold Salt added.	3a.
Tea.	Typhoo	1 Cup
Milk	S/Skimmed	1 Dessertspoon
Sugar	White	1½ Teaspoon.

MID MORNING - between breakfast time and lunch time

Food/Drink	Description and Preparation	Amount
Coffee	Maxwell House Instant ½ Water/½ S/Skimmed milk	1 Mug.
Sugar	White	1½ Teaspoons
Cake.	Homemade Date Cake.	16a.

LUNCH

Food/Drink	Description and Preparation	Amount
Gammon Steak	Microwaved	6oz.
Chips	Deep Fried in Oil (Crisp & Dry)	7a.
Peas	Birds Eye (Frozen)	12a.
Bread	Local Bakery White Unsliced	½ Slice ½" Thick
Apple Pie	Homemade	3B
Sugar	White - sprinkled on	1 Teaspoon
Custard	Birds - made with S/Skimmed milk	Small Fruit Dish.

TEA - between lunch time and the evening meal

Food/Drink	Description and Preparation	Amount
Tea.	Typhoo - tea bag.	1 Mug
Milk	S/Skimmed	1 Dessertspoon
Sugar	White	1½ Teaspoons
Biscuit	Chocolate Digestive Fox's	1

Appendix H: International Physical Activity Questionnaire (IPAQ).

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE (October 2002)

LONG LAST 7 DAYS SELF-ADMINISTERED FORMAT

FOR USE WITH YOUNG AND MIDDLE-AGED ADULTS (15-69 years)

The International Physical Activity Questionnaires (IPAQ) comprises a set of 4 questionnaires. Long (5 activity domains asked independently) and short (4 generic items) versions for use by either telephone or self-administered methods are available. The purpose of the questionnaires is to provide common instruments that can be used to obtain internationally comparable data on health–related physical activity.

Background on IPAQ
The development of an international measure for physical activity commenced in Geneva in 1998 and was followed by extensive reliability and validity testing undertaken across 12 countries (14 sites) during 2000. The final results suggest that these measures have acceptable measurement properties for use in many settings and in different languages, and are suitable for national population-based prevalence studies of participation in physical activity.

Using IPAQ
Use of the IPAQ instruments for monitoring and research purposes is encouraged. It is recommended that no changes be made to the order or wording of the questions as this will affect the psychometric properties of the instruments.

Translation from English and Cultural Adaptation
Translation from English is encouraged to facilitate worldwide use of IPAQ. Information on the availability of IPAQ in different languages can be obtained at www.ipaq.ki.se. If a new translation is undertaken we highly recommend using the prescribed back translation methods available on the IPAQ website. If possible please consider making your translated version of IPAQ available to others by contributing it to the IPAQ website. Further details on translation and cultural adaptation can be downloaded from the website.

Further Developments of IPAQ
International collaboration on IPAQ is on-going and an ***International Physical Activity Prevalence Study*** is in progress. For further information see the IPAQ website.

More Information
More detailed information on the IPAQ process and the research methods used in the development of IPAQ instruments is available at www.ipaq.ki.se and Booth, M.L. (2000). *Assessment of Physical Activity: An International Perspective.* Research Quarterly for Exercise and Sport, 71 (2): s114-20. Other scientific publications and presentations on the use of IPAQ are summarized on the website.

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the **last 7 days**. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Think about all the **vigorous** and **moderate** activities that you did in the **last 7 days**. **Vigorous** physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. **Moderate** activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal.

PART 1: JOB-RELATED PHYSICAL ACTIVITY

The first section is about your work. This includes paid jobs, farming, volunteer work, course work, and any other unpaid work that you did outside your home. Do not include unpaid work you might do around your home, like housework, yard work, general maintenance, and caring for your family. These are asked in Part 3.

1. Do you currently have a job or do any unpaid work outside your home?

☐ Yes

☐ No → ***Skip to PART 2: TRANSPORTATION***

The next questions are about all the physical activity you did in the **last 7 days** as part of your paid or unpaid work. This does not include traveling to and from work.

2. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, digging, heavy construction, or climbing up stairs **as part of your work**? Think about only those physical activities that you did for at least 10 minutes at a time.

_____ **days per week**

☐ No vigorous job-related physical activity → ***Skip to question 4***

3. How much time did you usually spend on one of those days doing **vigorous** physical activities as part of your work?

_____ **hours per day**
_____ **minutes per day**

4. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** physical activities like carrying light loads **as part of your work**? Please do not include walking.

_____ **days per week**

☐ No moderate job-related physical activity → ***Skip to question 6***

LONG LAST 7 DAYS SELF-ADMINISTERED version of the IPAQ. Revised October 2002.

5. How much time did you usually spend on one of those days doing **moderate** physical activities as part of your work?

   _____ **hours per day**
   _____ **minutes per day**

6. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time **as part of your work**? Please do not count any walking you did to travel to or from work.

   _____ **days per week**

   ☐ No job-related walking → ***Skip to PART 2: TRANSPORTATION***

7. How much time did you usually spend on one of those days **walking** as part of your work?

   _____ **hours per day**
   _____ **minutes per day**

PART 2: TRANSPORTATION PHYSICAL ACTIVITY

These questions are about how you traveled from place to place, including to places like work, stores, movies, and so on.

8. During the **last 7 days**, on how many days did you **travel in a motor vehicle** like a train, bus, car, or tram?

   _____ **days per week**

   ☐ No traveling in a motor vehicle → ***Skip to question 10***

9. How much time did you usually spend on one of those days **traveling** in a train, bus, car, tram, or other kind of motor vehicle?

   _____ **hours per day**
   _____ **minutes per day**

Now think only about the **bicycling** and **walking** you might have done to travel to and from work, to do errands, or to go from place to place.

10. During the **last 7 days**, on how many days did you **bicycle** for at least 10 minutes at a time to go **from place to place**?

    _____ **days per week**

    ☐ No bicycling from place to place → ***Skip to question 12***

11. How much time did you usually spend on one of those days to **bicycle** from place to place?

_____ **hours per day**
_____ **minutes per day**

12. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time to go **from place to place**?

_____ **days per week**

☐ No walking from place to place → ***Skip to PART 3: HOUSEWORK, HOUSE MAINTENANCE, AND CARING FOR FAMILY***

13. How much time did you usually spend on one of those days **walking** from place to place?

_____ **hours per day**
_____ **minutes per day**

PART 3: HOUSEWORK, HOUSE MAINTENANCE, AND CARING FOR FAMILY

This section is about some of the physical activities you might have done in the **last 7 days** in and around your home, like housework, gardening, yard work, general maintenance work, and caring for your family.

14. Think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, chopping wood, shoveling snow, or digging **in the garden or yard**?

_____ **days per week**

☐ No vigorous activity in garden or yard → ***Skip to question 16***

15. How much time did you usually spend on one of those days doing **vigorous** physical activities in the garden or yard?

_____ **hours per day**
_____ **minutes per day**

16. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** activities like carrying light loads, sweeping, washing windows, and raking **in the garden or yard**?

_____ **days per week**

☐ No moderate activity in garden or yard → ***Skip to question 18***

17. How much time did you usually spend on one of those days doing **moderate** physical activities in the garden or yard?

_____ **hours per day**
_____ **minutes per day**

18. Once again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** activities like carrying light loads, washing windows, scrubbing floors and sweeping **inside your home**?

_____ **days per week**

☐ No moderate activity inside home → ***Skip to PART 4: RECREATION, SPORT AND LEISURE-TIME PHYSICAL ACTIVITY***

19. How much time did you usually spend on one of those days doing **moderate** physical activities inside your home?

_____ **hours per day**
_____ **minutes per day**

PART 4: RECREATION, SPORT, AND LEISURE-TIME PHYSICAL ACTIVITY

This section is about all the physical activities that you did in the **last 7 days** solely for recreation, sport, exercise or leisure. Please do not include any activities you have already mentioned.

20. Not counting any walking you have already mentioned, during the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time **in your leisure time**?

_____ **days per week**

☐ No walking in leisure time → ***Skip to question 22***

21. How much time did you usually spend on one of those days **walking** in your leisure time?

_____ **hours per day**
_____ **minutes per day**

22. Think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **vigorous** physical activities like aerobics, running, fast bicycling, or fast swimming **in your leisure time**?

_____ **days per week**

☐ No vigorous activity in leisure time → ***Skip to question 24***

23. How much time did you usually spend on one of those days doing **vigorous** physical activities in your leisure time?

_____ **hours per day**
_____ **minutes per day**

24. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** physical activities like bicycling at a regular pace, swimming at a regular pace, and doubles tennis **in your leisure time**?

_____ **days per week**

☐ No moderate activity in leisure time → ***Skip to PART 5: TIME SPENT SITTING***

25. How much time did you usually spend on one of those days doing **moderate** physical activities in your leisure time?
_____ **hours per day**
_____ **minutes per day**

PART 5: TIME SPENT SITTING

The last questions are about the time you spend sitting while at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading or sitting or lying down to watch television. Do not include any time spent sitting in a motor vehicle that you have already told me about.

26. During the **last 7 days**, how much time did you usually spend **sitting** on a **weekday**?

_____ **hours per day**
_____ **minutes per day**

27. During the **last 7 days**, how much time did you usually spend **sitting** on a **weekend day**?

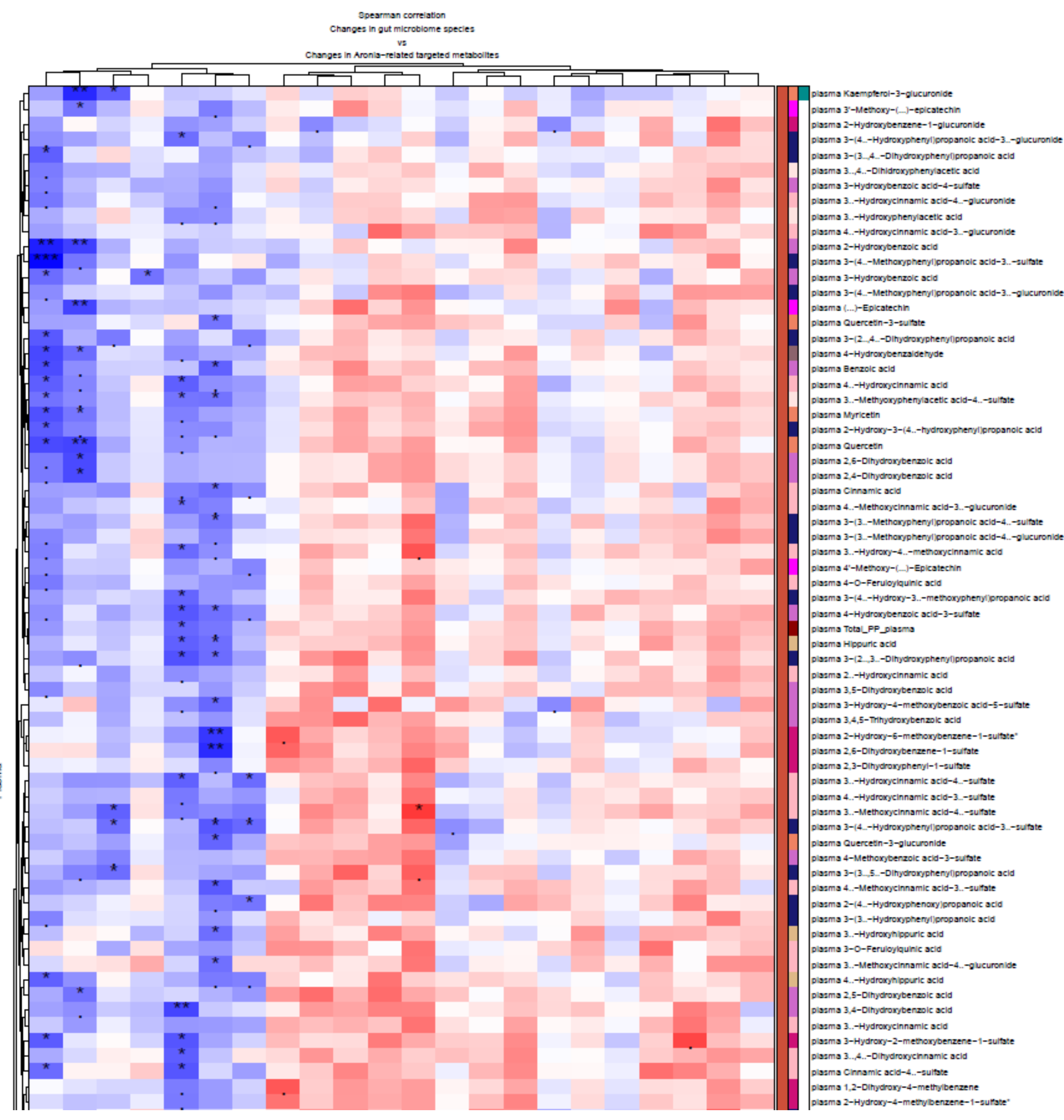
_____ **hours per day**
_____ **minutes per day**

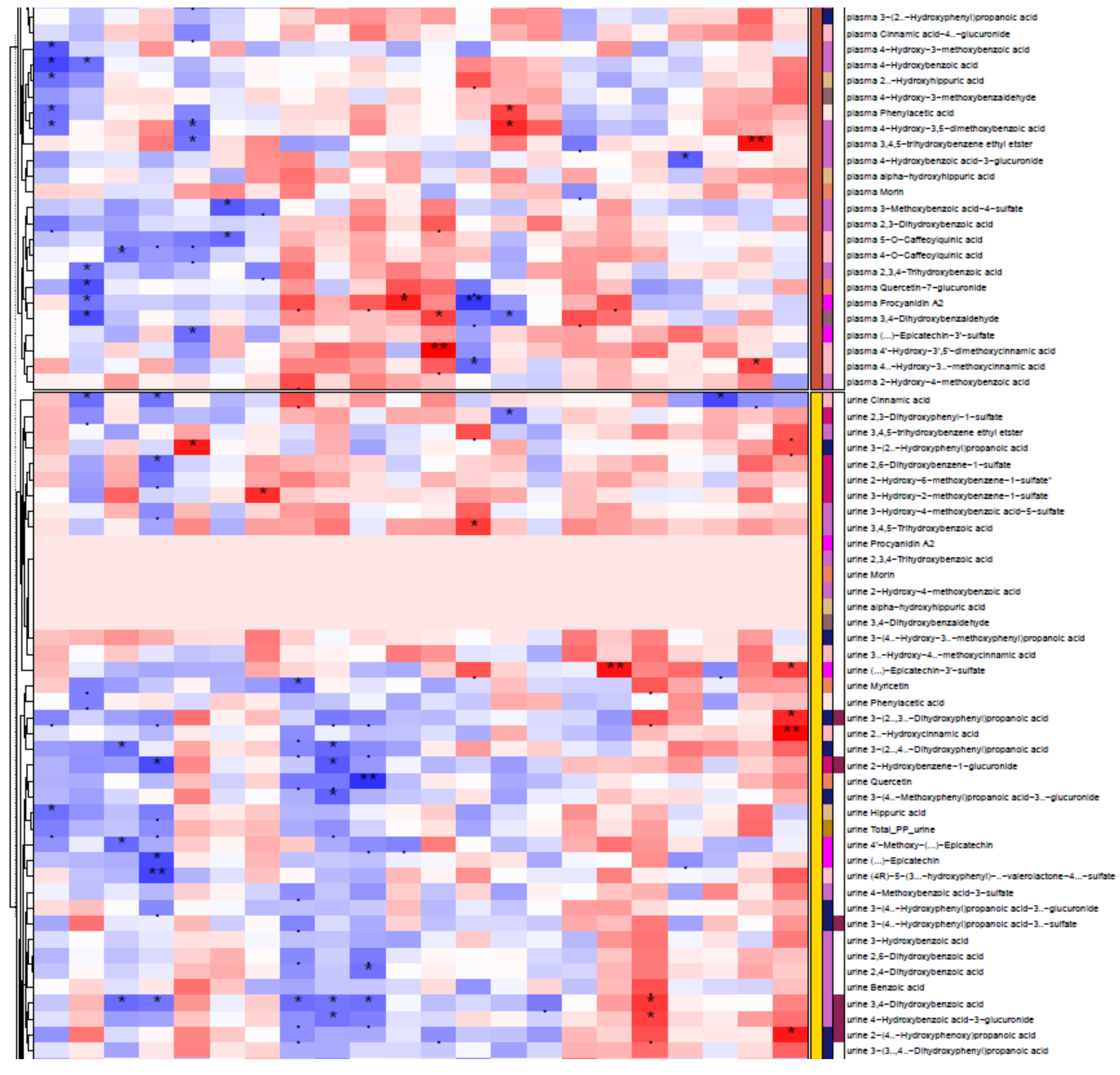
This is the end of the questionnaire, thank you for participating.

LONG LAST 7 DAYS SELF-ADMINISTERED version of the IPAQ. Revised October 2002.

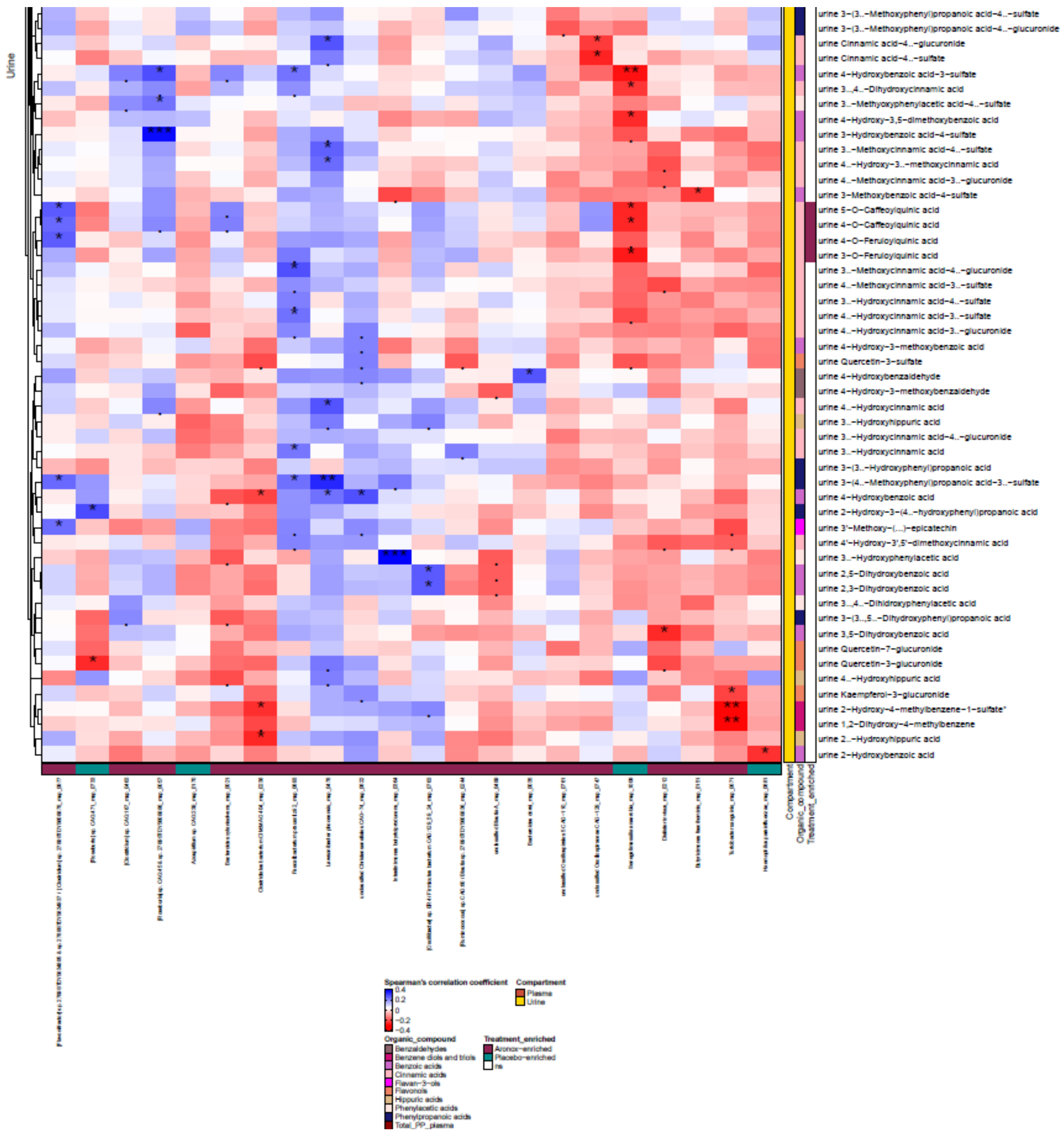
SUPPLEMENTARY MATERIAL

Supplementary Figure S1: Heatmap showing hierarchical Ward-linkage clustering of correlations between targeted Aronia-related compounds and significantly different features in gut microbiome and urinary and plasma metabolites (. < 0.1, *p< 0.05, **p< 0.01 and ***p< 0.001). The heatmap was visualised using the *ComplexHeatmap* R package.





plasma 3−(2..−Hydroxyphenyl)propanoic acid
plasma Cinnamic acid−4..−glucuronide
plasma 4−Hydroxy−3−methoxybenzoic acid
plasma 4−Hydroxybenzoic acid
plasma 2..−Hydroxyhippuric acid
plasma 4−Hydroxy−3−methoxybenzaldehyde
plasma Phenylacetic acid
plasma 4−Hydroxy−3,5−dimethoxybenzoic acid
plasma 3,4,5−trihydroxybenzene ethyl etster
plasma 4−Hydroxybenzoic acid−3−glucuronide
plasma alpha−hydroxyhippuric acid
plasma Morin
plasma 3−Methoxybenzoic acid−4−sulfate
plasma 2,3−Dihydroxybenzoic acid
plasma 5−O−Caffeoylquinic acid
plasma 4−O−Caffeoylquinic acid
plasma 2,3,4−Trihydroxybenzoic acid
plasma Quercetin−7−glucuronide
plasma Procyanidin A2
plasma 3,4−Dihydroxybenzaldehyde
plasma (....)−Epicatechin−3'−sulfate
plasma 4'−Hydroxy−3',5'−dimethoxycinnamic acid
plasma 4..−Hydroxy−3..−methoxycinnamic acid
plasma 2−Hydroxy−4−methoxybenzoic acid
urine Cinnamic acid
urine 2,3−Dihydroxyphenyl−1−sulfate
urine 3,4,5−trihydroxybenzene ethyl etster
urine 3−(2..−Hydroxyphenyl)propanoic acid
urine 2,6−Dihydroxybenzene−1−sulfate
urine 2−Hydroxy−6−methoxybenzene−1−sulfate'
urine 3−Hydroxy−2−methoxybenzene−1−sulfate
urine 3−Hydroxy−4−methoxybenzoic acid−5−sulfate
urine 3,4,5−Trihydroxybenzoic acid
urine Procyanidin A2
urine 2,3,4−Trihydroxybenzoic acid
urine Morin
urine 2−Hydroxy−4−methoxybenzoic acid
urine alpha−hydroxyhippuric acid
urine 3,4−Dihydroxybenzaldehyde
urine 3−(4..−Hydroxy−3..−methoxyphenyl)propanoic acid
urine 3..−Hydroxy−4..−methoxycinnamic acid
urine (....)−Epicatechin−3'−sulfate
urine Myricetin
urine Phenylacetic acid
urine 3−(2..,3..−Dihydroxyphenyl)propanoic acid
urine 2..−Hydroxycinnamic acid
urine 3−(2..,4..−Dihydroxyphenyl)propanoic acid
urine 2−Hydroxybenzene−1−glucuronide
urine Quercetin
urine 3−(4..−Methoxyphenyl)propanoic acid−3..−glucuronide
urine Hippuric acid
urine Total_PP_urine
urine 4'−Methoxy−(....)−Epicatechin
urine (....)−Epicatechin
urine (4R)−5−(3...−hydroxyphenyl)−..−valerolactone−4...−sulfate
urine 4−Methoxybenzoic acid−3−sulfate
urine 3−(4..−Hydroxyphenyl)propanoic acid−3..−glucuronide
urine 3−(4..−Hydroxyphenyl)propanoic acid−3..−sulfate
urine 3−Hydroxybenzoic acid
urine 2,6−Dihydroxybenzoic acid
urine 2,4−Dihydroxybenzoic acid
urine Benzoic acid
urine 3,4−Dihydroxybenzoic acid
urine 4−Hydroxybenzoic acid−3−glucuronide
urine 2−(4..−Hydroxyphenoxy)propanoic acid
urine 3−(3..,4..−Dihydroxyphenyl)propanoic acid


Urine
urine 3−(3..−Methoxyphenyl)propanoic acid−4..−sulfate
urine 3−(3..−Methoxyphenyl)propanoic acid−4..−glucuronide
urine Cinnamic acid−4..−glucuronide
urine Cinnamic acid−4..−sulfate
urine 4−Hydroxybenzoic acid−3−sulfate
urine 3..,4..−Dihydroxycinnamic acid
urine 3..−Methyoxyphenylacetic acid−4..−sulfate
urine 4−Hydroxy−3,5−dimethoxybenzoic acid
urine 3−Hydroxybenzoic acid−4−sulfate
urine 3..−Methoxycinnamic acid−4..−sulfate
urine 4..−Hydroxy−3..−methoxycinnamic acid
urine 4..−Methoxycinnamic acid−3..−glucuronide
urine 3−Methoxybenzoic acid−4−sulfate
urine 5−O−Caffeoylquinic acid
urine 4−O−Caffeoylquinic acid
urine 4−O−Feruloylquinic acid
urine 3−O−Feruloylquinic acid
urine 3..−Methoxycinnamic acid−4..−glucuronide
urine 4..−Methoxycinnamic acid−3..−sulfate
urine 3..−Hydroxycinnamic acid−4..−sulfate
urine 4..−Hydroxycinnamic acid−3..−sulfate
urine 4..−Hydroxycinnamic acid−3..−glucuronide
urine 4−Hydroxy−3−methoxybenzoic acid
urine Quercetin−3−sulfate
urine 4−Hydroxybenzaldehyde
urine 4−Hydroxy−3−methoxybenzaldehyde
urine 4..−Hydroxycinnamic acid
urine 3..−Hydroxyhippuric acid
urine 3..−Hydroxycinnamic acid−4..−glucuronide
urine 3..−Hydroxycinnamic acid
urine 3−(3..−Hydroxyphenyl)propanoic acid
urine 3−(4..−Methoxyphenyl)propanoic acid−3..−sulfate
urine 4−Hydroxybenzoic acid
urine 2−Hydroxy−3−(4..−hydroxyphenyl)propanoic acid
urine 3'−Methoxy−(....)−epicatechin
urine 4'−Hydroxy−3',5'−dimethoxycinnamic acid
urine 3..−Hydroxyphenylacetic acid
urine 2,5−Dihydroxybenzoic acid
urine 2,3−Dihydroxybenzoic acid
urine 3..,4..−Dihidroxyphenylacetic acid
urine 3−(3..,5..−Dihydroxyphenyl)propanoic acid
urine 3,5−Dihydroxybenzoic acid
urine Quercetin−7−glucuronide
urine Quercetin−3−glucuronide
urine 4..−Hydroxyhippuric acid
urine Kaempferol−3−glucuronide
urine 2−Hydroxy−4−methylbenzene−1−sulfate*
urine 1,2−Dihydroxy−4−methylbenzene
urine 2..−Hydroxyhippuric acid
urine 2−Hydroxybenzoic acid
Compartment
Organic_compound
Treatment_enriched
Spearman's correlation coefficient
0.4
0.2
0
−0.2
−0.4
Compartment
Plasma
Urine
Organic_compound
Benzaldehydes
Benzene diols and triols
Benzoic acids
Cinnamic acids
Flavan−3−ols
Flavonols
Hippuric acids
Phenylacetic acids
Phenylpropanoic acids
Total_PP_plasma
Treatment_enriched
Aronox−enriched
Placebo−enriched
ns

Supplementary Table S1. Multiple reaction monitoring (MRM) parameters of validated compounds and internal standards optimized by LC-ESI-MS/MS.

Compound name	Other usual name	Abbreviation	Source	RT	ESI mode (+/-)	Parent ion (m/z)	MRM transitions			Collision		
							Q (m/z)	q1 (m/z)	q2 (m/z)	Q (V)	q1 (V)	q2 (V)
Flavan-3-ols												
(–)-Epicatechin	-	EC	TRC	4.7	-	289	245	203	123	15	18	31
(–)-Epicatechin-3'-sulfate	-	EC-3'-S	GB	6.3	-	303	97	122	244	25	31	18
3'-Methoxy-(–)-epicatechin	-	3'-MeO-EC	GB	7.5	-	303	137	165	-	17	18	-
4'-Methoxy-(–)-Epicatechin	-	4'-MeO-EC	GB	4.2	-	369	289	97	231	31	18	20
Procyanidin A2	-	Procyanidin A2	SA	10.1	-	575	285	449	-	29	22	-
Flavonols												
Quercetin	-	Quercetin	SA	12.2	-	301	151	179	107	22	19	27
Quercetin-3-sulfate	-	Quercetin-3-S	TRC	7.2	-	477	301	-	-	22	-	-
Quercetin-3-glucuronide	-	Quercetin-3-GlcUA	TRC	7.8	-	477	301	-	-	23	-	-
Quercetin-7-glucuronide	-	Quercetin-7-GlcUA	TRC	6.2	-	381	301	179	-	17	29	-
Kaempferol-3-glucuronide	-	Kaempferol-3-GlcUA	ES	9.1	-	461	285	257	-	20	32	-
Myricetin	-	Myricetin	SA	11.8	-	301	151	149	125	19	28	19
Morin	-	Morin	SA	10.2	-	317	151	179	137	24	19	25
Benzene diols and triols												
1,2-Dihydroxy-4-methylbenzene	4-Methylcatechol	1,2-diOH-4-Me-Benz	IBET/ ITQB	3.5	-	123	108	95	-	21	15	-
2-Hydroxy-4-methylbenzene-1-sulfate*	4-Methylcatechol-1-sulfate	2-OH-4/5-Me-Benz-1-S*	IBET/ ITQB	3.5	-	203	123	122	80	22	32	21
2-Hydroxybenzene-1-glucuronide	Catechol-1-glucuronide	2-OH-Benz-1-GlcUA	IBET/ ITQB	3.4	-	285	109	113	-	30	13	-
2,3-Dihydroxybenzene-1-sulfate	Pyrogallol-1-O-sulfate	2,3-diOH-Benz-1-S	IBET/ ITQB	0.6	-	205	125	80	123	18	27	13
2,6-Dihydroxybenzene-1-sulfate	Pyrogallol-2-O-sulfate	2,6-diOH-Benz-1-S	IBET/ ITQB	1.0	-	205	125	80	123	18	27	13
2-Hydroxy-6-methoxybenzene-1-sulfate*	1-Methylpyrogallol-sulfate	2-OH-3/6-MeO-Benz-1-S*	IBET/ ITQB	2.8	-	219	124	139	-	25	15	-
3-Hydroxy-2-methoxybenzene-1-sulfate	2-Methylpyrogallol-sulfate	3-OH-2-MeO-Benz-1-S	IBET/ ITQB	1.5	-	219	124	-	-	25	-	-
Benzaldehydes												
3,4-Dihydroxybenzaldehyde	-	3,4-diOH-BAL	SA	4.1	-	121	92	93	-	24	22	-
4-Hydroxybenzaldehyde	-	4-OH-BAL	SA	3.3	-	177	108	92	81	23	25	21
4-Hydroxy-3-methoxybenzaldehyde	Vanillin	4-OH-3-MeO-BAL	TRC	5.1	-	151	136	92	108	16	22	23
Benzoic acids												
Benzoic acid	-	BA	SA	5.5	-	121	77	-	-	11	-	-
2-Hydroxybenzoic acid	-	2-OH-BA	SA	5.2	-	137	93	65	-	18	28	-
3-Hydroxybenzoic acid	-	3-OH-BA	SA	3.6	-	137	93	-	-	13	-	-
4-Hydroxybenzoic acid	-	4-OH-BA	SA	3.1	-	137	93	65	-	17	30	-
2,3-Dihydroxybenzoic acid	-	2,3-diOH-BA	SA	3.6	-	153	109	108	-	15	24	-
2,4-Dihydroxybenzoic acid	-	2,4/2,6-diOH-BA*	SA	3.4	-	153	67	65	109	16	17	21
2,5-Dihydroxybenzoic acid	-	2,5-diOH-BA	SA	2.9	-	153	109	108	-	15	21	-
2,6-Dihydroxybenzoic acid	-	2,6-diOH-BA	SA	3.3	-	153	135	65	109	17	21	18
3,4-Dihydroxybenzoic acid	Protocatechuic acid	3,4-diOH-BA	SA	1.7	-	153	108	109	-	15	24	-
3,5-Dihydroxybenzoic acid	-	3,5-diOH-BA	SA	1.4	-	153	109	65	-	14	13	-
2,3,4-Trihydroxybenzoic acid	-	2,3,4-triOH-BA	SA	1.8	-	169	151	107	123	16	21	21

2-Hydroxy-4-methoxybenzoic acid	-	2-OH-4-MeO-BA	SA	8.948	-	167.05	108.15	123.15	80.05	21	16	24
3-Hydroxybenzoic acid-4-sulfate	Protocatechuic acid-4-sulfate	3,4-diOH-BA-4-S	TRC	2.1	-	329	109	153	113	34	17	14
4-Hydroxybenzoic acid-3-sulfate	Protocatechuic acid-3-sulfate	3,4-diOH-BA-3-S	TRC	1.2	-	233	109	153	108	27	14	45
4-Hydroxybenzoic acid-3-glucuronide	Protocatechuic acid-3-glucuronide	3,4-diOH-BA-3-GlcUA	TRC	1.0	-	233	109	153	108	27	14	45
4-Hydroxy-3,5-dimethoxybenzoic acid	Syringic acid	4-OH-3,5-diMeO-BA	SA	4.5	-	197	182	123	-	12	21	-
3,4,5-trihydroxybenzene ethyl ester	Gallic acid ethyl ester	3,4,5-triOH-Benz-OEt	SA	4.8	-	197	124	125	169	22	22	17
3,4,5-Trihydroxybenzoic acid	Gallic acid	3,4,5-triOH-BA	SA	0.8	-	169	125	79	81	15	21	21
3-Hydroxy-4-methoxybenzoic acid-5-sulfate	4-Methylgallic acid-3- sulfate	3-OH-4-MeO-BA-5-S	TRC	1.6	-	263	168	183	124	22	15	30
4-Hydroxy-3-methoxybenzoic acid	Vanillic acid	4-OH-3-MeO-BA	SA	4.0	-	167	152	108	123	17	18	15
3-Methoxybenzoic acid-4-sulfate	Vanillic acid-4-sulfate	3-MeO-BA-4-S	IBET/ ITQB	2.3	-	247	167	152	-	15	25	-
4-Methoxybenzoic acid-3-sulfate	Isovanillic acid-3-sulfate	4-MeO-BA-3-S	TRC	3.2	-	247	167	152	108	15	23	31
Hippuric acids												
Hippuric acid	-	HA	SA	3.9	-	178	134	77	-	14	18	-
2'-Hydroxyhippuric acid	Salicyluric acid	2'-OH-HA	E	4.7	-	194	93	150	65	23	15	42
3'-Hydroxyhippuric acid	-	3'-OH-HA	E	3.1	-	194	150	93	92	14	17	30
4'-Hydroxyhippuric acid	-	4'-OH-HA	E	2.5	-	194	100	93	74	11	18	22
alpha-hydroxyhippuric acid	-	α-OH-HA	SA	3.0	-	194	73	-	-	10	-	-
Cinnamic acids												
Cinnamic acid	-	CA	SA	11.1	+	149	103	131	77	-15	-22	-32
3',4'-Dihydroxycinnamic acid	Caffeic acid	3',4'-diOH-CA	SA	4.0	-	179	134	135	-	17	25	-
3'-Hydroxycinnamic acid-4'-sulfate	Caffeic acid-4'-sulfate	3'-OH-CA-4'-S	TRC	3.5	-	259	179	135	134	15	26	40
4'-Hydroxycinnamic acid-3'-sulfate	Caffeic acid-3'-sulfate	4'-OH-CA-3'-S	TRC	3.2	-	259	179	135	134	15	26	40
3'-Hydroxycinnamic acid-4'-glucuronide	Caffeic acid-4'-glucuronide	3'-OH-CA-4'-GlcUA	TRC	3.8	-	355	135	179	-	35	20	-
4'-Hydroxycinnamic acid-3'-glucuronide	Caffeic acid-3'-glucuronide	4'-OH-CA-3'-GlcUA	TRC	3.5	-	355	179	135	-	16	35	-
4'-Hydroxy-3'-methoxycinnamic acid	trans-Ferulic acid	4'-OH-3'-MeO-CA	SA	4.0	-	193	134	178	133	17	14	25
3'-Methoxycinnamic acid-4'-sulfate	Ferulic acid-4'-sulfate	3'-MeO-CA-4'-S	TRC	4.0	-	273	193	178	134	14	23	29
3'-Methoxycinnamic acid-4'-glucuronide	Ferulic acid-4'-glucuronide	3'-MeO-CA-4'-GlcUA	TRC	4.7	-	369	134	193	113	35	17	14
3'-Hydroxy-4'-methoxycinnamic acid	Isoferulic acid	3'-OH-4'-MeO-CA	SA	6.0	-	193	134	-	-	15	-	-
4'-Methoxycinnamic acid-3'-sulfate	Isoferulic acid-3'-sulfate	4'-MeO-CA-3'-S	TRC	4.1	-	369	113	193	178	16	20	29
4'-Methoxycinnamic acid-3'-glucuronide	Isoferulic acid-3'-glucuronide	4'-MeO-CA-3'-GlcUA	TRC	4.5	-	273	193	178	134	16	22	28
4-O-Caffeoylquinic acid	Cryptochlorogenic acid	4-CQA	TRC	4.0	-	353	173	179	191	16	17	23
5-O-Caffeoylquinic acid	Chlorogenic acid	4/5-CQA*	SA	4.0	-	353	191	85	93	19	43	42
3-O-Feruloylquinic acid	-	3-FQA	TRC	5.4	-	367	191	93	134	15	27	34
4-O-Feruloylquinic acid	-	4-FQA	TRC	5.2	-	367	173	193	134	16	17	34
4'-Hydroxy-3',5'-dimethoxycinnamic acid	Sinapic acid	4'-OH-3',5'-diMeO-CA	SA	6.5	-	223	208	121	193	12	29	21
3'-Hydroxycinnamic acid	m-Coumaric acid	3'-OH-CA	SA	5.6	-	163	119	93	91	15	31	25
4'-Hydroxycinnamic acid	p-Coumaric acid	4'-OH-CA	SA	5.0	-	163	119	93	-	16	33	-
2'-Hydroxycinnamic acid	o-Coumaric acid	2'-OH-CA	SA	6.8	-	163	119	93	117	14	29	24

Cinnamic acid-4'-sulfate	p-Coumaric acid-4'-sulfate	CA-4'-S	TRC	3.7	-	243	119	-	-	25	-	-
Cinnamic acid-4'-glucuronide	p-Coumaric acid-4'-glucuronide	CA-4'-GlcUA	TRC	3.7	-	339	119	113	163	39	13	17
Phenylacetic acids	**-**											
Phenylacetic acid	-	PA	SA	5.7	-	135	91	-	-	9	-	-
3'-Hydroxyphenylacetic acid	-	3'-OH-PA	SA	3.9	-	151	107	-	-	12	-	-
3',4'-Dihidroxyphenylacetic acid	Homoprotocatechuic acid	3',4'-diOH-PA	TRC	2.8	-	167	123	-	-	10	-	-
3'-Methyoxyphenylacetic acid-4'-sulfate	Homovanillic acid-sulfate	3'-MeO-PA-4'-S	TRC	3.3	-	261	181	137	122	16	22	25
Phenylpropanoic acids												
2-(4'-Hydroxyphenoxy)propanoic acid	-	2-(4'-OH-ph-O)-PrA	SA	3.7	-	181	109	-	-	15	-	-
3-(2'-Hydroxyphenyl)propanoic acid	-	3-(2'-OH-ph)-PrA	TRC	5.5	-	165	121	106	-	15	21	-
3-(3'-Hydroxyphenyl)propanoic acid	-	3-(3'-OH-ph)-PrA	SA	4.9	-	165	121	119	-	14	16	-
3-(2',3'-Dihydroxyphenyl)propanoic acid	-	3-(2',3'-diOH-ph)-PrA	TRC	4.2	-	181	137	122	163	17	24	15
3-(2',4'-Dihydroxyphenyl)propanoic acid	-	3-(2',4'-diOH-ph)-PrA	SA	3.6	+	183	123	165	55	-11	-15	-16
3-(3',4'-Dihydroxyphenyl)propanoic acid	Dihydrocaffeic acid	3-(3',4'-diOH-ph)-PrA	SA	3.6	-	181	137	-	-	15	-	-
3-(3',5'-Dihydroxyphenyl)propanoic acid	-	3-(3',5'-diOH-ph)-PrA	SA	3.3	+	183	165	137	-	-12	-17	-
2-Hydroxy-3-(4'-hydroxyphenyl)propanoic acid	-	3-(4'-OH-ph)-2-OH-PrA	SA	2.9	-	181	163	135	-	16	17	-
3-(4'-Hydroxy-3'-methoxyphenyl)propanoic acid	Dihydroferulic acid	3-(4'-OH-3'-MeO-ph)-PrA	SA	5.2	-	195	136	121	-	15	27	-
3-(4'-Hydroxyphenyl)propanoic acid-3'-glucuronide	Dihydrocaffeic acid-3'-glucuronide	3-(4'-OH-ph)-PrA-3'-GlcUA	TRC	3.8	-	357	181	-	-	21	-	-
3-(4'-Hydroxyphenyl)propanoic acid-3'-sulfate	Dihydrocaffeic acid-3'-sulfate	3-(4'-OH-ph)-PrA-3'-S	TRC	3.4	-	261	181	137	-	17	23	-
3-(3'-Methoxyphenyl)propanoic acid-4'-sulfate	Dihydroferulic acid-4'-sulfate	3-(3'-MeO-ph)-PrA-4'-S	TRC	4.2	-	371	113	195	-	16	20	-
3-(3'-Methoxyphenyl)propanoic acid-4'-glucuronide	Dihydroferulic acid-4'-glucuronide	3-(3'-MeO-ph)-PrA-4'-GlcUA	TRC	3.9	-	275	195	136	135	17	23	35
3-(4'-Methoxyphenyl)propanoic acid-3'-sulfate	Dihydroisoferulic acid-3'-sulfate	3-(4'-MeO-ph)-PrA-3'-S	TRC	4.7	-	371	113	195	-	17	21	-
3-(4'-Methoxyphenyl)propanoic acid-3'-glucuronide	Dihydroisoferulic acid-3'-glucuronide	3-(4'-MeO-ph)-PrA-3'-GlcUA	TRC	4.2	-	275	195	136	135	18	25	35
Valerolactones												
(4R)-5-(3′-hydroxyphenyl)-γ-valerolactone-4′-sulfate	-	5-(3′-OH-ph)-γ-VL-4′-S	TRC	4.7	-	287	207	163	122	20	28	33
Internal standards												
Taxifolin	-	Taxifolin	SA	6.4	-	303	285	125	177	11	22	12

RT: retention time, ESI: electro-spray interface, MRM: multiple reaction monitoring, Q: transition used for quantification, q1,q2: qualifier transitions used for identification of each compound, RAq1, RAq2: relative ion abundance of q1 and q2, SA: Sigma-Aldrich (Steinheim, Germany), TRC: Toronto Research Chemicals (Toronto, Canada), IBET/ITQB: Institute for Experimental Biology and Technology/Instituto de Tecnologia Química e Biológica (Lisbon, Portugal), E: Enamine (Kiev, Ukraine), ES: Extrasynthese (Genay, France), GB: GERBU Biotechnik (Heidelberg, Germany), F:Fisher (Loughborough, UK), C: Cayman Chemical (Michigan, USA), RCG: ReseaChem GmbH (Burgdorf, Switzerland), CCB: ChemCruz™ Biochemicals (Huissen, Germany), DPS: Dalton Pharma Services (Toronto, Canada).* mix of isomers

Supplementary Table S2. Number of volunteers included in the BAB and BACO analysis for each parameter and each timepoint, in the ITT population.

