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Investigation into the effect of almond consumption on ectopic fat, endothelial function and other cardiometabolic disease risk factors
an observational study and a randomised controlled trial in UK adults

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Investigation into the effect of almond consumption on ectopic fat, endothelial function and other cardiometabolic disease risk factors: an observational study and a randomised controlled trial in UK adults

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Supervised by: Dr Wendy Hall and Dr Sarah Berry

A thesis submitted in partial fulfilment of the requirements for the degree of
DOCTOR OF PHILOSOPHY
(Nutritional Sciences Research)
August 2020

Departments of Nutritional Sciences
School of Life Course Sciences
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King's College London

To my family who has been giving me trust and courage incessantly to pursue my education and career abroad despite their wistful longing for being physically together as a family.

To my dad I admire so much for his hard work, dedication and persistence in life.

To my loving mom who is full of wisdom and always there for all of us.

To my sister who is the sunshine of this family.

Publications

Original research

Dikariyanto V, Berry SE, Pot GK, Francis L, Smith L, Hall WL. (2020) Tree nut snack consumption is associated with better diet quality and CVD risk in the UK adult population: National Diet and Nutrition Survey (NDNS) 2008-2014. *Public Health Nutr*, 1-10.

Dikariyanto V, Berry SE, Francis L, Smith L, Hall WL (2020) Whole almond consumption is associated with better diet quality and cardiovascular disease risk factors in the UK adult population: National Diet and Nutrition Survey (NDNS) 2008-2017. *Eur J Nutr*.

Dikariyanto V, Smith L, Francis L, Robertson M, Kusaslan E, O'Callaghan-Latham M, Palanche C, D'Annibale M, Christodoulou D, Basty N, Whitcher B, Shuaib H, Charles-Edwards G, Chowienczyk PJ, Ellis PR, Berry SEE*, Hall WL* (2020) Snacking on whole almonds for 6 weeks improves endothelial function and lowers LDL cholesterol but does not affect liver fat and other cardiometabolic risk factors in healthy adults: the ATTIS study, a randomized controlled trial. *Am J Clin Nutr* 111, 1178-1189.

Dikariyanto V, Smith L, Chowienczyk PJ, Berry SEE*, Hall WL* (2020) Snacking on whole almonds for six aeeks increases heart rate variability during mental stress in healthy adults: a randomized controlled trial. *Nutrients* 12.

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Abstracts

UK Nutrition Society Summer meeting 2017, London, UK.

Dikariyanto V, Hall WL, Berry SEE, Pot GK (2017) Associations between tree nut consumption and diet quality in the UK adult population based on National Diet and Nutrition Survey (NDNS) rolling programme 2008-2014. *Proceedings of the Nutrition Society* 76(OCE4), E161.

Dikariyanto V, Berry SEE, Pot GK, Hall WL (2017) Associations between tree nut consumption and cardiovascular disease risk markers in the UK adult population based on National Diet and Nutrition Survey (NDNS) rolling programme 2008-2014. *Proceedings of the Nutrition Society* 76(OCE4), E199.

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Smith L, **Dikariyanto V**, Francis L, Rokib M, Hall WL, Berry SEE. Determining the average nutrient profile from snack composition in the United Kingdom for development of a control in dietary snack intervention studies (P15-029). *Curr Dev Nutr* 2(11): nzy042.

13th European Nutrition Conference, FENS 2019, Dublin, Ireland.

Dikariyanto V, Berry SEE, Smith L, Francis L, Robertson M, Kusaslan E, O'Callaghan-Latham M, Ellis PR, Chowienczyk PJ, Hall WL (2019) Almond snack consumption improves endothelial function in adults with moderate risk of cardiovascular disease: a randomised, controlled, parallel trial. *Federation of European Nutrition Societies.*

UK Nutrition Society Winter meeting 2019, London, UK

Dikariyanto V, Berry SEE, Smith L, Francis L, Robertson M, Kusaslan E, O'Callaghan-Latham M, Ellis PR, Hall WL. The effects of whole almond snack consumption on fasting blood lipids and insulin sensitivity: a randomised controlled trial in adults. *Proceedings of the Nutrition Society* 79(OCE1), E5.

ASN Nutrition 2020, Seattle, USA
(the abstracts were accepted but the conference was cancelled due to the COVID-19 outbreak)

Dikariyanto V, Smith L, Francis L, Robertson M, Kusaslan E, O'Callaghan-Latham M, Palanche C, D'Annibale M, Christodoulou D, Basty N, Whitcher B, Shuaib H, Charles-Edwards G, Chowienczyk PJ, Ellis PR, Berry SEE, Hall WL. Snacking on whole almonds for 6 weeks improves endothelial function and lowers LDL cholesterol but does not affect liver fat and other cardiometabolic risk factors in healthy adults: a randomized controlled trial.

Dikariyanto V, Berry SEE, Smith L, O'Callaghan-Latham M, Chowienczyk PJ, Hall WL. Snacking on whole almonds for 6 weeks increases heart rate variability during a mental stress test in healthy adults: a randomized controlled trial.

Abstract

Background: Suboptimal dietary patterns associated with many snack foods, typically high-fat, high-refined carbohydrate foods, that are low in fibre and nutrients, present an easily identifiable target to improve cardio-metabolic health. Meta-analyses of epidemiological and interventional studies have reported that almonds are associated with lower intermediary risk of CVD. However, tree nut and almond consumption levels are unknown in the UK population, and the impact of almond snack consumption on endothelial function and liver fat, as key drivers of CVD, remains unclear.
Aim: 1) to investigate the prevalence of tree nut/almond consumption and its association with diet quality and CVD risk in the UK adult population, and 2) to study the effects of snacking on tree nuts, specifically whole almonds, on CVD risk markers, including endothelial function and liver fat as main outcomes, and other metabolic risk factors, in displacement of typical snacks.
Methods: In cross-sectional analysis using the UK National Diet and Nutrition Survey (NDNS) data from adult respondents (≥19y), the average consumption of almonds was determined and its association with intermediary CVD risk markers, including body composition and fasting blood lipids using analysis of covariance (ANCOVA) adjusted for covariates was explored. Following a 2-week run-in period consuming control snacks, a 6-week parallel arm trial was undertaken where participants were randomized to isoenergetic treatments: 1) control snacks (mini-muffins) replicating the UK average snack nutrient profile; or 2) dry-roasted whole almonds, providing 20% estimated energy requirement. Endothelial function (via flow-mediated dilation (FMD)), liver fat (via MRI/^{1}H-MRS), heart rate variability (HRV) and other risk measures were assessed at baseline and endpoint. A total of 107 participants (75 F, 32 M; mean age 56.2 y, SD 10.4) were randomized and 105 completed the trial.
Results: The NDNS analysis revealed that median intake in tree nut snack (TNS) and whole almond consumers was 6.5 g/d (IQR 10.8) and 5.0 g/d (IQR 9.3), respectively. TNS and whole almond consumers had significantly better diet quality and lower BMI and WC compared to non-consumers. Lower SBP and DBP and higher HDL were further observed in TNS consumers. From the RCT, almonds significantly increased FMD (mean difference 4.1%, 95% CI 2.2, 5.9), the long-phase HRV index, night-time very-low frequency power (mean difference 337 ms^2, 95% CI 12, 661), the high-frequency power in response to acute mental stress (mean difference mean difference 124 ms^2; 95% CI 11, 237) and plasma oleic acid levels (mean difference 228 µmol, 95% CI 7, 449) relative to control adjusted for baseline BMI and baseline dependent outcome values, but there were no treatment differences in ABP and subcutaneous, visceral, liver, muscle and pancreatic fat. Plasma LDL cholesterol levels were significantly decreased by almonds (mean difference -0.25 mmol/L, 95% CI -0.45, -0.04), but no differences were found in other blood lipids, insulin sensitivity, nor faecal SCFA levels.
Conclusion: Tree nut and whole almond consumption was low in the UK adult population, but it was an indicator of a healthier diet and associated with lower CVD risk. Whole almond consumption for 6 weeks in displacement of typical snacks increased endothelial function and HRV, suggesting that they may be cardioprotective by increasing availability of nitric oxide and improving cardiac autonomic function. The importance of including tree nuts in an overall healthy diet should be emphasized to help reduce CVD risk.

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production and NADPH oxidase activity increasing ROS production (Kim et al., 2006). Oxidative stress can stimulate inflammation via pro-inflammatory signalling. Inflammation downregulates eNOS via direct phosphorylation (Partovian et al., 2005) and increase production of endothelin-1, vascular endothelial growth factor (VEGF) – a vascular permeability factor, transforming growth factor (TGF)-β and plasminogen activator inhibitor (PAI)-1 – an inhibitor of fibrinolysis leading to atherosclerosis (Brownlee, 2005), intercellular adhesion molecule (ICAM), vascular cell adhesion molecule (VCAM) and selectin cell adhesion molecule expressed only in endothelial cells (E-selectin). These adhesion molecules play a role in the recruitment of leukocytes to atherosclerotic lesions (Brownlee, 2005; Huo & Ley, 2001). Furthermore, shared and interconnected mechanisms of glucotoxicity, lipotoxicity, and inflammation causes interlink between IR and ED that contributes to relationships between cardiovascular and metabolic diseases. This illustration is adapted from Kim et al. (2006). 36
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Acknowledgments

Firstly, I would like to thank my supervisors, Dr Wendy Hall and Dr Sarah Berry. I am very honoured to have had the opportunity to complete my PhD under their supervision. Their guidance challenged me to develop my scientific analytical thinking and produce publications and a thesis that I am proud of. I also thank them for putting me forward to present at conferences and scientific meetings as well as encouraging me to set up collaborations. I have learnt so much from them, and I appreciate all the efforts, patience and time they have invested in the supervision for me.

I would also like to thank Dr Geoffrey Charles-Edwards, for teaching me some magnetic resonance imaging/proton-magnetic resonance spectroscopy (MRI/^{1}H-MRS) analyses, and Haris Shuaib. Their technical support and expertise in magnetic resonance (MR) have been instrumental to the success of the ATTIS study. I would like to thank Alessandra Bisquera and Harimurti Buntaran for statistical advice and time spared to discuss statistical analyses with me.

I would like to thank Prof Peter Ellis and my colleagues, Leanne Smith, Dr Mayra Vera-Aviles, Timothy Wingham, Dr Ana Margarida Pinto, Dr Monica Castro-Acosta, Dr Olah Hakim, Dr Toyosi Bello, Alex Cheok, Wafa Alotaibi, Lucy Francis and Manal Al-Mughamisi who have continuously inspired and encouraged me. I will never forget their support and the trusting ears they had lent during the lows and highs of this journey. Leanne Smith has been an amazing friend throughout the ATTIS journey; I thank her for teaching me so much, being so willing to help me out whenever I got stuck and enduring the ATTIS roller coaster ride. Thanks to the ATTIS study team of NHS nurses and staff, MSc/BSc/intern students (Rachel Lee, Momena Rokib, Laure Verstraeten, Katie Patrick,

Charlotte Constable-Fernandez, Camille Palanché, Eugenia Rodrigues, Jane Powell, Elisabeth Cresta, Caroline Day, May Robertson, Jenny Cheung, Eslem Kusaslan, Inès Joffet, Laetitia Flottes de Pouzols, Molly O'Callaghan-Latham, Tamara Kaloti, Emma Barry and Joyce Shaw), laboratory technicians (Rosie Calokatsia, Anne-Catherine Perz, Dave Lincoln, Mary-Jo Searle, Robert Gray and Rose-Marie Minaisah) and Maria D'Annibale as well as ATTIS study participants who have made this project possible, it was an absolute pleasure to be a part of this team. Thanks also to other Office 4.46 members, Dr Haya Al-Khatib, Dr Geoffrey Istas, Yi Fan Xu, Mélanie Le Sayec, Sabine Hein, Eleanor (Elly) Wood, Zi Cheng Zhang, Michelle Gibbs, Aseel Alkoblan, Paul Young Tie Yang and Emily Prpa for making the office days colourful. I am also grateful for the financial support provided by the Almond Board of California that funded the ATTIS study and Indonesia Endowment Fund for Education that funded my PhD tuition fees and expenses.

I would also very much like to thank my loving family: my mother – Evi Sumiati, my father – Dikariyanto, and my sister – Vivien Lianna Antania Hanjani, who have supported me emotionally throughout this journey. Their incessant love and support overwhelm me every day. Everything I have achieved in my life is because of my parents' hard work and sacrifices; I am nothing without them. It is my pleasure and honour to hold my father's name and make him proud.

I would like to express my deepest gratitude to Dr Mayra Vera-Aviles, Asako Fujisawa and Joanne Cuff. They are my wonderful London big sisters. I thank them very much for being best pals in sharing London life stories and life lessons. Also, I would like to thank my very good friends, Mohammad Dimas, Fitri Maharani Indra, Samuel Sutiono, Titis

Apdini, Amanda Larasati, Rosi Destiani, Kunlanun Jittavanichprapha and Dewi Budianti, who have been my cheerleaders in the background and encouraged me to keep going even during the most difficult times.

Last but not least, I would like to thank my mentors and friends for life whom I have known since the life chapter in Sweden, Dr Elin Östman, Dr Anne Nilsson, Dr Cristina Teixeira, Dr Jonna Sandberg, Dr Anestis Dougkas, Dr Yoghatama Zanzer, Dr Nittaya Marungruang, Dr Cibele Furlan and Dr Ângela Batista. They are nutrition scientists who partly inspired me to do PhD.

This last paragraph is a tribute to the moment of COVID-19 (Coronavirus) outbreak – the global pandemic of the decade started in March here in the UK which I have been through during the completion of my thesis write-up. The anxiety and uncertainty due to COVID-19 outbreak have caused an enormous turmoil. I have been very up and down in managing the situation. My targets had been distressingly pushed forward that were beyond anybody's control. I am grateful for the online videocall presence of my family, Asako, Mayra, Toyosi, Manal and Wafa. It has definitely made my days brighter. I also thank my primary supervisor, Dr Wendy Hall, for sharing information on financial aid scheme. Plus, during this pandemic, I got a little accident causing my active arm broken which even made the situation even worse. Mayra and her husband, Antonio David Ortega, had been such angels who kindly helped me go through the recovery. I thank them so much.

Statement of contribution

Cross-sectional analyses using National Diet and Nutrition Survey (NDNS) were designed with valuable inputs during the initial state from Dr Gerda Pot, a health and nutrition researcher at Louis Bolk Institute, the Netherlands. Data were collected and prepared by Vita Dikariyanto and research assistants – Lucy Francis and Leanne Smith contributed to data collection. Vita Dikariyanto conducted data analyses and drafted manuscripts. Dr Wendy Hall and Dr Sarah Berry read, commented and contributed to subsequent versions. Dr Wendy Hall had responsibility for the final content of the manuscripts.

The ATTIS (**A**lmond **T**rial **T**argeting dietary **I**ntervention with **S**nacks) study was designed by Dr Wendy Hall and Dr Sarah Berry. Pilot study, recruitment and screening of participants as well as study visits were conducted by Leanne Smith and Vita Dikariyanto with assistance from MSc/BSc/intern students as a part of their thesis work or programme module: Momena Rokib, Rachel Lee, Laure Verstraeten, Katie Patrick, Charlotte Constable-Fernandez, Camille Palanché, Eugenia Rodrigues, Jane Powell, Elisabeth Cresta, Caroline Day, May Robertson, Jenny Cheung, Eslem Kusaslan, Molly O'Callaghan-Latham, Tamara Kaloti, Emma Barry, Joyce Shaw, Inès Joffet, Laetitia Flottes de Pouzols. Lab technicians – Mary-Jo Searle and Ann-Catherine Perz assisted in nutrient analysis and blood glucose analysis, respectively. A research assistant – Maria D'Annibale ran plasma fatty acid and faecal short chain fatty acid analysis at lab guided by a lab technician – Robert Gray. Blood lipid profiles and other circulating biomarkers were analysed by Affinity Biomarker Labs, UK, while nuclear magnetic resonance (NMR) was conducted by Nightingale Health, Finland. Dr Geoffrey Charles-Edward supervised the acquisition of MRI/^{1}H-MRS data and Haris Shuaib downloaded all

MRI/^{1}H-MRS data from the Guy's and St Thomas' NHS Foundation Trust system. Vita Dikariyanto conducted flow-mediated dilation (FMD) measurement and MRI/^{1}H-MRS data analysis for quantification of liver, pancreatic and muscle fat; statistical analysis and interpretation of other cardiometabolic data; and composed the present thesis in discussion with Dr Wendy Hall and Dr Sarah Berry. Dr Brandon Whitcher and Dr Nicolas Basty developed the MRI data analysis technique Vita Dikariyanto drafted manuscripts of cross-sectional analysis and the ATTIS study. Dr Wendy Hall and Dr Sarah Berry had responsibility for the final content of manuscripts.

List of abbreviations

^{1}H-MRS	proton magnetic resonance spectrometry
3D	3-dimentional
ABP	ambulatory blood pressure
ACh	acetylcholine
AGE	advanced glycation product
AKM	almond kernel only plus almond kernel in mixed nuts
AKO	almond kernel only
ALA	α-linolenic acid
ALP	alkaline phosphatase
ALT	alanine aminotransferase
ANCOVA	analysis of covariance
ANS	autonomic nervous system
ApEn	Approximate entropy
ATTIS	**A**lmond **T**rial **T**argeting dietary **I**ntervention with **S**nacks
BBC	British Broadcast System
BH_4	tetrahydrobiopterin
BMI	body mass index
BP	blood pressure
CAD	coronary artery disease
cBP	clinical blood pressure
CCK	cholecystokinin
cDBP	clinical diastolic blood pressure
CHD	coronary heart disease
COMA	Committee on Medical Aspects of Food Policy
CRF	Clinical Research Facility
CRP	C-reactive protein
cSBP	clinical systolic blood pressure
CT	computerised tomography
CV	coefficients of variance
CVD	cardiovascular disease
DASH	Dietary Approaches to Stop Hypertension
DBP	diastolic blood pressure
DEXA	dual-energy x-ray absorptiometry
DGA	Dietary Guidelines for Americans
DNL	*de novo* lipogenesis
E-selectin	selectin cell adhesion molecule expressed only in endothelial cells
ECG	electrocardiogram
ED	endothelial dysfunction
EDV	endothelium-dependent vasodilation
EER	estimated energy requirement
EI	energy intake
EMCL	extramyocellular lipid
eNOS	endothelium nitric oxide synthase
ET-1	endothelin-1
FA	fatty acid
FDA	Food and Drug Administration
FID	free-induction decay

FMD	flow-mediated dilation
HCA	heterocyclic aromatic amine
HDL	high density lipoprotein
HDS	Healthy Diet Score
HEI	Healthy Eating Index
HF	high-frequency power
HOMA-IR	homeostatic model assessment of insulin resistance
HR	heart rate
HRV	heart rate variability
GC	gas chromatography
GGT	gamma-glutamyl transferase
GI	glycaemic index
GL	glycaemic load
GLC	gas liquid chromatography
GLM	generalized linear model
GLP-1	glucagon-like peptide 1
GTN	glyceryl trinitrate
HC	high carbohydrate
HCA	heterocyclic aromatic amine
HDI	Heathy Diet Index
HF	high-frequency
HLSVD	Hankel Lanczos squares singular values decomposition
HSP	hexosamine biosynthetic pathway
IBI	interbeat intervals
ICAM	adhesion molecule
IDF	International Diabetes Foundation
IHD	ischaemic heart disease
IHL	intrahepatic lipid
IMCL	intramyocellular lipid
IPL	intrapancreatic lipid
IQR	Interquartile range
IR	insulin resistance
IRS	insulin receptor substrate
K	potassium
KCL	King's College London
LA	linoleic acid
LDL	low density lipoprotein
LF	low fat
LF	low-frequency
LPL	lipoprotein lipase
MD	Mediterranean diet
MDS	Mediterranean Diet Score
MetS	metabolic syndrome
MI	myocardial infarction
MR	magnetic resonance
MRI	magnetic resonance imaging
mRNA	messenger ribonucleic acid
MRU	Metabolic Research Unit
MUFA	monounsaturated fatty acids
Na	sodium

NADPH	nicotinamide adenine dinucleotide phosphate
NAFLD	non-alcoholic fatty liver disease
NASH	non-alcoholic steatohepatitis
NCD	non-communicable disease
NDNS	national diet and nutrition survey
NDNS-RP	National Diet and Nutrition Survey - Rolling Program
NE	norepinephrine
NEFA	non-esterified fatty acids
NHANES	national health and nutrition examination survey
NMR	nuclear magnetic resonance
NN	normal-to-normal
NO	nitric oxide
PAI-1	plasminogen activator inhibitor 1
PAL	physical activity level
PAT	peripheral arterial tonemetry
PDFF	proton density fat fraction
PI-3 kinase	phosphoinositide 3-kinase
PNS	parasympathetic nervous system
PREDIMED	Prevención con Dieta Mediterránea
PUFA	polyunsaturated fatty acids
PUI	polyunsaturation index
QCA	coronary epicardial vasoreactivity
RA	research assistant
RAAS	renin-aldosterone-angiotensin system
RCT	randomised controlled trial
RF	radio frequency
rMSSD	root mean square of successive differences of NN intervals
ROI	region of interest
SAT	subcutaneous adipose tissue
SBP	systolic blood pressure
SCFA	short chain fatty acids
SD	standard deviation
SDNN	standard deviations of the NN intervals
SFA	saturated fatty acids
SI	saturation index
SMC	smooth muscle cell
SNR	signal to noise ratio
SNS	sympathetic nervous system
SREBP	sterol regulatory element binding proteins
T2D	type-2 diabetes
TAG	triglycerides
TC	total cholesterol
TE	echo time
TEI	total energy intake
TGF-β	transforming growth factor β
TLR4	toll-like receptor 4
TNS	tree nut snacks
TNS-A	any amount of tree nut snacks
TNS-B	≥7.08 g tree nut snacks (equivalent to ¼ oz)
UI	unsaturation index

USDA	U. S. Department of Agriculture
VAT	visceral adipose tissue
VCAM	vascular adhesion molecule
VEGF	vascular endothelial growth factor
VLDL	very-low density lipoprotein
VLF	very low-frequency power
WC	waist circumference
WHO	World Health Organization

Preamble

This is a thesis incorporating publications outlining a series of work, detailed in every chapter, within a doctoral programme (1st July 2016 to 30th June 2020), comprising observational studies and a randomised controlled trial (the ATTIS study); a timeline is provided in **Appendix 1**.

The focus of the work was to:

1) Examine the levels of tree nut and whole almond consumption in the UK adult population and its associations with diet quality and cardiovascular disease (CVD) risk;
2) Investigate how whole almond consumption impacts endothelial function and liver fat as novel markers of CVD, with other cardiometabolic factors being also assessed to explore the putative mechanism.

Together, this allows for investigations into the impacts of tree nut consumption on cardiometabolic risk markers and its potential to be included as a part of the dietary strategy to reduce CVD risk.

Chapter 1: Introduction explores the relationship between endothelial dysfunction (ED) and liver fat accumulation, risk factors for atherosclerosis and non-alcoholic fatty liver disease (NAFLD), respectively. This chapter also covers the putative connection with the autonomic nervous system (ANS). Finally, current dietary guidelines addressing dietary strategies for the management of CVD and evidence for cardioprotective properties of tree nuts will be critically discussed, providing the overall hypothesis and individual aims and objectives of the studies conducted during the doctoral programme.

Chapter 2: Methods describes the methodology used in the observational studies and the randomised controlled trial (RCT), the ATTIS study. This includes statistical analysis used in the observational studies. Furthermore, this chapter also details all the tasks in the ATTIS study, such as control study meal development, feasibility and acceptability study of the control meals, measurements of primary outcomes of the RCT: endothelial function and liver fat concentrations, and of secondary outcomes including ambulatory blood pressure (ABP), 24 h ambulatory heart rate variability (HRV), acute mental stress HRV, fasting blood glucose and lipids, insulin sensitivity, other circulatory biomarkers, lipoprotein size via nuclear magnetic resonance (NMR) and an exploratory outcome from short chain fatty acids (SCFA) as results of colonic fermentation of whole almonds.

Chapter 3: Tree nut snack consumption is associated with better diet quality and cardiovascular disease risk in the UK adult population: National Diet and Nutrition Survey (NDNS) 2008-2014 presents cross-sectional analysis within this doctoral programme using data from the UK adult population (≥19 y) from the UK nationally representative database, i.e. the UK National Diet and Nutrition Survey (NDNS) 2008-2014 that aimed to examine the prevalence of tree nut consumption and its associations with diet quality and CVD. This work has been published in Public Health Nutrition (Dikariyanto *et al.*, 2020)

Chapter 4: Whole almonds is associated with better diet quality and cardiovascular disease risk in the UK adult population: National Diet and Nutrition Survey (NDNS) 2008-2017 presents another cross-sectional analysis of the UK adult population (≥19 y) in the UK NDNS 2008-2017 with similar design and aim as the Chapter 3 but for whole

almond consumption. This work has been published in European Journal of Nutrition (Dikariyanto *et al.*, 2020).

Chapter 5: Snacking on whole almonds for 6 weeks improves endothelial function and lowers LDL cholesterol but does not affect liver fat and other cardiometabolic risk factors in healthy adults, the ATTIS study, a randomised controlled trial discusses the findings of the ATTIS study that aimed to investigate whole almonds consumption for 20% of estimated energy requirement (EER) in displacement of isoenergetic typical snacks on endothelial function (via flow-mediated dilation (FMD)) and liver fat (via magnetic resonance imaging/spectrometry (MRI/^{1}H-MRS)) as the primary outcomes, and other cardiometabolic risk factors as the secondary outcomes, including body composition; subcutaneous, abdominal visceral and pancreatic fats (via MRI) and muscle fat (via ^{1}H-MRS); ambulatory blood pressure; 24 h heart rate variability (HRV); fasting blood glucose and lipids; insulin sensitivity and other circulatory markers; plasma fatty acids; short chain fatty acids; and lipoprotein size. The ATTIS study was the major part of the doctoral programme. It took over 3 years to complete the whole work which included the research ethics application, the development of control snacks, the feasibility/acceptability study of the control snacks, the training in FMD measurement, the learning process of multiple other measurement techniques; the preparations of all required documents for study participants; the participant recruitment; the study visits; the data analyses and interpretation; the statistical analysis and the writing phase of papers for publications. This work has been published in the American Journal of Clinical Nutrition (Dikariyanto *et al.*, 2020).

Chapter 6: Snacking on whole almonds for 6 weeks increases heart rate variability during a mental stress test in healthy adults: a randomized controlled trial presents specifically the findings from HRV during a mental stress test included in the ATTIS study as one of the secondary outcomes. Psychological stress inversely associated with HRV, an indicator of cardiac autonomic function and a predictor of risk of sudden cardiac death. At times of stress, people tend to favour high sugar and fatty foods, often as snacks, with potential adverse effects on cardiometabolic health. This chapter specifically discusses the implication of whole almond consumption on automatic nervous system function in response to mental stress. The interpretation of the link between mental stress, physiological responses including the impacts on cardiac autonomic function, and CVD was very complex, thereby this specific outcome is discussed separately from other outcomes of the ATTIS study. This work has been published in the Nutrients journal (Dikariyanto *et al.*, 2020).

Chapter 7: Discussions and conclusions consists of general discussion, strengths and limitations of the studies, future directions, conclusions and final remarks. The general discussion considers the greater integration of tree nut including almond consumption in the real-life context, its position as a part of solution scheme on CVD prevention and its impacts in other areas in the society. Suggested further work is discussed in the future directions.

Chapter 1: Introduction

1.1 Cardiovascular disease (CVD) and type-2 diabetes (T2D): an overview

Industrialised economy concomitantly occurring with consumerism and technology-led culture has created sedentary jobs with longer working hours and commutes, but less relaxing time. Stronger purchasing power driven by globalisation has also led people to consume a Western-style diet characterised by high intakes of energy, saturated fatty acids (SFA) and free sugars, and low dietary fibre intake. The phenomenon of physical inactivity and poor diet quality during the past century has been shown to be associated with the increased prevalence of non-communicable diseases (NCD) developed over the life course, including cardiovascular disease (CVD). In 2016, CVD caused 17.9 million global death cases in which this has made CVD the number one cause of worldwide mortality (WHO, 2017). Consequently, CVD has become the main hurdle of sustainable human development globally and burdened national economies.

CVD is also a prevalent comorbidity in type-2 diabetes (T2D) (Elnarson *et al.*, 2018; Shah *et al.*, 2015). The risk of cardiovascular mortality in type-2 diabetic people are doubled more than age-matched non-diabetic individuals (Sarwar *et al.*, 2010). Since CVD and T2D are well-established comorbidities, these diseases share similar risk factors. The complex pathological process occurs over many years and is influenced by a number of factors, both non-modifiable and modifiable parameters, as outlined in **Table 1.1** and discussed in more detail below. Lifestyle, one of the modifiable parameters, can be influenced by socioeconomic situations in which altogether these can cause metabolic disturbance leading to CVD and T2D (WHO, 2017).

Table 1.1. Cardiovascular disease (CVD) risk factors.

Non-modifiable risk factors	Modifiable risk factors	Socioeconomic aspects	Female-specific factors
• Age • Gender • Ethnicity • Biological inheritance	Lifestyle parameters • Smoking habit • Poor diet quality • Excess energy intake • Excess alcohol intake • Physical inactivity Psychological factors • Mental stress • Depression Metabolic and physiological parameters • Obesity • Hypertension • Dyslipidaemia • Hyperglycaemia • Diabetes	• Income • Education status • Globalisation • Mobilisation	• Pregnancy (hypertension, preeclampsia, eclampsia) • Gestational diabetes • Menopause

1.2 Cardiovascular disease (CVD) pathophysiology and risk measures

Individuals at high risk of developing T2D and CVD are often characterised by hypertension and metabolic syndrome (MetS). According to the International Diabetes Foundation (IDF), abdominal obesity and insulin resistance (IR) are prerequisites for MetS (IDF, 2006). Obesity is associated with a greater likelihood of metabolic disturbances such as hyperglycaemia, dyslipidaemia and inflammation. Accumulated low-density lipoprotein cholesterol (LDL) in the artery wall and oxidative stress can cause endothelial dysfunction (ED) and further progress into atherosclerosis. MetS may also lead to intrahepatic lipid (IHL) accumulation and non-alcoholic fatty liver diseases (NAFLD). Consequently, CVD, T2D and NAFLD are suggested to be interconnected. Within this subsection, these components of metabolic disorders that are attributable to CVD pathophysiology are discussed.

1.2.1 Dyslipidaemia

Dyslipidaemia is blood lipid abnormalities characterised by elevated total cholesterol (TC), triglycerides (TAG), non-esterified fatty acids (NEFA) and LDL, and low high-density lipoprotein cholesterol (HDL). Dyslipidaemia promotes atherosclerosis, the main cause of CVD, thereby it is known as a well-established CVD risk marker (Nelson, 2013). This phenomenon results from disrupted lipid metabolisms as illustrated in **Figure 1.1.**

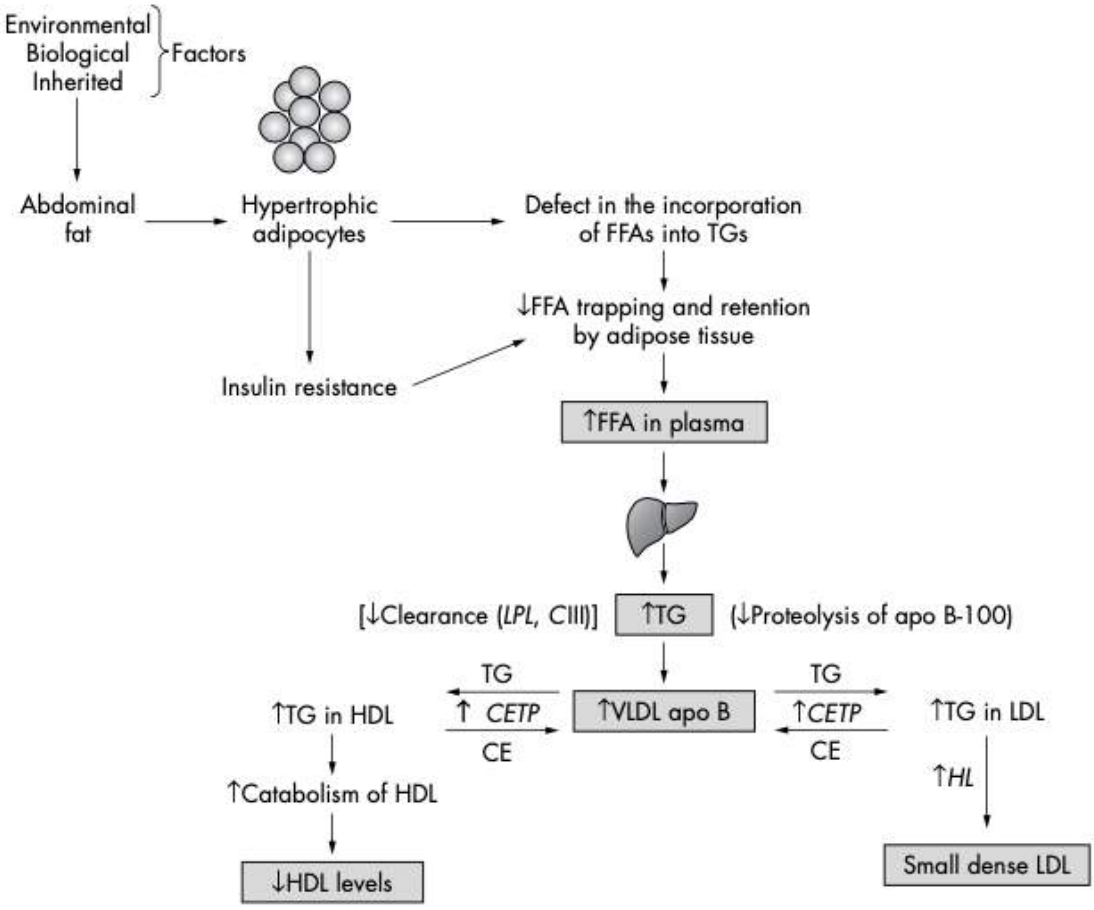


Figure 1.1. Schematic pathological mechanism of dyslipidaemia. Biological inheritance and environmental factors, including excess calorie intake, can lead to increased abdominal fat and insulin resistance. These induce elevated plasma NEFA and hepatic TAG resulting in VLDL overproduction, low HDL and increased small-dense LDL that are proatherogenic. This figure is adapted from Kolovou et al. *(2005).*

Dyslipidaemia is influenced by genetic predisposition, age, gender, medical conditions, e.g. central obesity and T2D, as well as environmental/lifestyle factors, e.g. diet, physical

activity, smoking habit and alcohol. Dietary factors influencing dyslipidaemia include excess calorie intake, high SFA and free fructose intake and low dietary fibre and phytosterols. Excess calorie intake elevates fasting and postprandial TAG and spillover NEFA (Klop *et al.*, 2013). High SFA intake increases LDL production (Mustad *et al.*, 1996; Woollett *et al.*, 1992), while high free fructose intake increases *de novo* lipogenesis (DNL) in hepatocytes, leading to elevated hepatic NEFA and TAG/IHL as well as very-low density lipoprotein (VLDL) (Alves-Bezerra & Cohen, 2017; Softic *et al.*, 2016). Furthermore, dietary fibre intake can lead to cholesterol and TAG reduction (Reynolds *et al.*, 2020). Combining these dietary factors into dietary strategies, such as Mediterranean diet, portfolio diet and plant-based dietary pattern have also been demonstrated to improve blood lipid profiles that is further discussed later in this chapter.