	Acute	Chronic			Acute on chronic
		24h	Awake	Asleep	
Ambulatory PSBP		97	94	94	
Ambulatory PDBP		97	94	94	
Office PSBP	102	97			97
Office PDBP	102	97			97
Office CSBP	101	96			96
Office CDBP	101	96			96
Office PWV	100	93			93
Office AIx	100	95			92
Office HR (seated)	102	97			97
Office HR (supine)	101	96			96
Ambulatory CSBP		80	79	77	
Ambulatory HR		97	97	94	
Ambulatory PWV		79	79	77	
Ambulatory AIx_{ao}		80	79	77	
Ambulatory AIx_{br}		80	79	77	
FMD	101	97			97
Blood flow velocity	100	97			96
QRISK®3 score		91			
Cortisol		90			
Total cholesterol		91			
Triglycerides		91			
HDL		91			
LDL		91			

Supplementary Table S3. Contribution of food groups and some specific foods to the intake of total (poly)phenols and (poly)phenol classes in the ABP study.

Polyphenols	Food category*
Total polyphenols	Coffee (29%), tea (27%) and fruits (11%)
Anthocyanins	Fruits (44%), alcoholic drinks (22%) and leafy vegetables (8%)
Proanthocyanidins	Fruits (34%), Teas (15%) and chocolate (9%)
Flavan-3-ols	Teas (88%), chocolate (4%) and fruits (2%)
Flavanones	Fruits (59%), fruit juices (23%) and soft drinks (11%)
Flavones	Tea (27%), roots, tubers and bulbs (23%) and fruit juices (14%)
Flavonols	Tea (43%), roots, tubers and bulbs (23%) and leafy vegetables (11%)
Isoflavonoids	Beans, peas and lentils (54%), breads (24%) and yoghurts (8%)
Total Flavonoids	Tea (46%), fruits (20%) and chocolate (5%)
Hydroxybenzoic acids	Teas (64%), nuts and seeds (13%) and fruits (5%)
Hydroxycinnamic acids	Coffee (82%), fruits (4%) and teas (3%)
Total Phenolic acids	Coffee (70%), teas (12%) and fruits (4%)
Lignans	Nuts and seeds (41%), oils (26%) and non-chocolate confectionary (15%)
Stilbenes	Alcoholic drinks (77%), fruits (15%) and beans, peas and lentils (2%)

* Contribution to polyphenol intake in the food group at baseline (percentages). Foods listed are the top 3 main food sources.

Supplementary Table S4. Effects of aronia berry extract after 12-week consumption on 24-hour, awake and asleep central PWV and peripheral and central AIx in the ITT population and each intervention group, following BACO analysis.

Parameter	*Unit*	CFB Aronia	CFB Control	CFC	p-value
24-hour PWV_{ao}	*m/sec*	-0.048 ± 0.110	0.111 ± 0.105	-0.159	0.301
Awake PWV_{ao}	*m/sec*	-0.153 ± 0.136	0.125 ± 0.134	-0.278	**0.049**
Asleep PWV_{ao}	*m/sec*	-0.079 ± 0.129	0.143 ± 0.128	-0.222	0.228
24-hour AIx_{ao}	*%*	-0.886 ± 0.796	2.183 ± 0.776	-3.069	**0.007**
Awake AIx_{ao}	*%*	-1.061 ± 0.982	1.860 ± 0.969	-2.921	**0.023**
Asleep AIx_{ao}	*%*	-0.701 ± 1.258	1.537 ± 1.241	-2.238	0.211
24-hour AIx_{br}	*%*	-2.169 ± 1.554	4.293 ± 1.515	-6.463	**0.004**
Awake AIx_{br}	*%*	-2.692 ± 1.985	3.578 ± 1.960	-6.270	**0.012**
Asleep AIx_{br}	*%*	-1.389 ± 2.485	3.029 ± 2.453	-4.418	0.211

Values expressed as mean ± SD. AIx, augmentation index; ao, aortic; br, brachial; CFB, changes from baseline; CFC, changes from Control; PWV, pulse wave velocity.

Supplementary Table S5. Post-hoc analysis of the power calculation of the main vascular parameters of the ABP study.

	Aronia		Control		Post-hoc power calculation (%)
	Changes from baseline	n	Changes from baseline	n	
24h heart rate (bpm)	-0.161 ± 0.900	49	-1.611 ± 0.909	48	100
24h SBP_{ao} (mmHg)	-1.444 ± 0.889	39	-0.276 ± 0.866	41	100
24h AIx_{ao} (%)	-0.985 ± 0.810	39	2.278 ± 0.789	41	100
24h AIx_{br} (%)	-2.330 ± 1.578	39	4.446 ± 1.539	41	100
24h PWV (m/s)	-0.045 ± 0.112	39	0.107 ± 0.108	41	100
Asleep SBP_{br} (mmHg)	-1.290 ± 1.056	47	-1.343 ± 1.056	47	4.3
Asleep DBP_{br} (mmHg)	-1.018 ± 0.785	47	-0.500 ± 0.785	47	89.2
Asleep heart rate (bpm)	0.170 ± 0.944	47	-0.291 ± 0.944	47	66
Asleep SBP_{ao} (mmHg)	-1.567 ± 1.400	38	0.017 ± 1.382	40	99.8
Asleep AIx_{ao} (%)	-0.966 ± 1.275	38	1.795 ± 1.258	40	100
Asleep AIx_{br} (%)	-1.913 ± 2.519	38	3.539 ± 2.486	40	100
Asleep PWV (m/s)	-0.112 ± 0.131	38	0.175 ± 0.129	40	100
Awake SBP_{br} (mmHg)	-1.965 ± 0.801	49	-1.797 ± 0.809	48	17.6
Awake DBP_{br} (mmHg)	-1.779 ± 0.588	49	-1.352 ± 0.595	48	94.5
Awake heart rate (bpm)	-0.089 ± 1.031	49	-1.860 ± 1.042	48	100
Awake SBP_{ao} (mmHg)	-1.685 ± 1.015	39	-1.031 ± 1.002	41	82.6
Awake AIx_{ao} (%)	-1.046 ± 1.006	39	1.845 ± 0.993	41	100
Awake AIx_{br} (%)	-2.621 ± 2.033	39	3.509 ± 2.007	41	100
Awake PWV (m/s)	-0.132 ± 0.138	39	0.105 ± 0.137	41	100
Office SBP (mmHg)	-2.378 ± 0.915	49	-0.01 ± 0.924	48	100
Office DBP (mmHg)	-0.909 ± 0.704	49	-1.020 ± 0.712	48	11.7
Office peripheral heart rate (bpm)	-0.471 ± 0.922	49	-2.228 ± 0.932	48	100
Office PWV (m/s)	0.334 ± 0.197	49	0.302 ± 0.204	46	11.8
Office AIx (%)	-0.902 ± 1.055	49	-0.560 ± 1.089	48	34.8
Office central SBP (mmHg)	-1.798 ± 0.932	49	-0.168 ± 0.952	48	100
Office central DBP (mmHg)	-0.208 ± 0.805	49	-1.038 ± 0.805	48	99.9
Office central heart rate (bpm)	-0.718 ± 0.869	49	-1.060 ± 0.888	48	48.3
Flow mediated dilation (%)	0.148 ± 0.208	49	0.564 ± 0.210	48	100
Blood flow velocity (cm/sec)	-0.984 ± 3.434	49	3.403 ± 3.488	48	100

AIx, augmentation index; ao, aortic; br, brachial; DBP, diastolic blood pressure; PWV, pulse wave velocity; R, responders; SBP, systolic blood pressure. Values presented as mean ± SD. Post-hoc power calculation using https://clincalc.com/stats/Power.aspx.

Supplementary Table S6. Urinary (poly)phenol metabolites at baseline and following acute, chronic, and acute on chronic consumption of aronia berries for Aronia and Control groups, and associated changes from Control.

	Aronia				Control				Chronic CFC (Aronia - Control)				
	Baseline (n= 51)		12 weeks (n=48)		Baseline (n= 51)		12 weeks (n=49)		Mean difference	SEM	95% CI		P
	Mean	SD	Mean	SD	Mean	SD	Mean	SD			Lower	Higher	
Total (poly)phenols	4906157	3120855	4176413	2549594	5268891	3081831	5191836	4680419	-922988	706565	-2325889	479912	0.356
Flavan-3-ols													
EC	123	178	56.6	114	182	363	137	295	-59.4	41.1	-141	22.2	0.691
EC-3'-S	712	797	655	598	663	710	622	698	14.9	131	-245	275	0.823
3'-MeO-EC	23.3	92.7	1.4	10.1	8.1	44.0	4.9	33.9	-3.3	5.1	-13.5	6.8	0.459
4'-MeO-EC	9.1	16.0	6.8	12.2	22.9	47.8	10.9	22.4	-4.2	3.7	-11.7	3.2	0.442
Procyanidin A2	-	-	-	-	-	-	-	-	-	-	-	-	
Flavonols													
Quercetin	20.9	83.7	8.3	31.2	83.5	532	24.1	161	-15.8	23.7	-62.8	31.2	0.983
Quercetin-3-S	143	144	125	124	163	166	168	170	-22.5	13.5	-49.4	4.3	0.191
Quercetin-3-GlcUA	200	383	150	215	178	222	187	382	-43.8	62.3	-167	79.8	0.931
Quercetin-7-GlcUA	8.2	29.2	5.5	17.9	8.5	21.4	7.6	21.5	-2.3	3.9	-10.1	5.5	0.665
Kaempferol-3-GlcUA	541	664	454	539	753	1220	541	792	-65.9	133	-329	197	0.965
Myricetin	83.1	147	78.2	133	204	831	84.0	183	-2.9	32.6	-67.7	61.8	0.283
Morin	-	-	-	-	-	-	-	-	-	-	-	-	
Benzene diols and triols													
1,2-diOH-4-Me-Benz	5712	4549	4522	3922	5445	4780	6645	8065	-2326	970	-4252	-400	0.054
2-OH-4/5-Me-Benz-1-S*	65715	61359	47076	46641	55617	50276	58334	62837	-16740	9049	-34706	1227	0.147
2-OH-Benz-1-GlcUA	2559	2331	2657	2241	3788	3451	4068	6069	-660	867	-2382	1062	0.225
2,3-diOH-Benz-1-S	1019	843	865	750	1289	1123	1524	1697	-527	243	-1010	-44	0.242
2,6-diOH-Benz-1-S	16167	23207	12012	14057	19148	21876	22161	38833	-9319	5537	-20313	1676	0.983
2-OH-3/6-MeO-Benz-1-S*	27828	37158	18218	17608	31495	35018	35947	58424	-17205	7895	-32881	-1529	0.184
3-OH-2-MeO-Benz-1-S	2584	5114	1392	1871	2680	3637	3642	8395	-2261	1045	-4336	-185	0.149
Benzaldehydes													
3,4-diOH-BAL	-	-	-	-	-	-	-	-	-	-	-	-	
4-OH-BAL	96.6	178	71.6	120	53.7	118	82.7	167	-21.7	29.1	-79.5	36.1	0.125
4-OH-3-MeO-BAL	77.9	439	-	-	-	-	73.5	509	-73.5	73.7	-220	72.8	0.085
Benzoic acids													
BA	16744	35288	10740	11975	10886	11962	7462	7058	1825	1547	-1246	4897	0.537
2-OH-BA	1603	2581	971	1098	2079	2898	2521	7452	-1404	1079	-3547	739	0.823
3-OH-BA	1284	1917	1022	818	996	1016	1012	1135	-40.8	195	-429	347	0.795
4-OH-BA	16284	18152	10822	7668	13900	8856	12891	10272	-2470	1782	-6009	1068	0.914
2,3-diOH-BA	134653	224300	94401	50583	84247	46896	89442	79441	2637	13657	-24479	29753	0.040
2,4/2,6-diOH-BA*	2238	2779	1575	972	1598	1002	1558	1177	-105	208	-517	308	0.413
2,5-diOH-BA	30330	56479	20472	11155	18270	10693	19642	18878	326	3176	-5980	6632	1.000
2,6-diOH-BA	1230	1511	892	485	898	513	879	612	-43.0	107	-256	170	0.574
3,4-diOH-BA	1289	825	1310	684	1286	628	1174	1195	122	185	-246	490	0.948
3,5-diOH-BA	15182	18064	12034	12317	15654	17787	17985	21872	-5496	3010	-11472	480	0.270
2,3,4-triOH-BA	-	-	-	-	-	-	-	-	-	-	-	-	-
2-OH-4-MeO-BA	-	-	-	-	-	-	-	-	-	-	-	-	-
3,4-diOH-BA-4-S	7299	5916	6461	3925	7068	3851	7813	8601	-1511	1318	-4129	1107	0.931
3,4-diOH-BA-3-S	4043	3421	4411	3757	3842	2718	5117	12450	-931	1844	-4593	2730	0.334
3,4-diOH-BA-3-GlcUA	113	85.4	116	64.5	121	82.8	109	104	9.4	16.5	-23.4	42.1	0.215
4-OH-3,5-diMeO-BA	967	711	798	492	911	603	818	724	-35.1	121	-274	204	0.756
3,4,5-triOH-Benz-OEt	35.2	120	26.5	107	5.0	28	24.4	119	-9.3	22.4	-53.8	35.3	0.670
3,4,5-triOH-BA	472	691	463	1014	549	1210	505	815	-16.1	174	-363	330	0.603
3-OH-4-MeO-BA-5-S	11477	12499	9202	13488	14115	26739	12401	15530	-2147	2468	-7046	2753	0.391
4-OH-3-MeO-BA	10672	6231	9065	6156	11531	6513	11326	9050	-1953	1419	-4770	864	0.507
3-MeO-BA-4-S	9059	10165	8049	7280	8773	6870	9375	8938	-1658	1435	-4508	1191	0.480
4-MeO-BA-3-S	4619	4000	4213	3557	4076	2404	4555	4237	-577	751	-2068	914	0.686
Hippuric acids													
HA	3553948	2529468	3009548	2120694	3949340	2585886	3812518	3307914	-670763	503808	-1671086	329559	0.411
2'-OH-HA	33702	25972	26745	18425	46701	58143	37665	40659	-6889	5862	-18527	4749	0.834
3'-OH-HA	240314	223269	239700	202259	271962	210083	320357	546724	-66210	79369	-223799	91378	0.559
4'-OH-HA	66733	69863	48929	31998	51932	22717	64047	61625	-18650	9841	-38190	890	0.091
α-OH-HA	-	-	-	-	-	-	-	-	-	-	-	-	

Cinnamic acids													
CA	-	-	27.1	190	110	555	204	1086	33.9	58.7	-82.8	150.5	0.548
3',4'-diOH-CA	503	410	422	223	520	301	509	658	-87	95	-275	101	0.801
3'-OH-CA-4'-S	4036	3991	3184	1645	3124	1838	3368	4001	-410	617	-1635	816	0.902
4'-OH-CA-3'-S	25049	26115	19963	9931	21114	14514	22490	29819	-3908	4382	-12608	4793	0.908
3'-OH-CA-4'-GlcUA	1955	1623	1600	1098	2023	1319	2315	2352	-712	360	-1427	2.9	0.130
4'-OH-CA-3'-GlcUA	556.5	498.6	442.6	266.2	508.1	415.6	559.4	780.9	-150.6	110.6	-370.1	69.0	0.449
4'-OH-3'-MeO-CA	109902	102415	90599	48814	103654	74577	100322	135279	-13549	19856	-52973	25876	0.823
3'-MeO-CA-4'-S	171891	147127	147215	77026	174754	123383	168934	207417	-24074	30062	-83762	35614	0.931
3'-MeO-CA-4'-GlcUA	31821	49032	27607	22451	33376	38064	36531	64553	-8916	9130	-27044	9212	0.598
3'-OH-4'-MeO-CA	13544	10268	10918	8421	12793	8489	12994	11149	-2522	1752	-6001	958	0.175
4'-MeO-CA-3'-S	2118	2838	1738	1291	1949	1696	2102	3380	-441	501	-1436	555	0.660
4'-MeO-CA-3'-GlcUA	7209	4607	6537	3602	8136	5474	8339	9963	-1486	1455	-4376	1403	0.948
4-CQA	165	225	224	245	266	388	217	459	46	71	-96	187	0.005
4/5-CQA*	1021	1306	1340	1448	1607	2289	1315	2716	250	422	-588	1088	0.005
3-FQA	2967	6380	3106	4139	3086	3645	2942	5964	138	1018	-1882	2159	0.020
4-FQA	1875	2530	2234	3282	2755	3169	2799	6190	82	906	-1717	1881	0.012
4'-OH-3',5'-diMeO-CA	802	1120	545	684	768	716	645	605	-105	129	-361	152	0.863
3'-OH-CA	1271	1466	1282	1116	1505	1849	1408	1759	-73	291	-652	505	0.521
4'-OH-CA	1376	1031	1394	864	1372	984	1307	1098	74	196	-315	462	0.403
2'-OH-CA	125	150	131	169	153	135	128	134	16	28	-40	72	0.382
CA-4'-S	2217	1428	2087	1223	1756	1517	2101	1601	-126	295	-711	459	0.106
CA-4'-GlcUA	680	703	632	469	503	433	598	560	7	105	-203	216	0.566
Phenylacetic acids													
PA	3232	4920	2620	4159	5599	11585	4094	5660	-1376	1020	-3402	649	0.566
3'-OH-PA	83213	61864	95992	102238	88972	57731	79667	51751	18552	15468	-12160	49263	0.391
3',4'-diOH-PA	13518	9176	12704	8961	13139	7482	12812	9569	-332	1683	-3673	3010	0.983
3'-MeO-PA-4'-S	2473	1863	2424	1774	2688	1520	2830	2899	-344	415	-1168	480	0.840
Phenylpropanoic acids													
2-(4'-OH-ph-O)-PrA	4960	5480	5539	5228	6368	5609	5734	8911	916	1133	-1335	3166	0.051
3-(2'-OH-ph)-PrA	232	331	162	238	232	259	197	263	-40	47	-132	53	0.789
3-(3'-OH-ph)-PrA	11852	18480	11651	14035	15485	21033	15217	26190	-2380	4057	-10435	5676	0.629
3-(2',3'-diOH-ph)-PrA	549	565	587	590	777	729	722	1055	-28	164	-353	297	0.175
3-(2',4'-diOH-ph)-PrA	1159	1114	726	695	1224	1268	966	1234	-217	186	-587	153	0.491
3-(3',4'-diOH-ph)-PrA	2358	2026	2175	1773	2666	1961	2528	3172	-122	408	-931	688	0.569
3-(3',5'-diOH-ph)-PrA	17452	15804	14711	15029	16427	12883	15784	11424	-1436	2440	-6281	3408	0.535
3-(4'-OH-ph)-2-OH-PrA	7839	6587	6411	4867	7737	5534	8063	7332	-1726	1050	-3810	359	0.306
3-(4'-OH-3'-MeO-ph)-PrA	25449	39792	21682	22277	27498	26334	27582	42225	-5340	6448	-18144	7463	0.629
3-(4'-OH-ph)-PrA-3'-GlcUA	1573	2704	2243	4211	1570	1493	1550	2300	649	677	-694	1992	0.395
3-(4'-OH-ph)-PrA-3'-S	18812	15384	22342	16715	21571	17359	22541	39765	1325	5105	-8812	11461	0.170
3-(3'-MeO-ph)-PrA-4'-S	3947	3054	4007	2819	4397	2697	4927	6837	-750	996	-2727	1228	0.773
3-(3'-MeO-ph)-PrA-4'-GlcUA	7096	6879	7679	6795	6452	5348	8859	21518	-2380	2694	-7730	2969	0.718
3-(4'-MeO-ph)-PrA-3'-S	4722	16868	2708	2975	2632	2285	3005	3647	-397	671	-1729	936	0.960
3-(4'-MeO-ph)-PrA-3'-GlcUA	7660	8374	8573	10046	8847	10594	7225	7870	1562	1707	-1827	4952	0.569
Valerolactones													
5-(3′-OH-ph)-γ-VL-4′-S	19030	18867	17899	18274	16222	14993	19923	24437	-4057	4031	-12061	3947	0.266

Differences were calculated from ANCOVA (Bonferroni) including baseline values as covariate, comparing Aronia with Control changes from baseline. 95% CI, 95% confidence interval; CFC, changes from Control; SD, standard deviation; SEM, standard error of mean. P-values (P) obtained following Mann-Whitney non-parametric test. * Mix of two isomers.

Supplementary Table S7. Plasma (poly)phenol metabolites at baseline and following acute, chronic, and acute on chronic consumption of aronia berries, showing data for Aronia and Control groups.

	Aronia								Control							
	Visit 1, 0h (n=47)		Visit 1, 2h (n=43)		Visit 2, 0h (n=47)		Visit 2, 2h (n=46)		Visit 1, 0h (n=50)		Visit 1, 2h (n=50)		Visit 2, 0h (n=48)		Visit 2, 2h (n=47)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Total (poly)phenols	353588	648724	289801	679444	530423	1120085	359181	663556	294836	462388	213013	245357	574121	1743646	359917	527399
Flavan-3-ols																
EC	101	382	47.4	72.6	56.3	76.9	121	487	90.5	206	73.3	124	243	982	145	338
EC-3'-S	12.3	40.0	11.2	31.5	15.6	62.3	12.9	33.8	8.7	31.3	6.6	24.6	27.0	118.7	11.2	35.7
3'-MeO-EC	54.3	154	35.8	67.4	68.7	196	87.7	309	55.1	152	63.3	145	172	594	105	278
4'-MeO-EC	12.5	20.4	14.7	32.5	14.4	20.2	14.0	21.7	11.0	11.4	10.1	9.8	13.0	18.0	16.1	22.4
Procyanidin A2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Flavonols																
Quercetin	40.4	94.8	46.5	152	40.6	84.8	46.7	111	26.2	52.6	25.9	44.8	69.6	225	47.6	89.3
Quercetin-3-S	6.0	3.4	6.2	3.0	12.3	41.5	7.1	8.9	9.0	13.8	9.4	20.1	7.6	7.6	7.4	6.9
Quercetin-3-GlcUA	9.1	32.2	13.5	27.2	13.1	28.4	24.4	54.1	9.5	20.3	4.9	10.5	23.5	110	95.2	614
Quercetin-7-GlcUA	0.6	2.1	0.5	1.8	0.5	2.2	1.1	5.0	2.0	6.7	2.2	9.6	1.8	5.0	4.9	21.2
Kaempferol-3-GlcUA	2.1	7.5	0.9	1.8	3.2	10.8	1.7	5.2	1.4	3.1	1.6	4.4	11.1	44.6	4.8	19.3
Myricetin	352	637	365	881	350	510	372	654	272	342	257	288	437	914	382	501
Morin	2.5	17.0	4.0	26.4	2.1	11.0	1.1	7.7	1.4	7.2	0.0	0.0	2.3	12.2	1.9	9.0
Benzene diols and triols																
1,2-diOH-4-Me-Benz	4460	8497	3774	9030	7344	20444	6074	15472	3576	5846	3345	6819	8188	25087	7068	14415
2-OH-4/5-Me-Benz-1-S*	46904	84223	40784	96681	80354	236395	67516	178994	39853	61926	35162	66812	83982	251009	56510	106657
2-OH-Benz-1-GlcUA	140	495	73.3	164	188	508	100	184	99.9	215	49.0	69.4	92.3	180	111	256
2,3-diOH-Benz-1-S	946	1388	1116	3765	2910	8225	1155	2609	1696	3340	1234	2783	1709	3397	1585	3257
2,6-diOH-Benz-1-S	1422	2048	1249	3867	3237	8471	1726	3440	2134	3921	3347	13608	3904	16281	2628	5363
2-OH-3/6-MeO-Benz-1-S*	2562	4139	3065	12577	5896	17070	3684	8556	3552	6358	5219	22056	6870	27219	4695	10275
3-OH-2-MeO-Benz-1-S	270	573	294	667	481	1378	251	492	244	407	291	815	404	982	292	604
Benzaldehydes																
3,4-diOH-BAL	6.6	37.7	6.3	30.4	1.2	7.9	4.8	16.4	2.6	10.8	0.0	0.0	9.1	34.1	5.2	18.6
4-OH-BAL	383.2	948.8	431.1	1077.0	529.6	1024.1	528.6	1156.4	304.3	544.0	331.2	571.0	625.6	1605.4	496.1	674.8
4-OH-3-MeO-BAL	143.9	264.7	133.7	324.3	169.4	310.5	149.6	289.2	98.2	122.9	105.8	162.9	191.3	572.1	146.7	232.5
Benzoic acids																
BA	6220	10581	5792	12238	10695	22298	7354	11920	6532	10884	5862	8328	16218	43490	9963	14569
2-OH-BA	1937	5753	1666	5067	1705	4060	1375	3014	665	1050	635	1080	1665	5100	1154	2376
3-OH-BA	1012	1715	1210	3404	927	1534	1381	3098	1220	2822	725	1040	2080	5621	1654	3949
4-OH-BA	12226	30152	7736	19010	8632	14578	6944	11758	8020	11562	5903	10869	17065	73063	7391	12749
2,3-diOH-BA	151	379	148	375	200	454	210	430	132	213	141	317	135	163	220	573
2,4/2,6-diOH-BA*	9378	21483	6628	15125	7016	11252	4778	6816	4889	7698	4214	5988	15512	63088	8906	18920
2,5-diOH-BA	7839	28371	6943	26808	4450	7896	3606	7281	2426	3611	1821	3311	5398	16212	3950	9673
2,6-diOH-BA	5119	11726	3618	8256	3830	6142	2608	3721	2669	4202	2300	3268	8467	34437	4861	10328
3,4-diOH-BA	354	827	438	1569	277	623	317	1026	194	316	159	282	670	2340	256	435
3,5-diOH-BA	676	1370	525	735	663	1030	688	1498	584	1106	458	643	1216	3148	908	1636
2,3,4-triOH-BA	7.2	29.3	10.3	49.6	6.4	26.4	8.1	25.2	4.1	14.4	3.6	16.8	10.9	40.1	6.8	15.5
2-OH-4-MeO-BA	1.7	7.5	0.6	2.5	1.3	3.5	0.9	3.2	1.4	4.3	0.6	2.0	1.1	3.3	1.8	6.7
3,4-diOH-BA-4-S	109	307	1665	2859	534	2500	5262	20212	310	1243	79.0	170	322	1058	128	266
3,4-diOH-BA-3-S	55.6	128	898	1287	176	498	1853	5686	82.3	264	31.6	40.2	91.1	242	59.6	184
3,4-diOH-BA-3-GlcUA	0.2	1.3	6.0	16.1	2.1	7.1	23.2	94.5	0.8	4.3	2.4	11.6	4.5	28.0	1.9	6.4
4-OH-3,5-diMeO-BA	73.6	129	117	362	110	180	153	388	69.2	119	76.9	191	123	275	119	221
3,4,5-triOH-Benz-OEt	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3,4,5-triOH-BA	9.6	32.4	3.6	9.4	6.8	16.1	6.7	20.2	9.6	36.0	5.8	14.7	17.3	67.4	10.5	22.6
3-OH-4-MeO-BA-5-S	107	208	80	129	103	258	203	628	184	554	165	619	1055	4679	160	350
4-OH-3-MeO-BA	396	1674	326	499	281	861	843	3110	386	752	157	679	527	1846	91.5	266
3-MeO-BA-4-S	76	317	137	162	102	329	363	1143	82.2	316	18.2	18.3	55.3	148	32.6	63.2
4-MeO-BA-3-S	3362	6097	2812	6635	5659	15791	3572	7066	2753	5131	2488	4960	7178	23735	3034	4569
Hippuric acids																
HA	176407	335436	136639	354981	284618	736998	155432	267277	157224	310989	90843	99093	276243	857259	162676	259267
2'-OH-HA	1076	3259	517	1060	730	1469	549	978	826	1512	569	1825	1867	8460	662	1330
3'-OH-HA	5946	18308	4783	14138	9175	18983	5139	8953	2803	3859	2515	4716	13848	59086	4641	9577
4'-OH-HA	1408	2816	1115	2198	2115	3786	1373	2957	748	1258	679	1501	1661	4672	957	1850
α-OH-HA	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cinnamic acids																
CA	98	196	156	457	193	538	195	523	86.0	139	95.4	171	167	510	156	300
3',4'-diOH-CA	16.9	35.2	13.5	34.6	22.6	54.9	19.5	39.3	11.9	21.8	9.5	11.1	26.8	77.3	23.1	48.1
3'-OH-CA-4'-S	13.1	20.1	24.6	41.6	26.3	45.7	77.8	312	34.9	142	14.8	31.5	36.2	101	121	652
4'-OH-CA-3'-S	125	325	511	1095	278	648	1073	3819	599	2875	234	929	409	1091	419	2033

3'-OH-CA-4'-GlcUA	199	337	162	245	324	765	216	430	207	415	163	275	372	1017	293	578
4'-OH-CA-3'-GlcUA	57.4	131	41.0	68.6	47.7	101	42.1	75.7	33.0	46.3	23.2	28.0	56.2	151	59.1	160
4'-OH-3'-MeO-CA	73.1	241	283	715	197	668	1084	4054	273	1333	118	526	598	3372	280	1813
3'-MeO-CA-4'-S	245	633	692	1638	491	1000	1414	4995	433	1398	170	331	465	1367	455	2190
3'-MeO-CA-4'-GlcUA	146	310	367	1304	369	907	417	1451	247	845	121	271	239	674	281	1297
3'-OH-4'-MeO-CA	146	486	234	479	187	299	284	496	117	185	66.6	92.6	182	397	187	630
4'-MeO-CA-3'-S	19.2	27.2	42.4	97.8	44.1	107	45.8	99.3	39.6	134	20.0	32.5	52.6	200	28.8	58.5
4'-MeO-CA-3'-GlcUA	79	204	169	379	216	443	248	696	115	295	57	110	129	234	116	369
4-CQA	1.7	7.9	13.0	25.5	5.4	18.6	25.6	75	5.8	30.5	2.9	16.2	2.3	6.5	3.1	16.5
4/5-CQA*	10.7	47.1	81.2	150	33.6	110	156	444	36.4	181	18.4	96.2	15.6	38.4	20.2	97.6
3-FQA	0.9	2.5	42.1	58.3	8.6	35.9	88.8	283	14.6	77.5	4.8	18.9	14.1	72.1	8.5	45.3
4-FQA	5.4	11	14	28.2	14.8	56.0	21.0	45.1	26.0	119	10.4	26.6	15.8	42.8	18.3	69.2
4'-OH-3',5'-diMeO-CA	0.3	1.6	2.9	11.7	0.3	1.3	0.2	1.3	0.6	4.0	1.2	7.9	0.3	1.9	1.3	4.9
3'-OH-CA	338	617	404	1182	397	685	508	1193	355	672	360	643	715	1875	569	923
4'-OH-CA	49.5	115	80.4	210	57.3	101	99.8	215	33.8	53.0	23.6	29.2	69.4	238	37.4	59.5
2'-OH-CA	24.3	47.5	20.3	33.8	33.7	67.1	37.2	85.4	26.7	54.5	25.0	55.0	59.7	167	36.4	72.2
CA-4'-S	29.7	75.0	40.5	102	29.5	46.9	51.7	151	13.9	21.8	10.5	14.7	36.8	100	18.8	27.9
CA-4'-GlcUA	30.5	59.8	31.3	55.2	39.5	98.8	46.4	116.7	29.6	96.3	13.3	22.9	36.5	84.7	38.3	93.4
Phenylacetic acids																
PA	11198	24195	10140	21246	9216	13857	11451	25523	6724	8325	6301	6587	17989	62988	14946	24440
3'-OH-PA	13771	23593	12230	22850	19523	32991	23682	66492	15651	35024	11070	13225	26596	84391	22563	45866
3',4'-diOH-PA	708	1239	680	1090	1041	2758	761	1689	627	1105	489	792	1389	4447	791	1064
3'-MeO-PA-4'-S	424	928	238	321	607	1479	343	669	395	762	271	329	597	1465	492	962
Phenylpropanoic acids																
2-(4'-OH-ph-O)-PrA	131	422	127	343	136	296	130	395	158	527	101	165	176	633	101	157
3-(2'-OH-ph)-PrA	412	863	328	1033	588	1297	393	689	376	833	243	500	692	2085	694	1341
3-(3'-OH-ph)-PrA	9038	26681	5406	16143	23415	75608	6823	11904	4151	6564	5200	13008	6313	14197	4005	6211
3-(2',3'-diOH-ph)-PrA	29.2	60.6	17.2	24.0	33.3	56.3	32.9	57.5	23.1	51.1	17.8	26.6	39.2	115.2	28.7	46.2
3-(2',4'-diOH-ph)-PrA	365	522	407	779	549	1098	604	1555	490	1175	398	658	1064	4204	573	852
3-(3',4'-diOH-ph)-PrA	130	245	134	321	222	483	225	600	147	346	128	272	249	802	234	565
3-(3',5'-diOH-ph)-PrA	682	1434	671	1639	899	1570	664	1036	562	1020	463	615	973	2631	829	1301
3-(4'-OH-ph)-2-OH-PrA	17028	29096	16612	34832	20194	31688	18465	31652	15240	22875	14802	26269	28296	70684	20489	28581
3-(4'-OH-3'-MeO-ph)-PrA	933	1726	760	1407	1412	2428	1091	2330	1097	1929	842	1083	4128	19459	1403	2753
3-(4'-OH-ph)-PrA-3'-GlcUA	39.3	88.5	32.1	45.0	56.1	118.7	56.5	141.3	37.0	73.3	44.8	75.8	66.5	181.0	51.7	83.2
3-(4'-OH-ph)-PrA-3'-S	362	864	243	688	1089	2978	380	907	532	1212	366	872	740	2587	302	526
3-(3'-MeO-ph)-PrA-4'-S	140	331	115	262	213	396	217	640	135	249	85.7	140	270	741	115	166
3-(3'-MeO-ph)-PrA-4'-GlcUA	216	403	184	569	584	1318	223	440	193	347	164	348	303	621	191	357
3-(4'-MeO-ph)-PrA-3'-S	2927	11919	1868	6192	1227	2731	734	1807	342	560	447	1130	977	2729	2294	12568
3-(4'-MeO-ph)-PrA-3'-GlcUA	252	582	572	3153	575	1353	236	579	163	272	104	152	195	358	138	215
Valerolactones																
5-(3'-OH-ph)-γ-VL-4'-S	1316	4994	328	1771	2124	7380	601	2091	197	622	526	1896	951	2673	306	1278

SD, standard deviation. * Mix of two isomers.

Supplementary Table S8. Plasma (poly)phenol metabolites' changes from Control following acute, chronic, and acute on chronic consumption of aronia berries.

	Acute CFC (Aronia - Control)				P	Chronic CFC (Aronia - Control)				P	Acute on chronic CFC (Aronia - Control)				P
	Mean difference	SEM	95% CI			Mean difference	SEM	95% CI			Mean difference	SEM	95% CI		
			Lower	Higher				Lower	Higher				Lower	Higher	
Total (poly)phenols	72651	103715	-133397	278699	0.644	-10004	316300	-638583	618576	0.674	790	124231	-246015	247596	0.132
Flavan-3-ols															
EC	-25.9	21.6	-68.8	17.0	0.893	-188	151	-488	112	0.683	-27.0	88.0	-202	148	0.333
EC-3'-S	4.2	5.8	-7.4	15.8	0.748	-10.6	20.5	-51.4	30.1	0.711	1.4	7.3	-13.0	15.9	0.961
3'-MeO-EC	-27.8	24.0	-75.5	20.0	0.431	-102	95.6	-292	87.9	0.933	-13.3	61.6	-136	109	0.980
4'-MeO-EC	4.5	4.9	-5.2	14.2	0.478	1.6	4.1	-6.6	9.8	0.628	-2.4	4.5	-11.4	6.6	0.048
Procyanidin A2	-	-	-	-		-	-	-	-		-	-	-	-	
Flavonols															
Quercetin	21.5	22.8	-23.8	66.7	0.784	-26.3	37.0	-99.7	47.2	0.589	-0.4	21.0	-42.3	41.4	0.389
Quercetin-3-S	-1.4	2.8	-6.9	4.2	0.206	6.8	6.4	-5.8	19.5	0.651	-0.5	1.6	-3.8	2.8	0.866
Quercetin-3-GlcUA	8.5	4.2	0.2	16.8	0.000	-10.5	17.3	-45.0	24.0	0.946	-17.6	28.4	-74.1	38.9	0.023
Quercetin-7-GlcUA	-1.0	1.4	-3.7	1.8	0.665	-0.8	0.7	-2.2	0.6	0.658	0.0	2.4	-4.8	4.8	0.329
Kaempferol-3-GlcUA	-0.8	0.6	-2.1	0.5	0.471	-8.7	6.9	-22.5	5.1	0.087	-2.7	3.0	-8.6	3.2	0.099
Myricetin	111	133	-155	376	0.829	-71.0	160.3	-389	247	0.880	-4.4	121	-245	236	0.197
Morin	4.1	3.7	-3.4	11.5	0.369	0.0	2.5	-5.0	4.9	0.712	-0.7	1.8	-4.2	2.8	0.718
Benzene diols and triols															
1,2-diOH-4-Me-Benz	105	1602	-3079	3288	0.817	-585	4943	-10407	9237	0.956	-810	2894	-6559	4939	0.275
2-OH-4/5-Me-Benz-1-S*	3187	16725	-30040	36414	0.945	-724	52565	-105185	103737	0.703	11790	26257	-40374	63955	0.381
2-OH-Benz-1-GlcUA	17.9	23.6	-29.1	64.8	0.817	106	81.7	-56.6	268	0.086	-18.6	46.5	-111	73.8	0.243
2,3-diOH-Benz-1-S	278	636	-984	1541	0.622	1658	1338	-1002	4317	0.886	-819	458	-1729	90.4	0.059
2,6-diOH-Benz-1-S	-809	1869	-4521	2904	0.488	768	2545	-4288	5825	0.975	-726	618	-1953	501	0.202
2-OH-3/6-MeO-Benz-1-S*	-438	3457	-7306	6430	0.508	616	4523	-8372	9604	0.849	-745	1478	-3681	2190	0.189
3-OH-2-MeO-Benz-1-S	-18.4	147.9	-312.3	275.5	0.988	65.2	248	-428	559	0.276	-48.9	112.9	-273	175	0.389
Benzaldehydes															
3,4-diOH-BAL	6.5	4.3	-2.1	15.1	0.194	-7.9	5.4	-18.6	2.7	0.301	0.1	3.7	-7.3	7.4	0.383
4-OH-BAL	100	177	-251	451	0.957	-73.7	291	-652	504	0.697	39.9	196	-349	429	0.134
4-OH-3-MeO-BAL	4.2	49.5	-94.1	103	0.455	-14.7	100	-214	184	0.727	2.8	54.7	-106	111	0.292
Benzoic acids															
BA	-64.0	2156	-4348	4219	0.982	-5246	7460	-20073	9580	0.662	-2108	2706	-7484	3268	0.216
2-OH-BA	205	556	-899	1309	0.988	155	1006	-1844	2153	0.899	210	522	-828	1247	0.210
3-OH-BA	504	504	-497	1505	0.666	-1166	889	-2933	601	0.781	-177	745	-1657	1303	0.424
4-OH-BA	1278	3154	-4989	7545	0.644	-8372	11472	-31171	14427	0.849	-300	2560	-5385	4785	0.303
2,3-diOH-BA	0.7	71.2	-141	142	0.673	67	72.7	-77.3	212	0.212	-52.4	95.9	-243	138	0.709
2,4/2,6-diOH-BA*	1990	2348	-2675	6655	0.441	-8151	9925	-27875	11572	0.763	-4009	2988	-9945	1926	0.210
2,5-diOH-BA	5004	3885	-2713	12721	0.347	-957	2782	-6486	4572	0.365	-297	1781	-3835	3241	0.265
2,6-diOH-BA	1086	1282	-1460	3633	0.441	-4449	5417	-15215	6317	0.763	-2188	1631	-5428	1051	0.210
3,4-diOH-BA	292	229	-163	747	0.732	-361	374	-1105	382	0.517	58.6	165	-269	386	0.290
3,5-diOH-BA	49.2	140	-229	327	1.000	-533	507	-1539	474	0.943	-168	326	-815	480	0.465
2,3,4-triOH-BA	7.0	7.5	-7.9	22.0	1.000	-4.0	7.4	-18.6	10.6	0.476	1.3	4.4	-7.4	9.9	0.875
2-OH-4-MeO-BA	0.1	0.5	-0.9	1.0	0.508	0.3	0.7	-1.2	1.8	0.512	-1.0	1.0	-3.0	1.1	0.475
3,4-diOH-BA-4-S	1599	409	787	2412	0.000	245	414	-579	1069	0.409	5183	2968	-714	11079	0.000
3,4-diOH-BA-3-S	877	182	516	1239	0.000	93.5	83.9	-73.3	260	0.249	1829	839	162	3496	0.000
3,4-diOH-BA-3-GlcUA	3.7	2.9	-2.1	9.4	0.037	-2.2	4.4	-11.1	6.6	0.183	21.3	13.9	-6.4	48.9	0.250
4-OH-3,5-diMeO-BA	35.9	58.4	-80.2	152.0	0.683	-9.9	50.1	-109	89.7	0.952	31.5	65.7	-99.1	162	0.683
3,4,5-triOH-Benz-OEt	-	-	-	-		-	-	-	-		-	-	-	-	
3,4,5-triOH-BA	-2.2	2.6	-7.3	3.0	0.592	-10.0	10.6	-31.2	11.1	0.588	-3.9	4.5	-12.9	5.0	0.383
3-OH-4-MeO-BA-5-S	-35.2	74.4	-183	113	0.775	-913	705	-2314	488	0.557	84.0	102	-119	287	0.010
4-OH-3-MeO-BA	168	126	-82.4	418	0.025	-235	311	-854	383	0.466	756	460	-157	1669	0.006
3-MeO-BA-4-S	119.0	23.2	72.5	165	0.000	51.2	54.7	-57.6	160	0.248	334	169	-0.5	669	0.000
4-MeO-BA-3-S	14.1	1119	-2208	2236	0.512	-1274	4359	-9937	7389	0.674	563	1236	-1891	3018	0.480
Hippuric acids															
HA	44154	52610	-60366	148673	0.493	25373	172237	-316912	367658	0.589	-7486	54751	-116258	101287	0.130
2'-OH-HA	-128	295	-715	459	0.740	-1180	1316	-3795	1435	0.918	-80.5	242	-562	401	0.645
3'-OH-HA	444	1633	-2801	3689	0.215	-4305	9577	-23337	14726	0.949	594	1924	-3229	4417	0.126
4'-OH-HA	136	351	-561	833	0.616	441	926	-1399	2281	0.739	388	513	-632	1407	0.205
α-OH-HA	-	-	-	-		-	-	-	-		-	-	-	-	

Cinnamic acids															
CA	58.0	70.1	-81.2	197.2	0.981	27.0	113	-197	251	0.590	31.2	84.4	-136	199	0.207
3',4'-diOH-CA	3.8	5.2	-6.6	14.2	0.328	-2.6	14.5	-31.5	26.2	0.372	-3.5	9.2	-21.7	14.7	0.551
3'-OH-CA-4'-S	13.4	6.7	0.1	26.6	0.124	-9.5	17.0	-43.2	24.2	0.544	-38.1	107	-250	174	0.315
4'-OH-CA-3'-S	421	162	99.6	742	0.000	-126	195	-513	261	0.671	712	632	-543	1968	0.000
3'-OH-CA-4'-GlcUA	-1.1	54.2	-109	107	0.746	-36.2	194	-421	349	0.662	-72.9	104	-280	135	0.078
4'-OH-CA-3'-GlcUA	16.9	10.8	-4.5	38.3	0.644	-6.4	28.0	-62.0	49.3	0.325	-16.6	26.1	-68.5	35.3	0.695
4'-OH-3'-MeO-CA	235	106	24.7	446	0.010	-408.9	529	-1460	642	1.000	812	655	-488	2113	0.002
3'-MeO-CA-4'-S	545	237	74.8	1016	0.000	42.1	259	-472	556	0.843	950	799	-637	2536	0.001
3'-MeO-CA-4'-GlcUA	264	188	-111	638	0.003	157	171	-184	497	0.410	105	286	-462	672	0.240
3'-OH-4'-MeO-CA	161	68.2	25.4	297	0.000	2.4	75.5	-148	152	0.473	93.6	113	-131	319	0.294
4'-MeO-CA-3'-S	25.0	14.6	-3.9	54.0	0.042	-7.9	34.9	-77.2	61.4	0.497	17.1	16.9	-16.6	50.7	0.433
4'-MeO-CA-3'-GlcUA	116	56.0	4.7	227	0.002	98.4	75.8	-52.2	249	0.329	127	117	-105	358	0.127
4-CQA	12.1	3.6	4.8	19.3	0.000	3.3	3.0	-2.7	9.3	0.546	22.5	11.3	0.0	45.0	0.000
4/5-CQA*	75.4	21.5	32.8	118	0.000	19.1	17.8	-16.2	54.4	0.446	135	67.2	1.4	268	0.000
3-FQA	40.5	8.4	23.9	57.2	0.000	-5.5	12.4	-30.1	19.2	0.511	81.3	42.0	-2.1	165	0.000
4-FQA	7.3	4.4	-1.4	16.0	0.004	0.1	10.8	-21.3	21.6	0.697	3.1	10.3	-17.4	23.6	0.067
4'-OH-3',5'-diMeO-CA	2.2	1.7	-1.2	5.7	0.856	0.1	0.2	-0.3	0.5	0.658	-1.1	0.7	-2.5	0.3	0.101
3'-OH-CA	44.3	194	-342	430	0.717	-309	304	-913	296	0.745	-30.2	222	-471	410	0.333
4'-OH-CA	57.5	30.3	-2.8	118	0.020	-11.3	39.9	-90.5	68.0	0.858	62.7	32.7	-2.3	128	0.210
2'-OH-CA	-4.6	9.6	-23.6	14.4	0.723	-25.5	27.6	-80.3	29.2	0.949	3.8	16.3	-28.5	36.1	0.461
CA-4'-S	26.3	14.7	-3.0	55.6	0.124	-4.9	17.1	-39.0	29.2	0.645	33.3	22.5	-11.4	78.0	0.569
CA-4'-GlcUA	18.0	8.6	1.0	35.1	0.871	5.1	19.8	-34.2	44.4	0.680	7.3	21.9	-36.1	50.7	0.460
Phenylacetic acids															
PA	3668	3217	-2722	10058	0.926	-8310	9962	-28109	11488	0.799	-3610	5232	-14005	6785	0.352
3'-OH-PA	1265	3781	-6247	8777	0.606	-7002	13838	-34503	20499	0.739	1064	11905	-22587	24715	0.113
3',4'-diOH-PA	174	195	-212	561	0.811	-294	797	-1879	1291	0.994	-23.4	294	-607	560	0.461
3'-MeO-PA-4'-S	-32.6	68.1	-168	103	0.459	37.3	317	-592	667	0.975	-149	173	-493	194	0.259
Phenylpropanoic acids															
2-(4'-OH-ph-O)-PrA	27.5	54.0	-79.7	135	0.820	-38.0	107	-250	174	0.760	29.0	62.5	-95.1	153	0.217
3-(2'-OH-ph)-PrA	84.2	166	-245	413	0.672	-88.6	374	-832	655	0.436	-274	202	-675	127	0.160
3-(3'-OH-ph)-PrA	-1953	2623	-7163	3258	0.488	18793	11745	-4548	42135	0.294	2534	1994	-1429	6496	0.023
3-(2',3'-diOH-ph)-PrA	-1.1	5.3	-11.6	9.3	0.517	-4.0	19.6	-42.9	35.0	0.855	4.5	10.8	-17.0	26.0	0.678
3-(2',4'-diOH-ph)-PrA	16.0	149	-281	313	0.926	-525	666	-1848	798	0.886	39.1	261	-480	558	0.554
3-(3',4'-diOH-ph)-PrA	5.8	61.8	-117	129	0.488	-17.2	143	-301	266	0.472	-6.5	121	-246	233	0.372
3-(3',5'-diOH-ph)-PrA	211	252	-289	711	0.548	-29.8	467	-959	899	0.994	-161	244	-647	324	0.222
3-(4'-OH-ph)-2-OH-PrA	1765	6394	-10937	14468	0.735	-7689	11836	-31211	15833	0.880	-1721	6288	-14212	10771	0.219
3-(4'-OH-3'-MeO-ph)-PrA	-76.2	260	-592	439	0.994	-2676	2998	-8633	3281	0.937	-250	531	-1305	805	0.939
3-(4'-OH-ph)-PrA-3'-GlcUA	-12.9	13.3	-39.3	13.5	0.652	-8.5	32.9	-74.0	56.9	0.679	5.5	24.0	-42.2	53.2	0.348
3-(4'-OH-ph)-PrA-3'-S	-105	164	-431	220	0.543	385	601	-810	1579	0.207	67.4	154	-238	373	0.095
3-(3'-MeO-ph)-PrA-4'-S	28.6	42.8	-56.4	113	0.969	-49.2	128	-304	205	0.793	103	97.1	-90.2	295	0.623
3-(3'-MeO-ph)-PrA-4'-GlcUA	16.1	96.6	-176	208	0.627	304	221	-135	742	0.387	13.5	83.2	-152	179	0.087
3-(4'-MeO-ph)-PrA-3'-S	935	856	-766	2637	0.841	252	594	-928	1431	0.578	-1698	1858	-5389	1993	0.265
3-(4'-MeO-ph)-PrA-3'-GlcUA	458	452	-440	1355	0.890	292	182	-69.7	653	0.270	59.1	90.1	-120	238	0.189
Valerolactones															
5-(3'-OH-ph)-γ-VL-4'-S	-252	389	-1025	521	0.215	1306	1205	-1090	3701	0.327	256	361	-461	972	0.052

Differences were calculated from ANCOVA (Bonferroni) including baseline values as covariate, comparing Aronia with Control changes from baseline. 95% CI, 95% confidence interval; CFC, changes from Control; SEM, standard error of mean. P-values (P) obtained following Mann-Whitney non-parametric test. * Mix of two isomers.

REFERENCES

2019 ESC/EAS guidelines for the management of dyslipidaemias: Lipid modification to reduce cardiovascular risk'. 2019. *Atherosclerosis*, 290: 140-205.

Adlercreutz, H., K. Höckerstedt, C. Bannwart, S. Bloigu, E. Hämäläinen, T. Fotsis, and A. Ollus. 1987. 'Effect of dietary components, including lignans and phytoestrogens, on enterohepatic circulation and liver metabolism of estrogens and on sex hormone binding globulin (SHBG)', *The Journal of Steroid Biochemistry and Molecular Biology*, 27: 1135-44.

Adlercreutz, H., and W. J. Mazur. 1997 'Phyto-oestrogens and Western diseases' *Annals of medicine.* 29: 95-120.

Afshin, A., P. J. Sur, K. A. Fay, L. Cornaby, G. Ferrara, J. S. Salama, E. C. Mullany, K. H. Abate, C. Abbafati, Z. Abebe, M. Afarideh, A. Aggarwal, S. Agrawal, T. Akinyemiju, F. Alahdab, U. Bacha, V. F. Bachman, H. Badali, A. Badawi, I. M. Bensenor, E. Bernabe, S. K. K. Biadgilign, S. H. Biryukov, L. E. Cahill, J. J. Carrero, K. M. Cercy, L. Dandona, R. Dandona, A. K. Dang, M. G. Degefa, M. El Sayed Zaki, A. Esteghamati, S. Esteghamati, J. Fanzo, C. Sofia e Sá Farinha, M. S. Farvid, F. Farzadfar, V. L. Feigin, J. C. Fernandes, L. Sorio Flor, N. A. Foigt, M. H. Forouzanfar, M. Ganji, J. M. Geleijnse, R. F. Gillum, A. C. Goulart, G. Grosso, I. Guessous, S. Hamidi, G. J. Hankey, S. Harikrishnan, H. Y. Hassen, S. I. Hay, C. L. Hoang, M. Horino, F. Islami, M. D. Jackson, S. L. James, L. Johansson, J. B. Jonas, A. Kasaeian, Y. Saleh Khader, I. A. Khalil, Y-H. Khang, R. W. Kimokoti, Y. Kokubo, G. A. Kumar, T. Lallukka, A. D. Lopez, S. Lorkowski, P. A. Lotufo, R. Lozano, R. Malekzadeh, W. März, T. Meier, Y. A. Melaku, W. Mendoza, G. B. M. Mensink, R. Micha, T. R. Miller, M. Mirarefin, V. Mohan, A. H. Mokdad, D. Mozaffarian, G. Nagel, M. Naghavi, C. T. Nguyen, M. R. Nixon, K. L. Ong, D. M. Pereira, H. Poustchi, M. Qorbani, R. Kumar Rai, C. Razo-García, C. D. Rehm, J. A. Rivera, S. Rodríguez-Ramírez, G. Roshandel, G. A. Roth, J. Sanabria, T. G. Sánchez-Pimienta, B Sartorius, J. Schmidhuber, A. E. Schutte, S. G. Sepanlou, M-J. Shin, R. J. D. Sorensen, M. Springmann, L. Szponar, A. L. Thorne-Lyman, A. G. Thrift, M. Touvier, B. Xuan Tran, S. Tyrovolas, K. N. Ukwaja, I. Ullah, O. A. Uthman, M. Vaezghasemi, T. Juhani Vasankari, S. E. Vollset, T. Vos, G. T. Vu, L. G. Vu, E. Weiderpass, A. Werdecker, T. Wijeratne, W. C.

Willett, J. H. Wu, G. Xu, N. Yonemoto, C. Yu, and C. J. L. Murray. 2019. 'Health effects of dietary risks in 195 countries, 1990-2013;2017: a systematic analysis for the Global Burden of Disease Study 2017', *The Lancet*, 393: 1958-72.

Ahles, S., Y. R. Stevens, P. J. Joris, D. Vauzour, J. Adam, E. de Groot, and J. Plat. 2020. 'The Effect of Long-Term Aronia melanocarpa Extract Supplementation on Cognitive Performance, Mood, and Vascular Function: A Randomized Controlled Trial in Healthy, Middle-Aged Individuals', *Nutrients*, 12: 2475.

Albert, M. A. 2011. 'Biomarkers and heart disease', *Journal of clinical sleep medicine*, 7: S9-S11.

Alexander, R. W.. 1995. 'Hypertension and the Pathogenesis of Atherosclerosis', *Hypertension*, 25: 155-61.

AlGhatrif, M., J. B. Strait, C. H. Morrell, M. Canepa, J. Wright, P. Elango, A. Scuteri, S. S. Najjar, L. Ferrucci, and E. G. Lakatta. 2013. 'Longitudinal trajectories of arterial stiffness and the role of blood pressure: the Baltimore Longitudinal Study of Aging', *Hypertension*, 62: 934-41.

Almeida, A., A. L. Mitchell, M. Boland, S. C. Forster, G. B. Gloor, A. Tarkowska, T. D. Lawley, and R. D. Finn. 2019. 'A new genomic blueprint of the human gut microbiota', *Nature*, 568: 499-504.

Almoosawi, S., C. Tsang, L. M. Ostertag, L. Fyfe, and E. A. Al-Dujaili. 2012. 'Differential effect of polyphenol-rich dark chocolate on biomarkers of glucose metabolism and cardiovascular risk factors in healthy, overweight and obese subjects: a randomized clinical trial', *Food and Function*, 3: 1035-43.

Alvarez, C. A., M. B. Jones, J. Hambor, and B. A. Cobb. 2020. 'Characterization of Polysaccharide A Response Reveals Interferon Responsive Gene Signature and Immunomodulatory Marker Expression', *Frontiers in immunology*, 11: 556813-13.

Alves-Santos, A. M., C. S. Araújo Sugizaki, G. Carielo Lima, and M. Margareth Veloso Naves. 2020. 'Prebiotic effect of dietary polyphenols: A systematic review', *Journal of Functional Foods*, 74: 104169.

Amin, H. P., C. Czank, K. S. Raheem, Q. Zhang, N. P. Botting, A. Cassidy, and C. D. Kay. 2015. 'Anthocyanin and its physiologically relevant metabolites

alter the expression of IL-6 and VCAM-1 in CD40L and oxidized LDL challenged vascular endothelial cells', *Molecular Nutrition & Food Research*, 59: 1095-106

Anand, S. S., C. Hawkes, R. J. de Souza, A. Mente, M. Dehghan, R. Nugent, M. A. Zulyniak, T. Weis, A. M. Bernstein, R. Krauss, D. Kromhout, D. J. A. Jenkins, V. Malik, M. A. Martinez-Gonzalez, D. Mozafarrian, S. Yusuf, W. C. Willett, and B. M. Popkin. 2015. 'Food Consumption and its impact on Cardiovascular Disease: Importance of Solutions focused on the globalized food system: A Report from the Workshop convened by the World Heart Federation', *Journal of the American College of Cardiology*, 66: 1590-614.

Anderson, T. J., J. Grégoire, G. J. Pearson, A. R. Barry, P. Couture, M. Dawes, G. A. Francis, J. Genest, Jr., S. Grover, M. Gupta, R. A. Hegele, D. C. Lau, L. A. Leiter, E. Lonn, G. B. Mancini, R. McPherson, D. Ngui, P. Poirier, J. L. Sievenpiper, J. A. Stone, G. Thanassoulis, and R. Ward. 2016. '2016 Canadian Cardiovascular Society Guidelines for the Management of Dyslipidemia for the Prevention of Cardiovascular Disease in the Adult', *The Canadian Journal of Cardiology*, 32: 1263-82.

Andersson, O., M. Lingman, A Himmelmann, R Sivertsson, and B Widgren. 2004. 'Prediction of future hypertension by casual blood pressure or invasive hemodynamics? A 30-year follow-up study', *Blood Pressure*, 13: 350-54.

Andreu, V. P., H. E. Augustijn, L. Chen, A. Zhernakova, J. Fu, M. A. Fischbach, D. Dodd, and M. H. Medema. 2021. 'A systematic analysis of metabolic pathways in the human gut microbiota', *bioRxiv*: 2021.02.25.432841.

Angelino, D., J. Godos, F. Ghelfi, M. Tieri, L. Titta, A. Lafranconi, S. Marventano, E. Alonzo, A. Gambera, S. Sciacca, S. Buscemi, S. Ray, F. Galvano, D. Del Rio, and G. Grosso. 2019. 'Fruit and vegetable consumption and health outcomes: an umbrella review of observational studies', *International Journal of Food Sciences and Nutrition,* 70: 652-67.

Anhê, F. F., D. Roy, G. Pilon, S. Dudonné, S. Matamoros, T. V. Varin, C. Garofalo, Q. Moine, Y. Desjardins, E. Levy, and A. Marette. 2015. 'A polyphenol-rich cranberry extract protects from diet-induced obesity, insulin resistance and intestinal inflammation in association with increased

Akkermansia spp. population in the gut microbiota of mice', *Gut*, 64: 872-83.

Anselmi, G., L. Gagliardi, G. Egidi, S. Leone, A. Gasbarrini, G. A. D. Miggiano, and L. Galiuto. 2020. 'Gut Microbiota And Cardiovascular Diseases: A Critical Review', *Cardiology in Review,* 29:195-204.

Aprikian, O., V. Duclos, S. Guyot, C. Besson, C. Manach, A. Bernalier, C. Morand, C. Rémésy, and C. Demigné. 2003. 'Apple pectin and a polyphenol-rich apple concentrate are more effective together than separately on cecal fermentations and plasma lipids in rats', *The Journal of Nutrition*, 133: 1860-5.

Arora, A., M. G. Nair, and G. M. Strasburg. 1998. 'Structure-activity relationships for antioxidant activities of a series of flavonoids in a liposomal system', *Free Radical Biology and Medicine*, 24: 1355-63.

Arumugam, M., J. Raes, E. Pelletier, D. Le Paslier, T. Yamada, D. R. Mende, G. R. Fernandes, J. Tap, T. Bruls, J. M. Batto, M. Bertalan, N. Borruel, F. Casellas, L. Fernandez, L. Gautier, T. Hansen, M. Hattori, T. Hayashi, M. Kleerebezem, K. Kurokawa, M. Leclerc, F. Levenez, C. Manichanh, H. B. Nielsen, T. Nielsen, N. Pons, J. Poulain, J. Qin, T. Sicheritz-Ponten, S. Tims, D. Torrents, E. Ugarte, E. G. Zoetendal, J. Wang, F. Guarner, O. Pedersen, W. M. de Vos, S. Brunak, J. Doré, M. Antolín, F. Artiguenave, H. M. Blottiere, M. Almeida, C. Brechot, C. Cara, C. Chervaux, A. Cultrone, C. Delorme, G. Denariaz, R. Dervyn, K. U. Foerstner, C. Friss, M. van de Guchte, E. Guedon, F. Haimet, W. Huber, J. van Hylckama-Vlieg, A. Jamet, C. Juste, G. Kaci, J. Knol, O. Lakhdari, S. Layec, K. Le Roux, E. Maguin, A. Mérieux, R. Melo Minardi, C. M'Rini, J. Muller, R. Oozeer, J. Parkhill, P. Renault, M. Rescigno, N. Sanchez, S. Sunagawa, A. Torrejon, K. Turner, G. Vandemeulebrouck, E. Varela, Y. Winogradsky, G. Zeller, J. Weissenbach, S. D. Ehrlich, and P. Bork. 2011. 'Enterotypes of the human gut microbiome', *Nature*, 473: 174-80.

Asmar, R., A. Benetos, G. London, C. Hugue, Y. Weiss, J. Topouchian, B. Laloux, and M. Safar. 1995. 'Aortic distensibility in normotensive, untreated and treated hypertensive patients', *Blood Pressure*, 4: 48-54.

Asnicar, F., S. E. Berry, A. M. Valdes, L. H. Nguyen, G. Piccinno, D. A. Drew, E. Leeming, R. Gibson, C. Le Roy, H. A. Khatib, L. Francis, M. Mazidi, O.

Mompeo, M. Valles-Colomer, A. Tett, F. Beghini, L. Dubois, D. Bazzani, A. M. Thomas, C. Mirzayi, A. Khleborodova, S. Oh, R. Hine, C. Bonnett, J. Capdevila, S. Danzanvilliers, F. Giordano, L. Geistlinger, L. Waldron, R. Davies, G. Hadjigeorgiou, J. Wolf, J. M. Ordovás, C. Gardner, P. W. Franks, A. T. Chan, C. Huttenhower, T. D. Spector, and N. Segata. 2021. 'Microbiome connections with host metabolism and habitual diet from 1,098 deeply phenotyped individuals', *Nature Medicine*, 27: 321-32.

Aune, D., E. Giovannucci, P. Boffetta, L. T. Fadnes, N. Keum, T. Norat, D. C. Greenwood, E. Riboli, L. J. Vatten, and S. Tonstad. 2017. 'Fruit and vegetable intake and the risk of cardiovascular disease, total cancer and all-cause mortality-a systematic review and dose-response meta-analysis of prospective studies', *International Journal of Epidemiology*, 46: 1029-56.

Aura, A-M., P. Martin-Lopez, K. Anne O'Leary, G. Williamson, K-M. Oksman-Caldentey, K. Poutanen, and C. Santos-Buelga. 2005. 'In vitro metabolism of anthocyanins by human gut microflora', *European Journal of Nutrition*, 44: 133-42.

Avolio, A. P., T. Kuznetsova, G. R. Heyndrickx, P. L. M. Kerkhof, and J. K. Li. 2018. 'Arterial Flow, Pulse Pressure and Pulse Wave Velocity in Men and Women at Various Ages', *Advances in Experimental Medicine and Biology*, 1065: 153-68.

Baars, A. E., J. R. Rubio-Valverde, Y. Hu, M. Bopp, H. Brønnum-Hansen, R. Kalediene, M. Leinsalu, P. Martikainen, E. Regidor, C. White, B. Wojtyniak, J. P. Mackenbach, and W. J. Nusselder. 2019. 'Fruit and vegetable consumption and its contribution to inequalities in life expectancy and disability-free life expectancy in ten European countries', *International Journal of Public Health*, 64: 861-72.

Badimón, L., G. Vilahur, and T. Padró. 2009. 'Lipoproteins, platelets and atherothrombosis', *Revista Española de Cardiología*, 62: 1161-78.

Bai, J., Y. Hu, and D. W. Bruner. 2019. 'Composition of gut microbiota and its association with body mass index and lifestyle factors in a cohort of 7-18 years old children from the American Gut Project', *Pediatric Obesity*, 14: e12480.

Bakogiannis, C., K. Stavropoulos, C. Papadopoulos, and V. Papademetriou. 2020. 'The Impact of Various Blood Pressure Measurements on Cardiovascular Outcomes', *Current vascular pharmacology*, 19: 313-322.

Baulmann, J., U. Schillings, S. Rickert, S. Uen, R. Düsing, M. Illyes, A. Cziraki, G. Nickenig, and T. Mengden. 2008. 'A new oscillometric method for assessment of arterial stiffness: comparison with tonometric and piezo-electronic methods', *Journal of Hypertension*, 26: 523-8

Bayram, B., A. González-Sarrías, G. Istas, M. Garcia-Aloy, C. Morand, K. Tuohy, R. García-Villalba, and P. Mena. 2018. 'Breakthroughs in the Health Effects of Plant Food Bioactives: A Perspective on Microbiomics, Nutri(epi)genomics, and Metabolomics', *Journal of Agricultural and Food Chemistry*, 66: 10686-92.

Bazzano, L. A., J. He, L. G. Ogden, C. M. Loria, S. Vupputuri, L. Myers, and P. K. Whelton. 2002. 'Fruit and vegetable intake and risk of cardiovascular disease in US adults: the first National Health and Nutrition Examination Survey Epidemiologic Follow-up Study', *The American Journal of Clinical Nutrition*, 76: 93-9.

Beattie, J., A. Crozier, and G. Duthie. 2005. 'Potential Health Benefits of Berries', *Current Nutrition & Food Science*, 1: 71-86.

Ben-Shlomo, Y., M. Spears, C. Boustred, M. May, S. G. Anderson, E. J. Benjamin, P. Boutouyrie, J. Cameron, C. H. Chen, J. K. Cruickshank, S. J. Hwang, E. G. Lakatta, S. Laurent, J. Maldonado, G. F. Mitchell, S. S. Najjar, A. B. Newman, M. Ohishi, B. Pannier, T. Pereira, R. S. Vasan, T. Shokawa, K. Sutton-Tyrell, F. Verbeke, K. L. Wang, D. J. Webb, T. Willum Hansen, S. Zoungas, C. M. McEniery, J. R. Cockcroft, and I. B. Wilkinson. 2014. 'Aortic pulse wave velocity improves cardiovascular event prediction: an individual participant meta-analysis of prospective observational data from 17,635 subjects', *Journal of the American College of Cardiology*, 63: 636-46.

Bendinelli, B., G. Masala, C. Saieva, S. Salvini, C. Calonico, C. Sacerdote, C. Agnoli, S. Grioni, G. Frasca, A. Mattiello, P. Chiodini, R. Tumino, P. Vineis, D. Palli, and S. Panico. 2011. 'Fruit, vegetables, and olive oil and risk of coronary heart disease in Italian women: the EPICOR Study', *The American Journal of Clinical Nutrition*, 93: 275-83.

Berry, S. E., A. M. Valdes, D. A. Drew, F. Asnicar, M. Mazidi, J. Wolf, J. Capdevila, G. Hadjigeorgiou, R. Davies, H. Al Khatib, C. Bonnett, S. Ganesh, E. Bakker, D. Hart, M. Mangino, J. Merino, I. Linenberg, P. Wyatt, J. M. Ordovas, C. D. Gardner, L. M. Delahanty, A. T. Chan, N. Segata, P. W. Franks, and T. D. Spector. 2020. 'Human postprandial responses to food and potential for precision nutrition', *Nature Medicine*, 26: 964-73.

Biagi, E., C. Franceschi, S. Rampelli, M. Severgnini, R. Ostan, S. Turroni, C. Consolandi, S. Quercia, M. Scurti, D. Monti, M. Capri, P. Brigidi, and M. Candela. 2016. 'Gut Microbiota and Extreme Longevity', *Current Biology*, 26: 1480-5.

Bian, K., and F. Murad. 2014. 'What is next in nitric oxide research? From cardiovascular system to cancer biology', *Nitric Oxide*, 43: 3-7.

Bindels, L. B., N. M. Delzenne, P. D. Cani, and J. Walter. 2015. 'Towards a more comprehensive concept for prebiotics', *Nature Reviews Gastroenterology & Hepatology*, 12: 303-10.

Binder, C. J., N. Papac-Milicevic, and J. L. Witztum. 2016. 'Innate sensing of oxidation-specific epitopes in health and disease', *Nature Reviews Immunology*, 16: 485-97.

Bingham, S. A., A. A. Welch, A. McTaggart, A. A. Mulligan, S. A. Runswick, R. Luben, S. Oakes, K. T. Khaw, N. Wareham, and N. E. Day. 2001. 'Nutritional methods in the European Prospective Investigation of Cancer in Norfolk', *Public Health Nutrition*, 4: 847-58.

Bischoff, S. C. 2016. 'Microbiota and aging', *Current Opinion in Clinical Nutrition & Metabolic Care*, 19: 26-30.

Bittner, K., S. Rzeppa, and H. U. Humpf. 2013. 'Distribution and quantification of flavan-3-ols and procyanidins with low degree of polymerization in nuts, cereals, and legumes', *Journal of Agricultural and Food Chemistry*, 61: 9148-54.

Bochkov, V. N., O. V. Oskolkova, K. G. Birukov, A. L. Levonen, C. J. Binder, and J. Stöckl. 2010. 'Generation and biological activities of oxidized phospholipids', *Antioxidants & Redox Signaling*, 12: 1009-59.

Bolca, S., M. Urpi-Sarda, P. Blondeel, N. Roche, L. Vanhaecke, S. Possemiers, N. Al-Maharik, N. Botting, D. De Keukeleire, M. Bracke, A. Heyerick, C. Manach, and H. Depypere. 2010. 'Disposition of soy isoflavones in normal

human breast tissue', *The American Journal of Clinical Nutrition*, 91: 976-84.

Bond, T., and E. Derbyshire. 2019. 'Tea Compounds and the Gut Microbiome: Findings from Trials and Mechanistic Studies', *Nutrients*, 11: 2364.

Borges, G., J. I. Ottaviani, J. J. J. van der Hooft, H. Schroeter, and A. Crozier. 2018. 'Absorption, metabolism, distribution and excretion of (-)-epicatechin: A review of recent findings', *Molecular Aspects of Medicine*, 61: 18-30.

Boto-Ordonez, M., M. Urpi-Sarda, M. I. Queipo-Ortuno, S. Tulipani, F. J. Tinahones, and C. Andres-Lacueva. 2014. 'High levels of Bifidobacteria are associated with increased levels of anthocyanin microbial metabolites: a randomized clinical trial', *Food and Function*, 5: 1932-8.

Boutouyrie, P., A. I. Tropeano, R. Asmar, I. Gautier, A. Benetos, P. Lacolley, and S. Laurent. 2002. 'Aortic stiffness is an independent predictor of primary coronary events in hypertensive patients: a longitudinal study', *Hypertension*, 39: 10-5.

Bowey, E., H. Adlercreutz, and I. Rowland. 2003. 'Metabolism of isoflavones and lignans by the gut microflora: a study in germ-free and human flora associated rats', *Food and Chemical Toxicology*, 41: 631-6.

Briasoulis, A., V. Agarwal, and F. H. Messerli. 2012. 'Alcohol consumption and the risk of hypertension in men and women: a systematic review and meta-analysis', *The Journal of Clinical Hypertension*, 14: 792-8.

British Heart Foundation. 'BHF Statistics Factsheet - UK ', Accessed 01/10/2020. https://www.bhf.org.uk/statistics.

British Heart Foundation. 'Facts and figures', Accessed 01/03/2021. https://tinyurl.com/ucu7955b.

Broncel, M., M. Kozirog, P. Duchnowicz, M. Koter-Michalak, J. Sikora, and J. Chojnowska-Jezierska. 2010. 'Aronia melanocarpa extract reduces blood pressure, serum endothelin, lipid, and oxidative stress marker levels in patients with metabolic syndrome', *Medical Science Monitor*, 16: Cr28-34.

Brown, B. G., K. H. Stukovsky, and X. Q. Zhao. 2006. 'Simultaneous low-density lipoprotein-C lowering and high-density lipoprotein-C elevation for optimum cardiovascular disease prevention with various drug classes, and

their combinations: a meta-analysis of 23 randomized lipid trials', *Current Opinion in Lipidology*, 17: 631-6.

Bruno, R. M., F. Stea, R. Sicari, L. Ghiadoni, S. Taddei, A. Ungar, U. Bonuccelli, G. Tognoni, S. Cintoli, S. Del Turco, S. Sbrana, L. Gargani, G. D'Angelo, L. Pratali, N. Berardi, L. Maffei, and E. Picano. 2018. 'Vascular Function Is Improved After an Environmental Enrichment Program: The Train the Brain-Mind the Vessel Study', *Hypertension*, 71: 1218-25.

Buda, V., M. Andor, A. Diana, F. Ardelean, I. Pavel, Cristina Dehelean, C. Soica, R. Folescu, F. Andrei, and Corina Danciu. 2020. "Cardioprotective Effects of Cultivated Black Chokeberries (Aronia spp.): Traditional Uses, Phytochemistry and Therapeutic Effects." *IntechOpen*, DOI: 10.5772/intechopen.92238

Bui, T. P. N., S. S. Anand, I. Lagkouvardos, J. Ritari, B. Chamlagain, F. P. Douillard, L. Paulin, V. Piironen, T. Clavel, C. M. Plugge, and W. M. de Vos. 2016. 'Comparative genomics and physiology of the butyrate-producing bacterium Intestinimonas butyriciproducens', *Environmental Microbiology Reports*, 8: 1024-37.

Calame, W., L. Street, and T. Hulshof. 2020. 'Vitamin D Serum Levels in the UK Population, including a Mathematical Approach to Evaluate the Impact of Vitamin D Fortified Ready-to-Eat Breakfast Cereals: Application of the NDNS Database', *Nutrients*, 12: 1868.

Calderón-Pérez, L., M. J. Gosalbes, S. Yuste, R. M. Valls, A. Pedret, E. Llauradó, N. Jimenez-Hernandez, A. Artacho, L. Pla-Pagà, J. Companys, I. Ludwig, M. P. Romero, L. Rubió, and R. Solà. 2020. 'Gut metagenomic and short chain fatty acids signature in hypertension: a cross-sectional study', *Scientifics Reports*, 10: 6436.

Canani, R. B., M. Di Costanzo, L. Leone, M. Pedata, R. Meli, and A. Calignano. 2011. 'Potential beneficial effects of butyrate in intestinal and extraintestinal diseases', *World Journal of Gastroenterology*, 17: 1519-28.

Cani, P. D., and A. Everard. 2016. 'Talking microbes: When gut bacteria interact with diet and host organs', *Molecular Nutrition & Food Research*, 60: 58-66.

Cardona, F., C. Andrés-Lacueva, S. Tulipani, F. J. Tinahones, and M. I. Queipo-Ortuño. 2013. 'Benefits of polyphenols on gut microbiota and implications in human health', *The Journal of Nutritional Biochemistry*, 24: 1415-22.