1.2.2 Blood pressure and hypertension

Blood pressure (BP) is the measure of force applied to arteries due to blood pumping by the heart to transport oxygen and nutrients, that can go up and down according to physiological and psychological demands. BP measurement consists of systolic BP (SBP), indicating the highest BP level when the heart beats/pumps the blood, as well as diastolic BP (DBP), the lowest BP level measured when the heart relaxes between beats. The BP readings for normotension is <140/90 mmHg, while hypertension has BP range 140-180/90-110 mmHg (British Heart Foundation, 2018). **Figure 1.2** illustrates the pathophysiology of hypertension.

Hypertension contributes to higher risk in developing T2D relative to normotensive and it worsens the morbidity and mortality rate majorly caused by CVD in T2D patients (Law *et al.*, 1991; Sowers *et al.*, 2001). For every 20 mmHg SBP or 10 mmHg DBP increase,

the mortality rate from ischaemic heart disease (IHD) and stroke doubles (Lewington *et al.*, 2002). Hypertension is associated with dyslipidaemia and IR which are also risk markers of atherosclerosis (Ormazabal *et al.*, 2018). Furthermore, hypertension aggravates the progression of atherosclerosis by inducing ED and destabilising atherosclerotic plaques (Schiffrin *et al.*, 2002).

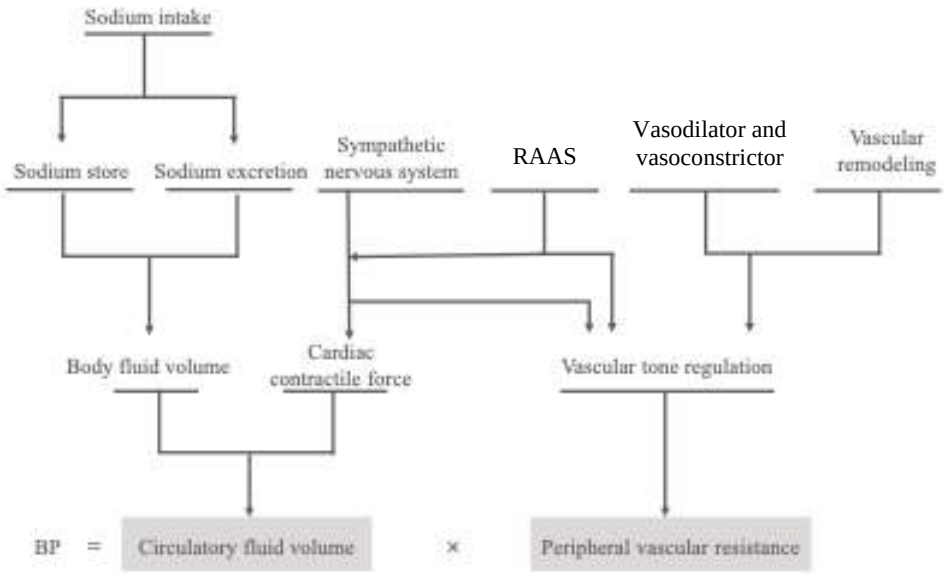


Figure 1.2. Pathophysiology of hypertension. BP is manifested by the relationship between circulatory fluid volume and peripheral vascular resistance. Circulatory fluid volume is controlled body fluid volume and cardiac contractile force. The balance between sodium retention and excretion influences body fluid volume. Cardiac contractile force is regulated by sympathetic nervous system (SNS) and mediated by renin-angiotensin-aldosterone system (RAAS). On the other hand, peripheral vascular resistance reflects vascular-tone regulation mediated by SNS, RAAS, vasodilator (e.g. nitric oxide (NO), vasoconstrictor (e.g. endothelin-1) and vascular remodeling. This illustration is adapted from Ohishi (2018).

Excess adiposity increases the risk of being hypertensive (Landsberg *et al.*, 2013). Other risk factors of hypertension include smoking, physical inactivity and high alcohol and salty, fatty and sugary food consumption (Beilin *et al.*, 1999; Kannel, 1996). Some dietary patterns have been shown to reduce BP, such as Dietary Approach to Stop Hypertension (DASH) diet and plant-based diet that are explained later in this chapter.

1.2.3 Obesity

Obesity is a chronic multifactorial disease manifested by a complex relationship between genes, age, sex, ethnicity and unhealthy environment and lifestyle (Nammi *et al.*, 2004). Environment and lifestyle parameters, including economic and educational status, psychological stress level, sleep deprivation and deficiency, physical inactivity and unhealthy eating behaviours, can all be modified. Dietary factors are discussed later in this chapter.

Obesity is associated with hypertension, dyslipidaemia, IR, T2D and increased fibrinogen and C-reactive protein (CRP) concentration. All of these metabolic risk markers and common comorbidities lead to the risk of developing CVD in which the pathophysiological pathway is illustrated in **Figure 1.3** (Din-Dzietham *et al.*, 2007; Ritchie & Connell, 2007). Evidence shows that the mortality and morbidity of CVD increase in individuals with central adipose tissue accumulation/abdominal obesity (Van Gaal *et al.*, 2006). Visceral adipose tissue (VAT), which is deposited around abdominal organs, is metabolically more active than subcutaneous adipose tissue (SAT). Excess central adiposity leads to higher production/secretion of adipokines, leptin and resistin, but less adiponectin (Lyon *et al.*, 2003). VAT is also associated with increased CRP levels, a systemic inflammatory marker (Sam *et al.*, 2009).

Circulating leptin concentrations are high in obesity, but eventually individuals may become resistant to the metabolic effects of leptin. Leptin is a peptide hormone coded by the 'ob' genes that reduces appetite and food intake, elevates energy expenditure and lower fat mass (Spiegelman & Flier, 2001). Leptin promotes insulin sensitivity, glucose uptake and use, and glycogen synthesis in the muscles via phosphoinositide 3-kinase (P1

3-kinase) pathway (D'Souza *et al.*, 2017); decreases *de novo* lipogenic and gluconeogenesis and increases fatty acid oxidation in the liver; and lowers lipogenesis and lipid esterification in adipose tissue (Kraus *et al.*, 2002; Muller *et al.*, 2004; Perez *et al.*, 2004; Walder *et al.*, 1997).

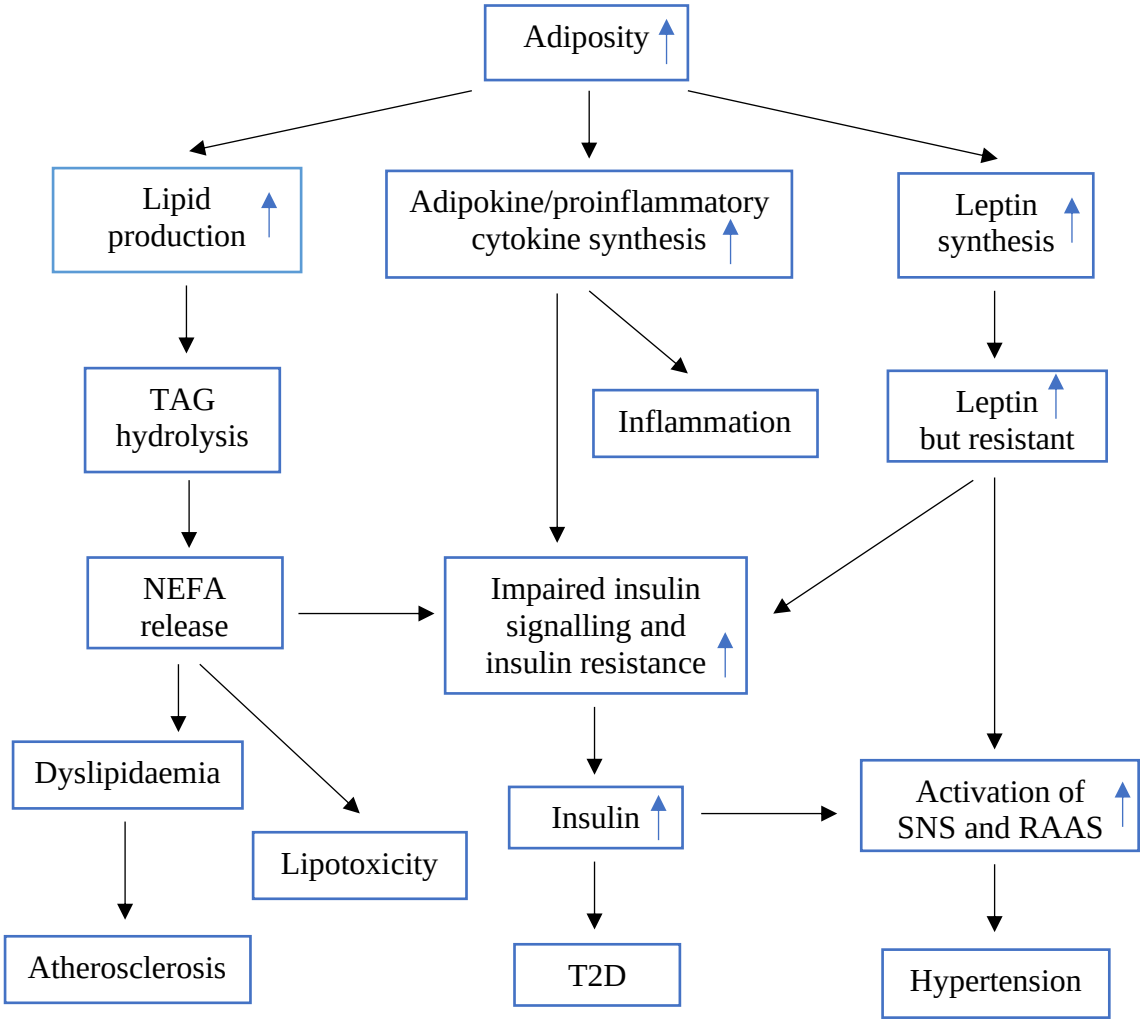


Figure 1.3. Obesity is characterised by excess adiposity deposition that leads to increased lipid production. High circulating NEFA causes lipotoxicity and dyslipidaemia including elevated LDL which is proatherogenic and oxidative stress-stimulating. Accumulated adipose tissue produces proinflammatory cytokines/adipokines, leptin and resistin, but less adiponectin (Lyon et al., *2003). Excessive secretion of adipokines results in inflammation. Leptin resistance and inflammatory marker secretion reduces insulin sensitivity (D'Souza* et al., *2017). Leptin and insulin resistance are also reported to induce SNS activation, leads to RAAS stimulation, angiotensin II secretion and increased BP (Hall* et al., *2010).*

Adiponectin also acts as an insulin sensitiser via its receptors expressed in skeletal muscle and liver, i.e. AdipoR1 and AdipoR2, that play a role in inhibition of fatty acids (FA) and TAG synthesis and promotion of glucose use and lipid oxidation (Kadowaki *et al.*, 2006). However, in obese individuals, depleted adiponectin occurs that together with leptin resistance and increased inflammatory markers leads to IR (Kadowaki *et al.*, 2006).

1.2.4 Insulin resistance

Insulin is a hormone secreted by pancreas following ingestion and increased blood glucose. Insulin further binds to the receptors and stimulates the downstream signalling cascade via insulin receptor substrate (IRS), inducing metabolic effects illustrated in **Figure 1.4**.

Defects within these signalling pathways lead the body to be unresponsive to metabolic effects of insulin. This phenomenon is called insulin resistance (IR) that can be genetically driven (leading to Type-1 diabetes) or caused by metabolic factors, such as endoplasmic reticulum stress-induced pancreatic β-cell failure and obesity, as discussed above. Furthermore, IR is well recognised driving factor of T2D (Muoio & Newgard, 2008).

As insulin also affects metabolic processes in endothelium shown by **Figure 1.4**, IR may result in imbalance of nitric oxide (NO) production and endothelin-1 secretion which leads to ED. Reduced endothelium-dependant vasodilation (EDV) was found along with IR (Steinberg *et al.*, 1996) and obesity (Hashimoto *et al.*, 1998; Williams *et al.*, 2005).

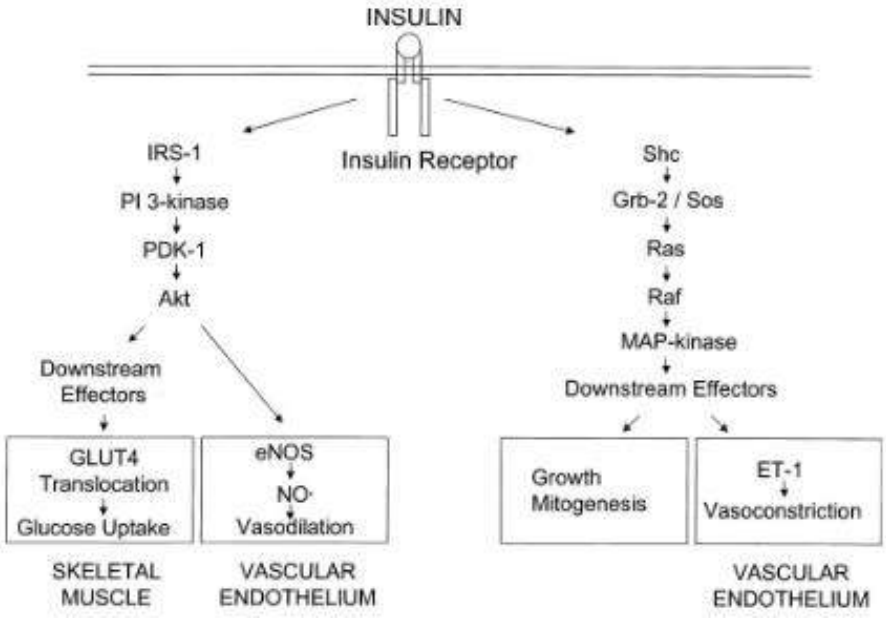


Figure 1.4. Insulin signaling via IRS-1, PI 3-kinase and Akt phosphorylation promotes glycogen synthesis through increment in glucose uptake in skeletal muscle. In parallel, insulin suppresses hepatic glucose production and release via inhibition of gluconeogenesis and glycogenolysis as well as stimulates lipid synthesis and diminishes lipid degradation in adipose tissues (Samuel & Shulman, 2012). The insulin signaling is also expressed in the endothelium and acts in several metabolic processes including the production of NO, a vasodilator (via PI 3-kinase/Akt), and the secretion of endothelin-1 (ET-1), a vasoconstrictor (via RAS/MAP-kinase) (Kim et al.*, 2006). This illustration is adapted from Kim* et al. *(2006).*

1.2.5 Endothelial dysfunction and atherosclerosis

ED, a key component of the pathology of atherosclerosis, is prevalent in individuals with dyslipidaemia, obesity and diabetes. Endothelial function reflects the regulation of vascular tone that is mediated by vasodilators (NO and prostacyclin) and vasoconstrictor (endothelin-1 and thromboxane A_2) secreted by endothelial cells. NO is the prime vasodilator and produced in endothelial cell involving L-arginine as a building block in which the electrons are carried by nicotinamide adenine dinucleotide phosphate (NADPH) and molecular oxygen (O_2), endothelial NO synthase (eNOS) as catalysing enzyme, and cofactor tetrahydrobiopterin (BH_4). BH_4 molecules assist in electron transfer for L-arginine oxidation. NO bioavailability depends on the balance of its production and degradation (**Figure 1.5**). The degradation of NO takes place as a result of its half-life

and reaction with reactive oxygen species (ROS) generated by NADPH oxidase activity (Andrew & Mayer, 1999). Peroxynitrite (OONO-), a product of the reaction between NO and superoxide (O_2-) generated by NADPH oxidase, can also reduce eNOS activation (Pacher *et al.*, 2007). In the shortage of BH_4, eNOS uncoupling may occur and also produce superoxide (Green *et al.*, 2004).

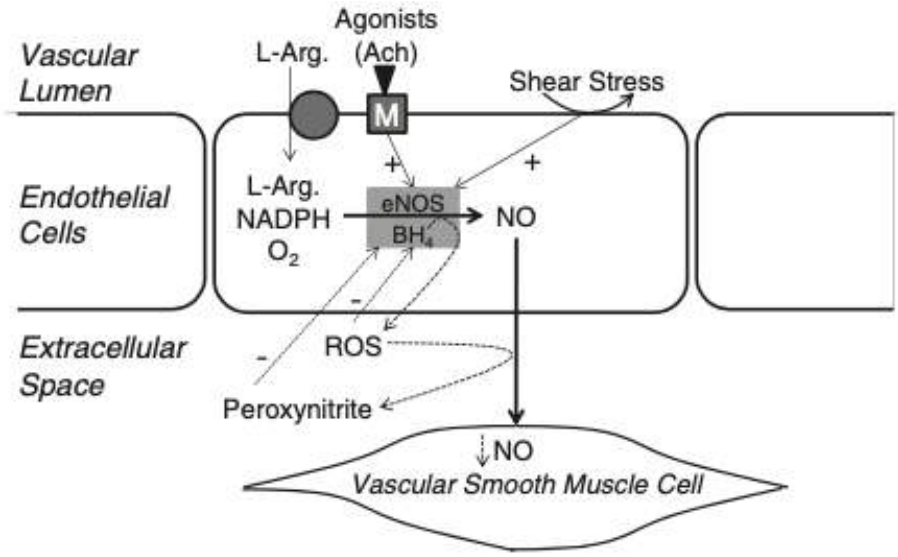


Figure 1.5. In- and outflow traffic of NO. NO synthesis involves L-arginine – the precursor, electrons from NADPH and O_2 as substrates; eNOS as the enzyme; and BH_4 as the cofactor. The NO bioavailability depends on the balance between the NO synthesis and the NO degradation. NO is degraded via direct reaction with ROS or BH_4 oxidation by ROS resulting in eNOS uncoupling thereby eNOS produces more ROS instead of NO. This illustration is adapted from Poitras and Pyke (2013).