Cassidy, A., K. J. Mukamal, L. Liu, M. Franz, A. H. Eliassen, and E. B. Rimm. 2013. 'High anthocyanin intake is associated with a reduced risk of myocardial infarction in young and middle-aged women', *Circulation*, 127: 188-96.

Cassidy, A., and A-M. Minihane. 2017. 'The role of metabolism (and the microbiome) in defining the clinical efficacy of dietary flavonoids', *The American Journal of Clinical Nutrition*, 105: 10-22.

Castelli, W. P., R. J. Garrison, P. W. Wilson, R. D. Abbott, S. Kalousdian, and W. B. Kannel. 1986. 'Incidence of coronary heart disease and lipoprotein cholesterol levels. The Framingham Study', *The Journal of the American Medical Association*, 256: 2835-8.

Cavalcante, J. L., J. A. Lima, A. Redheuil, and M. H. Al-Mallah. 2011. 'Aortic stiffness: current understanding and future directions', *Journal of the American College of Cardiology*, 57: 1511-22.

Celermajer, D. S., K. E. Sorensen, V. M. Gooch, D. J. Spiegelhalter, O. I. Miller, I. D. Sullivan, J. K. Lloyd, and J. E. Deanfield. 1992. 'Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis', *The Lancet*, 340: 1111-5.

Cerdá, B., F. A. Tomás-Barberán, and J. C. Carlos Espín. 2005. 'Metabolism of antioxidant and chemopreventive ellagitannins from strawberries, raspberries, walnuts, and oak-aged wine in humans: identification of biomarkers and individual variability', *Journal of Agricultural and Food Chemistry*, 53: 227-35.

Challa, H. J., M. A. Ameer, and K. R. Uppaluri. 2020. 'DASH Diet To Stop Hypertension.' in, *StatPearls* (StatPearls Publishing) 2021.

Chambers, E. S., T. Preston, G. Frost, and D. J. Morrison. 2018. 'Role of Gut Microbiota-Generated Short-Chain Fatty Acids in Metabolic and Cardiovascular Health', *Current nutrition reports*, 7: 198-206.

Charakida, M., S. Masi, T. F. Lüscher, J. J. Kastelein, and J. E. Deanfield. 2010. 'Assessment of atherosclerosis: the role of flow-mediated dilatation', *European Heart Journal*, 31: 2854-61.

Chassard, C., E. Delmas, P. A. Lawson, and A. Bernalier-Donadille. 2008. 'Bacteroides xylanisolvens sp. nov., a xylan-degrading bacterium isolated from human faeces', *International Journal of Systematic and Evolutionary Microbiology*, 58: 1008-13.

Chaudhry, S. I., H. M. Krumholz, and J. M. . Foody. 2004. 'Systolic Hypertension in Older Persons', *The Journal of the American Medical Association*, 292: 1074-80.

Chen, M., S. Liu, Kmsu Imam, L. Sun, Y. Wang, T. Gu, B. Wen, and F. Xin. 2020. 'The Effect of Xylooligosaccharide, Xylan, and Whole Wheat Bran on the Human Gut Bacteria', *Frontiers in Microbiology*, 11: 568457.

Chen, X. L., S. E. Varner, A. S. Rao, J. Y. Grey, S. Thomas, C. K. Cook, M. A. Wasserman, R. M. Medford, A. K. Jaiswal, and C. Kunsch. 2003. 'Laminar flow induction of antioxidant response element-mediated genes in endothelial cells. A novel anti-inflammatory mechanism', *Journal of Biological Chemistry*, 278: 703-11.

Chen, Y., H. Zheng, G. Zhang, F-I. Chen, L-D. Chen, and Z. Yang. 2020. 'High Oscillospira abundance indicates constipation and low BMI in the Guangdong Gut Microbiome Project', *Scientific Reports*, 10: 9364.

Cheng, X., R. C. Siow, and G. E. Mann. 2011. 'Impaired redox signaling and antioxidant gene expression in endothelial cells in diabetes: a role for mitochondria and the nuclear factor-E2-related factor 2-Kelch-like ECH-associated protein 1 defense pathway', *Antioxidants & Redox Signaling*, 14: 469-87.

Cheruvu, P. K., A. V. Finn, C. Gardner, J. Caplan, J. Goldstein, G. W. Stone, R. Virmani, and J. E. Muller. 2007. 'Frequency and distribution of thin-cap fibroatheroma and ruptured plaques in human coronary arteries: a pathologic study', *Journal of the American College of Cardiology*, 50: 940-9.

Cheynier, V. 2005. 'Polyphenols in foods are more complex than often thought', *The American Journal of Clinical Nutrition*, 81: 223s-29s.

Chiavaroli, L., S. K. Nishi, T. A. Khan, C. R. Braunstein, A. J. Glenn, S. B. Mejia, D. Rahelić, H. Kahleová, J. Salas-Salvadó, D. J. A. Jenkins, C. W. C. Kendall, and J. L. Sievenpiper. 2018. 'Portfolio Dietary Pattern and

Cardiovascular Disease: A Systematic Review and Meta-analysis of Controlled Trials', *Progress in Cardiovascular Diseases*, 61: 43-53.

Chiavaroli, L., E. Viguiliouk, S. K. Nishi, S. Blanco Mejia, D. Rahelić, H. Kahleová, J. Salas-Salvadó, C. W. Kendall, and J. L. Sievenpiper. 2019. 'DASH Dietary Pattern and Cardiometabolic Outcomes: An Umbrella Review of Systematic Reviews and Meta-Analyses', *Nutrients*, 11: 338.

Chrubasik, C., G. Li, and S. Chrubasik. 2010. 'The clinical effectiveness of chokeberry: a systematic review', *Phytotherapy Research*, 24: 1107-14.

Cifuentes-Gomez, T., A. Rodriguez-Mateos, I. Gonzalez-Salvador, M. E. Alañon, and J. P. E. Spencer. 2015. 'Factors Affecting the Absorption, Metabolism, and Excretion of Cocoa Flavanols in Humans', *Journal of Agricultural and Food Chemistry*, 63: 7615-23.

Cikiriz, N., I. Milosavljevic, B. Jakovljevic, S. Bolevich, J. Jeremic, T. R. Nikolic Turnic, M. Mitrovic, I. M. Srejovic, S. Bolevich, and V. Jakovljevic. 2020. 'The influences of chokeberry extract supplementation on redox status and body composition in handball players during competition phase', *Canadian Journal of Physiology and Pharmacology*, 99: 42-47.

Clarke, S. F., E. F. Murphy, O. O'Sullivan, A. J. Lucey, M. Humphreys, A. Hogan, P. Hayes, M. O'Reilly, I. B. Jeffery, R. Wood-Martin, D. M. Kerins, E. Quigley, R. P. Ross, P. W. O'Toole, M. G. Molloy, E. Falvey, F. Shanahan, and P. D. Cotter. 2014. 'Exercise and associated dietary extremes impact on gut microbial diversity', *Gut*, 63: 1913-20.

Clifford, M.N. 2000. 'Anthocyanins – nature, occurrence and dietary burden'. *Journal of the Science of Food and Agriculture*, 80: 1063-1072.

Collins, G. S., and D. G. Altman. 2009. 'An independent external validation and evaluation of QRISK cardiovascular risk prediction: a prospective open cohort study', *The BMJ*, 339: b2584.

Conroy, R. M., K. Pyörälä, A. P. Fitzgerald, S. Sans, A. Menotti, G. De Backer, D. De Bacquer, P. Ducimetière, P. Jousilahti, U. Keil, I. Njølstad, R. G. Oganov, T. Thomsen, H. Tunstall-Pedoe, A. Tverdal, H. Wedel, P. Whincup, L. Wilhelmsen, I. M. Graham, and Score project group on behalf of the. 2003. 'Estimation of ten-year risk of fatal cardiovascular disease in Europe: the SCORE project', *European Heart Journal*, 24: 987-1003.

Cook, N. R., J. Cohen, P. R. Hebert, J. O. Taylor, and C. H. Hennekens. 1995. 'Implications of Small Reductions in Diastolic Blood Pressure for Primary Prevention', *Archives of Internal Medicine*, 155: 701-09.

Corona, G., J. P. Spencer, and M. A. Dessì. 2009. 'Extra virgin olive oil phenolics: absorption, metabolism, and biological activities in the GI tract', *Toxicology and Industrial Health*, 25: 285-93.

Corretti, M. C., G. D. Plotnick, and R. A. Vogel. 1995. 'Technical aspects of evaluating brachial artery vasodilatation using high-frequency ultrasound', *American Journal of Physiology*, 268: H1397-404.

Corretti, M. C., T. J. Anderson, E. J. Benjamin, D. Celermajer, F. Charbonneau, M. A. Creager, J. Deanfield, H. Drexler, M. Gerhard-Herman, D. Herrington, P. Vallance, J. Vita, and R. A. Vogel. 2002. 'Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force', *Journal of the American College of Cardiology*, 39: 257-65.

Cortés-Martín, A., M. V. Selma, F. A. Tomás-Barberán, A. González-Sarrías, and J. C. Espín. 2020. 'Where to Look into the Puzzle of Polyphenols and Health? The Postbiotics and Gut Microbiota Associated with Human Metabotypes', *Molecular Nutrition & Food Research*, 64: e1900952.

Costa-Hong, V. A, H. Cotchi Simbo Muela, T. Andrade Macedo, A. Robson Kluser Sales, and L. Aparecido Bortolotto. 2018. 'Gender differences of aortic wave reflection and influence of menopause on central blood pressure in patients with arterial hypertension', *BMC cardiovascular disorders*, 18: 123.

Costello, E. K., C. L. Lauber, M. Hamady, N. Fierer, J. I. Gordon, and R. Knight. 2009. 'Bacterial community variation in human body habitats across space and time', *Science*, 326: 1694-7.

Cotillard, A., S. P. Kennedy, L. C. Kong, E. Prifti, N. Pons, E. Le Chatelier, M. Almeida, B. Quinquis, F. Levenez, N. Galleron, S. Gougis, S. Rizkalla, J. M. Batto, P. Renault, J. Doré, J. D. Zucker, K. Clément, and S. D. Ehrlich. 2013. 'Dietary intervention impact on gut microbial gene richness', *Nature*, 500: 585-8.

Craig, C. L., A. L. Marshall, M. Sjöström, A. E. Bauman, M. L. Booth, B. E. Ainsworth, M. Pratt, U. Ekelund, A. Yngve, J. F. Sallis, and P. Oja. 2003. 'International physical activity questionnaire: 12-country reliability and validity', *Medicine & Science in Sports & Exercise*, 35: 1381-95.

Crawford, A. A., S. Soderberg, C. Kirschbaum, L. Murphy, M. Eliasson, S. Ebrahim, G. Davey Smith, T. Olsson, N. Sattar, D. A. Lawlor, N. J. Timpson, R. M. Reynolds, and B. R. Walker. 2019. 'Morning plasma cortisol as a cardiovascular risk factor: findings from prospective cohort and Mendelian randomization studies', *European Journal of Endocrinology*, 181: 429-38.

Croft, K. D. 2016. 'Dietary polyphenols: Antioxidants or not?', *Archives of Biochemistry and Biophysics*, 595: 120-4.

Crozier, A., D. Del Rio, and M. N. Clifford. 2010. 'Bioavailability of dietary flavonoids and phenolic compounds', *Molecular Aspects of Medicine*, 31: 446-67.

Crozier, A., I. B. Jaganath, and M. N. Clifford. 2009. 'Dietary phenolics: chemistry, bioavailability and effects on health', *Natural Product Reports*, 26: 1001-43.

Crozier, A., M. E. J. Lean, M. S. McDonald, and C. Black. 1997. 'Quantitative Analysis of the Flavonoid Content of Commercial Tomatoes, Onions, Lettuce, and Celery', *Journal of Agricultural and Food Chemistry*, 45: 590-95.

Cruickshank, K., L. Riste, S. G. Anderson, J. S. Wright, G. Dunn, and R. G. Gosling. 2002. 'Aortic pulse-wave velocity and its relationship to mortality in diabetes and glucose intolerance: an integrated index of vascular function?', *Circulation*, 106: 2085-90.

Cueva, C., I. Gil-Sánchez, M. V. Moreno-Arribas, and B. Bartolomé. 2016. 'Interactions Between Wine Polyphenols and Gut Microbiota.' in M. V. Moreno-Arribas and B. Bartolomé Suáldea (eds.), *Wine Safety, Consumer Preference, and Human Health* (Springer International Publishing: Cham).

Curtis, P. J., J. Potter, P. A. Kroon, P. Wilson, K. Dhatariya, M. Sampson, and A. Cassidy. 2013. 'Vascular function and atherosclerosis progression after 1 y of flavonoid intake in statin-treated postmenopausal women with type 2

diabetes: a double-blind randomized controlled trial', *The American Journal of Clinical Nutrition*, 97: 936-42.

Curtis, P. J., V. van der Velpen, L. Berends, A. Jennings, M. Feelisch, A. M. Umpleby, M. Evans, B. O. Fernandez, M. S. Meiss, M. Minnion, J. Potter, A. M. Minihane, C. D. Kay, E. B. Rimm, and A. Cassidy. 2019. 'Blueberries improve biomarkers of cardiometabolic function in participants with metabolic syndrome-results from a 6-month, double-blind, randomized controlled trial', *The American Journal of Clinical Nutrition*, 109: 1535-45.

Czank, C., A. Cassidy, Q. Zhang, D. J. Morrison, T. Preston, P. A. Kroon, N. P. Botting, and C. D. Kay. 2013. 'Human metabolism and elimination of the anthocyanin, cyanidin-3-glucoside: a (13)C-tracer study', *The American Journal of Clinical Nutrition*, 97: 995-1003.

D'Archivio, M., C. Filesi, R. Di Benedetto, R. Gargiulo, C. Giovannini, and R. Masella. 2007. 'Polyphenols, dietary sources and bioavailability', *Annali dell'Istituto Superiore di Sanità*, 43: 348-61.

D'Archivio, M., C. Filesi, R. Varì, B. Scazzocchio, and R. Masella. 2010. 'Bioavailability of the polyphenols: status and controversies', *International Journal of Molecular Sciences*, 11: 1321-42.

Dai, L., L. Song, X. Li, Y. Yang, X. Zheng, Y. Wu, C. Li, H. Zhao, Y. Wang, S. Wu, and Y. Wang. 2018. 'Association of visit-to-visit blood pressure variability with the risk of all-cause mortality and cardiovascular events in general population', *The Journal of Clinical Hypertension*, 20: 280-88.

Dan, X., Z. Mushi, W. Baili, L. Han, W. Enqi, Z. Huanhu, and L. Shuchun. 2019. 'Differential Analysis of Hypertension-Associated Intestinal Microbiota', *International Journal of Medical Sciences*, 16: 872-81.

Dao, H. H., R. Essalihi, C. Bouvet, and P. Moreau. 2005. 'Evolution and modulation of age-related medial elastocalcinosis: impact on large artery stiffness and isolated systolic hypertension', *Cardiovascular Research*, 66: 307-17.

Dart A., M., C. D. Gatzka, B. A. Kingwell, W. Kristyn, J. D. Cameron, L. Yu-Lu, K. L. Berry, L. M. H. Wing, C. M. Reid, R. Philip yan, L. J. Beilin, G. L. R. Jennings, C. I. Johnston, J. J. McNeil, G. J. MacDonald, T. O. Morgan, and M. J. West. 2006. 'Brachial Blood Pressure But Not Carotid Arterial

Waveforms Predict Cardiovascular Events in Elderly Female Hypertensives', *Hypertension*, 47: 785-90.

David, L. A., C. F. Maurice, R. N. Carmody, D. B. Gootenberg, J. E. Button, B. E. Wolfe, A. V. Ling, A. S. Devlin, Y. Varma, M. A. Fischbach, S. B. Biddinger, R. J. Dutton, and P. J. Turnbaugh. 2014. 'Diet rapidly and reproducibly alters the human gut microbiome', *Nature*, 505: 559-63.

Dawson, E. A., M. A. Black, J. Pybis, N. T. Cable, and D. J. Green. 2009. 'The impact of exercise on derived measures of central pressure and augmentation index obtained from the SphygmoCor device', *Journal of Applied Physiology*, 106: 1896-901.

Day, A. J., F. J. Canada, J. C. Diaz, P. A. Kroon, R. McLauchlan, C. B. Faulds, G. W. Plumb, M. R. Morgan, and G. Williamson. 2000. 'Dietary flavonoid and isoflavone glycosides are hydrolysed by the lactase site of lactase phlorizin hydrolase', *FEBS Letters*, 468: 166-70.

Day, N., S. Oakes, R. Luben, K. T. Khaw, S. Bingham, A. Welch, and N. Wareham. 1999. 'EPIC-Norfolk: study design and characteristics of the cohort. European Prospective Investigation of Cancer', *British Journal of Cancer*, 80 Suppl 1: 95-103.

De Ferrars, R. M., C. Czank, Q. Zhang, N. P. Botting, P. A. Kroon, A. Cassidy, and C. D. Kay. 2014. 'The pharmacokinetics of anthocyanins and their metabolites in humans', *British Journal of Pharmacology*, 171: 3268-82.

De Filippo, C., D. Cavalieri, M. Di Paola, M. Ramazzotti, J. B. Poullet, S. Massart, S. Collini, G. Pieraccini, and P. Lionetti. 2010. 'Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa', *Proceedings of the National Academy of Sciences of the United States of America*, 107: 14691-6.

de la Cuesta-Zuluaga, J., S. T. Kelley, Y. Chen, J. S. Escobar, N. T. Mueller, R. E. Ley, D. McDonald, S. Huang, A. D. Swafford, R. Knight, and V. G. Thackray. 2019. 'Age- and Sex-Dependent Patterns of Gut Microbial Diversity in Human Adults', *mSystems*, 4: e00261-19.

de Toro-Martín, J., B. J. Arsenault, J-P. Després, and M-C. Vohl. 2017. 'Precision Nutrition: A Review of Personalized Nutritional Approaches for the Prevention and Management of Metabolic Syndrome', *Nutrients*, 9: 913.

Del Rio, D., G. Borges, and A. Crozier. 2010. 'Berry flavonoids and phenolics: bioavailability and evidence of protective effects', *British Journal of Nutrition*, 104 Suppl: S67-90.

Del Rio, D., A. Rodriguez-Mateos, J. P. Spencer, M. Tognolini, G. Borges, and A. Crozier. 2013. 'Dietary (poly)phenolics in human health: structures, bioavailability, and evidence of protective effects against chronic diseases', *Antioxidants & Redox Signaling*, 18: 1818-92.

Déprez, S., C. Brezillon, S. Rabot, C. Philippe, I. Mila, C. Lapierre, and A. Scalbert. 2000. 'Polymeric proanthocyanidins are catabolized by human colonic microflora into low-molecular-weight phenolic acids', *The Journal of Nutrition*, 130: 2733-8.

Derrien, M., C. Belzer, and W. M. de Vos. 2017. 'Akkermansia muciniphila and its role in regulating host functions', *Microbial Pathogenesis*, 106: 171-81.

Díez, J. 2007. 'Arterial stiffness and extracellular matrix', *Advances in Cardiology*, 44: 76-95.

Dobbie, L. J., S. T. Mackin, K. Hogarth, F. Lonergan, D. Kannenkeril, K. Brooksbank, and C. Delles. 2020. 'Validation of semi-automated flow-mediated dilation measurement in healthy volunteers', *Blood Pressure Monitoring*, 25: 216-23.

Dohadwala, M. M., M. Holbrook, N. M. Hamburg, S. M. Shenouda, W. B. Chung, M. Titas, M. A. Kluge, N. Wang, J. Palmisano, P. E. Milbury, J. B. Blumberg, and J. A. Vita. 2011. 'Effects of cranberry juice consumption on vascular function in patients with coronary artery disease', *The American Journal of Clinical Nutrition*, 93: 934-40.

Domínguez-Fernández, M., Y. Xu, P. Young Tie Yang, W. Alotaibi, R. Gibson, W. L. Hall, L. Barron, I. A. Ludwig, C. Cid, and A. Rodriguez-Mateos. 2020. 'Quantitative Assessment of Dietary (Poly)phenol Intake: A High-Throughput Targeted Metabolomics Method for Blood and Urine Samples', *Journal of Agricultural and Food Chemistry*.

Donovan, J. L., C. Manach, L. Rios, C. Morand, A. Scalbert, and C. Rémésy. 2002. 'Procyanidins are not bioavailable in rats fed a single meal containing a grapeseed extract or the procyanidin dimer B3', *British Journal of Nutrition*, 87: 299-306.

Dower, J. I., J. M. Geleijnse, L. Gijsbers, P. L. Zock, D. Kromhout, and P. C. H. Hollman. 2015. 'Effects of the pure flavonoids epicatechin and quercetin on vascular function and cardiometabolic health: a randomized, double-blind, placebo-controlled, crossover trial', *The American Journal of Clinical Nutrition*, 101: 914-21.

Dragsted, L. O. 2003. 'Antioxidant actions of polyphenols in humans', *International Journal for Vitamin and Nutrition Research*, 73: 112-9.

Draijer, R., Y. de Graaf, M. Slettenaar, E. de Groot, and C. I. Wright. 2015. 'Consumption of a polyphenol-rich grape-wine extract lowers ambulatory blood pressure in mildly hypertensive subjects', *Nutrients*, 7: 3138-53.

Dubois-Deruy, E., V. Peugnet, A. Turkieh, and F. Pinet. 2020. 'Oxidative Stress in Cardiovascular Diseases', *Antioxidants (Basel)*, 9: 2090.

Duchnowicz, P., A. Ziobro, E. Rapacka, M. Koter-Michalak, and B. Bukowska. 2018. 'Changes in Cholinesterase Activity in Blood of Adolescent with Metabolic Syndrome after Supplementation with Extract from Aronia melanocarpa', *BioMed Research International*, 2018: 5670145.

Duda-Chodak, A., T. Tarko, P. Satora, and P. Sroka. 2015. 'Interaction of dietary compounds, especially polyphenols, with the intestinal microbiota: a review', *European Journal of Nutrition*, 54: 325-41.

Duprez, D. A., D. R. Jacobs, Jr., P. L. Lutsey, D. A. Bluemke, L. C. Brumback, J. F. Polak, C. A. Peralta, P. Greenland, and R. A. Kronmal. 2011. 'Association of Small Artery Elasticity With Incident Cardiovascular Disease in Older Adults: The Multi-Ethnic Study of Atherosclerosis', *American Journal of Epidemiology*, 174: 528-36.

Durazzi, F., C. Sala, G. Castellani, G. Manfreda, D. Remondini, and A. De Cesare. 2021. 'Comparison between 16S rRNA and shotgun sequencing data for the taxonomic characterization of the gut microbiota', *Scientific Reports*, 11: 3030.

Duttaroy, A. K. 2021. 'Role of Gut Microbiota and Their Metabolites on Atherosclerosis, Hypertension and Human Blood Platelet Function: A Review', *Nutrients*, 13: 144.

Dwivedi, A. K., P. Dubey, D. P. Cistola, and S. Y. Reddy. 2020. 'Association Between Obesity and Cardiovascular Outcomes: Updated Evidence from Meta-analysis Studies', *Current Cardiology Reports*, 22: 25.

Eagles, S. K., A. S. Gross, and A. J. McLachlan. 2020. 'The Effects of Cruciferous Vegetable-Enriched Diets on Drug Metabolism: A Systematic Review and Meta-Analysis of Dietary Intervention Trials in Humans', *Clinical Pharmacology & Therapeutics*, 108: 212-27.

EFSA Panel on Dietetic Products, Nutrition, and Allergies. 2010. 'Scientific Opinion on the substantiation of health claims related to various food(s)/food constituent(s) and protection of cells from premature aging, antioxidant activity, antioxidant content and antioxidant properties, and protection of DNA, proteins and lipids from oxidative damage pursuant to Article 13(1) of Regulation (EC) No 1924/2006', *EFSA Journal*, 8: 1489.

EFSA 2011. 'Scientific Opinion on the substantiation of health claims related to the sugar replacers xylitol, sorbitol, mannitol, maltitol, lactitol, isomalt, erythritol, D-tagatose, isomaltulose, sucralose and polydextrose and maintenance of tooth mineralisation by decreasing tooth demineralisation (ID 463, 464, 563, 618, 647, 1182, 1591, 2907, 2921, 4300), and reduction of post-prandial glycaemic responses (ID 617, 619, 669, 1590, 1762, 2903, 2908, 2920) pursuant to Article 13(1) of Regulation (EC) No 1924/2006', *EFSA Journal*, 9: 2076.

Egert, S., A. Bosy-Westphal, J. Seiberl, C. Kürbitz, U. Settler, S. Plachta-Danielzik, A. E. Wagner, J. Frank, J. Schrezenmeir, G. Rimbach, S. Wolffram, and M. J. Müller. 2009. 'Quercetin reduces systolic blood pressure and plasma oxidised low-density lipoprotein concentrations in overweight subjects with a high-cardiovascular disease risk phenotype: a double-blinded, placebo-controlled cross-over study', *British Journal of Nutrition*, 102: 1065-74.

El Mohsen, M. A., J. Marks, G. Kuhnle, K. Moore, E. Debnam, S. Kaila Srai, C. Rice-Evans, and J. P. Spencer. 2006. 'Absorption, tissue distribution and excretion of pelargonidin and its metabolites following oral administration to rats', *British Journal of Nutrition*, 95: 51-8.

Ellwood, L., G. Torun, Z. Bahar, and R. Fernandez. 2019. 'Effects of flavonoid-rich fruits on hypertension in adults: a systematic review', JB*I Database of Systematic Reviews and Implementation Reports*, 17: 2075-105.

Eppinga, H., C. J. Sperna Weiland, H. B. Thio, C. J. van der Woude, T. E. Nijsten, M. P. Peppelenbosch, and S. R. Konstantinov. 2016. 'Similar Depletion of

Protective Faecalibacterium prausnitzii in Psoriasis and Inflammatory Bowel Disease, but not in Hidradenitis Suppurativa', *Journal of Crohn's and Colitis*, 10: 1067-75.

Erlund, I., R. Koli, G. Alfthan, J. Marniemi, P. Puukka, P. Mustonen, P. Mattila, and A. Jula. 2008. 'Favorable effects of berry consumption on platelet function, blood pressure, and HDL cholesterol', *The American Journal of Clinical Nutrition*, 87: 323-31.

Esler, M., E. Lambert, and M. Schlaich. 2010. 'Point: Chronic Activation of the Sympathetic Nervous System is the Dominant Contributor to Systemic Hypertension', *Journal of Applied Physiology*, 109: 1996-98.

Espin, J. C., A. Gonzalez-Sarrias, and F. A. Tomas-Barberan. 2017. 'The gut microbiota: A key factor in the therapeutic effects of (poly)phenols', *Biochemical Pharmacology*, 139: 82-93.

Estruch, R., E. Ros, J. Salas-Salvadó, M. I. Covas, D. Corella, F. Arós, E. Gómez-Gracia, V. Ruiz-Gutiérrez, M. Fiol, J. Lapetra, R. M. Lamuela-Raventos, L. Serra-Majem, X. Pintó, J. Basora, M. A. Muñoz, J. V. Sorlí, J. A. Martínez, M. Fitó, A. Gea, M. A. Hernán, and M. A. Martínez-González. 2018. 'Primary Prevention of Cardiovascular Disease with a Mediterranean Diet Supplemented with Extra-Virgin Olive Oil or Nuts', *The New England Journal of Medicine*, 378: e34.

Ettehad, D., C. A. Emdin, A. Kiran, S. G. Anderson, T. Callender, J. Emberson, J. Chalmers, A. Rodgers, and K. Rahimi. 2016. 'Blood pressure lowering for prevention of cardiovascular disease and death: a systematic review and meta-analysis', *The Lancet*, 387: 957-67.

European Commission. 'Summary of FBDG recommendations for fruit and vegetables for the EU, Iceland, Norway, Switzerland and the United Kingdom', Accessed 15/10/2020. https://ec.europa.eu/jrc/en/health-knowledge-gateway/promotion-prevention/nutrition/food-based-dietary-guidelines.

Eurostat. 2016. 'European Union Statistics', *News release*.

Fairlie-Jones, L., K. Davison, E. Fromentin, and A. M. Hill. 2017. 'The Effect of Anthocyanin-Rich Foods or Extracts on Vascular Function in Adults: A Systematic Review and Meta-Analysis of Randomised Controlled Trials', *Nutrients*, 9: 908.

Fantin, F., A. Mattocks, C. J. Bulpitt, W. Banya, and C. Rajkumar. 2007. 'Is augmentation index a good measure of vascular stiffness in the elderly?', *Age and Ageing*, 36: 43-48.

Faraci, F. M., and S. P. Didion. 2004. 'Vascular protection: superoxide dismutase isoforms in the vessel wall', *Arteriosclerosis, Thrombosis, and Vascular Biology*, 24: 1367-73.

Faraci, F. M., and D. D. Heistad. 1998. 'Regulation of the cerebral circulation: role of endothelium and potassium channels', *Physiological Reviews*, 78: 53-97.

Feliciano, R. P., C. E. Mills, G. Istas, C. Heiss, and A. Rodriguez-Mateos. 2017. 'Absorption, Metabolism and Excretion of Cranberry (Poly)phenols in Humans: A Dose Response Study and Assessment of Inter-Individual Variability', *Nutrients*, 9: 268.

Feliciano, R. P., G. Istas, C. Heiss, and A. Rodriguez-Mateos. 2016. 'Plasma and Urinary Phenolic Profiles after Acute and Repetitive Intake of Wild Blueberry', *Molecules,* 21: 1120-20.

Feliciano, R. P., A. Boeres, L. Massacessi, G. Istas, M. R. Ventura, C. Nunes dos Santos, C. Heiss, and A. Rodriguez-Mateos. 2016. 'Identification and quantification of novel cranberry-derived plasma and urinary (poly)phenols', *Archives of Biochemistry and Biophysics*, 599: 31-41.

Ference, B. A., W. Yoo, I. Alesh, N. Mahajan, K. K. Mirowska, A. Mewada, J, Kahn, L. Afonso, K. A. Williams, and J. M. Flack. 2012. 'Effect of Long-Term Exposure to Lower Low-Density Lipoprotein Cholesterol Beginning Early in Life on the Risk of Coronary Heart Disease: A Mendelian Randomization Analysis', *Journal of the American College of Cardiology*, 60: 2631-39.

Fernández-Pachón, M. S., G. Berná, E. Otaolaurruchi, A. M. Troncoso, F. Martín, and M. C. García-Parrilla. 2009. 'Changes in antioxidant endogenous enzymes (activity and gene expression levels) after repeated red wine intake', *Journal of Agricultural and Food Chemistry*, 57: 6578-83.

Fetics, B., E. Nevo, C. H. Chen, and D. A. Kass. 1999. 'Parametric model derivation of transfer function for noninvasive estimation of aortic pressure by radial tonometry', *IEEE Transactions on Biomedical Engineering*, 46: 698-706.

Fillmore, K. M., T. Stockwell, T. Chikritzhs, A. Bostrom, and W. Kerr. 2007. 'Moderate alcohol use and reduced mortality risk: systematic error in prospective studies and new hypotheses', *Annals of Epidemiology*, 17: S16-23.

Fisher, G., A. W. Brown, M. M. Bohan Brown, A. Alcorn, C. Noles, L. Winwood, H. Resuehr, B. George, M. M. Jeansonne, and D. B. Allison. 2015. 'High Intensity Interval- vs Moderate Intensity- Training for Improving Cardiometabolic Health in Overweight or Obese Males: A Randomized Controlled Trial', *PLoS One*, 10: e0138853.

Fisher, N. D. L., E. J. Orav, and G. Chang. 2018. 'Effects of alcohol consumption on blood pressure in hypertensive women', *The American Journal of Drug and Alcohol Abuse*, 44: 200-05.

Flammer, A. J., T. Anderson, D. S. Celermajer, M. A. Creager, J. Deanfield, P. Ganz, N. Hamburg, T. F. Lüscher, M. Shechter, S. Taddei, J. A. Vita, and A. Lerman. 2012. 'The Assessment of Endothelial Function – From Research into Clinical Practice', *Circulation*, 126: 753-67.

Fogliano, V., M. L. Corollaro, P. Vitaglione, A. Napolitano, R. Ferracane, F. Travaglia, M. Arlorio, A. Costabile, A. Klinder, and G. Gibson. 2011. 'In vitro bioaccessibility and gut biotransformation of polyphenols present in the water-insoluble cocoa fraction', *Molecular Nutrition & Food Research*, 55 Suppl 1: S44-55.

Forstermann, U., and T. Munzel. 2006. 'Endothelial nitric oxide synthase in vascular disease: from marvel to menace', *Circulation*, 113: 1708-14.

Fraga, C. G., K. D. Croft, D. O. Kennedy, and F. A. Tomás-Barberán. 2019. 'The effects of polyphenols and other bioactives on human health', *Food and Function*, 10: 514-28.

Francula-Zaninovic, S., and I. A. Nola. 2018. 'Management of Measurable Variable Cardiovascular Disease' Risk Factors', *Current Cardiology Reviews*, 14: 153-63.

Frank, S. M., J. Webster, B. McKenzie, P. Geldsetzer, J. Manne-Goehler, G. Andall-Brereton, C. Houehanou, D. Houinato, M. S. Gurung, B. W. Bicaba, R. W. McClure, A. Supiyev, Z. Zhumadilov, A. Stokes, D. Labadarios, A. M. Sibai, B. Norov, K. K. Aryal, K. B. Karki, G. B. Kagaruki, M. T. Mayige, J. S. Martins, R. Atun, T. Bärnighausen, S. Vollmer, and L. M. Jaacks.

2019. 'Consumption of Fruits and Vegetables Among Individuals 15 Years and Older in 28 Low- and Middle-Income Countries', *The Journal of Nutrition*, 149: 1252-59.

Franke, A. A., J. H. Hankin, M. C. Yu, G. Maskarinec, S-H. Low, and L. J. Custer. 1999. 'Isoflavone Levels in Soy Foods Consumed by Multiethnic Populations in Singapore and Hawaii', *Journal of Agricultural and Food Chemistry*, 47: 977-86.

Frankenfeld, C. L. 2017. 'Cardiometabolic risk and gut microbial phytoestrogen metabolite phenotypes', *Molecular Nutrition & Food Research*, 61: 1500900.

Franklin, S. S., W. th Gustin, N. D. Wong, M. G. Larson, M. A. Weber, W. B. Kannel, and D. Levy. 1997. 'Hemodynamic patterns of age-related changes in blood pressure. The Framingham Heart Study', *Circulation*, 96: 308-15.

Franzoni, F., L. Ghiadoni, F. Galetta, Y. Plantinga, V. Lubrano, Y. Huang, G. Salvetti, F. Regoli, S. Taddei, G. Santoro, and A. Salvetti. 2005. 'Physical activity, plasma antioxidant capacity, and endothelium-dependent vasodilation in young and older men', *American Journal of Hypertension*, 18: 510-6.

Frimodt-Møller, M., A. H. Nielsen, A. L. Kamper, and S. Strandgaard. 2008. 'Reproducibility of pulse-wave analysis and pulse-wave velocity determination in chronic kidney disease', *Nephrology Dialysis Transplantation*, 23: 594-600.

Furchgott, R. F., and J. V. Zawadzki. 1980. 'The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine', *Nature*, 288: 373-6.

Gao, K., A. Xu, C. Krul, K. Venema, Y. Liu, Y. Niu, J. Lu, L. Bensoussan, N. P. Seeram, D. Heber, and S. M. Henning. 2006. 'Of the major phenolic acids formed during human microbial fermentation of tea, citrus, and soy flavonoid supplements, only 3,4-dihydroxyphenylacetic acid has antiproliferative activity', *The Journal of Nutrition*, 136: 52-7.

Garcia-Alonso, M., A. M. Minihane, G. Rimbach, J. C. Rivas-Gonzalo, and S. de Pascual-Teresa. 2009. 'Red wine anthocyanins are rapidly absorbed in humans and affect monocyte chemoattractant protein 1 levels and

antioxidant capacity of plasma', *Journal of Nutritional Biochemistry*, 20: 521-9.

García-Conesa, M-T., K. Chambers, E. Combet, P. Pinto, M. Garcia-Aloy, C. Andrés-Lacueva, S. de Pascual-Teresa, P. Mena, A. Konic Ristic, W. J . Hollands, P. A. Kroon, A. Rodriguez-Mateos, G. Istas, C. A. Kontogiorgis, D. K. Rai, E. R. Gibney, C. Morand, J. C. Espin, and A. Gonzales-Sarrias. 2018. 'Meta-Analysis of the Effects of Foods and Derived Products Containing Ellagitannins and Anthocyanins on Cardiometabolic Biomarkers: Analysis of Factors Influencing Variability of the Individual Responses', *International Journal of Molecular Sciences*, 19: 694.

Gatzka, C. D., B. A. Kingwell, J. D. Cameron, K. L. Berry, Y. L. Liang, E. M. Dewar, C. M. Reid, G. L. Jennings, and A. M. Dart. 2001. 'Gender differences in the timing of arterial wave reflection beyond differences in body height', *Journal of Hypertension*, 19: 2197-203.

Gee, J. M., M. S. DuPont, A. J. Day, G. W. Plumb, G. Williamson, and I. T. Johnson. 2000. 'Intestinal transport of quercetin glycosides in rats involves both deglycosylation and interaction with the hexose transport pathway', *The Journal of Nutrition*, 130: 2765-71.

George, E. S., S. Marshall, H. L. Mayr, G. L. Trakman, O. A. Tatucu-Babet, A. M. Lassemillante, A. Bramley, A. J. Reddy, A. Forsyth, A. C. Tierney, C. J. Thomas, C. Itsiopoulos, and W. Marx. 2019. 'The effect of high-polyphenol extra virgin olive oil on cardiovascular risk factors: A systematic review and meta-analysis', *Critical Reviews in Food Science and Nutrition*, 59: 2772-95.

Geurts, L., V. Lazarevic, M. Derrien, A. Everard, M. Van Roye, C. Knauf, P. Valet, M. Girard, G. G. Muccioli, P. François, W. M. de Vos, J. Schrenzel, N. M. Delzenne, and P. D. Cani. 2011. 'Altered gut microbiota and endocannabinoid system tone in obese and diabetic leptin-resistant mice: impact on apelin regulation in adipose tissue', *Frontiers in Microbiology,* 2: 149.

Ghezzi, P., V. Jaquet, F. Marcucci, and Hhhw Schmidt. 2017. 'The oxidative stress theory of disease: levels of evidence and epistemological aspects', *British Journal of Pharmacology*, 174: 1784-96.

Ghimire, K., H. M. Altmann, A. C. Straub, and J. S. Isenberg. 2017. 'Nitric oxide: what's new to NO?', *American Journal of Physiology-Cell Physiology*, 312: C254-c62.

Gibney, E. R., D. Milenkovic, E. Combet, T. Ruskovska, A. Greyling, A. González-Sarrías, B. de Roos, F. Tomás-Barberán, C. Morand, and A. Rodriguez-Mateos. 2019. 'Factors influencing the cardiometabolic response to (poly)phenols and phytosterols: a review of the COST Action POSITIVe activities', *European Journal of Nutrition*, 58: 37-47.

Gil-Sánchez, I., A. Esteban-Fernández, D. González de Llano, M. Sanz-Buenhombre, A. Guadarrana, N. Salazar, M. Gueimonde, C. G. de los Reyes-Gaviláne, L. Martín Gómez, M. L. García Bermejo, B. Bartolomé, and M. V. Moreno-Arribas. 2018. 'Supplementation with grape pomace in healthy women: Changes in biochemical parameters, gut microbiota and related metabolic biomarkers', *Journal of Functional Foods*, 45: 34-46.

Gilani, M., D. R. Kaiser, C. W. Bratteli, C. Alinder, S. Rajala, A. J. Bank, and J. N. Cohn. 2007. 'Role of nitric oxide deficiency and its detection as a risk factor in pre-hypertension', *Journal of the American Society of Hypertension*, 1: 45-55.