ROS leading to oxidative stress is an important factor of reduced NO bioavailability and ED. Oxidative stress reflects the imbalance between ROS production and antioxidant availability. Chronic hyperglycaemia-induced glucotoxicity and dyslipidaemia-induced lipotoxicity stimulate oxidative stress through several pathways (see **Figure 1.6)**. Other factors that could lead to ROS production include exposure to fried food producing heterocyclic aromatic amine (HCA) arisen from cooking at high temperature (Moonen *et al.*, 2002), tobacco smoke, pollutants, pesticides and alcohol. These generate free radicals,

which also in turn reduce availability of antioxidants. Furthermore, hyperhomocysteinaemia is also suggested to contribute to the increment of ROS due to the superoxide anions ($O_2^{-\cdot}$) in its homocysteine molecule and the thiol group (Stamler *et al.*, 1993). Homocysteine is a building block of methionine in which the formation requires folate as a methyl donor during remethylation, thereby folate supply is important in homocysteine balance (Durand *et al.*, 2001).

Taking into account the pathophysiology of ED, dietary factors that may modulate endothelial function include L-arginine, antioxidant vitamins (such as vitamin C and E), folate, riboflavin and unsaturated fats as also reported by literature (Brown & Hu, 2001). Since ED is also mediated by components of metabolic disorders in obesity, such as hyperglycaemia, dyslipidaemia and IR (see **Figure 1.6**), dietary determinants of ED are suggested to be similar as dyslipidaemia and obesity.

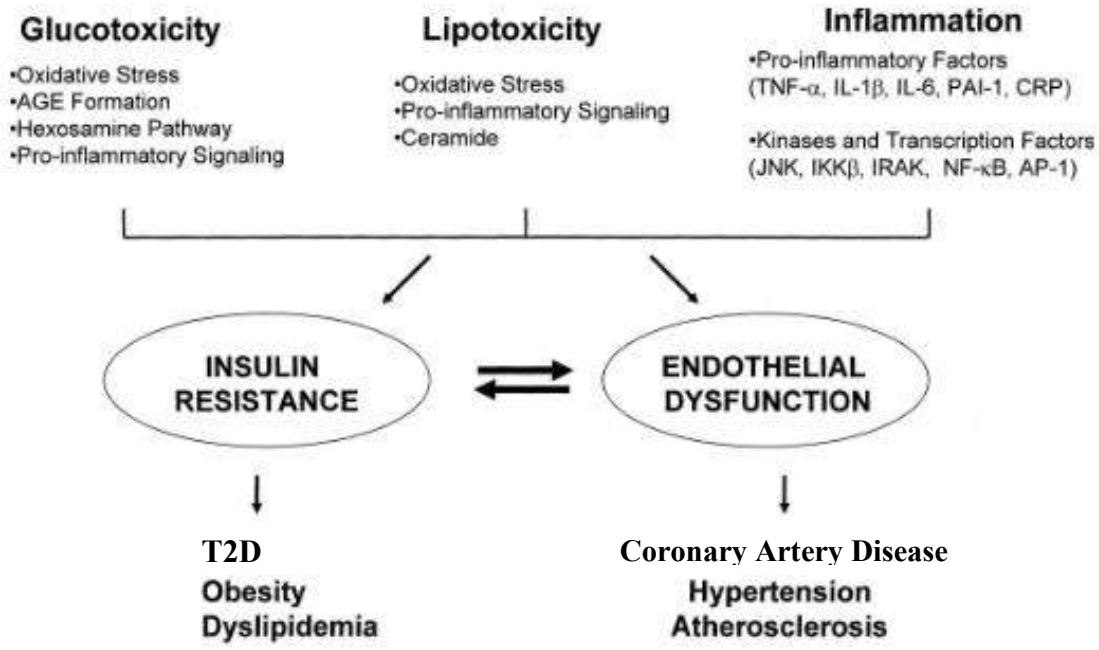


Figure 1.6. ROS-induced decrease in NO bioavailability is key in ED. Glucotoxicity and lipotoxicity lead to ROS production and oxidative stress. Glucotoxicity is mediated by hyperglycaemia-induced increment in hexosamine biosynthetic pathway (HSP) activity and advanced glycation product (AGE). Dyslipidaemia-induced lipotoxicity indicated by

NEFA accumulation may lead to ceramide production and NADPH oxidase activity increasing ROS production (Kim et al.*, 2006). Oxidative stress can stimulate inflammation via pro-inflammatory signalling. Inflammation downregulates eNOS via direct phosphorylation (Partovian* et al.*, 2005) and increase production of endothelin-1, vascular endothelial growth factor (VEGF) – a vascular permeability factor, transforming growth factor (TGF)-β and plasminogen activator inhibitor (PAI)-1 – an inhibitor of fibrinolysis leading to atherosclerosis (Brownlee, 2005), intercellular adhesion molecule (ICAM), vascular cell adhesion molecule (VCAM) and selectin cell adhesion molecule expressed only in endothelial cells (E-selectin). These adhesion molecules play a role in the recruitment of leukocytes to atherosclerotic lesions (Brownlee, 2005; Huo & Ley, 2001). Furthermore, shared and interconnected mechanisms of glucotoxicity, lipotoxicity, and inflammation causes interlink between IR and ED that contributes to relationships between cardiovascular and metabolic diseases. This illustration is adapted from Kim* et al. *(2006).*

ED is regarded as an early marker of atherosclerosis since impaired endothelial function indicates dysregulation of vasodilation and vasoconstriction, inhibition and stimulation of smooth muscle cell (SMC) proliferation and migration, and thrombogenesis and fibrinolysis in which all of these processes contribute to atherosclerotic plaque development (Kinlay *et al.*, 2001; Lüscher & Barton, 1997). The further developmental stages of atherosclerosis are described in **Table 1.2**, adapted from the atherosclerosis definition by American Heart Association, Insull (2009) and Virmani *et al.* (2000).

Therefore, in the development of ED and atherosclerosis, increased NEFA and LDL induced by dyslipidaemia, IR and obesity play important roles. Hypertension can also result from the imbalance of vasodilation and vasoconstrictor occurred in ED as well as stenosis led by atherosclerosis.

Table 1.2. Atherosclerotic lesion types.

Lesion name	Description
Reversable non-atherosclerotic intimal lesions	
1. Intimal thickening	LDL particles migrate from blood stream and are accumulated within the arterial intima and cytoplasm of intimal SMCs.
2. Intimal xanthoma (fatty streak)	• Oxidised LDL stimulates inflammation and immune response leading endothelial cells to produce growth factor and adhesion molecules (Insull, 2009). • SMCs interact with receptors of lymphocytes, monocytes, neutrophils and mast cells in the arterial walls causing migration of SMCs into the wall (Strong *et al.*, 1999). • Macrophages and dendritic cells bind the oxidised LDL which further are modified into multiple small foam cells forming fatty streak (Bentzon *et al.*, 2014; Yu *et al.*, 2013).
Progressive irreversible atherosclerotic lesions	
1a. Pathological intimal thickening	SMCs are surrounded by proteoglycan-rich matrix and extracellular lipid accumulation forming a plaque. Necrosis is absent.
1b. Erosion	Luminal thrombosis of the above plaque.
2a. Fibrous cap atheroma (fibroatheroma)	Necrosis occurs with covering collagen-proteoglycan-matrix-rich fibrous tissue cap. Inflammation further takes place leading to infiltration of macrophages and lymphocytes.
2b. Erosion	Luminal thrombosis of the fibroatheroma plaque.
3. Thin fibrous cap atheroma	Lipid-rich necrotic-cored fibrous caps can grow thin and weak for years due to SMCs gradual loss and macrophage infiltrations resulting in collagen-rich matrix degradation by proteolytic enzymes.
3a. Plaque rupture	Thin cap is susceptible towards rupture. Upon rupture, thrombogenic factors in the blood detect cap collagen and highly thrombogenic necrotic core leading to thrombosis (Bentzon *et al.*, 2014).
4. Calcified nodule	Apoptotic cells, extracellular matrix and necrotic core can serve as nidus for calcium microgranules.
5. Fibrocalcific plaque	Over years, calcified nodule can enlarge to greater collagen-rich calcium deposit lumps or plates with significant stenosis.

1.2.6 Non-alcoholic fatty liver disease (NAFLD)

NAFLD is a phenomenon marked by intrusion of fat in liver cells, appearing like liver injury caused by alcohol abuse but found in individuals who do not misuse alcohol. NAFLD is developed through a series of phases, fatty liver/steatosis, non-alcoholic steatohepatitis (NASH), fibrosis and cirrhosis as outlined in the **Table 1.3** (McCullough, 2004; Santos *et al.*, 2019; Schuppan & Afdhal, 2008). Fatty liver is associated with ED in epidemiological studies (Villanova *et al.*, 2005), indicating higher CVD risk in patients with NAFLD. For example, higher liver fat was positively associated with reduced flow-mediated dilation (FMD; a measurement technique of endothelial function) following adjustment for cohort, age; sex; status of smoking, menopause, hormone replacement therapy, hypertension, diabetes and lipid-lowering treatments; mean arterial pressure; heart rate; TC; TAG; HDL and fasting blood glucose (Long *et al.*, 2015).

The liver processes large amounts of FA but it can only store small quantities of TAG balanced with NEFA uptake from adiposity lipolysis, dietary fat and DNL; fatty acid β-oxidation; and incorporation into VLDL particles that are secreted into the bloodstream (Alves-Bezerra & Cohen, 2017). Elevated lipid delivery to the liver (e.g. by increased circulating NEFA) and synthesis in the liver, and reduced lipid oxidation may result in the development of fatty liver/steatosis. Steatosis is hepatic TAG deposition exceeding 5% causing increased size of liposomes (Heymsfield *et al.*, 2015). Steatosis is a feature of MetS and T2D, associated with IR and central obesity (McCullough, 2004; Santos *et al.*, 2019). Hepatic IR is also central to the pathogenesis of NAFLD as this causes a reduced suppression of lipolysis causing increased circulating NEFA for subsequent uptake in the liver and VAT accumulation. IR also diminishes hepatic β-oxidation of NEFA inducing IHL accumulation (Bi *et al.*, 2014). Since there is an interlinked

pathology between obesity, T2D, ED, atherosclerosis and fatty liver, mediated by increased NEFA, VAT accumulation, IR, oxidative stress and inflammation, the dietary determinants of fatty liver are similar.

Table 1.3. Different stages of non-alcoholic fatty liver disease (NAFLD) and assessment methods (McCullough, 2004; Santos et al.*, 2019; Schuppan & Afdhal, 2008).*

	Definition	Clinical characteristics	Assessment technique
Steatosis	≥5% liver fat deposition without hepatocyte injury	• Elevated plasma ALT • Overweight and obese without overconsumption of alcohol • MetS	• Hepatic ultrasound (widely available, non-invasive, very specific, but insensitive for <12% fat deposition) • Hepatic computed tomography (CT) (widely available, non-invasive, sensitive, but involving radiation) • Magnetic resonance imaging/spectroscopy (MRI/^{1}H-MRS) (non-invasive, very sensitive for small amount of fat deposit, but not widely available)
Steatohepatitis (NASH)	Steatosis with hepatic inflammation and hepatocyte injury (ballooning)	Similar as for steatosis	• Liver biopsy (invasive and expensive) • ALT test (insensitive and non-specific for NASH) • NASH test including age, body mass index (BMI), blood biomarkers (poor accuracy)
Fibrosis	Fibrosis (scarring)	>45 y subjects and T2D have elevated risk of developing fibrosis	Liver stiffness test via ultrasound transient elastography or magnetic resonance (MR) elastography (non-invasive)
Cirrhosis	Scarred and lumpy liver due to inflammation over years	• Elevated gamma-glutamyltransferase (GGT), more specific liver enzyme than ALT • Elevated bilirubin • Decreased albumin and hepatocytes	Liver biopsy

1.2.7 Cardiac autonomic function

Cardiac autonomic function includes complex neural and haemodynamic systems. Autonomic nervous system (ANS) is responsible for neural regulation of cardiac autonomic function, thereby ANS dysfunction is a predictor of sudden cardiac death. ANS function comprises the interplay between sympathetic and parasympathetic nervous system in which the responses are mediated by hormones regulated by hypothalamus (Dampney, 2016). The ANS modulates contractility of myocardium regulating heart rate and rhythm (Vaseghi & Shivkumar, 2008), while autonomic modulation to control circulatory homeostasis is regulated by baroreflex functioning (Eckberg & Sleight, 1992); ANS and baroreflex together with cardiac automaticity (intrinsic to pacemaker tissues) regulate heart rate (HR) (Eckel *et al.*, 2014; Lanfranchi & Somers, 2002). These allow cardiovascular system to respond physiological and psychological demands on daily basis (Vaseghi & Shivkumar, 2008).

Greater parasympathetic activity results in lower HR and longer RR intervals, mediated by synaptic release of acetylcholine (ACh), a neurotransmitter that has very short effect latency and high turnover rate. Therefore, the response is almost immediate (Draghici & Taylor, 2016). The sympathetic nervous system (SNS) mainly acts on the ventricular muscles and plays a role in increasing their contractility mediated by norepinephrine (NE) binding on cardiomyocytes, a hormone or neurotransmitter involved in stress. To respond a demand for a higher HR, the inhibitory effects of the parasympathetic nervous system (PNS) recede resulting in a predominance of the SNS. Therefore, PNS is the branch of the ANS that provides rapid alterations in cardiac beat-to-beat (Saul, 1990). Greater vagal tone (parasympathetic activity) lowers HR causing longer interbeat intervals and a greater capacity for beat-to-beat variability. In contrast, sympathetic tone generates shorter

interbeat interval and lower heart rate variability (HRV). Thus, ANS function can also be assessed by analysis of the sequence of interbeat intervals (Thayer *et al.*, 2012).

At times of stress, the increment in SNS activity generates shorter RR intervals, resulting in a reduced capacity for the interplay of SNS and PNS to adapt to physiological demands. Moreover, the sympathetic mediating neurotransmitter, NE, is slowly reabsorbed and metabolized, causing longer delays between the stimulus and resultant changes and potentially also causing longer effects, which further lower HRV (Draghici & Taylor, 2016). Evidence suggests that cognitive and emotional stress could induce a pathophysiological cascade of ANS dysfunction through activation and predominance of SNS (Won & Kim, 2016). Under resting conditions, parasympathetic activity prevails over sympathetic activity, and variations in the heart period are largely dependent on vagal modulation (Eckel *et al.*, 2014).

ANS also responds to stimulation from baroreflex function in a negative feedback fashion. When BP increases, afferent baroreflex nerve signals increase, efferent sympathetic nerve signal reduce, while efferent parasympathetic nerve signals are elevated, leading to decreased HR (Eckberg & Sleight, 1992). During stress, arterial pressure and carotid stiffness increase resulting in reduction of baroreflex functioning which generates autonomic imbalance (Lipman *et al.*, 2002).

Since the outcome of interplay between PNS and SNS induced by varied mental and physiological demands is fluctuation in a beat-to-beat length, HRV is used as indirect non-invasive method to examine ANS function. HRV measurement involves analysis of time and frequency domain parameters (see **Table 2.8** in the method chapter) (Task Force

of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). Low HRV has been demonstrated to be predictive of increased risk of sudden cardiac death and cardiovascular mortality (Kleiger *et al.*, 1987; La Rovere *et al.*, 1998). In addition, low HRV has also been shown in obese subjects that is suggested to be mediated by excess SNS activity (Karason *et al.*, 1999; Windham *et al*., 2012).

Therefore, sympathetic nerve tone induced by leptin resistance, IR, RAAS activation and ED plays a role in the pathological mechanisms of obesity, T2D and atherosclerosis. A greater risk of cardiac autonomic dysfunction is observed in NAFLD patients (Liu *et al.*, 2013). F**igure 1.7** illustrates the interlinked pathophysiology between CVD, NAFLD and cardiac autonomic dysfunction.

Regarding the dietary factors influencing HRV, scientific understanding of the impact and mechanism of dietary modifications on HRV is still very limited (Young & Benton, 2018), and mainly relates to the effects of omega-3 supplementation (Billman, 2013; Xin et al., 2013), energy imbalance (Lips *et al.*, 2013; Mouridsen *et al.*, 2013; Pinto *et al.*, 2019; Sjoberg *et al.*, 2011), vitamin D and B12 (Mann *et al.*, 2013; Sucharita *et al.*, 2014) which are not the main focus of this PhD. However, different types of dietary fat might influence HRV. The Mediterranean diet, constituted of a high content of unsaturated fats from oily fish, olive oil and nuts, has been reported to increase HRV (Dai *et al.*, 2010). Type of dietary carbohydrate may also have an impact as HRV was shown to be reduced following oral glucose loads (Kanaley *et al.*, 2007; Muscelli *et al.*, 1998; Paolisso *et al.*, 1997; Paolisso *et al.*, 2000). Furthermore, since sodium and potassium are associated with RAAS, these micronutrients can activate SNS and influence ANS and HRV.

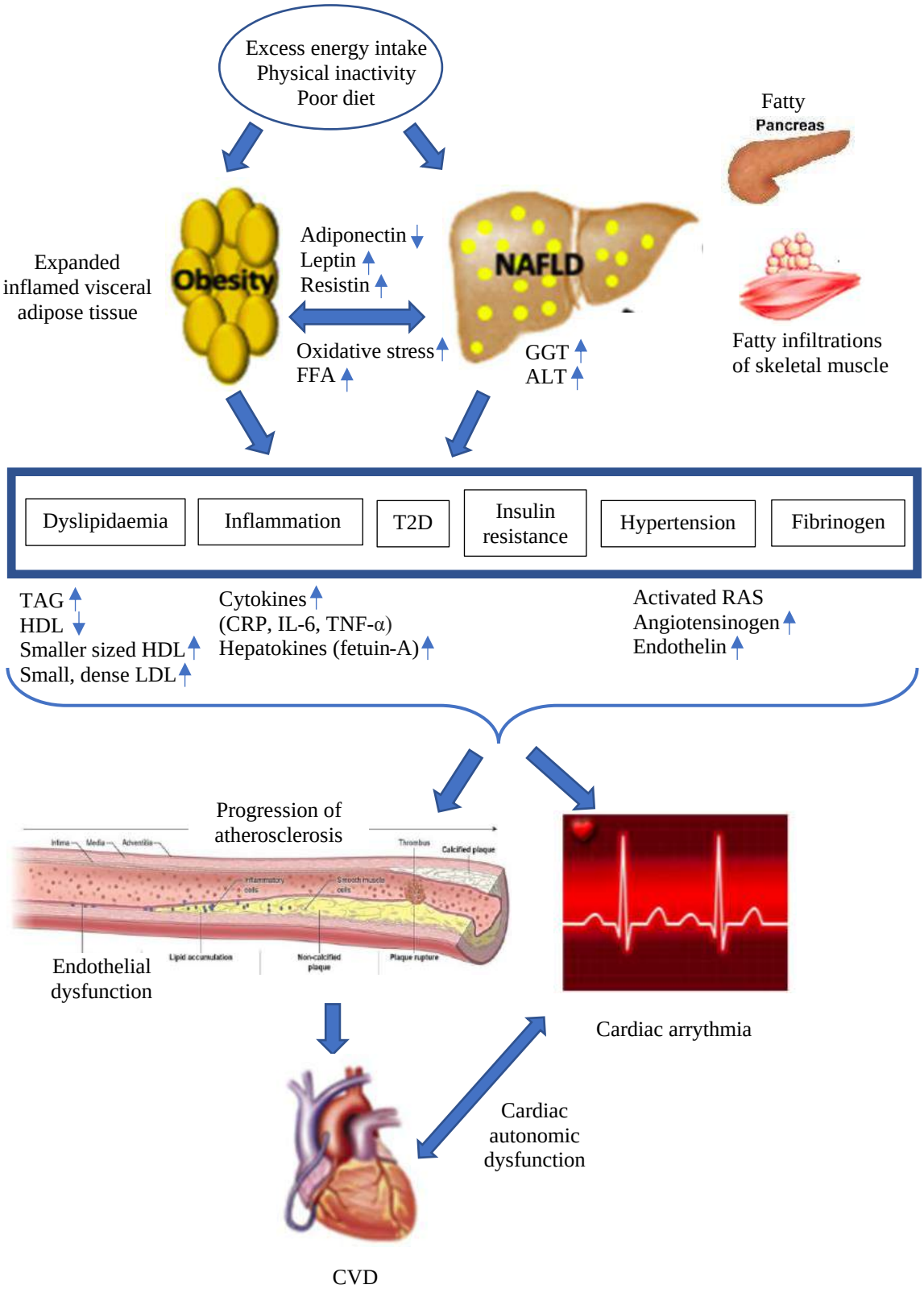


Figure 1.7. Putative mechanisms of complex inter-relationship among NAFLD and CVD including atherosclerosis and cardiac autonomic dysfunction. The pathophysiology may involve obesity, insulin resistance and inflammation. Excess energy intake alongside inadequate energy expenditure causing expanded fat storage leading to accumulation of adipose tissues and obesity; poor diet quality containing high saturated fat and high refined carbohydrates; leptin resistance; systemic and hepatic IR; increased lipolysis in

adipose tissues and circulating NEFA leading to dyslipidaemia; inhibition of glucose uptake in skeletal muscle; pancreatic fatty infiltration; modification of hepatic lipid metabolism: increased re-esterification and storage of NEFA in the form of triglycerides, reduced NEFA oxidation and stimulated DNL in which altogether induce IHL accumulation leading to steatosis; and lipotoxicity. VAT is metabolically active and secretes proinflammatory cytokines (adipokines). Lipotoxicity is stimulated by adipokines, oxidative stress and endoplasmic reticulum stress that may further progresses into NASH and fibrosis. Leptin and insulin activate angiotensinogen and endothelin-1 which are vasoconstrictor which may downregulate endothelial function and blood pressure. ED can also result from oxidative stress, IR and inflammation induced by hyperglycaemia and lipotoxicity. ED can initiate the atherosclerotic plaque formation. Progressive steps of atherosclerosis involve lipid accumulation, LDL oxidation and inflammation, fibroatheroma, calcification, plaque rupture and thrombosis. NAFLD, ED and atherosclerosis are associated with cardiac autonomic dysfunction, an imbalanced interaction between parasympathetic and sympathetic nervous system activities, although the underlying mechanism remains elusive. In combination with underlying coronary heart disease, cardiac autonomic dysfunction can cause cardiac arrythmia and increase the risk of sudden cardiac death.

1.3 Diet and cardiometabolic disease risk

1.3.1 Dietary guidelines

Dietary guidelines were developed to focus on nutrients related to undernutrition/malnutrition diseases (Mozaffarian *et al.*, 2018). This focus has now been shifted. Since the 20th century, the rates of chronic diet-related diseases have increased, including CVD, T2D and overweight and obesity. The dietary risk factors for these NCDs are dissimilar than those for nutrient deficiencies. NCDs are typically attributable to multiple dietary factors accumulated over a long period of time, including excess or insufficient intake leading to poor diet quality (GBD 2013 Mortality and Causes of Death Collaborators, 2015). This phenomenon occurs in many areas in the world in which the populations consume foods high in added sugars, SFA and salt, and have low proportion for fruits, vegetables and fibre as demonstrated by the reports of national surveys (Bates *et al.*, 2014; Pikosky *et al.*, 2019; Rehm *et al.*, 2016; Roberts *et al.*, 2018). Therefore, the evidence base is contingent on the relationship between chronic diseases, foods/food groups and the overall diet/dietary pattern (Hu, 2002; Malik *et al.*, 2010).

Diets emphasising certain nutrients have been demonstrated to elicit negative health impacts. For instance, low-fat/high-carbohydrate (LF/HC) diets focusing on total fat intake reduction leaving out the attention to the dietary fat quality/types/sources resulted in the excess intake of refined starch and free sugars that negatively affects cardiometabolic health (Yang *et al.,* 2014). Accordingly, there should be a room for dietary substitution taking into account macronutrient quality, including dietary fat and carbohydrate, within overall dietary pattern. Literature revealed that the impacts of certain macronutrient on the risk of NCD majorly rely on macronutrient replacement. For example, SFA replacing polyunsaturated fatty acids (PUFA) or whole grains are linked to higher CVD risk, but this relationship disappears when replacing refined carbohydrates (Li *et al.*, 2015). This nutrient-based diet approach also removes the attention to synergistic or antagonistic relationships between nutrients within dietary patterns (Sandström, 2001). Additionally, when it comes to diets, people's choices are based on foods, instead of nutrients.

Consequently, dietary guidelines should be developed based on dietary patterns or food choices to account for dietary substitution and alternative food sources. The UK's healthy eating model, the Eatwell Guide; the 2015-2020 Dietary Guidelines of Americans (DGA); and WHO healthy diet advice are food-based that consider appropriate intake proportions within calorie limits to achieve a well-balanced and healthy diet. Less intake of added sugar, SFA and sodium as well as higher intake of fibre and unsaturated fats are encouraged. The dietary recommendations emphasise more inclusion of whole fruits, vegetables, wholegrains and oily fish. In addition, the 2015-2020 DGA and WHO also encourage the consumption of nuts as one of sources of proteins and unsaturated fats

(Public Health England, 2018; U.S. Department of Health and Human Services and U.S. Department of Agriculture, 2015; WHO, 2018).

Besides the interaction of foods and food components, it is also essential to count in specific aspects of foods that may impact disease risk independent of the overall diet for special cases. For example, trans-fatty acid and heart diseases and folate intake for neural tube defect prevention. Tailored nutrient recommendations for at-risk populations, e.g. pregnant women and elderly people, are also considered important. In general, nutrient-focused research is still required to investigate the true causative agents and improve the understanding of food and diet impacts.

1.3.2 Dietary fatty acids and risk factors

Following a continued discussion regarding LF/HC diets, dietary fat quality has been a centre of research interest. Within human nutrition, a principal classification of dietary fat is based on the number and position of double bonds in the constituent FAs (Akoh & Min, 2002). SFA has no double bonds. It is ‘saturated’ with the maximum number of hydrogen atoms for a defined number of carbon atoms. Most animal fats have mostly SFA, such those derived from dairy products and fatty meats. On the other hand, unsaturated FA have double bonds and are further classified into monounsaturated fatty acids (MUFA) with a single double-bond or PUFA with two or more double bonds. Another classification of unsaturated FA considers the position of double bonds relative to the methyl end of the FA chain, the ‘n’ or ‘ω (omega)’ double bond, which can be on carbon 3 from the methyl terminus (n-3 or omega-3) on carbon 6 (n-6 or omega-6) (Akoh & Min, 2002). Although unsaturated FA is also found in red meat flesh and whole milk products, unsaturated FA are majorly sourced from plant-based food and marine sources.

Olives, vegetable oil, nuts and seeds are common sources of MUFA and n-6 PUFA. Oily fish is the main source of long chain n-3 PUFA (eicosapentaenoic acid and docosahexaenoic acid). However, n-3 PUFA (α-linolenic acid) is also found in walnuts, flaxseeds/flaxseed oil, soybean oil and canola oil in significant amounts (USDA, 2019). In general, unsaturated FA naturally has the cis (C-C single bonds adjacent to the double bonds in the same side) configuration as opposed to the trans ('opposite side'). Small amounts of trans-FA naturally occur in meat and milk fat including butter (National Research Council (US) Committee on Diet and Health, 1989). Industrial processing can produce artificial trans-FA through partial hydrogenation; for example, in the process of forming a semi-solid product from vegetable oil.

Saturated and unsaturated FAs modulate plasma cholesterols differently. SFA and trans-FA increase plasma LDL by augmenting LDL formation and reducing LDL turn-over. In addition to LDL increasing effects, trans-fatty acids also decrease HDL. These effects lead to increased risk of developing CVD (Sacks *et al.*, 2017). On the other hand, unsaturated fatty acids have cardioprotective effects (Zhang *et al.*, 2017).

Regulation of plasma LDL concentrations has been shown to involve LDL receptor activity, protein and messenger ribonucleic acid (mRNA) through animal studies. Mustad *et al* in a pig study demonstrated that SFA lowered the concentrations of LDL protein and the expression of related mRNA, leading to lower LDL receptor binding activity and increase LDL levels in blood circulation (Mustad *et al.*, 1996). Long-chain SFAs, e.g. myristic acid (C14:0), palmitic acid (C16:0) and stearic acid (C18:0), are suggested to result in greater CVD risk relative to short- and medium-chain SFAs (Mustad *et al.*, 1996). Woollett *et al* showed that lauric, myristic, and palmitic acids, relative to stearic

acids, had greater effects in increasing rate of LDL production (Woollett *et al.*, 1992). Evidence from metabolomic studies also suggests that palmitic acid is a strong pathologic contributor to atherosclerosis via inflammation from the activation of toll-like receptor 4 (TLR4) as well as oxidative stress from the increment of mitochondrial ROS leading to mitochondrial dysfunction. This also mediates vascular SMC apoptosis (Zhang *et al.*, 2017).

In contrast to SFA, unsaturated FA decrease LDL by increasing the number of LDL receptors in the liver and LDL turn-over (Fernandez *et al.*, 1992; Fernandez & West, 2005). PUFA still elevate the synthesis of cholesterols (Mattson & Grundy, 1985), but PUFA reduces plasma LDL via LDL receptor upregulation (Fernandez & McNamar, 1989). Therefore, lowering plasma cholesterol concentrations, especially proatherogenic LDL, can result from consumption of foods that are low in SFA and trans-FA, and higher unsaturated FA including PUFA.

N-6 and n-3 PUFAs favourably modulate plasma cholesterols with different mechanisms. N-6 PUFA increases the concentrations of LDL receptor mRNA and proteins, lowering LDL levels. N-6 PUFA also directly induces the expression of LXRα, a transcription factor in the liver which stimulates the activity of cholesterol 7α-hydroxylase (CYP7), an enzyme converting cholesterols to bile acids, thereby abolishing excess cholesterols. Furthermore, N-3 PUFA inhibits the expression of sterol regulatory element binding proteins (SREBP)-1, a regulator of protein-encoding genes expressed in lipogenesis and cholesterol synthesis, resulting in reduced lipogenesis and VLDL production. Animal studies also demonstrated that n-3 PUFA expedites chylomicrons clearance and increases

VLDL apo-B conversion to LDL apo-B via enhanced lipoprotein lipase (LPL) expression and attenuated apo-CIII concentrations (Fernandez & West, 2005).

Certain types of dietary fatty acids have also been studied and reported to contribute to liver injury differently. SFA, MUFA, PUFA and trans-fatty acids give significantly different effects in hepatic lipid metabolism showed by animal and human studies. *In vitro* experiments reported that SFA leads to mitochondrial depolarisation increasing ROS, IR and steatosis (de Ferranti & Mozaffarian, 2008; Li *et al.*, 2008). Low levels of PUFAs have been observed in NAFLD patients, especially for long chain n-3 and n-6 PUFAs, relative to individuals with normal liver function with similar diet (Araya *et al.*, 2004). Dietary fatty acid composition impacts the distribution of fatty acids in adipose tissue, but the distribution in the liver may not necessarily reflect diets. Therefore, in NAFLD patients, IHL is particularly saturated lipids and the molecular mechanism of this is still to be fully understood (Allard *et al.*, 2004; Baylin *et al.*, 2002). Regarding trans-FA, sedentary mice developed steatohepatitis following trans-fatty acid intake alone or also added with high fructose (Tetri *et al.*, 2008).

Therefore, to lower the risk of developing dyslipidaemia that may lead to obesity, T2D, CVD and fatty liver, replacing the intake of SFA and trans-FA with unsaturated FA may play an important role. There are various dietary patterns that include sources of unsaturated fats, such as Mediterranean diet, DASH diet, Nordic diet, portfolio diet and vegetarian/vegan diet that are discussed in latter part.

1.3.3 Dietary carbohydrate quality and risk factors

Apart from dietary fat, carbohydrate quality has also been a research interest related to cardiometabolic health. Carbohydrate is the only component which directly raises blood glucose, the major determinant of insulin secretion. Metabolic effects of carbohydrate consumption are not attributable to the length of the chain, but to the glycaemic index (GI), an empirical metric to differentiate foods based on their available carbohydrate impacting blood glucose per gram. Another metric that is also widely used and more practical to predict glucose response is glycaemic load (GL) referring to available carbohydrate in a typical portion of food. Prospective cohort studies have shown that energy adjusted for GI/GL is an independent risk factor of T2D and CVD morbidity and mortality (Dong *et al.*, 2012; Fan *et al.*, 2012; Livesey *et al.*, 2013; Mirrahimi *et al.*, 2012).

Within the context of foods, free sugars, refined grains/starch, whole grains, dietary fibre and resistant starch are types of carbohydrates that are largely discussed and influence dietary carbohydrate quality. Excess intake of free sugars, for example in the form of sugary drinks, plays a role in the obesity pandemic (WHO, 2015). Observational studies reported that modulation in sugary beverages is directly linked to modulation in energy intake and body weight as listed in **Table 1.4**. In the contrary, the consumption of minimally processed whole grains has been reported to positively impact health. Compared to refined grains, whole grains have lower GI and higher content of dietary fibre known for the beneficial metabolic effects as well as phytochemicals with antioxidative and anti-inflammatory potentials (Lattimer & Haub, 2010). Improved cardiometabolic risk factors has been shown following whole grain and dietary fibre consumption by previous studies (see **Table 1.4**). Therefore, choices of food containing

higher whole grain and dietary fibre and lower refined starch and free sugars are favourable and featured in some of dietary patterns benefitting cardiometabolic health as discussed below.

Table 1.4. Carbohydrates and evidence of cardiometabolic health benefits.