Gimbrone, M. A., Jr. 1999. 'Vascular Endothelium, Hemodynamic Forces, and Atherogenesis', *The American Journal of Pathology*, 155: 1-5.

Glenn, A. J., E. Viguiliouk, M. Seider, B. A. Boucher, T. A. Khan, S. Blanco Mejia, D. J. A. Jenkins, H. Kahleová, D. Rahelić, J. Salas-Salvadó, C. W. C. Kendall, and J. L. Sievenpiper. 2019. 'Relation of Vegetarian Dietary Patterns With Major Cardiovascular Outcomes: A Systematic Review and Meta-Analysis of Prospective Cohort Studies', *Frontiers in Nutrition*, 6: 80.

Goldstein, J. L., W. R. Hazzard, H. G. Schrott, E. L. Bierman, and A. G. Motulsky. 1973. 'Hyperlipidemia in coronary heart disease. I. Lipid levels in 500 survivors of myocardial infarction', *Journal of Clinical Investigation*, 52: 1533-43.

Goncharov, N. V., P. V. Avdonin, A. D. Nadeev, I. L. Zharkikh, and R. O. Jenkins. 2015. 'Reactive oxygen species in pathogenesis of atherosclerosis', *Current Pharmaceutical Design*, 21: 1134-46.

Gonthier, M-P., J. L. Donovan, O. Texier, C. Felgines, C. Remesy, and A. Scalbert. 2003. 'Metabolism of dietary procyanidins in rats', *Free Radical Biology and Medicine*, 35: 837-44.

González-Domínguez, R., O. Jáuregui, P. Mena, K. Hanhineva, F. J. Tinahones, D. Angelino, and C. Andrés-Lacueva. 2020. 'Quantifying the human diet in the crosstalk between nutrition and health by multi-targeted metabolomics of food and microbiota-derived metabolites', *International Journal of Obesity*, 44: 2372-81.

Gonzalez-Sanchez, J., L. Garcia-Ortiz, E. Rodriguez-Sanchez, J. A. Maderuelo-Fernandez, O. Tamayo-Morales, C. Lugones-Sanchez, J. I. Recio-Rodriguez, and M. A. Gomez-Marcos. 2020. 'The Relationship Between Alcohol Consumption With Vascular Structure and Arterial Stiffness in the Spanish Population: EVA Study', *Alcoholism: Clinical and Experimental Research*, 44: 1816-24.

González-Sarrías, A., J. C. Espín, and F. A. Tomás-Barberán. 2017. 'Non-extractable polyphenols produce gut microbiota metabolites that persist in circulation and show anti-inflammatory and free radical-scavenging effects', *Trend in food science and technology*, 69: 281-88.

González-Sarrías, A., M. Romo-Vaquero, R. García-Villalba, A. Cortés-Martín, M. V. Selma, and J. Carlos Espín. 2018. 'The endotoxemia marker lipopolysaccharide-binding protein is reduced in overweight-obese subjects consuming pomegranate extract by modulating the gut microbiota: A randomized clinical trial', *Molecular Nutrition & Food Research*, 62: 1800160.

Gooch, J. L., and A. C. Sharma. 2014. 'Targeting the immune system to treat hypertension: where are we?', *Current Opinion in Nephrology and Hypertension*, 23.

Gophna, U., T. Konikoff, and H. B. Nielsen. 2017. 'Oscillospira and related bacteria - From metagenomic species to metabolic features', *Environmental Microbiology*, 19: 835-41.

Grassi, D., G. Desideri, S. Necozione, P. di Giosia, R. Barnabei, L. Allegaert, H. Bernaert, and C. Ferri. 2015. 'Cocoa consumption dose-dependently improves flow-mediated dilation and arterial stiffness decreasing blood pressure in healthy individuals', *Journal of Hypertension*, 33: 294-303.

Green, D. J., E. A. Dawson, H. M. Groenewoud, H. Jones, and D. H. Thijssen. 2014. 'Is flow-mediated dilation nitric oxide mediated?: A meta-analysis', *Hypertension*, 63: 376-82.

Green, D. J., H. Jones, D. Thijssen, N. T. Cable, and G. Atkinson. 2011. 'Flow-mediated dilation and cardiovascular event prediction: does nitric oxide matter?', *Hypertension*, 57: 363-9.

Gross, G., D. M. Jacobs, S. Peters, S. Possemiers, J. van Duynhoven, E. E. Vaughan, and T. van de Wiele. 2010. 'In vitro bioconversion of polyphenols from black tea and red wine/grape juice by human intestinal microbiota displays strong interindividual variability', *Journal of Agricultural and Food Chemistry*, 58: 10236-46.

Grossini, E., P. Marotta, S. Farruggio, L. Sigaudo, F. Qoqaiche, G. Raina, V. de Giuli, D. Mary, G. Vacca, and F. Pollastro. 2015. 'Effects of Artemetin on Nitric Oxide Release and Protection against Peroxidative Injuries in Porcine Coronary Artery Endothelial Cells', *Phytotherapy Research*, 29: 1339-48.

Grosso, G., A. Micek, J. Godos, A. Pajak, S. Sciacca, F. Galvano, and E. L. Giovannucci. 2017. 'Dietary Flavonoid and Lignan Intake and Mortality in Prospective Cohort Studies: Systematic Review and Dose-Response Meta-Analysis', *American Journal of Epidemiology*, 185: 1304-16.

Grover-Paez, F., and A. B. Zavalza-Gomez. 2009. 'Endothelial dysfunction and cardiovascular risk factors', *Diabetes Research and Clinical Practice*, 84: 1-10.

Grundy, S. M., N. J. Stone, A. L. Bailey, C. Beam, K. K. Birtcher, R. S. Blumenthal, L. T. Braun, S. de Ferranti, J. Faiella-Tommasino, D. E. Forman, R. Goldberg, P. A. Heidenreich, M. A. Hlatky, D. W. Jones, D. Lloyd-Jones, N. Lopez-Pajares, C. E. Ndumele, C. E. Orringer, C. A. Peralta, J. J. Saseen, S. C. Smith, Jr., L. Sperling, S. S. Virani, and J. Yeboah. 2019. '2018 AHA/ACC/AACVPR/AAPA/ABC/ACPM/ADA/ AGS/APhA/ASPC/NLA/PCNA Guideline on the Management of Blood Cholesterol: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines', *Circulation*, 139: e1082-e143.

Gu, J., J. M. Thomas-Ahner, K. M. Riedl, M. T. Bailey, Y. Vodovotz, S. J. Schwartz, and S. K. Clinton. 2019. 'Dietary Black Raspberries Impact the Colonic Microbiome and Phytochemical Metabolites in Mice', *Molecular Nutrition & Food Research*, 63: e1800636.

Guasch-Ferré, M., S. N. Bhupathiraju, and F. B. Hu. 2018. 'Use of Metabolomics in Improving Assessment of Dietary Intake', *Clinical Chemistry*, 64: 82-98.

Gunnaiah, R., A. C. Kushalappa, R. Duggavathi, S. Fox, and D. J. Somers. 2012. 'Integrated Metabolo-Proteomic Approach to Decipher the Mechanisms by Which Wheat QTL (Fhb1) Contributes to Resistance against Fusarium graminearum', *PLoS One*, 7: e40695.

Guo, P., K. Zhang, X. Ma, and P. He. 2020. 'Clostridium species as probiotics: potentials and challenges', *Journal of Animal Science and Biotechnology*, 11: 24-24.

Guo, X., R. Tang, S. Yang, Y. Lu, J. Luo, and Z. Liu. 2018. 'Rutin and Its Combination With Inulin Attenuate Gut Dysbiosis, the Inflammatory Status and Endoplasmic Reticulum Stress in Paneth Cells of Obese Mice Induced by High-Fat Diet', *Frontiers in Microbiology*, 9: 2651.

Gupta, S., A. Rohatgi, C. R. Ayers, B. L. Willis, W. L. Haskell, A. Khera, M. H. Drazner, J. A. de Lemos, and J. D. Berry. 2011. 'Cardiorespiratory fitness and classification of risk of cardiovascular disease mortality', *Circulation*, 123: 1377-83.

Gwiazdowska, D., K. Juś, J. Jasnowska-Małecka, and K. Kluczyńska. 2015. 'The impact of polyphenols on Bifidobacterium growth', *Acta Biochimica Polonica*, 62: 895-901.

Haapala, E. A., E. Lee, and J. A. Laukkanen. 2020. 'Associations of cardiorespiratory fitness, physical activity, and BMI with arterial health in middle-aged men and women', *Physiological Reports*, 8: e14438.

Hackam, D. G., and S. S. Anand. 2003. 'Emerging risk factors for atherosclerotic vascular disease: a critical review of the evidence', *The Journal of the American Medical Association*, 290: 932-40.

Hadi, H. A. R., C. S. Carr, and J. Al Suwaidi. 2005. 'Endothelial dysfunction: cardiovascular risk factors, therapy, and outcome', *Vascular Health and Risk Management*, 1: 183-98.

Halliwell, B., J. Rafter, and A. Jenner. 2005. 'Health promotion by flavonoids, tocopherols, tocotrienols, and other phenols: direct or indirect effects? Antioxidant or not?', *The American Journal of Clinical Nutrition*, 81: 268s-76s.

Hanhineva, K., M. A. Lankinen, A. Pedret, U. Schwab, M. Kolehmainen, J. Paananen, V. de Mello, R. Sola, M. Lehtonen, K. Poutanen, M. Uusitupa, and H. Mykkanen. 2015. 'Nontargeted metabolite profiling discriminates diet-specific biomarkers for consumption of whole grains, fatty fish, and bilberries in a randomized controlled trial', *The Journal of Nutrition*, 145: 7-17.

Hansen, D., J. Niebauer, V. Cornelissen, O. Barna, D. Neunhäuserer, C. Stettler, C. Tonoli, E. Greco, R. Fagard, K. Coninx, L. Vanhees, M. F. Piepoli, R. Pedretti, G. R. Ruiz, U. Corrà, J. P. Schmid, C. H. Davos, F. Edelmann, A. Abreu, B. Rauch, M. Ambrosetti, S. S. Braga, P. Beckers, M. Bussotti, P. Faggiano, E. Garcia-Porrero, E. Kouidi, M. Lamotte, R. Reibis, M. A. Spruit, T. Takken, C. Vigorito, H. Völler, P. Doherty, and P. Dendale. 2018. 'Exercise Prescription in Patients with Different Combinations of Cardiovascular Disease Risk Factors: A Consensus Statement from the EXPERT Working Group', *Sports Medicine*, 48: 1781-97.

Harborne, J. B. 1995. 'The Flavonoids: Advances in Research Since 1986' (American Chemical Society). *Journal of Chemical Education* 72, DOI: 10.1021/ed072pA73.11.

Harborne, J. B., and Christine A. Williams. 2000. 'Advances in flavonoid research since 1992', *Phytochemistry*, 55: 481-504.

Haro, C., O. A. Rangel-Zúñiga, J. F. Alcalá-Díaz, F. Gómez-Delgado, P. Pérez-Martínez, J. Delgado-Lista, G. M. Quintana-Navarro, B. B. Landa, J. A. Navas-Cortés, M. Tena-Sempere, J. C. Clemente, J. López-Miranda, F. Pérez-Jiménez, and A. Camargo. 2016. 'Intestinal Microbiota Is Influenced by Gender and Body Mass Index', *PLoS One*, 11: e0154090.

Haro, C., S. Garcia-Carpintero, J. F. Alcala-Diaz, F. Gomez-Delgado, J. Delgado-Lista, P. Perez-Martinez, O. A. Rangel Zuñiga, G. M. Quintana-Navarro, B. B. Landa, J. C. Clemente, J. Lopez-Miranda, A. Camargo, and F. Perez-Jimenez. 2016. 'The gut microbial community in metabolic syndrome

patients is modified by diet', *The Journal of Nutritional Biochemistry*, 27: 27-31.

Hartley, L., E. Igbinedion, J. Holmes, N. Flowers, M. Thorogood, A. Clarke, S. Stranges, L. Hooper, and K. Rees. 2013. 'Increased consumption of fruit and vegetables for the primary prevention of cardiovascular diseases', *Cochrane Database Syst Rev*: Cd009874.

Hasan, N. A., B. A. Young, A. T. Minard-Smith, K. Saeed, H. Li, E. M. Heizer, N. J. McMillan, R. Isom, A. S. Abdullah, D. M. Bornman, S. A. Faith, S. Y. Choi, M. L. Dickens, T. A. Cebula, and R. R. Colwell. 2014. 'Microbial community profiling of human saliva using shotgun metagenomic sequencing', *PLoS One*, 9: e97699.

Hawkins, J., C. Hires, C. Baker, L. Keenan, and M. Bush. 2020. 'Daily supplementation with aronia melanocarpa (chokeberry) reduces blood pressure and cholesterol: a meta analysis of controlled clinical trials', *Journal of Dietary Supplements*, 1-14.

Heiss, C., A. Dejam, P. Kleinbongard, T. Schewe, H. Sies, and M. Kelm. 2003. 'Vascular effects of cocoa rich in flavan-3-ols', *The Journal of the American Medical Association*, 290: 1030-1.

Heiss, C., D. Finis, P. Kleinbongard, A. Hoffmann, T. Rassaf, M. Kelm, and H. Sies. 2007. 'Sustained increase in flow-mediated dilation after daily intake of high-flavanol cocoa drink over 1 week', *Journal of Cardiovascular Pharmacology and Therapeutics*, 49: 74-80.

Heiss, C., R. Sansone, H. Karimi, M. Krabbe, D. Schuler, A. Rodriguez-Mateos, T. Kraemer, M. M. Cortese-Krott, G. G. Kuhnle, J. P. Spencer, H. Schroeter, M. W. Merx, and M. Kelm. 2015. 'Impact of cocoa flavanol intake on age-dependent vascular stiffness in healthy men: a randomized, controlled, double-masked trial', *Age*, 37: 9794.

Heiss, C., P. Kleinbongard, A. Dejam, S. Perré, H. Schroeter, H. Sies, and M. Kelm. 2005. 'Acute consumption of flavanol-rich cocoa and the reversal of endothelial dysfunction in smokers', *Journal of the American College of Cardiology*, 46: 1276-83.

Heiss, C., A. Rodriguez-Mateos, and M. Kelm. 2015. 'Central Role of eNOS in the Maintenance of Endothelial Homeostasis', *Antioxidants & Redox Signaling*, 22: 1230-42.

Heneghan, C., M. Kiely, J. Lyons, and A. Lucey. 2018. 'The Effect of Berry-Based Food Interventions on Markers of Cardiovascular and Metabolic Health: A Systematic Review of Randomized Controlled Trials', *Molecular Nutrition & Food Research*, 62.

Henning, S. M., J. Yang, P. Shao, R-P. Lee, J, Huang, A. Ly, M. Hsu, Q-Y. Lu, G. Thames, D. Heber, and Z. Li. 2017. 'Health benefit of vegetable/fruit juice-based diet: Role of microbiome', *Sci Rep*, 7: 2167.

Hermann, M., A. Flammer, and T. F. Lüscher. 2006. 'Nitric oxide in hypertension', *The Journal of Clinical Hypertension*, 8: 17-29.

Hertog, M. G., E. J. Feskens, P. C. Hollman, M. B. Katan, and D. Kromhout. 1993. 'Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study', *The Lancet*, 342: 1007-11.

Hertog, M. G., E. J. Feskens, and D. Kromhout. 1997. 'Antioxidant flavonols and coronary heart disease risk', *The Lancet*, 349: 699.

Hervert-Hernández, D., C. Pintado, R. Rotger, and I. Goñi. 2009. 'Stimulatory role of grape pomace polyphenols on Lactobacillus acidophilus growth', *International Journal of Food Microbiology*, 136: 119-22.

Hester, S. N., A. Mastaloudis, R. Gray, J. M. Antony, M. Evans, and S. M. Wood. 2018. 'Efficacy of an Anthocyanin and Prebiotic Blend on Intestinal Environment in Obese Male and Female Subjects', *Journal of Nutrition and Metabolism*, 2018: 7497260.

Higashi, Y. 2015. 'Assessment of endothelial function. History, methodological aspects, and clinical perspectives', *International Heart Journal*, 56: 125-34.

Hills, R. D., Jr., B. A. Pontefract, H. R. Mishcon, C. A. Black, S. C. Sutton, and C. R. Theberge. 2019. 'Gut Microbiome: Profound Implications for Diet and Disease', *Nutrients*, 11: 1613.

Hollman, P. C., A. Cassidy, B. Comte, M. Heinonen, M. Richelle, E. Richling, M. Serafini, A. Scalbert, H. Sies, and S. Vidry. 2011. 'The biological relevance of direct antioxidant effects of polyphenols for cardiovascular health in humans is not established', *The Journal of Nutrition*, 141: 989s-1009s.

Hollman, P. C., J. M. van Trijp, M. N. Buysman, M. S. van der Gaag, M. J. Mengelers, J. H. de Vries, and M. B. Katan. 1997. 'Relative bioavailability

of the antioxidant flavonoid quercetin from various foods in man', *FEBS Letters*, 418: 152-6.

Hollman, P. C. 2014. 'Unravelling of the health effects of polyphenols is a complex puzzle complicated by metabolism', *Archives of Biochemistry and Biophysics*, 559: 100-05.

Hopkins, P. N. 2013. 'Molecular biology of atherosclerosis', *Physiological Reviews*, 93: 1317-542.

Hosoya, T., A. Maruyama, M. I. Kang, Y. Kawatani, T. Shibata, K. Uchida, E. Warabi, N. Noguchi, K. Itoh, and M. Yamamoto. 2005. 'Differential responses of the Nrf2-Keap1 system to laminar and oscillatory shear stresses in endothelial cells', *Journal of Biological Chemistry*, 280: 27244-50.

Huang, C. M., K. L. Wang, H. M. Cheng, S. Y. Chuang, S. H. Sung, W. C. Yu, C. T. Ting, E. G. Lakatta, F. C. Yin, P. Chou, and C. H. Chen. 2011. 'Central versus ambulatory blood pressure in the prediction of all-cause and cardiovascular mortalities', *Journal of Hypertension*, 29: 454-9.

Huang, H., G. Chen, D. Liao, Y. Zhu, and X. Xue. 2016. 'Effects of Berries Consumption on Cardiovascular Risk Factors: A Meta-analysis with Trial Sequential Analysis of Randomized Controlled Trials', *Scientific Reports*, 6: 23625-25.

Huang, J., J. Liao, Y. Fang, H. Deng, H. Yin, B. Shen, and M. Hu. 2020. 'Six-Week Exercise Training With Dietary Restriction Improves Central Hemodynamics Associated With Altered Gut Microbiota in Adolescents With Obesity', *Frontiers in Endocrinology*, 11: 569085-85.

Ibero-Baraibar, I., I. Abete, S. Navas-Carretero, A. Massis-Zaid, J. A. Martinez, and M. A. Zulet. 2014. 'Oxidised LDL levels decreases after the consumption of ready-to-eat meals supplemented with cocoa extract within a hypocaloric diet', *Nutrition, Metabolism and Cardiovascular Diseases*, 24: 416-22.

Igwe, E. O., K. E. Charlton, Y. C. Probst, K. Kent, and M. E. Netzel. 2019. 'A systematic literature review of the effect of anthocyanins on gut microbiota populations', *Journal of Human Nutrition and Dietetics*, 32: 53-62.

Inaba, Y., J. A. Chen, and S. R. Bergmann. 2010. 'Prediction of future cardiovascular outcomes by flow-mediated vasodilatation of brachial

artery: a meta-analysis', *The International Journal of Cardiovascular Imaging*, 26: 631-40.

Institute of Medicine Committee on Qualification of Biomarkers and Disease Surrogate Endpoints in Chronic. 2010. C. M. Micheel and J. R. Ball (eds.), *Evaluation of Biomarkers and Surrogate Endpoints in Chronic Disease* (National Academies Press (US)).

Insull, W., Jr. 2009. 'The pathology of atherosclerosis: plaque development and plaque responses to medical treatment', *The American Journal of Medicine*, 122: S3-s14.

International Physical Activity Questionnaire. 2005. 'Guidelines for Data Processing and Analysis of the International Physical Activity Questionnaire (IPAQ)', Accessed 01/03/2021. https://sites.google.com/site/theipaq/scoring-protocol.

Istas, G., R. P. Feliciano, T. Weber, R. Garcia-Villalba, F. Tomas-Barberan, C. Heiss, and A. Rodriguez-Mateos. 2018. 'Plasma urolithin metabolites correlate with improvements in endothelial function after red raspberry consumption: A double-blind randomized controlled trial', *Archives of Biochemistry and Biophysics*, 651: 43-51.

Istas, G., E. Wood, M. Le Sayec, C. Rawlings, J. Yoon, V. Dandavate, D. Cera, S. Rampelli, A. Costabile, E. Fromentin, and A. Rodriguez-Mateos. 2019. 'Effects of aronia berry (poly)phenols on vascular function and gut microbiota: a double-blind randomized controlled trial in adult men', *The American Journal of Clinical Nutrition*, 110: 316-29.

Ivey, K. L., J. M. Hodgson, K. D. Croft, J. R. Lewis, and R. L. Prince. 2015. 'Flavonoid intake and all-cause mortality', *The American Journal of Clinical Nutrition*, 101: 1012-20.

Jacobs, D. M., N. Deltimple, E. van Velzen, F. A. van Dorsten, M. Bingham, E. E. Vaughan, and J. van Duynhoven. 2008. '(1)H NMR metabolite profiling of feces as a tool to assess the impact of nutrition on the human microbiome', *NMR in Biomedicine*, 21: 615-26.

Jakobek, L., M. Drenjančević, V. Jukić, M. Šeruga, A. Turalija, and M. Milić. 2012. 'Polyphenols, anthocyanins and antiradical activity of chokeberries', *Electronic Journal of Environmental, Agricultural and Food Chemistry*, 11: 76-84.

Janner, J. H., N. S. Godtfredsen, S. Ladelund, J. Vestbo, and E. Prescott. 2010. 'Aortic augmentation index: reference values in a large unselected population by means of the SphygmoCor device', *American Journal of Hypertension*, 23: 180-5.

Janner, J. H., N. S. Godtfredsen, S. Ladelund, J. Vestbo, and E. Prescott. 2012. 'The association between aortic augmentation index and cardiovascular risk factors in a large unselected population', *Journal of Human Hypertension*, 26: 476-84.

Jaubert, M. P., Z. Jin, C. Russo, J. E. Schwartz, S. Homma, M. S. Elkind, T. Rundek, R. L. Sacco, and M. R. Di Tullio. 2014. 'Alcohol consumption and ambulatory blood pressure: a community-based study in an elderly cohort', *American Journal of Hypertension*, 27: 688-94.

Javadi, F., S. Eghtesadi, A. Ahmadzadeh, N. Aryaeian, M. Zabihiyeganeh, A. R. Foroushani, and S. Jazayeri. 2014. 'The effect of quercetin on plasma oxidative status, C-reactive protein and blood pressure in women with rheumatoid arthritis', *International Journal of Preventive Medicine*, 5: 293-301.

Jenkins, D. J. A., B. A. Boucher, F. D. Ashbury, M. Sloan, P. Brown, A. El-Sohemy, A. J. Hanley, W. Willett, M. Paquette, R. J. de Souza, C. Ireland, N. Kwan, A. Jenkins, S. C. Pichika, and N. Kreiger. 2017. 'Effect of Current Dietary Recommendations on Weight Loss and Cardiovascular Risk Factors', *Journal of the American College of Cardiology*, 69: 1103-12.

Jennings, A., A. A. Welch, S. J. Fairweather-Tait, C. Kay, A-M. Minihane, P. Chowienczyk, B. Jiang, M. Cecelja, T. Spector, A. Macgregor, and A. Cassidy. 2012. 'Higher anthocyanin intake is associated with lower arterial stiffness and central blood pressure in women', *The American Journal of Clinical Nutrition*, 96: 781-8.

Jiang, W., H. Wei, and B. He. 2014. 'Dietary flavonoids intake and the risk of coronary heart disease: A dose-response meta-analysis of 15 prospective studies', *Thrombosis Research*, 135: 459-63.

Jin, G., Y. Asou, K. Ishiyama, A. Okawa, T. Kanno, and Y. Niwano. 2018. 'Proanthocyanidin-Rich Grape Seed Extract Modulates Intestinal Microbiota in Ovariectomized Mice', *Journal of Food Science*, 83: 1149-52.

Jin, J. S., M. Touyama, T. Hisada, and Y. Benno. 2012. 'Effects of green tea consumption on human fecal microbiota with special reference to Bifidobacterium species', *Microbiology and Immunology*, 56: 729-39.

Jin, Y., D. Alimbetov, T. George, M. H. Gordon, and J. A. Lovegrove. 2011. 'A randomised trial to investigate the effects of acute consumption of a blackcurrant juice drink on markers of vascular reactivity and bioavailability of anthocyanins in human subjects', *European Journal Of Clinical Nutrition*, 65: 849-56.

Jones, D. P. 2006. 'Redefining oxidative stress', *Antioxid Redox Signal*, 8: 1865-79.

Joossens, M., G. Huys, M. Cnockaert, V. De Preter, K. Verbeke, P. Rutgeerts, P. Vandamme, and S. Vermeire. 2011. 'Dysbiosis of the faecal microbiota in patients with Crohn's disease and their unaffected relatives', *Gut*, 60: 631-7.

Joshipura, K. J., F. B. Hu, J. E. Manson, M. J. Stampfer, E. B. Rimm, F. E. Speizer, G. Colditz, A. Ascherio, B. Rosner, D. Spiegelman, and W. C. Willett. 2001. 'The effect of fruit and vegetable intake on risk for coronary heart disease', *Annals of Internal Medicine*, 134: 1106-14.

Juranović C., I., M. Zeiner, D. Mihajlov-Konanov, and G. Stingeder. 2017. 'Inorganic Macro- and Micronutrients in "Superberries" Black Chokeberries (Aronia melanocarpa) and Related Teas', *International Journal of Environmental Research and Public Health*, 14: 539.

Jurikova, T., J. Mlcek, S. Skrovankova, D. Sumczynski, J. Sochor, I. Hlavacova, L. Snopek, and J. Orsavova. 2017. 'Fruits of Black Chokeberry Aronia melanocarpa in the Prevention of Chronic Diseases', *Molecules*, 22: 944.

Kaess, B. M., J. Rong, M. G. Larson, N. M. Hamburg, J. A. Vita, D. Levy, E. J. Benjamin, R. S. Vasan, and G. F. Mitchell. 2012. 'Aortic stiffness, blood pressure progression, and incident hypertension', *The Journal of the American Medical Association*, 308: 875-81.

Kannel, W. B. 1999. 'Historic perspectives on the relative contributions of diastolic and systolic blood pressure elevation to cardiovascular risk profile', *Am Heart J*, 138: 205-10.

Kapci, B., E. Neradová, H. Čížková, M. Voldřich, A. Rajchl, and E. Capanoglu. 2013. 'Investigating the antioxidant potential of chokeberry (Aronia

melanocarpa) products', *Journal of Food and Nutrition Research*, 52: 219-29.

Kappus, R. M, C. D Curry, S. McAnulty, J. Welsh, D. Morris, D. C Nieman, J. Soukup, and S. R Collier. 2011. 'The effects of a multiflavonoid supplement on vascular and hemodynamic parameters following acute exercise', *Oxidative Medicine and Cellular Longevity*, 2011: 210798.

Karalliedde, J., A. Smith, L. DeAngelis, V. Mirenda, A. Kandra, J. Botha, P. Ferber, and G. Viberti. 2008. 'Valsartan improves arterial stiffness in type 2 diabetes independently of blood pressure lowering', *Hypertension*, 51: 1617-23.

Kardum, N., A. Konić-Ristić, K. Savikin, S. Spasić, A. Stefanović, J. Ivanišević, and M. Miljković. 2014. 'Effects of polyphenol-rich chokeberry juice on antioxidant/pro-oxidant status in healthy subjects', *Journal of Medicinal Food*, 17: 869-74.

Kardum, N., B. Milovanovic, K. Savikin, G. Zdunic, S. Mutavdzin, T. Gligorijevic, and S. Spasic. 2015. 'Beneficial Effects of Polyphenol-Rich Chokeberry Juice Consumption on Blood Pressure Level and Lipid Status in Hypertensive Subjects', *Journal of Medicinal Food*, 18: 1231-8.

Kargin, D., L. Tomaino, and L. Serra-Majem. 2019. 'Experimental Outcomes of the Mediterranean Diet: Lessons Learned from the Predimed Randomized Controlled Trial', *Nutrients*, 11: 2991.

Karlsson, F., V. Tremaroli, J. Nielsen, and F. Bäckhed. 2013. 'Assessing the human gut microbiota in metabolic diseases', *Diabetes*, 62: 3341-49.

Kasahara, K., K. A. Krautkramer, E. Org, K. A. Romano, R. L. Kerby, E. I. Vivas, M. Mehrabian, J. M. Denu, F. Bäckhed, A. J. Lusis, and F. E. Rey. 2018. 'Interactions between Roseburia intestinalis and diet modulate atherogenesis in a murine model', *Nature Microbiology*, 3: 1461-71.

Katsuyama, M. 2010. 'NOX/NADPH oxidase, the superoxide-generating enzyme: its transcriptional regulation and physiological roles', *Journal of Pharmacological Sciences*, 114: 134-46.

Katz, R. 2004. 'Biomarkers and surrogate markers: an FDA perspective', *NeuroRx*, 1: 189-95.

Kawabata, K., Y. Yoshioka, and J. Terao. 2019. 'Role of Intestinal Microbiota in the Bioavailability and Physiological Functions of Dietary Polyphenols', *Molecules*, 24: 370.

Kawabata, K., N. Baba, T. Sakano, Y. Hamano, S. Taira, A. Tamura, S. Baba, M. Natsume, T. Ishii, S. Murakami, and H. Ohigashi. 2018. 'Functional properties of anti-inflammatory substances from quercetin-treated Bifidobacterium adolescentis', *Bioscience, Biotechnology, and Biochemistry*, 82: 689-97.

Kawai, Y., S. Saito, T. Nishikawa, A. Ishisaka, K. Murota, and J. Terao. 2009. 'Different profiles of quercetin metabolites in rat plasma: comparison of two administration methods', *Bioscience, Biotechnology, and Biochemistry*, 73: 517-23.

Kay, C. D., G. J. Mazza, and B. J. Holub. 2005. 'Anthocyanins exist in the circulation primarily as metabolites in adult men', *The Journal of Nutrition*, 135: 2582-8.

Kay, C. D., G. Pereira-Caro, I. A. Ludwig, M. N. Clifford, and A. Crozier. 2017. 'Anthocyanins and Flavanones Are More Bioavailable than Previously Perceived: A Review of Recent Evidence', *Annual Review of Food Science and Technology*, 8: 155-80.

Keane, K. M., P. G. Bell, J. K. Lodge, C. L. Constantinou, S. E. Jenkinson, R. Bass, and G. Howatson. 2016. 'Phytochemical uptake following human consumption of Montmorency tart cherry (L. Prunus cerasus) and influence of phenolic acids on vascular smooth muscle cells in vitro', *European Journal of Nutrition*, 55: 1695-705.

Kelly, J. J., G. Mangos, P. M. Williamson, and J. A. Whitworth. 1998. 'Cortisol and hypertension', *Clinical and Experimental Pharmacology and Physiology*, 25: S51-6.

Kendall, C. W., and D. J. Jenkins. 2004. 'A dietary portfolio: maximal reduction of low-density lipoprotein cholesterol with diet', *Current Atherosclerosis Reports*, 6: 492-8.

Kennedy, D. O. 2014. 'Polyphenols and the human brain: plant “secondary metabolite” ecologic roles and endogenous signaling functions drive benefits', *Advences in Nutrition*, 5: 515-33.

Khan, F., S. Ray, A. M. Craigie, G. Kennedy, A. Hill, K. L. Barton, J. Broughton, and J. J. Belch. 2014. 'Lowering of oxidative stress improves endothelial function in healthy subjects with habitually low intake of fruit and vegetables: a randomized controlled trial of antioxidant- and polyphenol-rich blackcurrant juice', *Free Radical Biology and Medicine*, 72: 232-7.

Kidambi, S., T. Wang, T. Chelius, I. Nunuk, P. Agarwal, P. Laud, D. Mattson, A. W. Cowley, Jr., M. Liang, and T. Kotchen. 2020. 'Twenty-four-hour versus clinic blood pressure levels as predictors of long-term cardiovascular and renal disease outcomes among African Americans', *Scientific Reports*, 10: 11685.

Kim, H., L. E. Caulfield, V. Garcia-Larsen, L. M. Steffen, J. Coresh, and C. M. Rebholz. 2019. 'Plant-Based Diets Are Associated With a Lower Risk of Incident Cardiovascular Disease, Cardiovascular Disease Mortality, and All-Cause Mortality in a General Population of Middle-Aged Adults', *Journal of the American Heart Association*, 8: e012865.

Kim, H. L., and S. H. Kim. 2019. 'Pulse Wave Velocity in Atherosclerosis', *Frontiers in Cardiovascular Medicine*, 6: 41.

Kim, J. H., C. Auger, I. Kurita, E. Anselm, L. O. Rivoarilala, H. J. Lee, K. W. Lee, and V. B. Schini-Kerth. 2013. 'Aronia melanocarpa juice, a rich source of polyphenols, induces endothelium-dependent relaxations in porcine coronary arteries via the redox-sensitive activation of endothelial nitric oxide synthase', *Nitric Oxide*, 35: 54-64.

Kim, Y, and Y Je. 2017. 'Flavonoid intake and mortality from cardiovascular disease and all causes: A meta-analysis of prospective cohort studies', *Clinical Nutrition ESPEN*, 20: 68-77.

Kimble, R., K. M. Keane, J. K. Lodge, and G. Howatson. 2019. 'Dietary intake of anthocyanins and risk of cardiovascular disease: A systematic review and meta-analysis of prospective cohort studies', *Critical Reviews in Food Science and Nutrition*, 59: 3032-43.

Kjeldsen, S. E. 2018. 'Hypertension and cardiovascular risk: General aspects', *Pharmacological Research*, 129: 95-99.

Kodama, S., K. Saito, S. Tanaka, M. Maki, Y. Yachi, M. Asumi, A. Sugawara, K. Totsuka, H. Shimano, Y. Ohashi, N. Yamada, and H. Sone. 2009. 'Cardiorespiratory fitness as a quantitative predictor of all-cause mortality

and cardiovascular events in healthy men and women: a meta-analysis', *The Journal of the American Medical Association*, 301: 2024-35.

Kokotkiewicz, A., Z. Jaremicz, and M. Luczkiewicz. 2010. 'Aronia plants: a review of traditional use, biological activities, and perspectives for modern medicine', *Journal of Medicinal Food*, 13: 255-69.

Konturek, P. C., D. Haziri, T. Brzozowski, T. Hess, S. Heyman, S. Kwiecien, S. J. Konturek, and J. Koziel. 2015. 'Emerging role of fecal microbiota therapy in the treatment of gastrointestinal and extra-gastrointestinal diseases', *Journal of Physiology and Pharmacology*, 66: 483-91.

Koutsos, A., M. Lima, L. Conterno, M. Gasperotti, M. Bianchi, F. Fava, U. Vrhovsek, J. A. Lovegrove, and K. M. Tuohy. 2017. 'Effects of Commercial Apple Varieties on Human Gut Microbiota Composition and Metabolic Output Using an In Vitro Colonic Model', *Nutrients*, 9: 533.

Kowalczyk, E., P. Fijałkowski, M. Kura, P. Krzesiński, J. Błaszczyk, J. Kowalski, J. Smigielski, M. Rutkowski, and M. Kopff. 2005. '[The influence of anthocyanins from Aronia melanocarpa on selected parameters of oxidative stress and microelements contents in men with hypercholesterolemia]', *Polski Merkuriusz Lekarski*, 19: 651-3.

Kragel, A. H., S. G. Reddy, J. T. Wittes, and W. C. Roberts. 1989. 'Morphometric analysis of the composition of atherosclerotic plaques in the four major epicardial coronary arteries in acute myocardial infarction and in sudden coronary death', *Circulation*, 80: 1747-56.

Krauss, R. M., R. H. Eckel, B. Howard, L. J. Appel, S. R. Daniels, R. J. Deckelbaum, J. W. Erdman, Jr., P. Kris-Etherton, I. J. Goldberg, T. A. Kotchen, A. H. Lichtenstein, W. E. Mitch, R. Mullis, K. Robinson, J. Wylie-Rosett, S. St Jeor, J. Suttie, D. L. Tribble, and T. L. Bazzarre. 2000. 'AHA Dietary Guidelines: revision 2000: A statement for healthcare professionals from the Nutrition Committee of the American Heart Association', *Stroke*, 31: 2751-66.

Krga, I., D. Milenkovic, C. Morand, and L. E. Monfoulet. 2016. 'An update on the role of nutrigenomic modulations in mediating the cardiovascular protective effect of fruit polyphenols', *Food and Function*, 7: 3656-76.

Krogholm, K. S., L. Bredsdorff, S. Alinia, T. Christensen, S. E. Rasmussen, and L. O. Dragsted. 2010. 'Free fruit at workplace intervention increases total

fruit intake: a validation study using 24 h dietary recall and urinary flavonoid excretion', *European Journal of Clinical Nutrition*, 64: 1222-8.

Kuhnle, G. G. 2012. 'Nutritional biomarkers for objective dietary assessment', *Journal of the Science of Food and Agriculture*, 92: 1145-9.

Kulling, S. E., and H. M. Rawel. 2008. 'Chokeberry (Aronia melanocarpa)– A Review on the Characteristic Components and Potential Health Effects', *Planta Medica*, 74: 1625-34.

Kunutsor, S. K., S. J. L. Bakker, and R. P. F. Dullaart. 2017. 'Soluble Vascular Cell Adhesion Molecules May be Protective of Future Cardiovascular Disease Risk: Findings from the PREVEND Prospective Cohort Study', *Journal of Atherosclerosis and Thrombosis*, 24: 804-18.

Kutschera, M., W. Engst, M. Blaut, and A. Braune. 2011. 'Isolation of catechin-converting human intestinal bacteria', *Journal of Applied Microbiology*, 111: 165-75.

Kwon, G. P., J. L. Schroeder, M. J. Amar, A. T. Remaley, and R. S. Balaban. 2008. 'Contribution of macromolecular structure to the retention of low-density lipoprotein at arterial branch points', *Circulation*, 117: 2919-27.

La Rosa, S. L., M. L. Leth, L. Michalak, M. E. Hansen, N. A. Pudlo, R. Glowacki, G. Pereira, C. T. Workman, MØ Arntzen, P. B. Pope, E. C. Martens, M. A. Hachem, and B. Westereng. 2019. 'The human gut Firmicute Roseburia intestinalis is a primary degrader of dietary β-mannans', *Nature Communications*, 10: 905.

Lacombe, A., R. W. Li, D. Klimis-Zacas, A. S. Kristo, S. Tadepalli, E. Krauss, R. Young, and V. C. H. Wu. 2013. 'Lowbush Wild Blueberries have the Potential to Modify Gut Microbiota and Xenobiotic Metabolism in the Rat Colon', *PLoS One*, 8: e67497.

Lafay, S., A. Gil-Izquierdo, C. Manach, C. Morand, C. Besson, and A. Scalbert. 2006. 'Chlorogenic acid is absorbed in its intact form in the stomach of rats', *The Journal of Nutrition*, 136: 1192-97.

Lajemi, M., C. Labat, S. Gautier, P. Lacolley, M. Safar, R. Asmar, F. Cambien, and A. Benetos. 2001. 'Angiotensin II type 1 receptor-153A/G and 1166A/C gene polymorphisms and increase in aortic stiffness with age in hypertensive subjects', *Journal of Hypertension*, 19: 407-13.