Carbohydrate types	Definition	Evidence from epidemiological and clinical studies
Free sugars	• Free sugars include the sugars added in the food processing and preparation as well as sugars which are naturally in unsweetened fruit juices. Lactose from milk and sugars within cellular matrix in whole fruits and other foods are not counted (WHO, 2015).	• A pooled analysis of 5 RCTs in adults consuming *ad libitum* diets for 10 weeks to 8 months (Malik *et al.*, 2013): Decreased sugar intake up to 8% energy intake (EI) was associated with ↓ 0.80 kg body weight. • A meta-analysis of 16 prospective cohort studies with one-year follow up (Te Morenga *et al.*, 2013): Sugar sweetened drinks was associated with being overweight or obese. • A meta-analysis of 39 RCTs for 2 weeks-8-months in healthy, hyperlipidaemic or type-2 diabetic individuals (Te Morenga *et al.*, 2014): ↑ TC, TAG and LDL (relative to lower intake of free sugars). • A RCT in 47 overweight subjects for 6-month sugar sweetened drinks (regular cola) consumption relative to water (Maersk *et al.*, 2012): ↑ BP, TC, TAG, liver fat, skeletal muscle fat, visceral fat (but body weight did not change) • Increment in postprandial TAG and LDL was shown following fructose or high fructose corn syrup (HFCS) intake for 25% EI, but not glucose, in 16 adults (Stanhope *et al.*, 2011).
Whole grain	• Whole grains: bran, germ and endosperm are retained. • Refined grains: bran and germ are removed; only starchy endosperm remained (The Whole Grains Council, 2020).	• Pool analyses of RCTs (Hollænder *et al.*, 2015; Marventano *et al*., 2017; Pol *et al.*, 2013): Improved postprandial glucose and ↓ body fat percentage, TC, and LDL. • Prospective cohort studies (Aune *et al.*, 2013; Aune *et al.*, 2016; Hu *et al.*, 2012; Tang *et al.*, 2015; Zong *et al.*, 2016): ↓ T2D and CVD incidence with whole grain consumption. ↑ T2D incidence with refined grain consumption.
Dietary fibre	• Non-digestible polysaccharides	• A meta-analysis of 22 cohort studies with ≥3-year follow up within 1990-2013 (Threapleton *et al.*, 2013) as well as with T2D risk in 2 multi-country cohorts (Reynolds *et al.*, 2020):

	• Categories: soluble (viscous or non-viscous) and insoluble.	Soluble and insoluble fibre from cereals and vegetable sources was inversely associated with CAD and CVD risk. • A pooled analysis of 24 randomised placebo-controlled trials in >40 y subjects in soluble and insoluble fibre supplementation with average dose 15 g/d (Streppel *et al.*, 2005). ↓ SBP (-3.08 mmHg) and a dose-relationship -3.04 mmHg/g fibre supplement adjusted for age, sex and hypertensive status, study design (parallel vs crossover), study duration and fibre dose. • A pooled analysis of 42 trials in at-risk, prediabetic and T2D adults consuming 1-45 g/d dietary fibre and whole grain for 6-12 weeks (Reynolds *et al.*, 2020): ↓ TC (-0.34 mmol/L), LDL (-0.17 mmol/L) and TAG (-0.16 mmol/L). • A meta-analysis of 12 RCTs with soluble fibre supplements (3-34 g/d, 2-17-weeks) in overweight or obese adults ≥18 y relative to the placebo treatment (Thompson *et al.*, 2017): ↓ BMI (-0.84 kg/m^2), body weight (-2.52 kg), body fat (-0.41%), fasting glucose (-0.17 mmol/L), insulin (-15.99 pmol/L).

1.3.4 Dietary patterns and risk factors

Dietary patterns which feature foods rich in healthy fats and carbohydrates and low in poor dietary fat and carbohydrate sources include Mediterranean, DASH, Portfolio, Nordic and vegetarian/vegan diet. These dietary patterns have been demonstrated to be associated with lower risk of CVD and T2D incidence in diabetic and non-diabetic subjects by cohort studies (Chiavaroli *et al.*, 2019; Galbete *et al.*, 2018: Jannasch *et al.*, 2017; Kahleova *et al.*, 2019; Martínez-González *et al.*, 2019; Sofi *et al.*, 2010). Feeding trials also showed that these dietary patterns improved cardiometabolic health as listed in **Table 1.5**. The 2015-2020 DGA recommends the Mediterranean, DASH and vegetarian diet as healthy dietary patterns (U.S. Department of Health and Human Services and U.S. Department of Agriculture, 2015).

Mediterranean diet (MD) is one of the most largely observed, investigated and reported diet in literature. The PREDIMED trial (Prevención con Dieta Mediterránea) published in the New England Journal of Medicine in 2013 is one of these renowned studies. This study was a large-scale multi-centred parallel RCT conducted in Spain investigating the cardiovascular impacts of MD supplemented with 1 L/week extra virgin olive oil or 30 g/d nuts (a mixture of walnuts, hazelnuts and almonds) relative to low-fat control diet for 4.8 y in a very large adult cohort at high risk of developing CVD. The primary outcomes are the rate of major CVD incidents, such as myocardial infarction (MI) and stroke. A significantly substantial reduction of the incidence of major CVD events was observed in the intervention groups consuming the MD supplemented with olive oil and nuts (Estruch *et al.*, 2013; Estruch *et al.*, 2014). From this study, some sub analyses were conducted. When the health effects of olive oil or nuts alone as an individual diet component were tested using the same cohort data, increased frequency of olive oil or nut consumption

was shown to be associated with a significantly reduced risk of CVD mortality (Guasch-Ferre *et al.*, 2013; Guasch-Ferre *et al.*, 2014).

This study was retracted in 2018 due to error in the procedure of the randomisation leading to inconsistent randomised enrolment and imbalance participation. The researchers statistically reanalysed the data according to the corrected randomisation. In the republished study, these issues were stated, and the results were still similar. Both of the original and reanalysed studies revealed that there was approximately 30% decrease in CVD events in the groups consuming MD supplemented with olive oil and nuts relative to control.

However, the PREDIMED study still remains scientifically significant research that has shown strong evidence through the sustained results from its multicentre trials led by multiple principle investigators over many years. The positive impacts of the MD have also been shown to be consistent across a great number of studies investigating the effects on cardiometabolic risk factors. These virtues have signified the value of starting and conducting research on the effects of individual components of MD, such as nuts, a type of nuts and the nutrients contained therein on an array of cardiometabolic risk markers. Understanding the individual food contribution within the dietary patterns puts the dietary effects into context. Furthermore, alongside dietary patterns, it is also of importance to take dietary habit into account, including eating windows, snacking and time restricted feeding.

Table 1.5. Dietary patterns and evidence of cardiometabolic health benefits.

Dietary pattern	Components	Evidence from epidemiological and clinical studies
Mediterranean diet (MD) (Trichopoulou & Lagiou, 1997)	• Emphasis: high intake in fruits, vegetables, whole bread, grains and cereals, fish, legumes, nuts and olive oil; low to moderate in dairy products, eggs, poultry, and alcohol; and low in meat and meat products.	• A one-year multicentre RCT in 5 European countries in 561 healthy males: ↓ SBP (Jennings *et al.*, 2019). • A meta-analysis of 14 RCTs (duration: 4 weeks to 2.3 years): ↑ FMD by 1.66% (Shannon *et al.*, 2020). • A RCT for 2 years in 90 subjects with MetS relative to control with similar macronutrient proportion: ↓ body weight, IR, CRP and IL-6, ↑ endothelial function (Esposito *et al.*, 2004). • A cross-over RCT for 6 weeks in 12 non-diabetic adults with NAFLD relative to control, a low-fat high carbohydrate diet: ↓ IR and steatosis measured by ^{1}H-MRS (Ryan *et al.*, 2013). • A cross-sectional analysis of 73 adults with NAFLD in Greece: ↓ NAFLD, ↑ insulin sensitivity in adherence to MD (Kontogianni *et al.*, 2014). • Twins Heart Study cohort of 180 twin pairs: ↑ HRV (Dai *at al.*, 2010).
DASH diet (Sacks *et al.*, 1995)	• A nutritional programme targeting a reduction of BP and prevention of hypertension. • Emphasis: variety of nutritious food including vegetables and fruits, low-fat dairy products, whole grains, poultry, fish, and nuts that is simultaneously aligned with US DGA. • Low in fat, meat, sweets, and sugar-loaded beverages. • More calcium, potassium, magnesium, and dietary fibre and less fat, SFA, cholesterol, and	• The RCT including 3-week control Western diet consumption followed by 8-week intervention: ↓ SBP and DBP in both of normotensive and hypertensive subjects (Conlin *et al.*, 2000; Sacks *et al.*, 1995). • An umbrella review of 4 meta-analysis of 31 RCTs: ↓ body weight, SBP and DBP, TC, LDL, fasting insulin (Chiavaroli *et al.*, 2019). • A RCT for 8 weeks in 30 overweight adults with NAFLD, relative to a calorie-restricted control diet:

	sodium relative to the typical Western diet ensuring adequate micronutrient intake.	↓ body weight, BMI, TAG, VLDL, liver enzymes (alanine aminotransferase (ALT) and alkaline phosphatase (ALP)), fasting insulin, CRP (Zade *et al.*, 2016).
Vegan/vegetarian diet (Satija & Hu, 2018; Tonstad *et al.*, 2009)	• Emphasis: more dietary fibre, plant protein, phytosterols and phytochemicals: a variety of whole grains, legume, nuts, fruits and vegetables. • Vegans: no all animal sourced food. • Lacto-ovo vegetarians: no all animal products, except dairy and eggs. • Pescatarians: no animal products, except fish.	• A meta-analysis of 9 RCTs in vegans and lacto-ovo vegetarian diet in diabetic subjects for 4-74-weeks (Kahleova *et al.*, 2019): ↓ body weight, WC, fasting blood glucose, LDL, non-HDL. • A meta-analysis of 7 RCTs (≥6-weeks, healthy population) and 32 observational studies (follow up after ≥1 year) in vegan/vegetarian diet relative to omnivorous diet (Yokoyama *et al.*, 2014): ↓ SBP and DBP.
The Portfolio diet (Jenkins *et al.*, 2003)	• A part of National Cholesterol Education Programme (NCEP) Step II diet. • Emphasis: nuts, plant protein from soybeans and other legumes, soluble fibre and phytosterols as key cholesterol-lowering food.	A meta-analysis of 7 RCTs in 439 hyperlipidaemic subjects: ↓ LDL, non-HDL, TC, TAG, SBP, DBP, CRP (Chiavaroli *et al.*, 2018).
The Nordic diet (Bere & Brug, 2009)	Emphasis: vegetables, fruits, whole grains, legumes, fatty fish, seafood and low-fat dairy.	A pooled study of 15 feeding trials for 6-26 weeks: ↓ TC, LDL, BP, CRP, fasting blood glucose, insulin sensitivity (but the effects on blood glucose and insulin sensitivity disappeared after adjustment for weight loss) (Berild *et al.*, 2017).
Liquid meal replacements (Noronha *et al.*, 2019)	A part of a weight loss diet in diabetic patients comprising a combination of macro- and micronutrients as replacement of one or two meals on daily basis, and usually consumed with fruits, vegetables and nuts.	A meta-analysis of 9 RCTs in overweight/T2D subjects for ≥2-weeks: ↓ body weight, BMI, body fat, WC, fasting blood glucose and insulin, SBP, DPB (but no effects on blood lipids) (Noronha *et al.*, 2019).

1.3.4.1 Snacking

Snacking is a dietary habit that can affect the association of diets and health. Snacks can be defined as either foods consumed between main meals (based on timing) or foods containing 15-25% total energy intake (TEI) (based on calorie content). In nutritional research, snacks are typically defined as foods consumed between main meals. Analyses of national diet and nutrient surveys in many countries show that many populations consume snacks frequently, 2-3 snacks/d in average (Health Canada, 2011; McGuire, 2016; Potter *et al.*, 2018). In the US and Canadian adult populations, the daily snacking prevalence is 97% and 80.4% respectively, and French adult population eats at least one snack every day. Among adult population, snacks contribute to 19-22% daily EI in the UK (Murakami & Livingstone, 2016), 18-24% EI in the US (Piernas & Popkin, 2010), 23% EI in Canada (Garriguet, 2007; Vatanparast *et al.*, 2019) and 18.5% in France (Bellisle *et al.*, 2003).

Snacking is often regarded to be associated with a sedentary lifestyle, excessive eating and choices of low-quality foods. Energy derived from snacks was reported to be associated with increased body weight and lower diet quality assessed using Healthy Diet Index (HDI) and Mediterranean Diet Score (MDS) in the UK adult population (Murakami & Livingstone, 2016). Investigations using national diet and nutrition surveys revealed that most eaten snacks are biscuits, cakes, crisps and chocolate confectionery in the UK (Murakami & Livingstone, 2016; Summerbell *et al.*, 1995); desserts, salty foods and sweetened drinks the US (Piernas & Popkin, 2010); fatty and salty foods in Canada (Garriguet, 2007); and sweets, cereal bars, biscuits and sodas in France (Bellisle *et al.*, 2003). These common snack foods are characterised by high free sugars, SFA and salt and low dietary fibre, which are dietary determinants of developing cardiometabolic risk.

An excess intake of free sugars, SFA and sodium in the UK adult population was observed, as showed by the findings from the UK National Diet and Nutrition Survey (NDNS) 2008-2019, relative to values recommended by the dietary guidelines (Ashford *et al.*, 2020; Bates *et al.*, 2019). This highlights the need for dietary modification to improve population diet quality. Snacking is a convenient target of dietary modification considering the rapid urbanisation and lifestyle changes in current fast-paced society that have led to a decline in traditional mealtimes and formal dining occasions.

Based on examination using the UK NDNS 2008-2012, snacks which made up 20% daily TEI consisted of 55.30% total carbohydrate (23.78% free sugars), protein 9.84% and 35.55% total fat (13.47% saturated fat) (Smith *et al.*, 2017). **Figure 1.8** showed the comparison between UK recommended intake, UK dietary average intake and UK dietary average contribution from snacks.

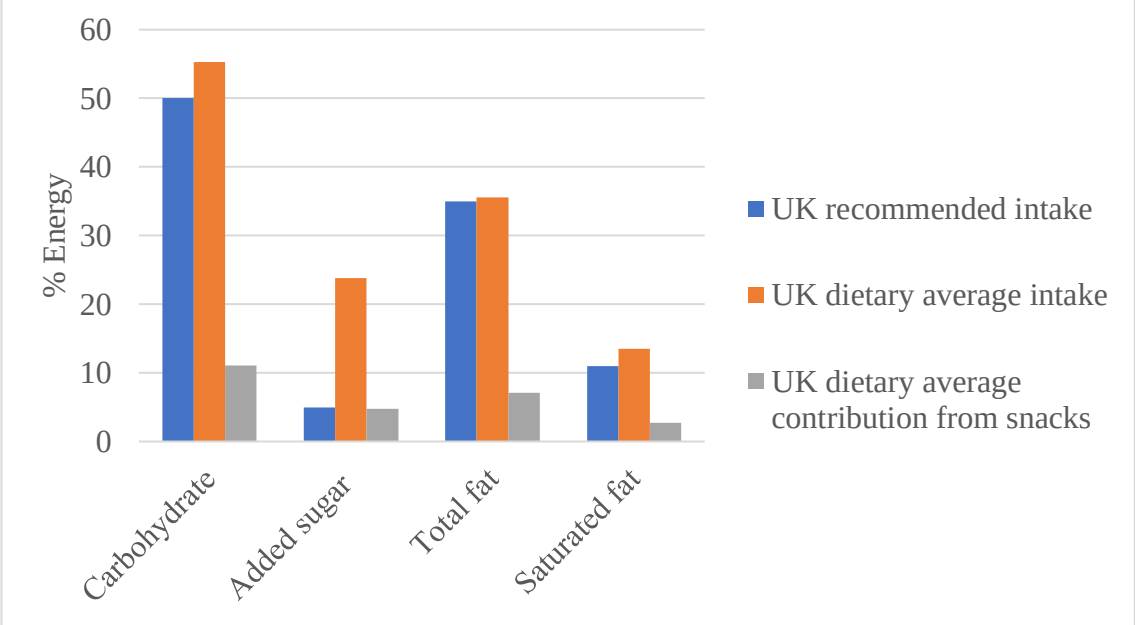


Figure 1.8. Comparison of UK recommended intake, dietary average intake and dietary average contribution from snacks on carbohydrate, added sugar, total fat and saturated fat, adapted from Smith et al. *(2017).*

Of this 20% daily EI from the snacks, based on NDNS analysis, SFA in snacks contribute 3% of total energy. The current average daily intake of SFA is 13.47% while the UK dietary guideline recommends SFA intake to be <11% (Public Health England, 2016). If this excess of 2.47% is replaced by healthier alternative dietary fat, e.g. unsaturated fats, the recommended daily average intake is almost achieved. Similarly, for added sugars, the dietary recommendation is <5% of TEI (Public Health England, 2016), and the current average intake exceeds that by 18.78% energy. If snack sugars are replaced with an alternative energy source, it will also help achieve the target to some extent. Therefore, if typically consumed snacks are replaced by healthier snack food choices, snacking can contribute to lower the overall intake of SFA and free sugars, benefiting cardiometabolic health instead. Indeed, there are some studies reporting that snacks can contribute to nutrient quality of the diets. Having 1-2 nutrient-dense and caloric-balanced snacks between meals have been shown to play a role in meeting the dietary recommendations, such as intake levels of fruits for dietary fibre, vitamins and minerals (Lloyd-Williams *et al.*, 2009; Zizza *et al.*, 2010). Snacking can also avoid severe hunger leading to excess food intake and metabolic overload at mealtimes (Marangoni *et al.*, 2019; Njike *et al.*, 2016). Choosing healthier snack foods, such as tree nuts which are nutrient-dense foods with favourable cardiometabolic effects, is as simple dietary strategy that can give positive health impacts.