Lakenbrink, C., S. Lapczynski, B. Maiwald, and U. H. Engelhardt. 2000. 'Flavonoids and other polyphenols in consumer brews of tea and other caffeinated beverages', *Journal of Agricultural, and Food Chemistry,* 48: 2848-52.

Lakshminarayanan, B., C. Stanton, P. W. O'Toole, and R. P. Ross. 2014. 'Compositional dynamics of the human intestinal microbiota with aging: implications for health', *The Journal of Nutrition, Health and Aging*, 18: 773-86.

Lancrajan, I. 2012. 'Aronia melanocarpa, a potential therapeutic agent', *Studia Universitatis "Vasile Goldis" Arad. Seria Stiintele Vietii (Life Sciences Series)*, 22: 389-94.

Landete, J. M. 2011. 'Ellagitannins, ellagic acid and their derived metabolites: A review about source, metabolism, functions and health', *Food Research International*, 44: 1150-60.

Laurent, S., P. Boutouyrie, R. Asmar, I. Gautier, B. Laloux, L. Guize, P. Ducimetiere, and A. Benetos. 2001. 'Aortic stiffness is an independent predictor of all-cause and cardiovascular mortality in hypertensive patients', *Hypertension*, 37: 1236-41.

Laurent, S., J. Cockcroft, L. Van Bortel, P. Boutouyrie, C. Giannattasio, D. Hayoz, B. Pannier, C. Vlachopoulos, I. Wilkinson, and H. Struijker-Boudier. 2006. 'Expert consensus document on arterial stiffness: methodological issues and clinical applications', *European Heart Journal*, 27: 2588-605.

Laurent, S., J. Sharman, and P. Boutouyrie. 2016. 'Central versus peripheral blood pressure: finding a solution', *Journal of Hypertension*, 34: 1497-99.

Lavefve, L., L. R. Howard, and F. Carbonero. 2020. 'Berry polyphenols metabolism and impact on human gut microbiota and health', *Food and Function*, 11: 45-65.

Le Chatelier, E., T. Nielsen, J. Qin, E. Prifti, F. Hildebrand, G. Falony, M. Almeida, M. Arumugam, J. M. Batto, S. Kennedy, P. Leonard, J. Li, K. Burgdorf, N. Grarup, T. Jørgensen, I. Brandslund, H. B. Nielsen, A. S. Juncker, M. Bertalan, F. Levenez, N. Pons, S. Rasmussen, S. Sunagawa, J. Tap, S. Tims, E. G. Zoetendal, S. Brunak, K. Clément, J. Doré, M. Kleerebezem, K. Kristiansen, P. Renault, T. Sicheritz-Ponten, W. M. de Vos, J. D. Zucker, J. Raes, T. Hansen, P. Bork, J. Wang, S. D. Ehrlich, and O.

Pedersen. 2013. 'Richness of human gut microbiome correlates with metabolic markers', *Nature*, 500: 541-6.

Le Roy, T., P. Van der Smissen, A. Paquot, N. Delzenne, G. G. Muccioli, J. F. Collet, and P. D. Cani. 2020. 'Dysosmobacter welbionis gen. nov., sp. nov., isolated from human faeces and emended description of the genus Oscillibacter', *International Journal of Systematic and Evolutionary Microbiology*, 70: 4851-58.

Lee, H. C., A. M. Jenner, C. Seng Low, and Y. K. Lee. 2006. 'Effect of tea phenolics and their aromatic fecal bacterial metabolites on intestinal microbiota', *Research in Microbiology*, 157: 876-84.

Leeming, E. R., A. J. Johnson, T. D. Spector, and C. I. Le Roy. 2019. 'Effect of Diet on the Gut Microbiota: Rethinking Intervention Duration', *Nutrients*, 11: 2862.

Leeming, E. R., P. Louca, R. Gibson, C. Menni, T. D. Spector, and C. I. Le Roy. 2021. 'The complexities of the diet-microbiome relationship: advances and perspectives', *Genome medicine*, 13: 10.

Leonard, P. J., Brand, M. H., Connolly, B. A., Obae, S. G.,. 2013. 'Investigation of the Origin of Aronia mitschurinii using Amplified Fragment Length Polymorphism Analysis', *Horticultural Science*, 48: 520-24

Leonarduzzi, G., G. Testa, B. Sottero, P. Gamba, and G. Poli. 2010. 'Design and development of nanovehicle-based delivery systems for preventive or therapeutic supplementation with flavonoids', *Current Medicinal Chemistry*, 17: 74-95.

Leopold, J. A., and J. Loscalzo. 2009. 'Oxidative risk for atherothrombotic cardiovascular disease', *Free Radical Biology and Medicine,* 47: 1673-706.

Li, C., J. He, S. Li, W. Chen, L. Bazzano, X. Sun, Luqi Shen, X. Gu, and T. Kelly. 2018. 'Abstract MP65: Metabolomics Associated With Augmentation Index and Pulse Wave Velocity: Findings From the Bogalusa Heart Study', *Circulation*, 137: AMP65-AMP65.

Li, G., B. Yin, J. Li, J. Wang, W. Wei, D. I. Bolnick, X. Wan, B. Zhu, and Z. Zhang. 2020. 'Host-microbiota interaction helps to explain the bottom-up effects of climate change on a small rodent species', *The ISME Journal*, 14: 1795-808.

Li, H., N. Xia, S. Hasselwander, and A. Daiber. 2019. 'Resveratrol and Vascular Function', *International Journal of Molecular Sciences*, 20: 2155.

Li, J., F. Zhao, Y. Wang, J. Chen, J. Tao, G. Tian, S. Wu, W. Liu, Q. Cui, B. Geng, W. Zhang, R. Weldon, K. Auguste, L. Yang, X. Liu, L. Chen, X. Yang, B. Zhu, and J. Cai. 2017. 'Gut microbiota dysbiosis contributes to the development of hypertension', *Microbiome*, 5: 14.

Li, N., X. Wu, W. Zhuang, L. Xia, Y. Chen, R. Zhao, M. Yi, Q. Wan, L. Du, and Y. Zhou. 2020. 'Soy and Isoflavone Consumption and Multiple Health Outcomes: Umbrella Review of Systematic Reviews and Meta-Analyses of Observational Studies and Randomized Trials in Humans', *Molecular Nutrition & Food Research*, 64: e1900751.

Libby, P., J. E. Buring, L. Badimon, G. K. Hansson, J. Deanfield, M. S. Bittencourt, L. Tokgözoğlu, and E. F. Lewis. 2019. 'Atherosclerosis', *Nature Reviews Disease Primers*, 5: 56.

Libby, P. 2012. 'Inflammation in atherosclerosis', *Arteriosclerosis, Thrombosis, and Vascular Biology*, 32: 2045-51.

Lin, J., K. M. Rexrode, F. Hu, C. M. Albert, C. U. Chae, E. B. Rimm, M. J. Stampfer, and J. E. Manson. 2007. 'Dietary intakes of flavonols and flavones and coronary heart disease in US women', *American Journal of Epidemiology*, 165: 1305-13.

Lin, X., I. Zhang, A. Li, J. E. Manson, H. D. Sesso, L. Wang, and S. Liu. 2016. 'Cocoa Flavanol Intake and Biomarkers for Cardiometabolic Health: A Systematic Review and Meta-Analysis of Randomized Controlled Trials', *The Journal of Nutrition*, 146: 2325-33.

Litescu, S. C., S. Eremia, and G. L. Radu. 2010. 'Methods for the determination of antioxidant capacity in food and raw materials', *Advances in Experimental Medicine and Biology*, 698: 241-9.

Liu, R. H. 2013. 'Health-promoting components of fruits and vegetables in the diet', *Advances in Nutrition*, 4: 384s-92s.

Liu, X. M., Y. J. Liu, Y. Huang, H. J. Yu, S. Yuan, B. W. Tang, P. G. Wang, and Q. Q. He. 2017. 'Dietary total flavonoids intake and risk of mortality from all causes and cardiovascular disease in the general population: A systematic review and meta-analysis of cohort studies', *Molecular Nutrition & Food Research*, 61: 1601003.

Liu, X., D. A. Martin, J. C. Valdez, S. Sudakaran, F. Rey, and B. W. Bolling. 2021. 'Aronia berry polyphenols have matrix-dependent effects on the gut microbiota', *Food Chemistry*, 359: 129831.

Liu, X., B. Mao, J. Gu, J. Wu, S. Cui, G. Wang, J. Zhao, H. Zhang, and W. Chen. 2021. 'Blautia—a new functional genus with potential probiotic properties?', *Gut Microbes*, 13: 1-21.

Liu, Y-X., Y. Qin, T. Chen, M. Lu, X. Qian, X. Guo, and Y. Bai. 2021. 'A practical guide to amplicon and metagenomic analysis of microbiome data', *Protein & Cell*, 12: 315-30.

Liu, Z., and M. Hu. 2007. 'Natural polyphenol disposition via coupled metabolic pathways', *Expert Opinion on Drug Metabolism & Toxicology*, 3: 389-406.

Lock, K., J. Pomerleau, L. Causer, D. R. Altmann, and M. McKee. 2005. 'The global burden of disease attributable to low consumption of fruit and vegetables: implications for the global strategy on diet', *Bulletin of the World Health Organization*, 83: 100-8.

London, G. M., J. Blacher, B. Pannier, A. P. Guérin, S. J. Marchais, and M. E. Safar. 2001. 'Arterial wave reflections and survival in end-stage renal failure', *Hypertension*, 38: 434-8.

Loo, B. M., I. Erlund, R. Koli, P. Puukka, J. Hellstrom, K. Wahala, P. Mattila, and A. Jula. 2016. 'Consumption of chokeberry (Aronia mitschurinii) products modestly lowered blood pressure and reduced low-grade inflammation in patients with mildly elevated blood pressure', *Nutrition Research*, 36: 1222-30.

Lopez-Siles, M., S. H. Duncan, L. J. Garcia-Gil, and M. Martinez-Medina. 2017. 'Faecalibacterium prausnitzii: from microbiology to diagnostics and prognostics', *The ISME Journal*, 11: 841-52.

Louis, P., and H. J Flint. 2017. 'Formation of propionate and butyrate by the human colonic microbiota', *Environmental Microbiology*, 19: 29-41.

Louis, X. L., P. Raj, L. Chan, S. Zieroth, T. Netticadan, and J. T. Wigle. 2019. 'Are the cardioprotective effects of the phytoestrogen resveratrol sex-dependent?', *Canadian Journal of Physiology and Pharmacology*, 97: 503-14.

Luca, S. V., I. Macovei, A. Bujor, A. Miron, K. Skalicka-Woźniak, A. C. Aprotosoaie, and A. Trifan. 2020. 'Bioactivity of dietary polyphenols: The

role of metabolites', *Critical Reviews in Food Science and Nutrition*, 60: 626-59.

Ludmer, P. L., A. P. Selwyn, T. L. Shook, R. R. Wayne, G. H. Mudge, R. W. Alexander, and P. Ganz. 1986. 'Paradoxical Vasoconstriction Induced by Acetylcholine in Atherosclerotic Coronary Arteries', *The New England Journal of Medicine*, 315: 1046-51.

Luo, D., Y. Cheng, H. Zhang, M. Ba, P. Chen, H. Li, K. Chen, W. Sha, C. Zhang, and H. Chen. 2020. 'Association between high blood pressure and long term cardiovascular events in young adults: systematic review and meta-analysis', *The BMJ*, 370: m3222.

Lüscher, T. F., S. Taddei, J. C. Kaski, J. W. Jukema, D. Kallend, T. Münzel, J. J. Kastelein, and J. E. Deanfield. 2012. 'Vascular effects and safety of dalcetrapib in patients with or at risk of coronary heart disease: the dal-VESSEL randomized clinical trial', *European Heart Journal*, 33: 857-65.

Lusis, A. J. 2000. 'Atherosclerosis', *Nature*, 407: 233-41.

Lyu, M., Y. F. Wang, G. W. Fan, X. Y. Wang, S. Y. Xu, and Y. Zhu. 2017. 'Balancing Herbal Medicine and Functional Food for Prevention and Treatment of Cardiometabolic Diseases through Modulating Gut Microbiota', *Frontiers in Microbiology*, 8: 2146.

Magne, F., M. Gotteland, L. Gauthier, A. Zazueta, S. Pesoa, P. Navarrete, and R. Balamurugan. 2020. 'The Firmicutes/Bacteroidetes Ratio: A Relevant Marker of Gut Dysbiosis in Obese Patients?', *Nutrients*, 12: 1474.

Maki, J. J., D. W. Nielsen, and T. Looft. 2020. ' Complete Genome Sequence and Annotation for Turicibacter sanguinis MOL361T (DSM 14220)', *Microbiology Resource Announcements*, 9: e00475-20.

Manach, C., D. Milenkovic, T. Van de Wiele, A. Rodriguez-Mateos, B. de Roos, M. T. Garcia-Conesa, R. Landberg, E. R. Gibney, M. Heinonen, F. Tomás-Barberán, and C. Morand. 2017. 'Addressing the inter-individual variation in response to consumption of plant food bioactives: Towards a better understanding of their role in healthy aging and cardiometabolic risk reduction', *Molecular Nutrition & Food Research*, 61: 1600557.

Manach, C., G. Williamson, C. Morand, A. Scalbert, and C. Remesy. 2005. 'Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97

bioavailability studies', *The American Journal of Clinical Nutrition*, 81: 230s-42s.

Manach, C., A. Scalbert, C. Morand, C. Remesy, and L. Jimenez. 2004. 'Polyphenols: food sources and bioavailability', *The American Journal of Clinical Nutrition*, 79: 727-47.

Manzano, S., and G. Williamson. 2010. 'Polyphenols and phenolic acids from strawberry and apple decrease glucose uptake and transport by human intestinal Caco-2 cells', *Molecular Nutrition & Food Research*, 54: 1773-80.

Marchesi, C., P. Paradis, and E. L. Schiffrin. 2008. 'Role of the renin-angiotensin system in vascular inflammation', *Trends in Pharmacological Sciences*, 29: 367-74.

Marchesi, J. R., D. H. Adams, F. Fava, G. D. Hermes, G. M. Hirschfield, G. Hold, M. N. Quraishi, J. Kinross, H. Smidt, K. M. Tuohy, L. V. Thomas, E. G. Zoetendal, and A. Hart. 2016. 'The gut microbiota and host health: a new clinical frontier', *Gut*, 65: 330-9.

Marín, L., E. M. Miguélez, C. J. Villar, and F. Lombó. 2015. 'Bioavailability of dietary polyphenols and gut microbiota metabolism: antimicrobial properties', *BioMed Research International*, 2015: 905215-15.

Marques, C., I. Fernandes, M. Meireles, A. Faria, J. P. E. Spencer, N. Mateus, and C. Calhau. 2018. 'Gut microbiota modulation accounts for the neuroprotective properties of anthocyanins', *Scientific Reports*, 8: 11341.

Martínez-López, S., B. Sarriá, R. Mateos, and L. Bravo-Clemente. 2019. 'Moderate consumption of a soluble green/roasted coffee rich in caffeoylquinic acids reduces cardiovascular risk markers: results from a randomized, cross-over, controlled trial in healthy and hypercholesterolemic subjects', *European Journal of Nutrition*, 58: 865-78.

Martini, D., M. Marino, D. Angelino, C. Del Bo, D. Del Rio, P. Riso, and M. Porrini. 2020. 'Role of berries in vascular function: a systematic review of human intervention studies', *Nutrition Reviews*, 78: 189-206.

Maruhashi, T., Y. Kihara, and Y. Higashi. 2018. 'Assessment of endothelium-independent vasodilation: from methodology to clinical perspectives', *Journal of Hypertension*, 36: 1460-67.

Masumoto, S., A. Terao, Y. Yamamoto, T. Mukai, T. Miura, and T. Shoji. 2016. 'Non-absorbable apple procyanidins prevent obesity associated with gut microbial and metabolomic changes', *Scientific Reports*, 6: 31208.

Matsuzawa, Y., T. G. Kwon, R. J. Lennon, L. O. Lerman, and A. Lerman. 2015. 'Prognostic Value of Flow-Mediated Vasodilation in Brachial Artery and Fingertip Artery for Cardiovascular Events: A Systematic Review and Meta-Analysis', *Journal of the American Heart Association*, 4: e002270-e70.

Mattace-Raso, F. U., T. J. van der Cammen, A. P. van den Elzen, M. A. Schalekamp, R. Asmar, R. S. Reneman, A. P. Hoeks, A. Hofman, and J. C. Witteman. 2005. 'Moderate alcohol consumption is associated with reduced arterial stiffness in older adults: the Rotterdam study', *The Journals of Gerontology Series A Biological Sciences and Medical Sciences*, 60: 1479-83.

Mazmanian, S. K., J. L. Round, and D. L. Kasper. 2008. 'A microbial symbiosis factor prevents intestinal inflammatory disease', *Nature*, 453: 620-5.

McAnulty, L. S., S. R. Collier, M. J. Landram, D. S. Whittaker, S. E. Isaacs, J. M. Klemka, S. L. Cheek, J. C. Arms, and S. R. McAnulty. 2014. 'Six weeks daily ingestion of whole blueberry powder increases natural killer cell counts and reduces arterial stiffness in sedentary males and females', *Nutrition research*, 34: 577-84.

McCullough, M. L., J. J. Peterson, R. Patel, P. F. Jacques, R. Shah, and J. T. Dwyer. 2012. 'Flavonoid intake and cardiovascular disease mortality in a prospective cohort of US adults', *The American Journal of Clinical Nutrition*, 95: 454-64.

McEniery, C. M., B. J. McDonnell, A. So, S. Aitken, C. E. Bolton, M. Munnery, S. S. Hickson, Yasmin, K. M. Maki-Petaja, J. R. Cockcroft, A. K. Dixon, and I. B. Wilkinson. 2009. 'Aortic calcification is associated with aortic stiffness and isolated systolic hypertension in healthy individuals', *Hypertension*, 53: 524-31.

McEniery, C. M., S. Wallace, I. S. Mackenzie, B. McDonnell, Yasmin, D. E. Newby, J. R. Cockcroft, and I. B. Wilkinson. 2006. 'Endothelial function is associated with pulse pressure, pulse wave velocity, and augmentation index in healthy humans', *Hypertension*, 48: 602-8.

McEniery, C. M., Yasmin, I. R. Hall, A. Qasem, I. B. Wilkinson, and J. R. Cockcroft. 2005. 'Normal vascular aging: differential effects on wave reflection and aortic pulse wave velocity: the Anglo-Cardiff Collaborative Trial (ACCT)', *Journal of the American College of Cardiology*, 46: 1753-60.

McIntyre, T. M., and S. L. Hazen. 2010. 'Lipid oxidation and cardiovascular disease: introduction to a review series', *Circulation Research*, 107: 1167-9.

Mecha, E., R. P. Feliciano, A. Rodriguez-Mateos, S. D. Silva, M. E. Figueira, M. C. Vaz Patto, and M. R. Bronze. 2020. 'Human bioavailability of phenolic compounds found in common beans: the use of high-resolution MS to evaluate inter-individual variability', *British Journal of Nutrition*, 123: 273-92.

Mena, L. J., V. G. Felix, J. D. Melgarejo, and G. E. Maestre. 2017. '24-Hour Blood Pressure Variability Assessed by Average Real Variability: A Systematic Review and Meta-Analysis', *Journal of the American Heart Association*, 6: e006895.

Mena, P., L. Calani, R. Bruni, and D. Del Rio. 2015. 'Chapter 6 - Bioactivation of High-Molecular-Weight Polyphenols by the Gut Microbiome.' in K. Tuohy and D. Del Rio (eds.), *Diet-Microbe Interactions in the Gut* (Academic Press: San Diego).

Menezes, R., A. Rodriguez-Mateos, A. Kaltsatou, A. González-Sarrías, A. Greyling, C. Giannaki, C. Andres-Lacueva, D. Milenkovic, E. R. Gibney, J. Dumont, M. Schär, M. Garcia-Aloy, S. A. Palma-Duran, T. Ruskovska, V. Maksimova, E. Combet, and P. Pinto. 2017. 'Impact of Flavonols on Cardiometabolic Biomarkers: A Meta Analysis of Randomized Controlled Human Trials to Explore the Role of Inter-Individual Variability', *Nutrients*, 9: 117.

Menni, C., C. Lin, M. Cecelja, M. Mangino, M. L. Matey-Hernandez, L. Keehn, R. P. Mohney, C. J. Steves, T. D. Spector, C. F. Kuo, P. Chowienczyk, and A. M. Valdes. 2018. 'Gut microbial diversity is associated with lower arterial stiffness in women', *European Heart Journal*, 39: 2390-97.

Menni, C., M. Mangino, M. Cecelja, M. Psatha, M. J. Brosnan, J. Trimmer, R. P. Mohney, P. Chowienczyk, S. Padmanabhan, T. D. Spector, and A. M.

Valdes. 2015. 'Metabolomic study of carotid–femoral pulse-wave velocity in women', *Journal of Hypertension*, 33: 791-6.

Mertens-Talcott, S. U., J. Rios, P. Jilma-Stohlawetz, L. A. Pacheco-Palencia, B. Meibohm, S. T. Talcott, and H. Derendorf. 2008. 'Pharmacokinetics of anthocyanins and antioxidant effects after the consumption of anthocyanin-rich acai juice and pulp (Euterpe oleracea Mart.) in human healthy volunteers', *Journal of Agricultural and Food Chemistry*, 56: 7796-802.

Meslier, V., M. Laiola, H. M. Roager, F. De Filippis, H. Roume, B. Quinquis, R. Giacco, I. Mennella, R. Ferracane, N. Pons, E. Pasolli, A. Rivellese, L. O. Dragsted, P. Vitaglione, S. D. Ehrlich, and D. Ercolini. 2020. 'Mediterranean diet intervention in overweight and obese subjects lowers plasma cholesterol and causes changes in the gut microbiome and metabolome independently of energy intake', *Gut*, 69: 1258-68.

Micha, R., S. Kalantarian, P. al Wirojratana, T. Byers, G. Danaei, I. Elmadfa, E. Ding, E. Giovannucci, J. Powles, and S. Smith-Warner. 2012. 'Estimating the global and regional burden of suboptimal nutrition on chronic disease: methods and inputs to the analysis', *European Journal of Clinical Nutrition*, 66: 119-29.

Micha, R., M. L. Shulkin, J. L. Peñalvo, S. Khatibzadeh, G. M. Singh, M. Rao, S. Fahimi, J. Powles, and D. Mozaffarian. 2017. 'Etiologic effects and optimal intakes of foods and nutrients for risk of cardiovascular diseases and diabetes: Systematic reviews and meta-analyses from the Nutrition and Chronic Diseases Expert Group (NutriCoDE)', *PLoS One*, 12: e0175149.

Mikael, L. R., A. M. G. Paiva, M. M. Gomes, A. L. L. Sousa, Pcbv Jardim, P. V. O. Vitorino, M. B. Euzébio, W. M. Sousa, and W. K. S. Barroso. 2017. 'Vascular Aging and Arterial Stiffness', *Arquivos Brasileiros de Cardiologia*, 109: 253-58.

Mikulic-Petkovsek, M., V. Schmitzer, A. Slatnar, F. Stampar, and R. Veberic. 2012. 'Composition of Sugars, Organic Acids, and Total Phenolics in 25 Wild or Cultivated Berry Species', *Journal of Food Science*, 77: C1064-C70.

Milenkovic, D., C. Morand, A. Cassidy, A. Konic-Ristic, F. Tomás-Barberán, J. M. Ordovas, P. Kroon, R. De Caterina, and A. Rodriguez-Mateos. 2017.

'Interindividual Variability in Biomarkers of Cardiometabolic Health after Consumption of Major Plant-Food Bioactive Compounds and the Determinants Involved', *Advances in Nutrition*, 8: 558-70.

Milenkovic, D., C. Deval, E. Gouranton, J-F. Landrier, A. Scalbert, C. Morand, and A. Mazur. 2012. 'Modulation of miRNA expression by dietary polyphenols in apoE deficient mice: a new mechanism of the action of polyphenols', *PLoS One*, 7: e29837-e37.

Milenkovic, D., B. Jude, and C. Morand. 2013. 'miRNA as molecular target of polyphenols underlying their biological effects', *Free Radical Biology and Medicine*, 64: 40-51.

Miller, K., W. Feucht, and M. Schmid. 2019. 'Bioactive Compounds of Strawberry and Blueberry and Their Potential Health Effects Based on Human Intervention Studies: A Brief Overview', *Nutrients*, 11: 1510.

Miller, V., S. Yusuf, C. K. Chow, M. Dehghan, D. J. Corsi, K. Lock, B. Popkin, S. Rangarajan, R. Khatib, S. A. Lear, P. Mony, M. Kaur, V. Mohan, K. Vijayakumar, R. Gupta, A. Kruger, L. Tsolekile, N. Mohammadifard, O. Rahman, A. Rosengren, A. Avezum, A. Orlandini, N. Ismail, P. Lopez-Jaramillo, A. Yusufali, K. Karsidag, R. Iqbal, J. Chifamba, S. M. Oakley, F. Ariffin, K. Zatonska, P. Poirier, L. Wei, B. Jian, C. Hui, L. Xu, B. Xiulin, K. Teo, and A. Mente. 2016. 'Availability, affordability, and consumption of fruits and vegetables in 18 countries across income levels: findings from the Prospective Urban Rural Epidemiology (PURE) study', *The Lancet Global Health*, 4: e695-703.

Mills, C. E., V. Govoni, L. Faconti, M. L. Casagrande, S. V. Morant, H. Crickmore, F. Iqbal, P. Maskell, A. Masani, E. Nanino, A. J. Webb, and J. K. Cruickshank. 2020. 'A randomised, factorial trial to reduce arterial stiffness independently of blood pressure: Proof of concept? The VaSera trial testing dietary nitrate and spironolactone', *British Journal of Clinical Pharmacology*, 86: 891-902.

Milutinovic, M., R. V. Radovanovic, K. Savikin, S. Radenkovic, M. Arvandi, M. Pesic, M. Kostic, B. Miladinovic, S. Brankovic, and D. Kitic. 2019. 'Chokeberry juice supplementation in type 2 diabetic patients - impact on health status', *Journal of Applied Biomedicine*, 17: 218-24.

Mink, P. J., C. G. Scrafford, L. M. Barraj, L. Harnack, C-P. Hong, J. A. Nettleton, and D. R. Jacobs. 2007. 'Flavonoid intake and cardiovascular disease mortality: a prospective study in postmenopausal women', *The American Journal of Clinical Nutrition*, 85: 895-909.

Miquel, S., R. Martín, C. Bridonneau, V. Robert, H. Sokol, L. G. Bermúdez-Humarán, M. Thomas, and P. Langella. 2014. 'Ecology and metabolism of the beneficial intestinal commensal bacterium Faecalibacterium prausnitzii', *Gut Microbes*, 5: 146-51.

Mitchell, G. F., S. J. Hwang, M. G. Larson, N. M. Hamburg, E. J. Benjamin, R. S. Vasan, D. Levy, and J. A. Vita. 2016. 'Transfer function-derived central pressure and cardiovascular disease events: the Framingham Heart Study', *Journal of Hypertension*, 34: 1528-34.

Mitchell, G. F., S. J. Hwang, R. S. Vasan, M. G. Larson, M. J. Pencina, N. M. Hamburg, J. A. Vita, D. Levy, and E. J. Benjamin. 2010. 'Arterial stiffness and cardiovascular events: the Framingham Heart Study', *Circulation*, 121: 505-11.

Moco, S., F-P. J. Martin, and S. Rezzi. 2012. 'Metabolomics View on Gut Microbiome Modulation by Polyphenol-rich Foods', *Journal of Proteome Research*, 11: 4781-90.

Molan, A-L., Z. Liu, and M. Kruger. 2010. 'The ability of blackcurrant extracts to positively modulate key markers of gastrointestinal function in rats', *World Journal of Microbiology and Biotechnology*, 26: 1735-43.

Molinaro, A., P. Bel Lassen, M. Henricsson, H. Wu, S. Adriouch, E. Belda, R. Chakaroun, T. Nielsen, P. O. Bergh, C. Rouault, S. André, F. Marquet, F. Andreelli, J. E. Salem, K. Assmann, J. P. Bastard, S. Forslund, E. Le Chatelier, G. Falony, N. Pons, E. Prifti, B. Quinquis, H. Roume, S. Vieira-Silva, T. H. Hansen, H. K. Pedersen, C. Lewinter, N. B. Sønderskov, L. Køber, H. Vestergaard, T. Hansen, J. D. Zucker, P. Galan, M. E. Dumas, J. Raes, J. M. Oppert, I. Letunic, J. Nielsen, P. Bork, S. D. Ehrlich, M. Stumvoll, O. Pedersen, J. Aron-Wisnewsky, K. Clément, and F. Bäckhed. 2020. 'Imidazole propionate is increased in diabetes and associated with dietary patterns and altered microbial ecology', *Nature Communications*, 11: 5881.

Monagas, M., M. Urpi-Sarda, F. Sanchez-Patan, R. Llorach, I. Garrido, C. Gomez-Cordoves, C. Andres-Lacueva, and B. Bartolome. 2010. 'Insights into the metabolism and microbial biotransformation of dietary flavan-3-ols and the bioactivity of their metabolites', *Food and Function*, 1: 233-53.

Monagas, M., J. E. Quintanilla-López, C. Gómez-Cordovés, B. Bartolomé, and R. Lebrón-Aguilar. 2010. 'MALDI-TOF MS analysis of plant proanthocyanidins', *Journal of Pharmaceutical and Biomedical Analysis*, 51: 358-72.

Moncada, S., and J. D. Erusalimsky. 2002. 'Does nitric oxide modulate mitochondrial energy generation and apoptosis?', *Nature Reviews Molecular Cell Biology*, 3: 214.

Morelli, L., M. L. Callegari, and V. Patrone. 2018. 'Chapter 17 - Prebiotics, Probiotics, and Synbiotics: A Bifidobacterial View.' in Paola Mattarelli, Bruno Biavati, Wilhelm H. Holzapfel and Brian J. B. Wood (eds.), *The Bifidobacteria and Related Organisms* (Academic Press).

Moreno-Indias, I., L. Sánchez-Alcoholado, P. Pérez-Martínez, C. Andrés-Lacueva, F. Cardona, F. Tinahones, and M. I. Queipo-Ortuño. 2016. 'Red wine polyphenols modulate fecal microbiota and reduce markers of the metabolic syndrome in obese patients', *Food and Function*, 7: 1775-87.

Mortensen, R. N., T. A. Gerds, J. L. Jeppesen, and C. Torp-Pedersen. 2017. 'Office blood pressure or ambulatory blood pressure for the prediction of cardiovascular events', *European Heart Journal*, 38: 3296-304.

Mosele, J. I., M. J. Gosalbes, A. Macià, L. Rubió, J. F. Vázquez-Castellanos, N. Jiménez Hernández, A. Moya, A. Latorre, and M. J. Motilva. 2015. 'Effect of daily intake of pomegranate juice on fecal microbiota and feces metabolites from healthy volunteers', *Molecular Nutrition & Food Research*, 59: 1942-53.

Mulder, T. P, A. G Rietveld, and J. M van Amelsvoort. 2005. 'Consumption of both black tea and green tea results in an increase in the excretion of hippuric acid into urine', *The American Journal of Clinical Nutrition*, 81: 256S-60S.

Mulligan, A. A., R. N. Luben, A. Bhaniani, D. J. Parry-Smith, L. O'Connor, A. P. Khawaja, N. G. Forouhi, and K. T. Khaw. 2014. 'A new tool for converting

food frequency questionnaire data into nutrient and food group values: FETA research methods and availability', *BMJ Open*, 4: e004503.

Muñoz-Tamayo, R., B. Laroche, E. Walter, J. Doré, S. H. Duncan, H. J. Flint, and M. Leclerc. 2011. 'Kinetic modelling of lactate utilization and butyrate production by key human colonic bacterial species', *FEMS Microbiology Ecology*, 76: 615-24.

Muntner, P., D. Shimbo, R. M. Carey, J. B. Charleston, T. Gaillard, S. Misra, M. G. Myers, G. Ogedegbe, J. E. Schwartz, R. R. Townsend, E. M. Urbina, A. J. Viera, W. B. White, and J. T. Wright. 2019. 'Measurement of Blood Pressure in Humans: A Scientific Statement From the American Heart Association', *Hypertension*, 73: e35-e66.

Murota, K., Y. Nakamura, and M. Uehara. 2018. 'Flavonoid metabolism: the interaction of metabolites and gut microbiota', *Bioscience, Biotechnology, and Biochemistry*, 82: 600-10.

Nabel, E. G., A. P. Selwyn, and P. Ganz. 1990. 'Large coronary arteries in humans are responsive to changing blood flow: An endothelium-dependent mechanism that fails in patients with atherosclerosis', *Journal of the American College of Cardiology*, 16: 349-56.

Nachvak, S. M., S. Moradi, J. Anjom-Shoae, J. Rahmani, M. Nasiri, V. Maleki, and O. Sadeghi. 2019. 'Soy, Soy Isoflavones, and Protein Intake in Relation to Mortality from All Causes, Cancers, and Cardiovascular Diseases: A Systematic Review and Dose-Response Meta-Analysis of Prospective Cohort Studies', *Journal of the Academy of Nutrition and Dietetics*, 119: 1483-500.e17.

Najda, A., and H. Aabuda. 2013. 'Content of phenolic compounds and antioxidant properties of fruits of selected orchard shrub species', *Modern Phytomorphology*, 3: 105-09.

Nakamura, T., S. Fujii, J. Hoshino, Y. Saito, H. Mizuno, Y. Saito, and M. Kurabayashi. 2005. 'Selective angiotensin receptor antagonism with valsartan decreases arterial stiffness independently of blood pressure lowering in hypertensive patients', *Hypertension Research*, 28: 937-43.

Namasivayam, M., A. Adji, and M. F. O'Rourke. 2010. 'Aortic Augmentation Index and Aging: Mathematical Resolution of a Physiological Dilemma?', *Hypertension*, 56: e9-e10.

Napoli, C., V. Crudele, A. Soricelli, M. Al-Omran, N. Vitale, T. Infante, and F. P. Mancini. 2012. 'Primary prevention of atherosclerosis: a clinical challenge for the reversal of epigenetic mechanisms?', *Circulation*, 125: 2363-73.

Naruszewicz, M., I. Laniewska, B. Millo, and M. Dluzniewski. 2007. 'Combination therapy of statin with flavonoids rich extract from chokeberry fruits enhanced reduction in cardiovascular risk markers in patients after myocardial infraction (MI)', *Atherosclerosis*, 194: e179-84.

Nash, V., C. S. Ranadheera, E. N. Georgousopoulou, D. D. Mellor, D. B. Panagiotakos, A. J. McKune, J. Kellett, and N. Naumovski. 2018. 'The effects of grape and red wine polyphenols on gut microbiota - A systematic review', *Food Research International*, 113: 277-87.

National Health Service. 2018. ' Health Survey for England, 2018: Adults' health related behaviours report (version 3)', Accessed 12/11/2020. https://digital.nhs.uk/data-and information/publications/statistical/ health-survey-for-england/2018#resources

Navar, L. G. 2010. 'Counterpoint: Activation of the Intrarenal Renin-Angiotensin System is the Dominant Contributor to Systemic Hypertension', *Journal of Applied Physiology*, 109: 1998-2000.

Naveed, M., V. Hejazi, M. Abbas, A. A. Kamboh, G. J. Khan, M. Shumzaid, F. Ahmad, D. Babazadeh, X. FangFang, F. Modarresi-Ghazani, L. WenHua, and Z. XiaoHui. 2018. 'Chlorogenic acid (CGA): A pharmacological review and call for further research', *Biomedicine and Pharmacotherapy*, 97: 67-74.

Neveu, V., J. Perez-Jimenez, F. Vos, V. Crespy, L. du Chaffaut, L. Mennen, C. Knox, R. Eisner, J. Cruz, D. Wishart, and A. Scalbert. 2010. 'Phenol-Explorer: an online comprehensive database on polyphenol contents in foods', *Database*, 2010: bap024.

Nguyen, T. T. T., K. Oshima, H. Toh, A. Khasnobish, Y. Fujii, K. Arakawa, and H. Morita. 2019. 'Draft Genome Sequence of Butyricimonas faecihominis 30A1, Isolated from Feces of a Japanese Alzheimer's Disease Patient', *Microbiology Resource Announcements*, 8: e00462-19.

Nishiwaki, M., N. Kora, and N. Matsumoto. 2017. 'Ingesting a small amount of beer reduces arterial stiffness in healthy humans', *Physiological Reports*, 5(15): e13381.

Norat, T, D Chan, R Lau, D Aune, and R Vieira. 2010. 'The associations between food, nutrition and physical activity and the risk of colorectal cancer: WCRF/AICR Systematic Literature Review Continuous Update Project Report', *Imperial College London: WCRF/AICR*.

Nürnberger, J., R. Michalski, T. R. Türk, A. Opazo Saez, O. Witzke, and A. Kribben. 2011. 'Can arterial stiffness parameters be measured in the sitting position?', *Hypertension Research*, 34: 202-8.

Nutrient Data Laboratory, Beltsville Human Nutrition Research Center, ARS, USDA. 2015. 'USDA Database for the Isoflavone Content of Selected Foods Release 2.1', Accessed 09/11/2020. https://doi.org/10.15482/USDA.ADC/1324538.

Nutrient Data Laboratory. Beltsville Human Nutrition Research Center, ARS, USDA 2015. 'USDA Database for the Proanthocyanidin Content of Selected Foods Release 2', Accessed 09/11/2020. https://doi.org/10.15482/USDA.ADC/1324

Nutrient Data Laboratory. Beltsville Human Nutrition Research Center, ARS, USDA 2016. 'USDA Database for the Flavonoid Content of Selected Foods Release 3.2', Accessed 09/11/2020. https://doi.org/10.15482/USDA.ADC/1324465.

Nutritics. 2020. 'Nutritics Databases', Accessed 07/12/2020. https://www.nutritics.com/p/userguide&c=523.

Oberoi, S., U. J. Schoepf, M. Meyer, T. Henzler, G. W. Rowe, P. Costello, and J. W. Nance. 2013. 'Progression of arterial stiffness and coronary atherosclerosis: longitudinal evaluation by cardiac CT', *American Journal of Roentgenology*, 200: 798-804.

Ochmian I., J. Oszmianski, and K. Skupien. 2009. 'Chemical composition, phenolics, and firmness of small black fruits', *Journal of Applied Botany and Food Quality*, 83: 64-69.

Ochmian I. D., J. Grajkowski, and M. Smolik. 2012. 'Comparison of Some Morphological Features, Quality and Chemical Content of Four Cultivars of Chokeberry Fruits (Aronia melanocarpa)', *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, 40: 253-60.

Oja, P., P. Kelly, E. M. Murtagh, M. H. Murphy, C. Foster, and S. Titze. 2018. 'Effects of frequency, intensity, duration and volume of walking

interventions on CVD risk factors: a systematic review and meta-regression analysis of randomised controlled trials among inactive healthy adults', *British Journal of Sports Medicine*, 52: 769-75.

Okumura, K., H. Yasue, K. Matsuyama, H. Ogawa, Y. Morikami, K. Obata, and N. Sakaino. 1992. 'Effect of acetylcholine on the highly stenotic coronary artery: Difference between the constrictor response of the infarct-related coronary artery and that of the noninfarct-related artery', *Journal of the American College of Cardiology*, 19: 752-58.

Olas, B., B. Wachowicz, A. Tomczak, J. Erler, A. Stochmal, and W. Oleszek. 2008. 'Comparative anti-platelet and antioxidant properties of polyphenol-rich extracts from: berries of Aronia melanocarpa, seeds of grape and bark of Yucca schidigera in vitro', *Platelets*, 19: 70-7.