1.3.5 The roles of tree nuts in cardioprotective mechanism

Tree nuts are generally energy- and nutrient-dense, and botanically defined as any dry fruits coming from trees with generally a single seed surrounded by hard wall at maturity (Alasalvar & Shahidi, 2009). Edible tree nuts that are widely available and largely consumed worldwide include almonds, Brazil nuts, cashews, hazelnuts/filberts/cobnuts,

macadamia nuts, pecans, pine nuts, pistachios, walnuts and chestnuts. However, relative to other edible tree nuts listed above, chestnuts are less energy-dense and less nutrient-rich (USDA, 2019). Other edible tree nuts include acorn nuts, beech nuts, betel nuts, heartnuts and hickory nuts, but they are not largely produced and eaten (Alasalvar & Shahidi, 2009). The US Food and Drug Administration (FDA) includes coconuts as a tree nut. Nevertheless, based on the botanical category, coconuts are a drupe (Stern, 1997), not a nut.

Tree nut consumption is associated with reduced risk of CVD. A meta-analysis of 3 large prospective cohort studies with a total 210,836 adult subjects (25-75 y males and females, healthy or with medical conditions, such as CVD and cancer) reported that tree nut (≥2 times/week) and walnuts (≥1 times/week) consumption was associated with a 13-19% lower risk of total CVD and 15-23% lower risk of coronary heart disease (CHD) (Guasch-Ferré *et al.,* 2017). Another meta-analysis of 29 prospective cohort studies from US, Europe, Asia and Australia also revealed that higher tree nut intake was associated with lower risk of diabetes, CVD and cancer (Aune *et al.,* 2016). Cardioprotective properties of tree nuts are attributable to their distinct nutrient and non-nutrient compositions, especially the unsaturated FA, dietary fibre, micronutrients, phytochemicals and phytosterols. **Table 1.6** shows nutrient composition of variety types of tree nuts and **Table 1.7** provides information about components of tree nuts that have been largely discussed to be associated with CVD.

Tree nuts are rich in unsaturated FA, with MUFA (oleic acid) accounting for >50% of fatty acids, and low in SFA (USDA, 2019). It is worth noting that almonds have the lowest SFA content of all nuts and walnuts have mostly PUFA, i.e. linoleic acid (LA, C18:2n-6)

and α-linolenic acid (ALA, C18:3n-3); of all edible plants, walnuts have the highest content of ALA (USDA, 2019). Given these unique fat constituents leading to high ratio of unsaturated and saturated FAs, tree nuts have been further investigated and demonstrated to favourably alter blood lipid profile by lowering TC and LDL by some observational and dietary interventional studies as discussed later.

Table 1.6. Nutrient composition of some varieties of tree nuts per one-ounce portion (USDA, 2019).

	Almond	**Brazil nut**	**Cashew**	**Hazelnut**	**Macadamia**	**Pecan**	**Pine nut**	**Pistachio**	**Walnut**
Calories, kcal	164	187	157	178	**204**	196	191	159	185
Protein, g	**6.0**	4.1	5.2	4.2	2.2	2.6	3.9	5.8	4.3
Total fat, g	14.2	18.8	12.4	17.2	**21.5**	20.4	19.4	12.8	18.5
SFA, g	1.1	**4.6**	2.2	1.3	3.4	1.8	1.4	1.7	1.7
PUFA, g	3.5	6.9	2.2	2.2	0.4	6.1	9.7	4.1	**13.4**
MUFA, g	8.9	6.8	6.8	12.9	**16.7**	11.6	5.3	6.6	2.5
Carbohydrates, g	6.1	3.3	**8.6**	4.7	3.9	3.9	3.7	7.7	3.9
Dietary fibre, g	**3.5**	2.1	0.9	2.8	2.4	2.7	1.1	3.0	1.9
Potassium, mg	208	187	187	193	104	116	169	**291**	125
Magnesium, mg	76.5	**107.0**	82.8	46.2	36.9	34.3	71.2	34.3	44.8
Zinc, mg	0.9	1.2	**1.6**	0.7	0.4	1.3	1.8	0.6	0.9
Copper, mg	0.3	0.5	**0.6**	0.5	0.2	0.3	0.4	0.4	0.5
Manganese, mg	0.6	0.3	0.5	**1.8**	1.2	1.3	2.5	0.3	1.0
Selenium, µg	1.2	**543**	5.6	0.7	1.0	1.1	0.2	2.0	1.4
Pantothenic acid, mg	0.1	0.1	0.2	**0.3**	0.2	0.2	0.1	0.1	0.2
Vitamin B6, mg	0	0	0.1	0.2	0.1	0.1	0	**0.5**	0.2
Folate, µg	12.5	6.2	7.1	**32.0**	3.1	6.2	9.6	14.5	27.8
Thiamin, mg	**0.6**	0.2	0.1	0.2	0.3	0.2	0.1	0.2	0.1
Riboflavin, mg	**0.3**	0	0	0	0	0	0.1	0	0
Niacin, mg	**1.0**	0.1	0.3	0.5	0.7	0.3	1.2	0.4	0.3
Vit E, α-tocopherol, mg	**7.3**	1.6	0.3	4.3	0.2	0.4	2.6	0.8	0.2
Calcium, mg	**76.3**	45.4	10.5	32.3	24.1	19.8	4.5	29.8	27.8
Iron, mg	1.1	0.7	**1.9**	1.3	1.1	0.7	1.6	1.1	0.8
Phosphorus, mg	136.0	**206.0**	168.0	82.2	53.3	78.5	163.0	139.0	98.1
Sodium, mg	0.284	0.851	**3.4**	0	1.4	0	0.6	0.3	0.6
Stigmasterol, mg	1.1	**1.7**	0	0.3	0	0.9	0	1.4	0
Campesterol, mg	1.4	0.6	2.6	2.0	2.3	1.7	**5.7**	2.8	1.42
Beta-sitosterol, mg	36.9	18.1	32.0	28.9	30.6	33.2	37.4	**56.1**	24.7

Table 1.7. Cardioprotective mechanisms of tree nuts.

Nutrients in nuts	**Potential mechanism**
MUFA and PUFA	Lower TC and LDL
Plant-based protein (amino acids): high in arginine	Arginine is a precursor of NO
Dietary fibre	Delay gastric emptying Lower LDL
Vitamin E	Lower LDL oxidation
Folates and riboflavin	Reduce blood homocysteine
Magnesium	Prevent ventricle arrhythmia
Phytochemicals, e.g. tannins, flavonoids, phenolic acids, tocotrienols, resveratrol	Provide antioxidative properties
Phytosterols	Lower TC and LDL

Although total fat contents in tree nuts are relatively high rendering them energy-dense, the bioaccessibility of the fats has been shown to be low (Baer & Novotny, 2018; Berry *et al.*, 2008; Mandalari *et al.*, 2008; McArthur & Mattes, 2020). For example, due to the botanical structure of almonds, the fats are encapsulated within cell walls and mastication does not disrupt the cell wall structure completely (Ellis *et al.*, 2004). An *in vitro* digestion model demonstrated that fats in whole almonds, whole blanched almonds and finely ground almonds have been shown to have 9.9%, 13.0% and 39.3% bio-accessibility, respectively (Mandalari *et al.*, 2008). An *in vivo* study, i.e. cross-over RCT, in 20 healthy men also showed that whole almonds attenuate postprandial lipaemia (an independent risk factor for CVD (Bansal *et al.*, 2007; Karpe *et al.*, 1994; Miller *et al.*, 2011; Nordestgaard *et al.*, 2007; Zilversmit, 1979) compared with ground almonds or a control oil (Berry *et al.*, 2008). This actual metabolisable energy of almonds may also explain almonds properties leading to reduction in postprandial glycaemia and lipaemia leading

to gradual insulin release that may result in sustained insulin response, increased insulin sensitivity and longer satiety cascade (Berry *et al.*, 2008; Josse *et al.*, 2007).

Almond particles with intact cell walls were found in stool and suggested to be susceptible towards bacterial fermentation (Ellis *et al.*, 2004), which may confer additional beneficial health effects mediated by the microbiome. Therefore, the botanical structure of almonds, as well as their nutrient composition beneficially impacts health.

The metabolisable fats from walnuts and pistachios were also shown to be different from Atwater factors (Baer *et al.*, 2012; Baer *et al.*, 2016), as reported by a recent chewing study involving 7 healthy volunteers followed by *in vitro* digestion model reported (McArthur & Mattes, 2020). This could be due to the microstructure of these nuts that limits the lipid bioaccessibility (McArthur & Mattes, 2020). Cashews were also shown to have lower metabolisable energy than predicted due to the same phenomenon as occurred in almonds, walnuts and pistachios (Baer & Novotny, 2018). For other types of tree nuts, the information on lipid bioaccessibility is still lacking.

Tree nuts are not only beneficial due to their dietary fat quality and botanical structure, but also due to their fibre content. Dietary fibre in tree nuts is mostly insoluble comprising cellulose, hemicellulose and lignin. Pectin makes up the proportion of soluble fibre in tree nuts (Marlett, 1992). Soluble fibre increases bulk, generates viscosity and slows down macronutrient absorption in stomach and small intestine, thereby delaying gastric emptying and subsequent meal ingestion, inducing satiety, decreasing postprandial glycaemia and potentially leading to improved insulin sensitivity (Jenkins *et al.*, 1976; Schneeman, 1987). Due to this increasing intestinal viscosity characteristic, dietary fibre

has been proposed to have bile acid binding capacity leading to reducing bile acid reabsorption, stimulating *de novo* hepatic catabolism of cholesterol as a bile acid building block and declining cholesterol concentration in the blood (Brown *et al.*, 1999; Kritchevsky & Story, 1974).

Insoluble fibre is inert and low-glycaemic, and has been shown in a cross-over RCT investigating 33 g insoluble fibre cereal intake to have a direct effect in inducing subjective satiety and reducing meal intake (Samra & Anderson, 2007) with the potential mechanism is related with the inverse association of a satiety hormone, cholecystokinin (CCK), with glycaemic and insulin responses to carbohydrate meals (Holt *et al.*, 1992). Insoluble fibre increases meal transit time in stomach and small intestine and finally goes to the colon. Colonic microflora digests it through fermentation producing short chain fatty acids (SCFA), such as acetic acids, propionic acids and butyric acids, that have been suggested to provide cardiometabolic health benefits. Animal studies reported that SCFA elevates proglucagon mRNA in rats (Tappenden *et al.*, 1996) and glucagon-like peptide 1 (GLP-1) production in dogs (Massimino *et al.*, 1998), and human studies showed that SCFA decrease gastric tone, postprandial glycaemia, hepatic glucose production and serum free fatty acids (Brighenti *et al.*, 2006; Ropert *et al.*, 1996; Wolever *et al.*, 1989; Thorburn *et al.*, 1993). Very few studies were conducted to investigate the amount of SCFA produced by tree nut consumption using in vitro digestion models. Mandalari *et al.* reported that propionate and butyrate productions significantly elevated after 8 h incubation fed with finely ground almonds and defatted finely ground almonds and reached the highest concentration after 24 h, while elevated acetate production was observed after 24 h (Mandalari *et al.*, 2008).

Apart from having favourable macronutrient quality, tree nuts are also rich in micronutrients, e.g. riboflavin, folate, vitamin E, potassium and magnesium, phytosterols and bioactive phytochemicals, e.g. polyphenols and other phenolic compounds (Bolling *et al.*, 2010; USDA, 2019). Riboflavin and folate play a role in lowering the concentration of homocysteine that is associated with arterial and venous thrombosis potentially leading to vascular dysfunction (Cheng *et al.*, 2009; Jacques *et al.*, 2010).

Vitamin E and bioactive phytochemicals are antioxidants. Phytochemicals are majorly degraded by gut microbiota in the colon; however, they may still benefit though their antioxidative properties. Antioxidants are mostly located in the skins. If the skins are removed, >50% of antioxidants will be lost (Blomhoff *et al.*, 2006). Processing also reduces antioxidant concentrations in tree nuts, for example, bleaching, blanching and roasting of pistachios or almonds. However, roasting almonds retains more antioxidants compared to bleaching and blanching (Garrido *et al.*, 2008; Seeram *et al.*, 2006). On the other hand, walnuts are mostly consumed raw with skin. Among all types of tree nuts, almonds have the highest content of vitamin E. Regarding the content of minerals, tree nuts contain calcium, magnesium, potassium, selenium, zinc and copper in significant amount, but very little sodium. This composition promotes the prevention of bone demineralisation and arterial hypertension. If tree nut products are salted, however, it will negate the benefits of low sodium content of tree nuts.

Phytosterols provide an advantageous virtue to lower cholesterol absorption. Phytosterols have similar chemical structure as cholesterols. However, given more sizeable hydrocarbon molecule, phytosterols have more affinity for micelles relative to cholesterols. In the intestinal lumen, cholesterols are consequently displaced by

phytosterols from micelles binding resulting in less cholesterols absorbed. This mechanism renders phytosterols cholesterol-lowering capacity (Thompson & Grundy, 2005). A pooled analysis of 124 studies with ≥2-week duration and <10 g/d dose of phytosterols reported that LDL-lowering efficacy by 2% was already observed with ≥2.0 dose <2.5 g/d (Ras *et al*., 2014). Among all types of tree nuts, pistachios contain the highest levels of phytosterols, 214 mg/100 g (USDA, 2019). However, it is unlikely that individuals would reach the required phytosterol intake level from tree nuts alone to reduce LDL.

Regarding the cardiovascular effects of magnesium, literature suggests that it shows anti-arrhythmic action. The putative mechanism is suggested to be related to the function of magnesium as a cofactor of the membrane Na-K pump. The lack of magnesium could lead to reduction of the pump's activity, partial depolarisation and alteration in the activities of cardiac ion channels, including cardiac K^+ ion channel functioning (Agus & Agus, 2001). However, studies reported inconsistency related to this effect of magnesium. A pooled analysis of 12 RCTs conducted by Onalan *et al.* found that magnesium significantly impacts the regulation of cardiac rhythm favourably (Onalan *et al.*, 2007). Magnesium is shown to decrease the incidence risk of postoperative atrial fibrillation through a meta-analysis of 7 double-blind, placebo controlled RCTs (Gu *et al*., 2012). However, a meta-analysis of 10 RCTs conducted by Ho *et al.* showed that magnesium provides insignificant effects to treat atrial fibrillation relative to placebo or other anti-arrhythmic drugs (Ho *et al.*, 2007).

Due to these beneficial components, the associations between tree nut consumption and CVD risk factors as well as their impacts on key drivers of obesity, T2D and CVD have

been investigated in the current research. The following sections describe studies that have been conducted in relation with body weight management, insulin sensitivity, blood lipids and endothelial function, the individual studies are summarised in **Table 1.8**.

1.3.5.1 Studies on tree nut consumption and body weight management

Evidence shows that consumption of tree nuts is not associated with weight gain as shown by four large-cohort epidemiology studies involving >25 y respondents without chronic diseases, i.e. the Adventist Health study) (Fraser *et al.*, 1992), Iowa Women's Health Study (Ellsworth *et al.*, 2001), Nurse's Health Study (Hu *et al.*, 1998) and Physicians' Health Study (Hshieh *et al.*, 2015), although the strengths of the studies were questioned as the dose of nuts consumed was not reported. Tree nut intake with dose ≥¼ ounce/day in >19 y adults was associated with lowered BMI and waist circumference (WC) revealed by cross-sectional analysis using the US National Health and Nutrition Examination Survey (NHANES) 2005-2010 database (O'Neil *et al.*, 2015). However, the results from feeding trials are inconsistent according to the findings from previous studies (Fraser *et al.*, 2002; Sabate *et al.*, 2005; Wien *et al.*, 2003) that are detailed in **Table 1.8**.

1.3.5.2 Studies on tree nut consumption and insulin sensitivity

Tree nuts are low-glycaemic due to the high content of complex carbohydrates as well as low amount of carbohydrate relative to the fat content. In addition to lowered BMI and WC, the cross-sectional analysis using the US NHANES 2005-2010 database also reported that >¼ ounce/day of tree nut consumption was associated with reduced Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) (O'Neil *et al.*, 2015). However, the findings in insulin sensitivity from clinical trials are inconsistent (Tindall *et al.*, 2019; Viguiliouk *et al.*, 2014) as outlined in **Table 1.8**.

1.3.5.3 Studies on tree nut consumption and blood lipids

It has been well established that tree nuts have cholesterol-lowering effects based on observational studies and feeding trials. Tree nut consumption was associated with increased HDL in the US adult population (O'Neil *et al.*, 2015). Improvement in blood lipids was also observed in Iranian adult population (Askari *et al.*, 2013). A recent meta-analysis of 61 interventional studies by Del Gobbo *et al.* with an array of study subjects' health status, i.e. healthy, hypercholesterolaemic, overweight/obese and type-2 diabetic; 5-100 g/d dosage; and 3-26 weeks of study duration showed significant reductions in TC (-4.7 mg/dL), LDL (-4.8 mg/dL), TAG (-2.2 mg/dL) were observed but HDL was maintained (Del Gobbo *et al.*, 2015) Similar findings were also reported by another recent meta-analysis (Mejia *et al.*, 2014), detailed in **Table 1.8**.