Omura, Y., A. Y. Lee, S. L. Beckman, R. Simon, M. Lorberboym, H. Duvvi, S. I. Heller, and C. Urich. 1996. '177 cardiovascular risk factors, classified in 10 categories, to be considered in the prevention of cardiovascular diseases: an update of the original 1982 article containing 96 risk factors', *Acupuncture & Electro-Therapeutics Research*, 21: 21-76.

Oroian, M., and I. Escriche. 2015. 'Antioxidants: Characterization, natural sources, extraction and analysis', *Food Research Intrenational*, 74: 10-36.

Ostertag, L. M., P. A. Kroon, S. Wood, G. W. Horgan, E. Cienfuegos-Jovellanos, S. Saha, G. G. Duthie, and B. de Roos. 2013. 'Flavan-3-ol-enriched dark chocolate and white chocolate improve acute measures of platelet function in a gender-specific way--a randomized-controlled human intervention trial', *Molecular Nutrition & Food Research*, 57: 191-202.

Oszmianski, J., and A. Wojdylo. 2005. 'Aronia melanocarpa phenolics and their antioxidant activity', *European Food Research and Technology*, 221: 809-13.

Otake, Y., and T. Walle. 2002. 'Oxidation of the flavonoids galangin and kaempferide by human liver microsomes and CYP1A1, CYP1A2, and CYP2C9', *Drug Metabolism and Disposition*, 30: 103-5.

Oteiza, P. I., C. G. Fraga, D. A. Mills, and D. H. Taft. 2018. 'Flavonoids and the gastrointestinal tract: Local and systemic effects', *Molecular Aspects of Medicine*, 61: 41-49.

Otsuka, F., M. C. A. Kramer, P. Woudstra, K. Yahagi, E. Ladich, A. V. Finn, R. J. de Winter, F. D. Kolodgie, T. N. Wight, H. R. Davis, M. Joner, and R. Virmani. 2015. 'Natural progression of atherosclerosis from pathologic intimal thickening to late fibroatheroma in human coronary arteries: A pathology study', *Atherosclerosis*, 241: 772-82.

Ottaviani, J. I., T. Y. Momma, G. K. Kuhnle, C. L. Keen, and H. Schroeter. 2012. 'Structurally related (-)-epicatechin metabolites in humans: assessment using de novo chemically synthesized authentic standards', *Free Radical Biology and Medicine*, 52: 1403-12.

Ottaviani, J. I., G. Borges, T. Y. Momma, J. P. E. Spencer, C. L. Keen, A. Crozier, and H. Schroeter. 2016. 'The metabolome of [2-(14)C](-)-epicatechin in humans: implications for the assessment of efficacy, safety, and mechanisms of action of polyphenolic bioactives.' *Scientific Reports*, 6: 29034.

Ottaviani, J. I., R. Y. Fong, G. Borges, H. Schroeter, and A. Crozier. 2018. 'Use of LC-MS for the quantitative analysis of (poly)phenol metabolites does not necessarily yield accurate results: Implications for assessing existing data and conducting future research', *Free Radical Biology and Medicine*, 124: 97-103.

Ottesen, A., P. Ramachandran, E. Reed, J. R. White, N. Hasan, P. Subramanian, G. Ryan, K. Jarvis, C. Grim, N. Daquiqan, D. Hanes, M. Allard, R. Colwell, E. Brown, and Y. Chen. 2016. 'Enrichment dynamics of Listeria monocytogenes and the associated microbiome from naturally contaminated ice cream linked to a listeriosis outbreak', *BMC Microbiology*, 16: 275.

Ou, K, and L Gu. 2014. 'Absorption and metabolism of proanthocyanidins', *Journal of Functional Foods*, 7: 43-53.

Overall, J., S. Bonney, M. Wilson, A. Beermann, M. Grace, D. Esposito, M. Lila, and S. Komarnytsky. 2017. 'Metabolic Effects of Berries with Structurally Diverse Anthocyanins', *International Journal of Molecular Sciences,* 18: 422.

Palmnäs, M., C. Brunius, L. Shi, A. Rostgaard-Hansen, N. E. Torres, R. González-Domínguez, R. Zamora-Ros, Y. L. Ye, J. Halkjær, A. Tjønneland, G. Riccardi, R. Giacco, G. Costabile, C. Vetrani, J. Nielsen,

C. Andres-Lacueva, and R. Landberg. 2020. 'Perspective: Metabotyping-A Potential Personalized Nutrition Strategy for Precision Prevention of Cardiometabolic Disease', *Advances in Nutrition*, 11: 524-32.

Pan, P., V. Lam, N. Salzman, Y. W. Huang, J. Yu, J. Zhang, and L. S. Wang. 2017. 'Black Raspberries and Their Anthocyanin and Fiber Fractions Alter the Composition and Diversity of Gut Microbiota in F-344 Rats', *Nutrition and Cancer*, 69: 943-51.

Paniker, N. V., S. K. Srivastava, and E. Beutler. 1970. 'Glutathione metabolism of the red cells. Effect of glutathione reductase deficiency on the stimulation of hexose monophosphate shunt under oxidative stress', *Biochimica et Biophysica Acta*, 215: 456-60.

Parati, G., G. Stergiou, E. O'Brien, R. Asmar, L. Beilin, G. Bilo, D. Clement, A. de la Sierra, P. de Leeuw, E. Dolan, R. Fagard, J. Graves, G. A. Head, Y. Imai, K. Kario, E. Lurbe, J. M. Mallion, G. Mancia, T. Mengden, M. Myers, G. Ogedegbe, T. Ohkubo, S. Omboni, P. Palatini, J. Redon, L. M. Ruilope, A. Shennan, J. A. Staessen, G. vanMontfrans, P. Verdecchia, B. Waeber, J. Wang, A. Zanchetti, and Y. Zhang. 2014. 'European Society of Hypertension practice guidelines for ambulatory blood pressure monitoring', *Journal of Hypertension*, 32: 1359-66.

Parati, G., G. S. Stergiou, E. Dolan, and G. Bilo. 2018. 'Blood pressure variability: clinical relevance and application', *The Journal of Clinical Hypertension*, 20: 1133-37.

Parkar, S. G., T. M. Trower, and D. E. Stevenson. 2013. 'Fecal microbial metabolism of polyphenols and its effects on human gut microbiota', *Anaerobe*, 23: 12-9.

Passamonti, S., U. Vrhovsek, A. Vanzo, and F. Mattivi. 2003. 'The stomach as a site for anthocyanins absorption from food', *FEBS Letters*, 544: 210-3.

Paturi, G., T. Mandimika, C. A. Butts, S. Zhu, N. C. Roy, W. C. McNabb, and J. Ansell. 2012. 'Influence of dietary blueberry and broccoli on cecal microbiota activity and colon morphology in mdr1a(-/-) mice, a model of inflammatory bowel diseases', *Nutrition*, 28: 324-30.

Payvandi, L., A. Dyer, D. McPherson, P. Ades, J. Stein, K. Liu, L. Ferrucci, M. H. Criqui, J. M. Guralnik, D. Lloyd-Jones, M. R. Kibbe, S. T. Liang, B. Kane, W. H. Pearce, M. Verta, W. J. McCarthy, J. R. Schneider, A. Shroff, and

M. M. McDermott. 2009. 'Physical activity during daily life and brachial artery flow-mediated dilation in peripheral arterial disease', *Vascular Medicine*, 14: 193-201.

Pereira-Caro, G., G. Borges, J. van der Hooft, M. N. Clifford, D. Del Rio, M. E. Lean, S. A. Roberts, M. B. Kellerhals, and A. Crozier. 2014. 'Orange juice (poly)phenols are highly bioavailable in humans', *The American Journal of Clinical Nutrition*, 100: 1378-84.

Pérez-Jiménez, J., V. Neveu, F. Vos, and A. Scalbert. 2010. 'Identification of the 100 richest dietary sources of polyphenols: an application of the Phenol-Explorer database', *European Journal of Clinical Nutrition*, 64 Suppl 3: S112-20.

Pérez Martínez, G., C. Bäuerl, and M. C. Collado. 2014. 'Understanding gut microbiota in elderly's health will enable intervention through probiotics', *Beneficial Microbes*, 5: 235-46.

Peters, S. A., D. E. Grobbee, and M. L. Bots. 2011. 'Carotid intima–media thickness: a suitable alternative for cardiovascular risk as outcome?', *European Journal of Preventive Cardiology*, 18: 167-74.

Petersen, C., U. D. Wankhade, D. Bharat, K. Wong, J. E. Mueller, S. V. Chintapalli, B. D. Piccolo, T. Jalili, Z. Jia, J. D. Symons, K. Shankar, and P. V. Anandh Babu. 2019. 'Dietary supplementation with strawberry induces marked changes in the composition and functional potential of the gut microbiome in diabetic mice', *Journal of Nutritional Biochemistry*, 66: 63-69.

Phenol Explorer. 'Foods with Polyphenol Retention Factors', Accessed 01/03/2021. http://phenol-explorer.eu/food-processing/foods.

Phenol Explorer. 'Polyphenol classes - classification', Accessed 05/10/2020. http://phenol-explorer.eu/compounds/classification.

Piepoli, M. F., A. W. Hoes, S. Agewall, C. Albus, C. Brotons, A. L. Catapano, M. T. Cooney, U. Corrà, B. Cosyns, C. Deaton, I. Graham, M. S. Hall, F. D. R. Hobbs, M. L. Løchen, H. Löllgen, P. Marques-Vidal, J. Perk, E. Prescott, J. Redon, D. J. Richter, N. Sattar, Y. Smulders, M. Tiberi, H. B. van der Worp, I. van Dis, W. M. M. Verschuren, and S. Binno. 2016. '2016 European Guidelines on cardiovascular disease prevention in clinical practice: The Sixth Joint Task Force of the European Society of Cardiology

and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (constituted by representatives of 10 societies and by invited experts)Developed with the special contribution of the European Association for Cardiovascular Prevention & Rehabilitation (EACPR)', *European Heart Journal*, 37: 2315-81.

Pimpao, R. C., M. R. Ventura, R. B. Ferreira, G. Williamson, and C. N. Santos. 2015. 'Phenolic sulfates as new and highly abundant metabolites in human plasma after ingestion of a mixed berry fruit puree', *British Journal of Nutrition*, 113: 454-63.

Pokimica, B., M. T. García-Conesa, M. Zec, J. Debeljak-Martačić, S. Ranković, N. Vidović, G. Petrović-Oggiano, A. Konić-Ristić, and M. Glibetić. 2019. 'Chokeberry Juice Containing Polyphenols Does Not Affect Cholesterol or Blood Pressure but Modifies the Composition of Plasma Phospholipids Fatty Acids in Individuals at Cardiovascular Risk', *Nutrients*, 11: 850.

Pollard, S. E., G. G. Kuhnle, D. Vauzour, K. Vafeiadou, X. Tzounis, M. Whiteman, C. Rice-Evans, and J. P. Spencer. 2006. 'The reaction of flavonoid metabolites with peroxynitrite', *Biochemical and Biophysical Research Communications*, 350: 960-8.

Ponnusamy, D., E. V. Kozlova, J. Sha, T. E. Erova, S. R. Azar, E. C. Fitts, M. L. Kirtley, B. L. Tiner, J. A. Andersson, C. J. Grim, R. P. Isom, N. A. Hasan, R. R. Colwell, and A. K. Chopra. 2016. 'Cross-talk among flesh-eating Aeromonas hydrophila strains in mixed infection leading to necrotizing fasciitis', *Proceedings of the National Academy of Sciences of the United States of America*, 113: 722-7.

Popescu, E. 2018. 'Results from testing of pruning operations in plants of chokeberry (Aronia melanocarpa L.)', *Journal of Mountain Agriculture on the Balkans*, 21: 141-51.

Poreba, R., A. Skoczynska, P. Gac, M. Poreba, I. Jedrychowska, A. Affelska-Jercha, B. Turczyn, A. Wojakowska, J. Oszmianski, and R. Andrzejak. 2009. 'Drinking of chokeberry juice from the ecological farm Dzieciolowo and distensibility of brachial artery in men with mild hypercholesterolemia', *Annals of Agricultural and Environmental Medicine*, 16: 305-8.

Potì, F., D. Santi, G. Spaggiari, F. Zimetti, and I. Zanotti. 2019. 'Polyphenol Health Effects on Cardiovascular and Neurodegenerative Disorders: A Review and Meta-Analysis', *International Journal of Molecular Sciences*, 20: 351.

Prentice, R. L. 1989. 'Surrogate endpoints in clinical trials: definition and operational criteria', *Statistics in Medicine*, 8: 431-40.

Public Health England. 2016. 'Eatwell guide'. Accessed 26/11/2020. https://www.gov.ukgovernment/publications/the-eatwell-guide

Queipo-Ortuno, M. I., M. Boto-Ordonez, M. Murri, J. M. Gomez-Zumaquero, M. Clemente-Postigo, R. Estruch, F. Cardona Diaz, C. Andres-Lacueva, and F. J. Tinahones. 2012. 'Influence of red wine polyphenols and ethanol on the gut microbiota ecology and biochemical biomarkers', *The American Journal of Clinical Nutrition*, 95: 1323-34.

Rajzer, M. W., W. Wojciechowska, M. Klocek, I. Palka, M. Brzozowska-Kiszka, and K. Kawecka-Jaszcz. 2008. 'Comparison of aortic pulse wave velocity measured by three techniques: Complior, SphygmoCor and Arteriograph', *Journal of Hypertension*, 26: 2001-7.

Raman, G., E. E. Avendano, S. Chen, J. Wang, J. Matson, B. Gayer, J. A. Novotny, and A. Cassidy. 2019. 'Dietary intakes of flavan-3-ols and cardiometabolic health: systematic review and meta-analysis of randomized trials and prospective cohort studies', *The American Journal of Clinical Nutrition*, 110: 1067-78.

Raman, G., M. Shams-White, E. E. Avendano, F. Chen, J. A. Novotny, and A. Cassidy. 2018. 'Dietary intakes of flavan-3-ols and cardiovascular health: a field synopsis using evidence mapping of randomized trials and prospective cohort studies', *Systematic reviews*, 7: 100-00.

Ras, R. T., P. L. Zock, Y. E. Zebregs, N. R. Johnston, D. J. Webb, and R. Draijer. 2013. 'Effect of polyphenol-rich grape seed extract on ambulatory blood pressure in subjects with pre- and stage I hypertension', *British Journal of Nutrition*, 110: 2234-41.

Ras, R, T., M. T. Streppel, R. Draijer, and P. L. Zock. 2013. 'Flow-mediated dilation and cardiovascular risk prediction: A systematic review with meta-analysis', *International Journal of Cardiology*, 168: 344-51.

Razungles, A., J. A. N. Oszmianski, and J-C. Sapis. 1989. 'Determination of Carotenoids in Fruits of Rosa sp. (Rosa Canina and Rosa Rugosa) and of Chokeberry (Aronia Melanocarpa)', *Journal of Food Science*, 54: 774-75.

Rees, K., A. Takeda, N. Martin, L. Ellis, D. Wijesekara, A. Vepa, A. Das, L. Hartley, and S. Stranges. 2019. 'Mediterranean-style diet for the primary and secondary prevention of cardiovascular disease', *Cochrane Database of Systematic Reviews*, 3: Cd009825.

Reino-Gonzalez, S., S. Pita-Fernández, T. Seoane-Pillado, B. López-Calviño, and S. Pértega Díaz. 2017. 'How in-office and ambulatory BP monitoring compare: A systematic review and meta-analysis', *The Journal of Family Practice*, 66: E5-e12.

Remely, M., I. Tesar, B. Hippe, S. Gnauer, P. Rust, and A. G. Haslberger. 2015. 'Gut microbiota composition correlates with changes in body fat content due to weight loss', *Beneficial Microbes*, 6: 431-9.

Reygner, J., and N. Kapel. 2019. 'Chapter 16 - Current Status of Fecal Microbiota Transplantation.' in Joel Faintuch and Salomao Faintuch (eds.), *Microbiome and Metabolome in Diagnosis, Therapy, and other Strategic Applications* (Academic Press).

Rezai, M. R., G. Goudot, C. Winters, J. D. Finn, F. C. Wu, and J. K. Cruickshank. 2011. 'Calibration mode influences central blood pressure differences between SphygmoCor and two newer devices, the Arteriograph and Omron HEM-9000', *Hypertension Research*, 34: 1046-51.

Rice-Evans, C. 1995. 'Plant polyphenols: free radical scavengers or chain-breaking antioxidants?', *Biochemical Society Symposia*, 61: 103-16.

Ricotta, C., and J. Podani. 2017. 'On some properties of the Bray-Curtis dissimilarity and their ecological meaning', *Ecological Complexity*, 31: 201-05.

Ridaura, V. K., J. J. Faith, F. E. Rey, J. Cheng, A. E. Duncan, A, L. Kau, N, W. Griffin, V. Lombard, B. Henrissat, J. R. Bain, M. J. Muehlbauer, O. Ilkayeva, C. F. Semenkovich, K. Funai, D. K. Hayashi, B, J. Lyle, M. C. Martini, L. K. Ursell, J. C. Clemente, W. Van Treuren, W. A. Walters, R. Knight, C. B. Newgard, A. C. Heath, and J. I. Gordon. 2013. 'Gut Microbiota from Twins Discordant for Obesity Modulate Metabolism in Mice', *Science*, 341: 1241214.

Ried, K., P. Fakler, and N. P. Stocks. 2017. 'Effect of cocoa on blood pressure', *Cochrane Database of Systematic Reviews*, 4: Cd008893.

Rigotti, N. A., and C. Clair. 2013. 'Managing tobacco use: the neglected cardiovascular disease risk factor', *European Heart Journal*, 34: 3259-67.

Rimm, E. B., M. B. Katan, A. Ascherio, M. J. Stampfer, and W. C. Willett. 1996. 'Relation between intake of flavonoids and risk for coronary heart disease in male health professionals', *Annals of Internal Medicine*, 125: 384-9.

Rinninella, E., P. Raoul, M. Cintoni, F. Franceschi, G. A. D. Miggiano, A. Gasbarrini, and A. Cristina Mele. 2019. 'What is the Healthy Gut Microbiota Composition? A Changing Ecosystem across Age, Environment, Diet, and Diseases', *Microorganisms*, 7: 14.

Rios, L. Y., R. N. Bennett, S. A. Lazarus, C. Rémésy, A. Scalbert, and G. Williamson. 2002. 'Cocoa procyanidins are stable during gastric transit in humans', *The American Journal of Clinical Nutrition*, 76: 1106-10.

Rios, L. Y., M. P. Gonthier, C. Remesy, I. Mila, C. Lapierre, S. A. Lazarus, G. Williamson, and A. Scalbert. 2003. 'Chocolate intake increases urinary excretion of polyphenol-derived phenolic acids in healthy human subjects', *The American Journal of Clinical Nutrition*, 77: 912-8.

Rodriguez-Castaño, Gina Paola, Federico E. Rey, Alejandro Caro-Quintero, and Alejandro Acosta-González. 2020. 'Gut-derived Flavonifractor species variants are differentially enriched during in vitro incubation with quercetin', *PLoS One*, 15: e0227724-e24.

Rodríguez-Lagunas, M. J., F. Vicente, P. Pereira, M. Castell, and F. J. Pérez-Cano. 2019. 'Relationship between Cocoa Intake and Healthy Status: A Pilot Study in University Students', *Molecules*, 24: 812.

Rodriguez-Mateos, A., M. Hezel, H. Aydin, M. Kelm, J. O. Lundberg, E. Weitzberg, J. P. Spencer, and C. Heiss. 2015. 'Interactions between cocoa flavanols and inorganic nitrate: additive effects on endothelial function at achievable dietary amounts', *Free Radical Biology and Medicine*, 80: 121-8.

Rodriguez-Mateos, A., G. Istas, L. Boschek, R. P. Feliciano, C. E. Mills, C. Boby, S. Gomez-Alonso, D. Milenkovic, and C. Heiss. 2019. 'Circulating Anthocyanin Metabolites Mediate Vascular Benefits of Blueberries: Insights From Randomized Controlled Trials, Metabolomics, and

Nutrigenomics', *The Journals of Gerontology Series A Biological Sciences and Medical Sciences*, 74: 967-76.

Rodriguez-Mateos, A., C. Rendeiro, T. Bergillos-Meca, S. Tabatabaee, T. W. George, C. Heiss, and J. P. Spencer. 2013. 'Intake and time dependence of blueberry flavonoid-induced improvements in vascular function: a randomized, controlled, double-blind, crossover intervention study with mechanistic insights into biological activity', *The American Journal of Clinical Nutrition*, 98: 1179-91.

Rodriguez-Mateos, A., D. Vauzour, C. G. Krueger, D. Shanmuganayagam, J. Reed, L. Calani, P. Mena, D. Del Rio, and A. Crozier. 2014. 'Bioavailability, bioactivity and impact on health of dietary flavonoids and related compounds: an update', *Archives of Toxicology*, 88: 1803-53.

Rodriguez-Mateos, A., R. P. Feliciano, A. Boeres, T. Weber, C. Nunes dos Santos, M. R. Ventura, and C. Heiss. 2016. 'Cranberry (poly)phenol metabolites correlate with improvements in vascular function: A double-blind, randomized, controlled, dose-response, crossover study', *Molecular Nutrition & Food Research*, 60: 2130-2140.

Rodriguez-Mateos, A., C. Heiss, G. Borges, and A. Crozier. 2014. 'Berry (poly)phenols and cardiovascular health', *Journal of Agricultural and Food Chemistry*, 62: 3842-51.

Roe, M., H. Pinchen, S. Church, and P. Finglas. 2015. 'McCance and Widdowson's The Composition of Foods Seventh Summary Edition and updated Composition of Foods Integrated Dataset', *Nutrition Bulletin*, 40: 36-39.

Rondanelli, M., A. Giacosa, M. A. Faliva, S. Perna, F. Allieri, and A. M. Castellazzi. 2015. 'Review on microbiota and effectiveness of probiotics use in older', *World Journal of Clinical Cases*, 3: 156-62.

Roos, G., L. Johansson, A. Kasmel, J. Klumbiené, and R. Prättälä. 2001. 'Disparities in vegetable and fruit consumption: European cases from the north to the south', *Public Health Nutrition*, 4: 35-43.

Rosi, A., G. Paolella, B. Biasini, and F. Scazzina. 2019. 'Dietary habits of adolescents living in North America, Europe or Oceania: A review on fruit, vegetable and legume consumption, sodium intake, and adherence to the

Mediterranean Diet', *Nutrition, Metabolism and Cardiovascular Diseases*, 29: 544-60.

Rosmond, R. 2005. 'Role of stress in the pathogenesis of the metabolic syndrome', *Psychoneuroendocrinology*, 30: 1-10.

Ross, R. 1999. 'Atherosclerosis--an inflammatory disease', *The New England Journal of Medicine*, 340: 115-26.

Rossi, R., A. Nuzzo, G. Origliani, and M. Grazia Modena. 2008. 'Prognostic role of flow-mediated dilation and cardiac risk factors in post-menopausal women', *Journal of the American College of Cardiology*, 51: 997-1002.

Roth, G. A., C. Johnson, A. Abajobir, F. Abd-Allah, S. F. Abera, G. Abyu, M. Ahmed, B. Aksut, T. Alam, K. Alam, F. Alla, N. Alvis-Guzman, S. Amrock, H. Ansari, J. Ärnlöv, H. Asayesh, T. Mehari Atey, L. Avila-Burgos, A. Awasthi, A. Banerjee, A. Barac, T. Bärnighausen, L. Barregard, N. Bedi, E. Belay Ketema, D. Bennett, G. Berhe, Z. Bhutta, S. Bitew, J. Carapetis, J. J. Carrero, D. Carvalho Malta, C. A. Castañeda-Orjuela, J. Castillo-Rivas, F. Catalá-López, J-Y. Choi, H Christensen, M. Cirillo, L. Cooper, M. Criqui, D. Cundiff, A. Damasceno, L. Dandona, R. Dandona, K. Davletov, S. Dharmaratne, P. Dorairaj, M. Dubey, R. Ehrenkranz, M. El Sayed Zaki, A. Esteghamati, E. J. A. Faraon, T. Farid, M. Farvid, V. Feigin, E. L. Ding, G. Fowkes, T. Gebrehiwot, R. Gillum, A. Gold, P. Gona, R. Gupta, T. Dejenie Habtewold, N. Hafezi-Nejad, T. Hailu, G. Bugssa Hailu, G. Hankey, H. Yimam Hassen, K. Hassen Abate, R. Havmoeller, S. I. Hay, M. Horino, P. J. Hotez, K. Jacobsen, S. James, M. Javanbakht, P. Jeemon, D. John, J. Jonas, Y. Kalkonde, C. Karimkhani, A. Kasaeian, Y. Khader, A. Khan, Y-H. Khang, S. Khera, A. T. Khoja, J. Khubchandani, D. Kim, D. Kolte, S. Kosen, K. J. Krohn, G. A. Kumar, G. F. Kwan, D. Kumar Lal, A. Larsson, S. Linn, A. Lopez, P. A. Lotufo, H. Magdy Abd El Razek, R. Malekzadeh, M. Mazidi, T. Meier, K. G. Meles, G. Mensah, A. Meretoja, H. Mezgebe, T. Miller, E. Mirrakhimov, S. Mohammed, A. E. Moran, K. I. Musa, J. Narula, B. Neal, F. Ngalesoni, G. Nguyen, C. M. Obermeyer, M. Owolabi, G. Patton, J. Pedro, D. Qato, M. Qorbani, K. Rahimi, R. K. Rai, S. Rawaf, A. Ribeiro, S. Safiri, J. A. Salomon, I. Santos, M. Santric Milicevic, B. Sartorius, A. Schutte, S. Sepanlou, M. A. Shaikh, M-J. Shin, M. Shishehbor, H. Shore, D. A. Santos Silva, E. Sobngwi, S. Stranges, S.

Swaminathan, R. Tabarés-Seisdedos, N. Tadele Atnafu, F. Tesfay, J. S. Thakur, A. Thrift, R. Topor-Madry, T. Truelsen, S. Tyrovolas, K. N. Ukwaja, O. Uthman, T. Vasankari, V. Vlassov, S. E. Vollset, T. Wakayo, D. Watkins, R. Weintraub, A. Werdecker, R. Westerman, C. S. Wiysonge, C. Wolfe, A. Workicho, G. Xu, Y. Yano, P. Yip, N. Yonemoto, M. Younis, C. Yu, T. Vos, M. Naghavi, and C. Murray. 2017. 'Global, Regional, and National Burden of Cardiovascular Diseases for 10 Causes, 1990 to 2015', *Journal of the American College of Cardiology*, 70: 1-25.

Rothwell, J. A., M. Urpi-Sarda, M. Boto-Ordoñez, R. Llorach, A. Farran-Codina, D. K. Barupal, V. Neveu, C. Manach, C, Andres-Lacueva, and A. Scalbert. 2016. 'Systematic analysis of the polyphenol metabolome using the Phenol-Explorer database', *Molecular Nutrition & Food Research*, 60: 203-11.

Ruskovska, T., V. Maksimova, and D. Milenkovic. 2020. 'Polyphenols in human nutrition: from the in vitro antioxidant capacity to the beneficial effects on cardiometabolic health and related inter-individual variability - an overview and perspective', *British Journal of Nutrition*, 123: 241-54.

Sacks, F. M., E. Obarzanek, M. M. Windhauser, L. P. Svetkey, W. M. Vollmer, M. McCullough, N. Karanja, P-H. Lin, P. Steele, M. A. Proschan, M. A. Evans, L. J. Appel, G. A. Bray, T. M. Vogt, T. J. Moore, and DASH Investigators. 1995. 'Rationale and design of the Dietary Approaches to Stop Hypertension trial (DASH)', *Annals of Epidemiology*, 5: 108-18.

Sadeghi Ekbatan, S., L. Sleno, K. Sabally, J. Khairallah, B. Azadi, L. Rodes, S. Prakash, D. J. Donnelly, and S. Kubow. 2016. 'Biotransformation of polyphenols in a dynamic multistage gastrointestinal model', *Food Chemistry*, 204: 453-62.

Safar, M. E, and P. Jankowski. 2009. 'Central blood pressure and hypertension: role in cardiovascular risk assessment', *Clinical Science*, 116: 273-82.

Sahebkar, A. 2017. 'Effects of quercetin supplementation on lipid profile: A systematic review and meta-analysis of randomized controlled trials', *Critical Reviews in Food Science and Nutrition*, 57: 666-76.

Santhakumar, A. B., M. Battino, and J. M. Alvarez-Suarez. 2018. 'Dietary polyphenols: Structures, bioavailability and protective effects against atherosclerosis', *Food and Chemical Toxicology*, 113: 49-65.

Sasaki, S., E. Yoshioka, Y. Saijo, T. Kita, E. Okada, A. Tamakoshi, and R. Kishi. 2013. 'Relation between alcohol consumption and arterial stiffness: A cross-sectional study of middle-aged Japanese women and men', *Alcohol*, 47: 643-9.

Saulnier, D. M., S. Kolida, and G. R. Gibson. 2009. 'Microbiology of the human intestinal tract and approaches for its dietary modulation', *Current Pharmaceutical Design*, 15: 1403-14.

Scalbert, A., and G. Williamson. 2000. 'Dietary intake and bioavailability of polyphenols', *Journal of Nutrition*, 130: 2073s-85s.

Schechter, A. N., and M. T. Gladwin. 2003. 'Hemoglobin and the paracrine and endocrine functions of nitric oxide', *The New England Journal of Medicine*, 348: 1483-5.

Scheelbeek, P., R. Green, K. Papier, A. Knuppel, C. Alae-Carew, A. Balkwill, T. J. Key, V. Beral, and A. D. Dangour. 2020. 'Health impacts and environmental footprints of diets that meet the Eatwell Guide recommendations: analyses of multiple UK studies', *BMJ Open*, 10: e037554.

Schröder, K. 2010. 'Isoform specific functions of Nox protein-derived reactive oxygen species in the vasculature', *Curr Opin Pharmacol*, 10: 122-6.

Selma, M. V., D. Beltran, R. Garcia-Villalba, J. C. Espin, and F. A. Tomas-Barberan. 2014. 'Description of urolithin production capacity from ellagic acid of two human intestinal Gordonibacter species', *Food and Function*, 5: 1779-84.

Selma, M. V., A. González-Sarrías, J. Salas-Salvadó, C. Andrés-Lacueva, C. Alasalvar, A. Örem, F. A. Tomás-Barberán, and J. C. Espín. 2018. 'The gut microbiota metabolism of pomegranate or walnut ellagitannins yields two urolithin-metabotypes that correlate with cardiometabolic risk biomarkers: Comparison between normoweight, overweight-obesity and metabolic syndrome', *Clin Nutr*, 37: 897-905.

Selma, M. V., J. C. Espín, and F. A. Tomás-Barberán. 2009. 'Interaction between Phenolics and Gut Microbiota: Role in Human Health', *Journal of Agricultural and Food Chemistry*, 57: 6485-501.

Serban, M. C., A. Sahebkar, A. Zanchetti, D. P. Mikhailidis, G. Howard, D. Antal, F. Andrica, A. Ahmed, W. S. Aronow, P. Muntner, G. Y. Lip, I. Graham, N.

Wong, J. Rysz, and M. Banach. 2016. 'Effects of Quercetin on Blood Pressure: A Systematic Review and Meta-Analysis of Randomized Controlled Trials', *Journal of the American Heart Association*, 5: e002713.

Shechter, M., A. Shechter, N. Koren-Morag, M. S. Feinberg, and L. Hiersch. 2014. 'Usefulness of brachial artery flow-mediated dilation to predict long-term cardiovascular events in subjects without heart disease', *The American Journal of Cardiology*, 113: 162-7.

Shen, Y., K. D Croft, J. M Hodgson, R. Kyle, I-L. E Lee, Y. Wang, R. Stocker, and N. C. Ward. 2012. 'Quercetin and its metabolites improve vessel function by inducing eNOS activity via phosphorylation of AMPK', *Biochemical Pharmacology*, 84: 1036-44.

Shiina, K., H. Tomiyama, C. Matsumoto, S. Komatsu, M. Natsume, C. Oba, Y. Ohshiba, T. Yamaji, T. Chikamori, and A. Yamashina. 2019. 'Gender difference in the effects of cacao polyphenols on blood pressure and glucose/lipid metabolism in prediabetic subjects: a double-blinded, randomized, placebo-controlled crossover trial', *Hypertenstion Research*, 42: 1083-85.

Sidor, A., and A. Gramza-Michałowska. 2019. 'Black Chokeberry Aronia melanocarpa L.-A Qualitative Composition, Phenolic Profile and Antioxidant Potential', *Molecules*, 24: 3710.

Sies, H., and E. Cadenas. 1985. 'Oxidative stress: damage to intact cells and organs', *Philosophical Transactions of The Royal Society B Biological Sciences*, 311: 617-31.

Sies, H. 2010. 'Polyphenols and health: Update and perspectives', *Archives of Biochemistry and Biophysics*, 501: 2-5.

Sikora, J., M. Broncel, and E. Mikiciuk-Olasik. 2014. 'Aronia melanocarpa Elliot reduces the activity of angiotensin i-converting enzyme-in vitro and ex vivo studies', *Oxidative Medicine and Cellular Longevity*, 2014: 739721.

Silveira-Nunes, G., D. F. Durso, Lrao Jr, E. H. M. Cunha, T. U. Maioli, A. T. Vieira, E. Speziali, R. Corrêa-Oliveira, O. A. Martins-Filho, A. Teixeira-Carvalho, C. Franceschi, S. Rampelli, S. Turroni, P. Brigidi, and A. M. C. Faria. 2020. 'Hypertension Is Associated With Intestinal Microbiota Dysbiosis and Inflammation in a Brazilian Population', *Frontiers in Pharmacology*, 11: 258.

Simeonov, S. B., N. P. Botushanov, E. B. Karahanian, M. B. Pavlova, H. K. Husianitis, and D. M. Troev. 2002. 'Effects of Aronia melanocarpa juice as part of the dietary regimen in patients with diabetes mellitus', *Folia Medica*, 44: 20-3.

Singh, M., M. Arseneault, T. Sanderson, V. Murthy, and C. Ramassamy. 2008. 'Challenges for research on polyphenols from foods in Alzheimer's disease: bioavailability, metabolism, and cellular and molecular mechanisms', *Journal of Agricultural and Food Chemistry*, 56: 4855-73.

Sitia, S., L. Tomasoni, F. Atzeni, G. Ambrosio, C. Cordiano, A. Catapano, S. Tramontana, F. Perticone, P. Naccarato, P. Camici, E. Picano, L. Cortigiani, M. Bevilacqua, L. Milazzo, D. Cusi, C. Barlassina, P. Sarzi-Puttini, and M. Turiel. 2010. 'From endothelial dysfunction to atherosclerosis', *Autoimmunity Reviews*, 9: 830-4.

Skoczyńska, A., I. Jedrychowska, R. Poręba, A. Affelska-Jercha, B. Turczyn, A. Wojakowska, and R. Andrzejak. 2007. 'Influence of chokeberry juice on arterial blood pressure and lipid parameters in men with mild hypercholesterolemia', *Pharmacological Reports*, 59: 177-82.

Skrede, G., B. K. Martinsen, A-B. Wold, S-E. Birkeland, and K. Aaby. 2012. 'Variation in quality parameters between and within 14 Nordic tree fruit and berry species', *Acta Agriculturae Scandinavica, Section B — Soil & Plant Science*, 62: 193-208.

Skupień, K., I. Ochmian, and J. Grajkowski. 2008. "Influence of mineral fertilization on selected physical features and chemical composition of aronia fruit." In. Skupień K, Ochmian I, Grajkowski J. 2008. 'Influence of mineral fertilization on selected physical features and chemical composition of aronia fruit', *Acta Agrophysica*, 11: 213-226.

Smulyan, H., S. J. Marchais, B. Pannier, A. P. Guerin, M. E. Safar, and G. M. London. 1998. 'Influence of Body Height on Pulsatile Arterial Hemodynamic Data', *Journal of the American College of Cardiology*, 31: 1103-09.

Soltani, S., A. Jayedi, S. Shab-Bidar, N. Becerra-Tomás, and J. Salas-Salvadó. 2019. 'Adherence to the Mediterranean Diet in Relation to All-Cause Mortality: A Systematic Review and Dose-Response Meta-Analysis of Prospective Cohort Studies', *Advances in Nutrition*, 10: 1029-39.

Spencer, J. P., G. Chowrimootoo, R. Choudhury, E. S. Debnam, S. K. Srai, and C. Rice-Evans. 1999. 'The small intestine can both absorb and glucuronidate luminal flavonoids', *FEBS Letters*, 458: 224-30.

Stalmach, A., W. Mullen, D. Barron, K. Uchida, T. Yokota, C. Cavin, H. Steiling, G. Williamson, and A. Crozier. 2009. 'Metabolite profiling of hydroxycinnamate derivatives in plasma and urine after the ingestion of coffee by humans: identification of biomarkers of coffee consumption', *Drug Metabolism and Disposition*, 37: 1749-58.

Stalmach, A., W. Mullen, M. Pecorari, M. Serafini, and A. Crozier. 2009. 'Bioavailability of C-linked dihydrochalcone and flavanone glucosides in humans following ingestion of unfermented and fermented rooibos teas', *Journal of Agricultural and Food Chemistry*, 57: 7104-11.

Stalmach, A., H. Steiling, G. Williamson, and A. Crozier. 2010. 'Bioavailability of chlorogenic acids following acute ingestion of coffee by humans with an ileostomy', *Archives of Biochemistry and Biophysics*, 501: 98-105.

Stalmach, A., C. A. Edwards, J. D Wightman, and A. Crozier. 2013. 'Colonic catabolism of dietary phenolic and polyphenolic compounds from Concord grape juice', *Food and Function*, 4: 52-62.

Stea, T. H., O. Nordheim, E. Bere, P. Stornes, and T. A. Eikemo. 2020. 'Fruit and vegetable consumption in Europe according to gender, educational attainment and regional affiliation-A cross-sectional study in 21 European countries', *PLoS One*, 15: e0232521.

Steffen, Y., T. Schewe, and H. Sies. 2007. '(-)-Epicatechin elevates nitric oxide in endothelial cells via inhibition of NADPH oxidase', *Biochemical and Biophysical Research Communications*, 359: 828-33.

Steffensen, L. B., M. B. Mortensen, M. Kjolby, M. K. Hagensen, C. Oxvig, and J. F. Bentzon. 2015. 'Disturbed Laminar Blood Flow Vastly Augments Lipoprotein Retention in the Artery Wall: A Key Mechanism Distinguishing Susceptible From Resistant Sites', *Arteriosclerosis, Thrombosis, and Vascular Biology*, 35: 1928-35.

Stevens, S. L., S. Wood, C. Koshiaris, K. Law, P. Glasziou, R. J. Stevens, and R. J. McManus. 2016. 'Blood pressure variability and cardiovascular disease: systematic review and meta-analysis', *The BMJ*, 354: i4098.

Stocker, R., and J. F. Keaney, Jr. 2004. 'Role of oxidative modifications in atherosclerosis', *Physiological Reviews*, 84: 1381-478.

Stoupi, S., G. Williamson, J. W. Drynan, D. Barron, and M. N. Clifford. 2010. 'A comparison of the in vitro biotransformation of (–)-epicatechin and procyanidin B2 by human faecal microbiota', *Molecular Nutrition & Food Research*, 54: 747-59.

Strandwitz, P.. 2018. 'Neurotransmitter modulation by the gut microbiota', *Brain Research*, 1693: 128-33.

Strong, J. P., G. T. Malcom, C. A. McMahan, R. E. Tracy, W. P. Newman, 3rd, E. E. Herderick, and J. F. Cornhill. 1999. 'Prevalence and extent of atherosclerosis in adolescents and young adults: implications for prevention from the Pathobiological Determinants of Atherosclerosis in Youth Study', *The Journal of the American Medical Association,* 281: 727-35.

Struijs, K., J. P. Vincken, and H. Gruppen. 2009. 'Bacterial conversion of secoisolariciresinol and anhydrosecoisolariciresinol', *Journal of Applied Microbiology*, 107: 308-17.

Subbotina, E., A. Sierra, Z. Zhu, Z. Gao, S. R. Koganti, S. Reyes, E. Stepniak, S. A. Walsh, M. R. Acevedo, C. M. Perez-Terzic, D. M. Hodgson-Zingman, and L. V. Zingman. 2015. 'Musclin is an activity-stimulated myokine that enhances physical endurance', *Proceedings of the National Academy of Sciences of the United States of America*, 112: 16042-7.

Sugiura, T., Y. Dohi, H. Takase, S. Yamashita, S. Fujii, and N. Ohte. 2017. 'Oxidative Stress is Closely Associated with Increased Arterial Stiffness, Especially in Aged Male Smokers without Previous Cardiovascular Events: A Cross-Sectional Study', *Journal of Atherosclerosis and Thrombosis*, 24: 1186-98.

Sun, Z. 2015. 'Aging, arterial stiffness, and hypertension', *Hypertension*, 65: 252-56.

Supiano, M. A., L. Lovato, W. T. Ambrosius, J. Bates, S. Beddhu, P. Drawz, J. P. Dwyer, N. M. Hamburg, D. Kitzman, J. Lash, E. Lustigova, C. M. Miracle, S. Oparil, D. S. Raj, D. E. Weiner, A. Taylor, J. A. Vita, R. Yunis, G. M. Chertow, and M. Chonchol. 2018. 'Pulse wave velocity and central aortic

pressure in systolic blood pressure intervention trial participants', *PLoS One*, 13: e0203305.

Suzuki, A., N. Yamamoto, H. Jokura, M. Yamamoto, A. Fujii, I. Tokimitsu, and I. Saito. 2006. 'Chlorogenic acid attenuates hypertension and improves endothelial function in spontaneously hypertensive rats', *Journal of Hypertension*, 24: 1065-73.

Tabas, I. 2010. 'Macrophage death and defective inflammation resolution in atherosclerosis', *Nature Reviews Immunology*, 10: 36-46.

Taheri, R., B. A. Connolly, M. H. Brand, and B. W. Bolling. 2013. 'Underutilized Chokeberry (Aronia melanocarpa, Aronia arbutifolia, Aronia prunifolia) Accessions Are Rich Sources of Anthocyanins, Flavonoids, Hydroxycinnamic Acids, and Proanthocyanidins', *Journal of Agricultural and Food Chemistry*, 61: 8581-88.

Takachi, R., M. Inoue, J. Ishihara, N. Kurahashi, M. Iwasaki, S. Sasazuki, H. Iso, Y. Tsubono, and S. Tsugane. 2008. 'Fruit and vegetable intake and risk of total cancer and cardiovascular disease: Japan Public Health Center-Based Prospective Study', *American Journal of Epidemiology*, 167: 59-70.

Talavera, S., C. Felgines, O. Texier, C. Besson, J-L. Lamaison, and C. Rémésy. 2003. 'Anthocyanins are efficiently absorbed from the stomach in anesthetized rats', *The Journal of Nutrition*, 133: 4178-82.

Tamanai-Shacoori, Z., I. Smida, L. Bousarghin, O. Loreal, V. Meuric, S. B. Fong, M. Bonnaure-Mallet, and A. Jolivet-Gougeon. 2017. 'Roseburia spp.: a marker of health?', *Future Microbiology*, 12: 157-70.

Tao, W., Y. Zhang, X. Shen, Y. Cao, J. Shi, X. Ye, and S. Chen. 2019. 'Rethinking the Mechanism of the Health Benefits of Proanthocyanidins: Absorption, Metabolism, and Interaction with Gut Microbiota', *Comprehensive Reviews in Food Science and Food Safety*, 18: 971-85.

Thijssen, D. H. J., R. M. Bruno, Accm van Mil, S. M. Holder, F. Faita, A. Greyling, P. L. Zock, S. Taddei, J. E. Deanfield, T. Luscher, D. J. Green, and L. Ghiadoni. 2019. 'Expert consensus and evidence-based recommendations for the assessment of flow-mediated dilation in humans', *European Heart Journal*, 40: 2534-47.

Tierney, A. C., J. McMonagle, D. I. Shaw, H. L. Gulseth, O. Helal, W. H. Saris, J. A. Paniagua, I. Gołąbek-Leszczyñska, C. Defoort, C. M. Williams, B.

Karsltröm, B. Vessby, A. Dembinska-Kiec, J. López-Miranda, E. E. Blaak, C. A. Drevon, M. J. Gibney, J. A. Lovegrove, and H. M. Roche. 2011. 'Effects of dietary fat modification on insulin sensitivity and on other risk factors of the metabolic syndrome--LIPGENE: a European randomized dietary intervention study', *International Journal of Obesity*, 35: 800-9.

Tjelle, T. E., L. Holtung, S. K. Bohn, K. Aaby, M. Thoresen, S. A. Wiik, I. Paur, A. S. Karlsen, K. Retterstol, P. O. Iversen, and R. Blomhoff. 2015. 'Polyphenol-rich juices reduce blood pressure measures in a randomised controlled trial in high normal and hypertensive volunteers', *British Journal of Nutrition*, 114: 1054-63.

Tomás-Barberán, F. A., R. Garcia-Villalba, A. Gonzalez-Sarrias, M. V. Selma, and J. C. Espin. 2014. 'Ellagic acid metabolism by human gut microbiota: consistent observation of three urolithin phenotypes in intervention trials, independent of food source, age, and health status', *Journal of Agricultural and Food Chemistry*, 62: 6535-8.

Tomás-Barberán, F. A., M. V. Selma, and J. C. Espin. 2016. 'Interactions of gut microbiota with dietary polyphenols and consequences to human health', *Current Opinion in Clinical Nutrition & Metabolic Care*, 19: 471-76.

Tomás-Barberán, F. A., and M. N. Clifford. 2000. 'Flavanones, chalcones and dihydrochalcones – nature, occurrence and dietary burden', *Journal of the Science of Food and Agriculture*, 80: 1073-80.

Torres, N., M. Guevara-Cruz, L. A. Velázquez-Villegas, and A. R. Tovar. 2015. 'Nutrition and Atherosclerosis', *Archives of Medical Research*, 46: 408-26.

Tsao, C. W., K. M. Pencina, J. M. Massaro, E. J. Benjamin, D. Levy, R. S. Vasan, U. Hoffmann, C. J. O'Donnell, and G. F. Mitchell. 2014. 'Cross-sectional relations of arterial stiffness, pressure pulsatility, wave reflection, and arterial calcification', *Arteriosclerosis, Thrombosis, and Vascular Biology*, 34: 2495-500.

Tzounis, X., A. Rodriguez-Mateos, J. Vulevic, G. R. Gibson, C. Kwik-Uribe, and J. P. E. Spencer. 2011. 'Prebiotic evaluation of cocoa-derived flavanols in healthy humans by using a randomized, controlled, double-blind, crossover intervention study', *The American Journal of Clinical Nutrition*, 93: 62-72.

U.S. Department of Health, Human Services, U.S. Department of Agriculture. 2015 – 2020. 'Dietary Guidelines for Americans', 8th Edition.

USDA. 'U.S. National Plant Germplasm System Germplasm Resources Information Network (GRIN-Taxonomy),' Accessed 29/10/2020. https://npgsweb.ars-grin.gov/gringlobal/taxon/taxonomydetail?id=51.

USDA. 2014. 'USDA database for the flavonoid content of selected foods release', USDA, Beltsville, Release 3.1.

Vaiserman, A. M., A. K. Koliada, and F. Marotta. 2017. 'Gut microbiota: A player in aging and a target for anti-aging intervention', *Ageing Research Reviews*, 35: 36-45.

Vaiserman, A., M. Romanenko, L. Piven, V. Moseiko, O. Lushchak, N. Kryzhanovska, V. Guryanov, and A. Koliada. 2020. 'Differences in the gut Firmicutes to Bacteroidetes ratio across age groups in healthy Ukrainian population', *BMC Microbiology*, 20: 221.

Valdes, A. M, J. Walter, E. Segal, and T. D. Spector. 2018. 'Role of the gut microbiota in nutrition and health', *The BMJ*, 361: k2179.

Vallée, A., A. Gabet, V. Deschamps, J. Blacher, and V. Olié. 2019. 'Relationship between Nutrition and Alcohol Consumption with Blood Pressure: The ESTEBAN Survey', *Nutrients*, 11: 1433.

van der Heijden-Spek, J. J., J. A. Staessen, R. H. Fagard, A. P. Hoeks, H. A. Boudier, and L. M. van Bortel. 2000. 'Effect of age on brachial artery wall properties differs from the aorta and is gender dependent: a population study', *Hypertension*, 35: 637-42.

van Dorsten, F. A., C. H. Grun, E. J. van Velzen, D. M. Jacobs, R. Draijer, and J. P. van Duynhoven. 2010. 'The metabolic fate of red wine and grape juice polyphenols in humans assessed by metabolomics', *Molecular Nutrition & Food Research*, 54: 897-908.

van Popele, N. M., D. E. Grobbee, M. L. Bots, R. Asmar, J. Topouchian, R. S. Reneman, A. P. Hoeks, D. A. van der Kuip, A. Hofman, and J. C. Witteman. 2001. 'Association between arterial stiffness and atherosclerosis: the Rotterdam Study', *Stroke*, 32: 454-60.

van Zanden, J. J., H. van der Woude, J. Vaessen, M. Usta, H. M. Wortelboer, N. H. Cnubben, and I. M. Rietjens. 2007. 'The effect of quercetin phase II

metabolism on its MRP1 and MRP2 inhibiting potential', *Biochemical Pharmacology*, 74: 345-51.

Varela, C. E., E. Fromentin, M. Roller, F. Villarreal, and I. Ramirez-Sanchez. 2016. 'Effects of a natural extract of Aronia Melanocarpa berry on endothelial cell nitric oxide production', *Journal of Food Biochemistry*, 40: 404-10.

Vauzour, D., A. Rodriguez-Mateos, G. Corona, M. J. Oruna-Concha, and J. P. E. Spencer. 2010. 'Polyphenols and human health: prevention of disease and mechanisms of action', *Nutrients*, 2: 1106-31.

Vendrame, S., S. Guglielmetti, P. Riso, S. Arioli, D. Klimis-Zacas, and M. Porrini. 2011. 'Six-week consumption of a wild blueberry powder drink increases bifidobacteria in the human gut', *Journal of Agricultural and Food Chemistry*, 59: 12815-20.

Vendrame, S., and D. Klimis-Zacas. 2019. 'Potential Factors Influencing the Effects of Anthocyanins on Blood Pressure Regulation in Humans: A Review', *Nutrients*, 11: 1431.

Verhaar, B. J. H., D. Collard, A. Prodan, J. H. M. Levels, A. H. Zwinderman, F. Bäckhed, L. Vogt, M. J. L. Peters, M. Muller, M. Nieuwdorp, and B. H. van den Born. 2020. 'Associations between gut microbiota, faecal short-chain fatty acids, and blood pressure across ethnic groups: the HELIUS study', *European Heart Journal*, 41: 4259-67.

Verhaar, M. C., M. L. Honing, T. van Dam, M. Zwart, H. A. Koomans, J. J. Kastelein, and T. J. Rabelink. 1999. 'Nifedipine improves endothelial function in hypercholesterolemia, independently of an effect on blood pressure or plasma lipids', *Cardiovascular Research*, 42: 752-60.

Versari, D., E. Daghini, A. Virdis, L. Ghiadoni, and S. Taddei. 2009. 'Endothelium-dependent contractions and endothelial dysfunction in human hypertension', *British Journal of Pharmacology*, 157: 527-36.

Vetrani, C., A. A. Rivellese, G. Annuzzi, M. Adiels, J. Borén, I. Mattila, M. Orešič, and A. M. Aura. 2016. 'Metabolic transformations of dietary polyphenols: comparison between in vitro colonic and hepatic models and in vivo urinary metabolites', *Journal of Nutritional Biochemistry*, 33: 111-8.

Violi, F., V. Sanguigni, R. Carnevale, A. Plebani, P. Rossi, A. Finocchi, C. Pignata, D. De Mattia, B. Martire, M. C. Pietrogrande, S. Martino, E.

Gambineri, A. R. Soresina, P. Pignatelli, F. Martino, S. Basili, and L. Loffredo. 2009. 'Hereditary deficiency of gp91(phox) is associated with enhanced arterial dilatation: results of a multicenter study', *Circulation*, 120: 1616-22.

Virmani, R., F. D. Kolodgie, A. P. Burke, A. Farb, and S. M. Schwartz. 2000. 'Lessons from sudden coronary death: a comprehensive morphological classification scheme for atherosclerotic lesions', *Arteriosclerosis, Thrombosis, and Vascular Biology*, 20: 1262-75.

Vitaglione, P., G. Donnarumma, A. Napolitano, F. Galvano, A. Gallo, L. Scalfi, and V. Fogliano. 2007. 'Protocatechuic acid is the major human metabolite of cyanidin-glucosides', *The Journal of Nutrition*, 137: 2043-8.

Vitale, M., R. Giacco, M. Laiola, G. Della Pepa, D. Luongo, A. Mangione, D. Salamone, P. Vitaglione, D. Ercolini, and A. Albarosa Rivellese. 2021. 'Acute and chronic improvement in postprandial glucose metabolism by a diet resembling the traditional Mediterranean dietary pattern: Can SCFAs play a role?', *Clinical Nutrition*, 40: 428-37.

Vitrac, X., J-P. Monti, J. Vercauteren, G. Deffieux, and J-M. Mérillon. 2002. 'Direct liquid chromatographic analysis of resveratrol derivatives and flavanonols in wines with absorbance and fluorescence detection', *Analytica Chimica Acta,* 458: 103-10.

Vlachopoulos, C., K. Aznaouridis, and C. Stefanadis. 2010. 'Prediction of cardiovascular events and all-cause mortality with arterial stiffness: a systematic review and meta-analysis', *Journal of the American College of Cardiology*, 55: 1318-27.

Wadhera, R. K., D. L. Steen, I. Khan, R. P. Giugliano, and J. M. Foody. 2016. 'A review of low-density lipoprotein cholesterol, treatment strategies, and its impact on cardiovascular disease morbidity and mortality', *Journal of Clinical Lipidology*, 10: 472-89.

Wang, H. Y., H. Wang, J. H. Wang, Q. Wang, Q. F. Ma, and Y. Y. Chen. 2015. 'Protocatechuic Acid Inhibits Inflammatory Responses in LPS-Stimulated BV2 Microglia via NF-κB and MAPKs Signaling Pathways', *Neurochemical Research*, 40: 1655-60.

Wang K. L., H. M. Cheng, S. H. Sung, S. Y. Chuang, C. H. Li, H. A. Spurgeon, C. T. Ting, S. S. Najjar, E. G. Lakatta, F. C. Yin, P. Chou, and C. H Chen

. 2010. 'Wave Reflection and Arterial Stiffness in the Prediction of 15-Year All-Cause and Cardiovascular Mortalities', *Hypertension*, 55: 799-805.

Wang, N., J. Fulcher, N. Abeysuriya, L. Park, S. Kumar, G. L. Di Tanna, I. Wilcox, A. Keech, A. Rodgers, and S. Lal. 2020. 'Intensive LDL cholesterol-lowering treatment beyond current recommendations for the prevention of major vascular events: a systematic review and meta-analysis of randomised trials including 327 037 participants', *The Lancet Diabetes & Endocrinology*, 8: 36-49.

Wang Q., F. Li, B. Liang, Y. Liang, S. Chen, X. Mo, Y. Ju, H. Zhao, H. Jia, T. D. Spector, H. Xie, and R. Guo. 2018. 'A metagenome-wide association study of gut microbiota in asthma in UK adults', *BMC Microbiology*, 18: 114-14.

Wang, X., Y. Ouyang, J. Liu, M. Zhu, G. Zhao, W. Bao, and F. B. Hu. 2014. 'Fruit and vegetable consumption and mortality from all causes, cardiovascular disease, and cancer: systematic review and dose-response meta-analysis of prospective cohort studies', *The BJM*, 349: g4490.

Wangensteen, H., M. Bräunlich, V. Nikolic, K. Egil Malterud, R. Slimestad, and H. Barsett. 2014. 'Anthocyanins, proanthocyanidins and total phenolics in four cultivars of aronia: Antioxidant and enzyme inhibitory effects', *Journal of Functional Foods*, 7: 746-52.

Waters, J. L., and R. E. Ley. 2019. 'The human gut bacteria Christensenellaceae are widespread, heritable, and associated with health', *BMC Biology*, 17: 83.

Weber, C., and H. Noels. 2011. 'Atherosclerosis: current pathogenesis and therapeutic options', *Nature Medicine*, 17: 1410-22.

Weber, T., J. Auer, M. F. O'Rourke, E. Kvas, E. Lassnig, G. Lamm, N. Stark, M. Rammer, and B. Eber. 2005. 'Increased arterial wave reflections predict severe cardiovascular events in patients undergoing percutaneous coronary interventions', *European Heart Journal*, 26: 2657-63.

Weber, T., M. F. O'Rourke, E. Lassnig, M. Porodko, M. Ammer, M. Rammer, and B. Eber. 2010. 'Pulse waveform characteristics predict cardiovascular events and mortality in patients undergoing coronary angiography', *Journal of Hypertension*, 28: 797-805.

Wentzel, J. J., Y. S. Chatzizisis, F. J. H. Gijsen, G. D. Giannoglou, C. L. Feldman, and P. H. Stone. 2012. 'Endothelial shear stress in the evolution of

coronary atherosclerotic plaque and vascular remodelling: current understanding and remaining questions', *Cardiovascular Research*, 96: 234-43.

West, D. G., M. D. McIntyre, M. J. Piotrowski, N. Poupin, D. L. Miller, A. G. Preston, P. Wagner, L. F. Groves, and A. C. Skulas-Ray. 2014. 'Effects of dark chocolate and cocoa consumption on endothelial function and arterial stiffness in overweight adults', *The British Journal of Nutrition*, 111: 653-61.

Whelton, P. K., J. He, L. J. Appel, J. A. Cutler, S. Havas, T. A. Kotchen, E. J. Roccella, R. Stout, C. Vallbona, M. C. Winston, and J. Karimbakas. 2002. 'Primary prevention of hypertension: clinical and public health advisory from The National High Blood Pressure Education Program', *JAMA: The Journal of the American Medical Association*, 288: 1882-8.

Wiczkowski, W., E. Romaszko, and M. K. Piskula. 2010. 'Bioavailability of cyanidin glycosides from natural chokeberry (Aronia melanocarpa) juice with dietary-relevant dose of anthocyanins in humans', *Journal of Agricultural and Food Chemistry*, 58: 12130-36.

Wierzbicki, A. S. 2008. 'Surrogate markers, atherosclerosis and cardiovascular disease prevention', *International Journal of Clinical Practice*, 62: 981-7.

Wilkes, K., L. R. Howard, C. Brownmiller, and R. L. Prior. 2014. 'Changes in Chokeberry (Aronia melanocarpa L.) Polyphenols during Juice Processing and Storage', *Journal of Agricultural and Food Chemistry*, 62: 4018-25.

Wilkinson, I. B., H. MacCallum, L. Flint, J. R. Cockcroft, D. E. Newby, and D. J. Webb. 2000. 'The influence of heart rate on augmentation index and central arterial pressure in humans', *The Journal of Physiology*, 525 Pt 1: 263-70.

Wilkinson, I. B., S. S. Franklin, and J. R. Cockcroft. 2004. 'Nitric oxide and the regulation of large artery stiffness', *Hypertension*, 44: 112-16.

Willett, W. C., F. Sacks, A. Trichopoulou, G. Drescher, A. Ferro-Luzzi, E. Helsing, and D. Trichopoulos. 1995. 'Mediterranean diet pyramid: a cultural model for healthy eating', *The American Journal of Clinical Nutrition,* 61: 1402s-06s.

Williams, K. J., and I. Tabas. 1995. 'The response-to-retention hypothesis of early atherogenesis', *Arteriosclerosis, Thrombosis, and Vascular Biology*, 15: 551-61.

Williamson, G., and M. N. Clifford. 2010. 'Colonic metabolites of berry polyphenols: the missing link to biological activity?', *Br J Nutr*, 104 Suppl 3: S48-66.

Willum-Hansen, T., J. A. Staessen, C. Torp-Pedersen, S. Rasmussen, L. Thijs, H. Ibsen, and J. Jeppesen. 2006. 'Prognostic value of aortic pulse wave velocity as index of arterial stiffness in the general population', *Circulation*, 113: 664-70.

Wilson, P. W., R. B. D'Agostino, D. Levy, A. M. Belanger, H. Silbershatz, and W. B. Kannel. 1998. 'Prediction of coronary heart disease using risk factor categories', *Circulation*, 97: 1837-47.

Witteman, J. C., D. E. Grobbee, H. A. Valkenburg, A. M. van Hemert, T. Stijnen, H. Burger, and A. Hofman. 1994. 'J-shaped relation between change in diastolic blood pressure and progression of aortic atherosclerosis', *The Lancet*, 343: 504-7.

Wolinsky, H., and S. Glagov. 1967. 'A Lamellar Unit of Aortic Medial Structure and Function in Mammals', *Circulation Research*, 20: 99-111.

Wood, A. M., S. Kaptoge, A. S. Butterworth, P. Willeit, S. Warnakula, T. Bolton, E. Paige, D. S. Paul, M. Sweeting, S. Burgess, S. Bell, W. Astle, D. Stevens, A. Koulman, R. M. Selmer, W. M. M. Verschuren, S. Sato, I. Njølstad, M. Woodward, V. Salomaa, B. G. Nordestgaard, B. B. Yeap, A. Fletcher, O. Melander, L. H. Kuller, B. Balkau, M. Marmot, W. Koenig, E. Casiglia, C. Cooper, V. Arndt, O. H. Franco, P. Wennberg, J. Gallacher, A. G. de la Cámara, H. Völzke, C. C. Dahm, C. E. Dale, M. M. Bergmann, C. J. Crespo, Y. T. van der Schouw, R. Kaaks, L. A. Simons, P. Lagiou, J. D. Schoufour, J. M. A. Boer, T. J. Key, B. Rodriguez, C. Moreno-Iribas, K. W. Davidson, J. O. Taylor, C. Sacerdote, R. B. Wallace, J. R. Quiros, R. Tumino, D. G. Blazer, A. Linneberg, M. Daimon, S. Panico, B. Howard, G. Skeie, T. Strandberg, E. Weiderpass, P. J. Nietert, B. M. Psaty, D. Kromhout, E. Salamanca-Fernandez, S. Kiechl, H. M. Krumholz, S. Grioni, D. Palli, J. M. Huerta, J. Price, J. Sundström, L. Arriola, H. Arima, R. C. Travis, D. B. Panagiotakos, A. Karakatsani, A. Trichopoulou, T. Kühn, D.

E. Grobbee, E. Barrett-Connor, N. van Schoor, H. Boeing, K. Overvad, J. Kauhanen, N. Wareham, C. Langenberg, N. Forouhi, M. Wennberg, J. P. Després, M. Cushman, J. A. Cooper, C. J. Rodriguez, M. Sakurai, J. E. Shaw, M. Knuiman, T. Voortman, C. Meisinger, A. Tjønneland, H. Brenner, L. Palmieri, J. Dallongeville, E. J. Brunner, G. Assmann, M. Trevisan, R. F. Gillum, I. Ford, N. Sattar, M. Lazo, S. G. Thompson, P. Ferrari, D. A. Leon, G. D. Smith, R. Peto, R. Jackson, E. Banks, E. Di Angelantonio, and J. Danesh. 2018. 'Risk thresholds for alcohol consumption: combined analysis of individual-participant data for 599 912 current drinkers in 83 prospective studies', *The Lancet*, 391: 1513-23.

Wood, E., S. Hein, C. Heiss, C. Williams, and A. Rodriguez-Mateos. 2019. 'Blueberries and cardiovascular disease prevention', *Food and Function*, 10: 7621-33.

World Cancer Research Fund. 2018. 'Diet, Nutrition, Physical Activity and Cancer: a Global Perspective-Continuous Update Project Expert Report'. Accessed 10/03/2021, https://www.wcrf.org/diet-and-cancer/.

World Health Organisation. 2017. 'The top 10 causes of death worldwide': Accessed 5/11/2018, http://www.who.int/mediacentre/factsheets/fs3.

World Health Organization. 1990. "Diet, Nutrition, and the Prevention of Chronic Diseases. Report of a WHO Study Group." In *WHO Technical Report Series*.

World Health Organization. 2004. *Vitamin and mineral requirements in human nutrition* (World Health Organization). Accessed 19/02/2021. https://apps.who.int/iris/handle/10665/42716.

Wright, J. T., Jr., J. D. Williamson, P. K. Whelton, J. K. Snyder, K. M. Sink, M. V. Rocco, D. M. Reboussin, M. Rahman, S. Oparil, C. E. Lewis, P. L. Kimmel, K. C. Johnson, D. C. Goff, Jr., L. J. Fine, J. A. Cutler, W. C. Cushman, A. K. Cheung, and W. T. Ambrosius. 2015. 'A Randomized Trial of Intensive versus Standard Blood-Pressure Control', *The New England Journal of Medicine*, 373: 2103-16.

Wu, S., R. Hu, H. Nakano, K. Chen, M. Liu, X. He, H. Zhang, J. He, and D. X. Hou. 2018. 'Modulation of Gut Microbiota by Lonicera caerulea L. Berry Polyphenols in a Mouse Model of Fatty Liver Induced by High Fat Diet', *Molecules*, 23: 3213.

Wu, T., C. Grootaert, J. Pitart, N. K. Vidovic, S. Kamiloglu, S. Possemiers, M. Glibetic, G. Smagghe, K. Raes, T. Van de Wiele, and J. Van Camp. 2018. 'Aronia (Aronia melanocarpa) Polyphenols Modulate the Microbial Community in a Simulator of the Human Intestinal Microbial Ecosystem (SHIME) and Decrease Secretion of Proinflammatory Markers in a Caco-2/endothelial Cell Coculture Model', *Molecular Nutrition & Food Research*, 62: e1800607.

Wu, X., T. T. Y. Wang, R. L. Prior, and P. R. Pehrsson. 2018. 'Prevention of Atherosclerosis by Berries: The Case of Blueberries', *Journal of Agricultural and Food Chemistry*, 66: 9172-88.

Wu, X., G. Cao, and R. L. Prior. 2002. 'Absorption and Metabolism of Anthocyanins in Elderly Women after Consumption of Elderberry or Blueberry', *The Journal of Nutrition*, 132: 1865-71.

Wu, X., L. Gu, R. L. Prior, and S. McKay. 2004. 'Characterization of Anthocyanins and Proanthocyanidins in Some Cultivars of Ribes, Aronia, and Sambucus and Their Antioxidant Capacity', *Journal of Agricultural and Food Chemistry*, 52: 7846-56.

Xie, L., T. Vance, B. Kim, S. G. Lee, C. Caceres, Y. Wang, P. A. Hubert, J. Y. Lee, O. K. Chun, and B. W. Bolling. 2017. 'Aronia berry polyphenol consumption reduces plasma total and low-density lipoprotein cholesterol in former smokers without lowering biomarkers of inflammation and oxidative stress: a randomized controlled trial', *Nutrition Research*, 37: 67-77.

Xie, L., S. G. Lee, T. M. Vance, Y. Wang, B. Kim, J-Y.Lee, O. K. Chun, and B. W. Bolling. 2016. 'Bioavailability of anthocyanins and colonic polyphenol metabolites following consumption of aronia berry extract', *Food Chemistry*, 211: 860-68.

Xu, Y., R. C. Arora, B. M. Hiebert, B. Lerner, A. Szwajcer, K. McDonald, C. Rigatto, P. Komenda, M. M. Sood, and N. Tangri. 2014. 'Non-invasive endothelial function testing and the risk of adverse outcomes: a systematic review and meta-analysis', *European Heart Journal: Cardiovascular Imaging*, 15: 736-46.

Yahfoufi, N., N. Alsadi, M. Jambi, and C. Matar. 2018. 'The Immunomodulatory and Anti-Inflammatory Role of Polyphenols', *Nutrients*, 10: 1618.

Yahya, H. M., A. Day, C. Lawton, K. Myrissa, F. Croden, L. Dye, and G. Williamson. 2016. 'Dietary intake of 20 polyphenol subclasses in a cohort of UK women', *European Journal of Nutrition*, 55: 1839-47.

Yan, L., S. Haiting, H. Yufen, Y. Anqi, W. Ping, H. Jiao, Q. Dingding, Z. Lixia, T. Mei, W. Chen, J. Fangfang, Z. Jinyang, S. Silong, R. L. Augusto, X. Xuefeng, S. Xin, and X. Lize. 2021. 'Gut metagenomics discriminates unique microbial signatures in diverse symptomatic profiles with attention-deficit/hyperactivity disorder', *Research Square, DOI: 10.21203/rs.3.rs-28862/v1*.

Yan, Q., Y. Gu, X. Li, W. Yang, L. Jia, C. Chen, X. Han, Y. Huang, L. Zhao, P. Li, Z. Fang, J. Zhou, X. Guan, Y. Ding, S. Wang, M. Khan, Y. Xin, S. Li, and Y. Ma. 2017. 'Alterations of the Gut Microbiome in Hypertension', *Frontiers in Cellular and Infection Microbiology*, 7: 381.

Yan, X., J. Jin, X. Su, X. Yin, J. Gao, X. Wang, S. Zhang, P. Bu, M. Wang, Y. Zhang, Z. Wang, and Q. Zhang. 2020. 'Intestinal Flora Modulates Blood Pressure by Regulating the Synthesis of Intestinal-Derived Corticosterone in High Salt-Induced Hypertension', *Circulation Research*, 126: 839-53.

Yang, L., W. Ling, Z. Du, Y. Chen, D. Li, S. Deng, Z. Liu, and L. Yang. 2017. 'Effects of Anthocyanins on Cardiometabolic Health: A Systematic Review and Meta-Analysis of Randomized Controlled Trials', *Advances in Nutrition*, 8: 684-93.

Yang, T., M. M. Santisteban, V. Rodriguez, E. Li, N. Ahmari, J. M. Carvajal, M. Zadeh, M. Gong, Y. Qi, J. Zubcevic, B. Sahay, C. J. Pepine, M. K. Raizada, and M. Mohamadzadeh. 2015. 'Gut dysbiosis is linked to hypertension', *Hypertension*, 65: 1331-40.

Yang, W. Y., J. D. Melgarejo, L. Thijs, Z. Y. Zhang, J. Boggia, F. F. Wei, T. W. Hansen, K. Asayama, T. Ohkubo, J. Jeppesen, E. Dolan, K. Stolarz-Skrzypek, S. Malyutina, E. Casiglia, L. Lind, J. Filipovský, G. E. Maestre, Y. Li, J. G. Wang, Y. Imai, K. Kawecka-Jaszcz, E. Sandoya, K. Narkiewicz, E. O'Brien, P. Verhamme, and J. A. Staessen. 2019. 'Association of Office and Ambulatory Blood Pressure With Mortality and Cardiovascular Outcomes', T*he Journal of the American Medical Association*, 322: 409-20.

Yang, Y., J. Z. Xu, Y. Wang, X. F. Tang, and P. J. Gao. 2014. 'Brachial flow-mediated dilation predicts subclinical target organ damage progression in essential hypertensive patients: a 3-year follow-up study', *Journal of Hypertension*, 32: 2393-400.

Yatsunenko, T., F. E. Rey, M. J. Manary, I. Trehan, M. G. Dominguez-Bello, M. Contreras, M. Magris, G. Hidalgo, R. N. Baldassano, A. P. Anokhin, A. C. Heath, B. Warner, J. Reeder, J. Kuczynski, J. G. Caporaso, C. A. Lozupone, C. Lauber, J. C. Clemente, D. Knights, R. Knight, and J. I. Gordon. 2012. 'Human gut microbiome viewed across age and geography', *Nature*, 486: 222-7.

Yeboah, J., J. R. Crouse, F. C. Hsu, G. L. Burke, and D. M. Herrington. 2007. 'Brachial flow-mediated dilation predicts incident cardiovascular events in older adults: the Cardiovascular Health Study', *Circulation*, 115: 2390-7.

Yoshida, N., T. Emoto, T. Yamashita, H. Watanabe, T. Hayashi, T. Tabata, N. Hoshi, N. Hatano, G. Ozawa, N. Sasaki, T. Mizoguchi, H. Z. Amin, Y. Hirota, W. Ogawa, T. Yamada, and K. I. Hirata. 2018. 'Bacteroides vulgatus and Bacteroides dorei Reduce Gut Microbial Lipopolysaccharide Production and Inhibit Atherosclerosis', *Circulation*, 138: 2486-98.

Yu, D., X. Zhang, Y. T. Gao, H. Li, G. Yang, J. Huang, W. Zheng, Y. B. Xiang, and X. O. Shu. 2014. 'Fruit and vegetable intake and risk of CHD: results from prospective cohort studies of Chinese adults in Shanghai', *British Journal of Nutrition*, 111: 353-62.

Yun, Y., H. N. Kim, S. E. Kim, S. G. Heo, Y. Chang, S. Ryu, H. Shin, and H. L. Kim. 2017. 'Comparative analysis of gut microbiota associated with body mass index in a large Korean cohort', *BMC Microbiology*, 17: 151.

Zamora-Ros, R., D. Achaintre, J. A. Rothwell, S. Rinaldi, N. Assi, P. Ferrari, M. Leitzmann, M. C. Boutron-Ruault, G. Fagherazzi, A. Auffret, T. Kühn, V. Katzke, H. Boeing, A. Trichopoulou, A. Naska, E. Vasilopoulou, D. Palli, S. Grioni, A. Mattiello, R. Tumino, F. Ricceri, N. Slimani, I. Romieu, and A. Scalbert. 2016. 'Urinary excretions of 34 dietary polyphenols and their associations with lifestyle factors in the EPIC cohort study', *Scientific Reports*, 6: 26905.

Zamora-Ros, R., V. Knaze, J. A. Rothwell, B. Hémon, A. Moskal, K. Overvad, A. Tjønneland, C. Kyrø, G. Fagherazzi, M-C. Boutron-Ruault, M. Touillaud,

V. Katzke, T. Kühn, H. Boeing, J. Förster, A. Trichopoulou, E. Valanou, E. Peppa, D. Palli, C. Agnoli, F. Ricceri, R. Tumino, M. Santucci de Magistris, P. H. M. Peeters, H. B. Bueno-de-Mesquita, D. Engeset, G. Skeie, A. Hjartåker, V. Menéndez, A. Agudo, E. Molina-Montes, J. M. Huerta, A. Barricarte, P. Amiano, E. Sonestedt, L. Maria Nilsson, R. Landberg, T. J. Key, K-T. Khaw, N. J. Wareham, Y. Lu, N. Slimani, I. Romieu, E. Riboli, and A. Scalbert. 2016. 'Dietary polyphenol intake in Europe: the European Prospective Investigation into Cancer and Nutrition (EPIC) study', *European Journal of Nutrition*, 55: 1359-75.

Zamora-Ros, R., M. Touillaud, J. A. Rothwell, I. Romieu, and A. Scalbert. 2014. 'Measuring exposure to the polyphenol metabolome in observational epidemiologic studies: current tools and applications and their limits', *The American Journal of Clinical Nutrition*, 100: 11-26.

Zanotti, I., M. Dall'Asta, P. Mena, L. Mele, R. Bruni, S. Ray, and D. Del Rio. 2015. 'Atheroprotective effects of (poly)phenols: a focus on cell cholesterol metabolism', *Food and Function*, 6: 13-31.

Zapolska-Downar, D., D. Bryk, M. Małecki, K. Hajdukiewicz, and D. Sitkiewicz. 2012. 'Aronia melanocarpa fruit extract exhibits anti-inflammatory activity in human aortic endothelial cells', *European Journal of Nutrition*, 51: 563-72.

Zduńczyk, Z., J. Juśkiewicz, and I. Estrella. 2006. 'Cecal parameters of rats fed diets containing grapefruit polyphenols and inulin as single supplements or in a combination', *Nutrition*, 22: 898-904.

Zhan, J., Y. J. Liu, L. B. Cai, F. R. Xu, T. Xie, and Q. Q. He. 2017. 'Fruit and vegetable consumption and risk of cardiovascular disease: A meta-analysis of prospective cohort studies', *Critical Reviews in Food Science and Nutrition*, 57: 1650-63.

Zhang, T., X. Liang, L. Shi, L. Wang, J. Chen, C. Kang, J. Zhu, and M. Mi. 2013. 'Estrogen receptor and PI3K/Akt signaling pathway involvement in S-(-)equol-induced activation of Nrf2/ARE in endothelial cells', *PLoS One*, 8: e79075.

Zhao, C. N., X. Meng, Y. Li, S. Li, Q. Liu, G. Y. Tang, and H. B. Li. 2017. 'Fruits for Prevention and Treatment of Cardiovascular Diseases', *Nutrients*, 9: 598.

Zheng, A., H. Yi, F. Li, L. Han, J. Yu, X. Cheng, H. Su, K. Hong, and J. Li. 2019. 'Changes in Gut Microbiome Structure and Function of Rats with Isoproterenol-Induced Heart Failure', *International Heart Journal*, 60: 1176-83.

Zheng, J., Y. Zhou, S. Li, P. Zhang, T. Zhou, D-P. Xu, and H-B. Li. 2017. 'Effects and Mechanisms of Fruit and Vegetable Juices on Cardiovascular Diseases', *International Journal of Molecular Sciences*, 18: 555.

Zheng, W., and S. Y. Wang. 2003. 'Oxygen Radical Absorbing Capacity of Phenolics in Blueberries, Cranberries, Chokeberries, and Lingonberries', *Journal of Agricultural and Food Chemistry*, 51: 502-09.

Zhong, Q., M. J. Hu, Y. J. Cui, L. Liang, M. M. Zhou, Y. W. Yang, and F. Huang. 2018. 'Carotid-Femoral Pulse Wave Velocity in the Prediction of Cardiovascular Events and Mortality: An Updated Systematic Review and Meta-Analysis', *Angiology*, 69: 617-29.

Zhu, Y., J. Y. Zhang, Y. L. Wei, J. Y. Hao, Y. Q. Lei, W. B. Zhao, Y. H. Xiao, and A. D. Sun. 2020. 'The polyphenol-rich extract from chokeberry (Aronia melanocarpa L.) modulates gut microbiota and improves lipid metabolism in diet-induced obese rats', A*pplied Physiology, Nutrition, and Metabolism*, 17: 54.

Zhu, Y., Y. Bo, X. Wang, W. Lu, X. Wang, Z. Han, and C. Qiu. 2016. 'The Effect of Anthocyanins on Blood Pressure: A PRISMA-Compliant Meta-Analysis of Randomized Clinical Trials.', *Medicine*, 95: e3380.

Ziauddeen, N., A. Rosi, D. Del Rio, B. Amoutzopoulos, S. Nicholson, P. Page, F. Scazzina, F. Brighenti, S. Ray, and P. Mena. 2019. 'Dietary intake of (poly)phenols in children and adults: cross-sectional analysis of UK National Diet and Nutrition Survey Rolling Programme (2008-2014)', *European Journal of Nutrition*, 58: 3183-98.

Zieman, S. J., V. Melenovsky, and D. A. Kass. 2005. 'Mechanisms, pathophysiology, and therapy of arterial stiffness', *Arteriosclerosis, Thrombosis, and Vascular Biology*, 25: 932-43.

Zureik, M., J. M. Bureau, M. Temmar, C. Adamopoulos , D. Courbon, K. Bean, P. J. Touboul, A. Benetos, and P. Ducimetière. 2003. 'Echogenic carotid plaques are associated with aortic arterial stiffness in subjects with subclinical carotid atherosclerosis', *Hypertension*, 41: 519-27.

La vie est courte,

La Science est longue,

L'occasion fugitive,

L'expérience trompeuse,

Le jugement difficile.

Hippocrate