1.3.5.4 Studies on tree nut consumption and blood pressure and endothelial function

Although tree nuts have shown an established capacity in lowering blood cholesterol, the findings of the consumption effects on BP is inconsistent. Cross-sectional analysis in the US adult population showed that tree nut consumption was associated with lowered SBP (O'Neil *et al.*, 2015), but the recent meta-analyses conducted by Mejia *et al.* and Del Gobbo *et al.* that include diverse varieties of tree nuts reported that tree nut consumption did not change BP (Del Gobbo *et al.*, 2015; Mejia *et al.*, 2014). However, lowered BP was observed following the consumption of almonds, pistachios or cashews alone (Jalali *et al.*, 2020; Lee-Bravatti *et al.*, 2019; Mohammadifard *et al.*, 2015), outlined in **Table 1.8**.

Even though there are conflicting results in BP, the impacts of tree nut consumption on vascular function have been investigated considering their contents of L-arginine, polyphenols and unsaturated fats that are suggested to contribute to improvement of vascular function. Due to the distinctly rich α-linolenic acid and polyphenol content, walnuts have been a major type of tree nuts investigated for this outcome. In a study involving animals of accelerated atherosclerosis which were fed with a walnut-enriched diet, reduction in endothelin-1 expression was reported and it was suggested that the fat components in walnut could contribute to this effect (Davis *et al.*, 2006). Three clinical trials in healthy, at-risk and diabetic adults showed that walnut consumption with daily dose 36 g/d in *ad libitum* diet for 8 weeks or 6 months increased FMD relative to control (*ad libitum* diet without walnuts) (Katz *et al.*, 2012; Ma *et al.*, 2010; Nijke *et al.*, 2015). Walnuts as replacement of MUFA (32% EI) in MD also improved FMD in 20 hypercholesterolaemic subjects relative to isocaloric MD without walnuts (Ros *et al.*, 2004).

A very limited number of studies have been conducted in almond, pistachio or hazelnut consumption. No study has yet been conducted to investigate the effects of almond consumption on endothelial function by FMD of the brachial artery, the EDV standard measurement, in individuals who are healthy or at risk of developing CVD. In 45 CAD patients, almond consumption for 85 g/d did not improve FMD after 22 weeks (Chen *et al.*, 2015); however, the participants were on many medications which may have masked the effects of the almond consumption on vascular function. Vascular reactivity measured by venous occlusion plethysmography significantly increased by 3.7% following daily snack substitution with 50 g/d almonds for 4 weeks in 20 healthy adult males relative to control with habitual diets (Choudury *et al.*, 2014). Daily pistachio consumption for 20%

EI for 12 weeks did not alter FMD in 30 T2D patients relative to isocaloric control diet (Sauder *et al.*, 2015), although pistachio is rich in phytosterols (USDA, 2019). On the other hand, following daily consumption of hazelnuts for 18-20% EI for 4 weeks, endothelial function improved in 21 hypercholesterolaemic individuals relative to isoenergetic control diet (Orem *et al.*, 2013).

1.3.5.5 Studies on tree nut consumption and heart rate variability

To the latest knowledge, HRV has only been studied following a pistachio intervention (Sauder *et al.*, 2014). A diet with moderate fat (33% fat) containing pistachios (20% EI) for 4 weeks increased HRV during acute stress tasks in T2D patients relative to a LF control diet (27% fat) containing LF/HC snacks. The study suggested that the effects of nutrient displacement may be responsible for the increase in HRV. SFA and sodium from the control diet snacks, such as pretzels and string cheese, were replaced by unsaturated fats and potassium of pistachios (Sauder *et al.*, 2014). Therefore, it is of interest to study the impacts of other types of tree nut consumption on HRV, especially almonds, the most widely consumed tree nut variety type, across the globe and Europe, in which the UK is ono of the leading markets (International Nut & Dried Fruit Council, 2019).

1.3.6 Almond and current evidence on its cardiovascular health benefits

As outlined above, tree nuts are cardioprotective and almonds are the most consumed and available tree nuts in the markets worldwide. The consumption of almond, mainly as snacks, is increasing every year globally (International Nut & Dried Fruit Council, 2019). Nevertheless, there have been no observational and interventional studies investigating

the impacts of almond consumption on cardiometabolic risk factors in the UK healthy adult population, especially as a substitution of suboptimal diet, e.g. snacks.

1.3.6.1 Observational studies on almond consumption and health outcomes

To date, the only observational study published investigating whole almond consumption and health impacts is a cross analysis of the US NHANES 2001-2010 data from 24,808 adults (≥ 19 y) (O'Neil *et al.*, 2016). The study reported that almond consumption (mean intake 29.5 ± 1.5 g/d) was associated with better nutrient intake and diet quality via Healthy Eating Index 2010 (HEI-2010) and lower BMI and WC (O'Neil *et al.*, 2016). However, epidemiological approaches provide insights in the association of the consumption and the cardiometabolic risk factors but are not enough to report causal-effect relationship. In addition, BMI and WC do not represent regional adiposity, e.g. central adiposity related to VAT accumulation, which is a significant factor influencing MetS/obesity. VAT and ectopic fat, such as IHL and IMCL, measured by the most advanced measurement techniques, e.g. MRI/^{1}H-MRS, would be useful to study the effects of almond consumption in regional fat distribution. Therefore, human clinical trials are needed to study the impact of almond consumption on these risk markers.

1.3.6.2 Interventional studies on almond consumption and cardiovascular disease risk factors

Previous RCTs investigating the chronic effects of almond consumption on body weight, BP, fasting blood lipids, insulin sensitivity, endothelial function and liver fat are summarised in **Table 1.8**. Body weight has been shown to decrease after an almond intervention (Wien *et al.*, 2003), however the effects of almond consumption on insulin

sensitivity are inconsistent (Jenkins *et al.*, 2008; Li *et al.*, 2011; Lovejoy *et al.*, 2002). Recent pooled analyses conducted by Lee-Bravatii *et al.* and Musa-Veloso *et al.* reported that 25-168 g/d almond consumption for 4-24 weeks decreased TC, LDL, TAG, body weight and apo-B and maintained HDL in adults who were healthy or at-risk of CVD (Lee-Bravatti *et al.*, 2019; Musa-Veloso *et al.*, 2016), detailed in **Table 1.8**. Information on almond consumption and liver fat is limited. The only study looking at this showed that there was no difference in liver fat observed after 6-week almond (56 g/d) consumption T2D patients (Bowen *et al.*, 2019) as detailed in **Table 1.8**. However, improvement in liver fat following almond consumption is indeed expected to see since previous observational studies investigating Mediterranean-style diet reported that higher adherence was inversely associated with prevalence of fatty liver (Aller *et al.*, 2015; Baratta *et al.*, 2017; Chan *et al.*, 2015; Corte *et al.*, 2017).

To summarise the whole sections above, the worldwide prevalence of T2D and CVD is increasing. These NCDs are also suggested to be inter-related with fatty liver and ANS reported by literature. There are a number of factors influencing the development of these diseases, including lifestyle. Excess calorie intake as well as diet high in SFA, refined starch and free sugars and low in dietary fibre, micronutrients and phytochemicals are attributable to metabolic disturbances leading to these chronic diseases. To reduce the risk of these NCDs, food-based dietary guidelines were developed based on scientific evaluation on overall diet related ill-health. However, according to the UK NDNS report, excess intake of SFA and free sugars occurs in the population. This implicates that lifestyle modification as a scheme of disease prevention is a challenging task. Snacking that contributes to 19-22% TEI can be a target of lifestyle modification. Tree nuts that are widely eaten as snacks have cardioprotective components and show a well-established

cholesterol-lowering capacity and potential in helping manage body weight. However, more investigations into the consumption of almonds – the most consumed tree nuts worldwide are required to reveal its impacts on a broader array of cardiometabolic risk markers leading to more understanding of its potential in reducing CVD risk. The current research gap on almonds, dietary challenges and CVD prevention presents in the next section.

Table 1.8. Studies investigating the effects of tree nut and almond consumption on cardiometabolic risk factors.

Author, year	Study design	Study duration	Number of subject	Subjects characteristics	Dosage	Findings	Comments
Tree nuts: Satiety, body weight management and obesity							
Fraser *et al.*, 1992	The Adventist Study, a prospective cohort study	6 years (1977-1982)	31208 US males and females	Age >25 y, white, non-hispanic, non-diabetic, no heart diseases	>4 times/week	↓ BMI	• Peanuts were included • Dose of nuts consumed was not reported
Hu *et al.*, 1998	Nurse's Health Study, a prospective cohort study	14 years (1980-1993)	83818 US females	Age 34-59 y; no CHD, stroke or cancer	>5 ounce/week	• In individuals with baseline BMI <25 kg/m^2: ↓ BMI of frequent tree nut consumption In individuals with baseline BMI ≥25 kg/m^2: ↓ BMI trend	
Ellsworth *et al.*, 2001	Iowa Women's Health Study, a prospective cohort study	12 years (1986-1998)	34111 US females	Age 55-69 y, postmenopausal, no cancer and heart disease	≥2 servings/weeks (one serving: 28.5 g) vs <1 serving/week	↓ BMI and waist-to-hip ratio of higher frequency of nut intake	This inverse association was not statistically significant
Hshieh *et al.*, 2014	Physicians' Health Study, a	4 years (1999-2002)	20742 US males	Age ≥50 y, no history of serious illness.	Up to ≥5 servings/week	↓ baseline BMI of more frequent nut	

	prospective cohort study					consumers, relative to non-consumers	
O'Neil *et al.*, 2014	US NHANES 2005-2010 cross-sectional analysis		14386 males and females	Age >19 y	≥¼ ounce/day	↓ BMI, WC, HOMA-IR. ↑ HDL	Adjustment for age, sex, ethnicity, poverty index, physical activity level (PAL), smoking status and alcohol intake
Sabate *et al.*, 2005	Cross-over RCT	6 months	90 (50 females and 40 males)	Age 30-72y (mean age 54.3 y), healthy	12% EI walnuts vs control (no-walnut)	Significant weight gain of average 0.4 kg (SD 0.1) adjusted for EI	
Almonds: Satiety, body weight management and obesity							
Fraser *et al.*, 2002	Cross-over RCT	6 months	81 (38 females and 43 males)	Mean age 40.4 y, healthy or overweight	15% EI of almonds vs control (no almonds)	No difference in body weight, relative to the control	
Wien *et al.*, 2013	Parallel RCT	6 months	65 (37 females and 28 males)	Age 27-79 y (mean age 55 y), overweight or obese	Low-calorie diet added with 84 g/d almonds vs isocaloric complex carbohydrate-enriched low-calorie diet.	• 62% greater reductions of body weight • ↓ BMI, fat mass, SBP and insulin sensitivity measured by HOMA-IR • ↓ DBP, fasting blood glucose, insulin, TC,	• Overestimation of subjects' PAL may introduce error in the analysis of the result • There was 6-7% difference in

						TAG, LDL and LDL:HDL in both treatment groups to similar extent	carbohydrate intake between groups that may deviate the isocaloric concept of the study leading to error in findings
Tree nuts: Insulin sensitivity							
Viguiliouk *et al.*, 2014	Meta-analysis of 12 RCTs	1-20 months	1080 males and females	Mean age 51-66 y, obese or T2D subjects	28-113 g/d of tree nuts (almonds, Brazil nuts, cashews, hazelnuts, macadamia nuts, pecans, pine nuts, pistachios and walnuts)	• Fasting blood glucose decreased by 0.15 mmol/L • No differences in fasting insulin and HOMA-IR	
Tindall *et al.*, 2019	Meta-analysis of 40 RCTs	1-12 months	2832 males and females	Mean age 24-66 y; healthy, at risk of developing CVD or T2D (overweight, dyslipidaemia or prediabetic) or T2D patients	28-128 g/d peanuts and tree nuts (almonds, cashews, hazelnuts, mixed nuts, pecans, pistachios and walnuts)	• No difference in fasting blood glucose • ↓ HOMA-IR (WMD -0.23) and fasting insulin (WMD -0.40 μIU/mL)	
Almonds: Insulin sensitivity							
Jenkins *et al.*, 2008	Cross-over RCT	4 weeks	27 (12 females	Age 48-86 y (mean age 64y), men and postmenopausal	A muffin phase (control) vs 1 full-dose almond	• ↓ Body weight • No differences in fasting blood	

			and 15 males)	women who were hyperlipidemic	(22.2% of TEI (73 ± 3 g/d)) vs half-dose almond plus half-dose muffin	glucose, insulin and HOMA-IR
Li *et al.*, 2011	Cross-over RCT	4 weeks	20 (11 females and 9 males)	Mean age 58 y, T2D patients	20% EI unsalted whole almonds in NCEP Step II diet with carbohydrate 56%, protein 17%, and fat 27%, (SFA <7% and PUFA <10%) vs NCEP Step II control diet	↓ Body fat, fasting blood glucose, insulin, HOMA-IR, TC, LDL, LDL:HDL, NEFA, Apo-B and Apo-B:Apo-A1
Lovejoy e*t al.*, 2002	Open-labelled	4 weeks		Age 20-50 y (mean age 25.1 y), healthy with normal glucose tolerance	100 g/d	• No difference in insulin sensitivity • ↓ TC and LDL • ↑ Body weight
Tree nuts: Blood lipids and blood pressure						
Askari *et al.*, 2013	the Isfahan Healthy Heart Program, cross-sectional analysis	1 year (in 2007)	9660 males and females	Age ≥19 y	≥4 times/week of intakes of four types of nuts: walnuts, almonds, pistachios and hazelnuts	↓ TC, TAG, LDL and apo B:apo A in females ↓ TAG, LDL and apoB:apoA in males

Mejia *et al.*, 2014	A meta-analysis of 49 RCTs	3 weeks-18 months	2226 males and females	Median age 50.2 y; healthy, dyslipidaemia, MetS or T2D subjects	25-100 g/d tree nuts (walnuts, almonds, pistachios, cashews, pecans, hazelnuts and macadamia nuts)	• ↓ TAG (-0.06 mmol/L) • HDL was maintained • No effects on BP
Del Gobbo *et al.*, 2015	A meta-analysis of 61 dietary interventions (RCT and non-RCT)	3-26 weeks	2582 males and females	Median age 45 y; healthy, hypercholesterolaemia, overweight/obese or T2D subjects	5-100 g/d tree nuts (walnuts, almonds, pistachios, cashews, pecans, hazelnuts, macadamia nuts and Brazil nuts). Each trial included was standardised to dosage 1 ounce/d (28.4 g/d) for the effect size	• ↓ TC (-4.7 mg/dL), LDL (-4.8 mg/dL) and TAG (-2.2 mg/dL) • HDL was maintained • ↓ TC and HDL in each sub-analysis for walnuts, pistachios, macadamia nuts, almonds, hazelnuts • ↓ TAG in sub-analysis for almond • No effects on BP
Mohammadifard *et al.*, 2015	A subanalysis of 3 RCTs for pistachios from a meta-	4 weeks	75 males and females	Age 21-75 y; healthy or dyslipidaemic subjects	30-100 g/d pistachios (raw, roasted, salted, unsalted)	• ↓ SBP (-1.82 mmHg) and DBP (-0.80 mmHg) in sub-analysis for pistachios

	analysis of 21 RCTs						
Jalali *et al.*, 2020	A meta-analysis of 3 RCTs	4-12 weeks	392 males and females	Mean age 45-56.8 y; healthy, MetS or T2D patients	30-42 g/d cashews	• ↓ SBP (-3.39 mmHg)	One of the studies did not report the dose
Almonds: Blood lipids and blood pressure							
Musa-Velosso *et al.*, 2016	A meta-analysis of 18 RCTs	4-24 weeks	837 males and females	Mean age 22-66 y; healthy, at-risk of developing T2D (pre-diabetic or dyslipidaemic) or diabetic	25-168 g/d (unroasted, unsalted, roasted, whole, or powder forms; in meals or as snacks)	• ↓ TC, LDL and TAG • HDL were maintained	
Lee-Bravatti *et al.*, 2019	A meta-analysis of 15 RCTs	4-16 weeks	669 males and females	Mean age 24-64 y; healthy or at-risk of developing CVD (hypertensive, dyslipidaemia or diabetic)	25-100 g/d	• ↓ TC, LDL-C, body weight and apo-B • ↓ DBP with >42.5 g/d consumption vs ≤42.5 g/d	
Tree nuts: Endothelial function							
Ros *et al.*, 2004	Cross-over RCT	4 weeks	20 (12 females and 8 males)	Age 26-75 y (mean age 55 y), moderate hypercholesterolemia but no chronic illness	Walnut-enriched MD (walnuts replaced 32%EI from MUFA) vs isocaloric MD control without walnuts	↑ FMD (Baseline 3.4 ± 3.7% vs endpoint of treatment 5.9 ± 3.3% and control 3.6 ± 3.3%)	In this RCT, the changes in FMD was also shown to be inversely correlated with TC:HDL

Ma *et al.*, 2010	Cross-over RCT	8 weeks	24 (14 females and 10 males)	Age 30-75 y (mean age 58 y), T2D patients	56 g/d walnut-enriched *ad libitum* diet vs *ad libitum* diet control without walnuts	↑ FMD (2.2 ± 1.7% vs control 1.2 ± 1.6%, from baseline)
Katz *et al.*, 2012	Cross-over RCT	8 weeks	46 (28 females and 18 males)	Age 30-75 y (mean age 57.4 y); BMI >25 kg/m^2 with elevated WC and ≥1 additional MetS signs	56 g/d walnut-enriched ad libitum diet vs *ad libitum* diet control without walnuts	↑ FMD (treatment effect 1.1%)
Nijke *et al.*, 2015	Parallel RCT	6 months	112 (81 females and 31 males)	Age 25-75 y (mean age 54.5 y), at risk of developing T2D	56 g/d walnut-enriched *ad libitum* diet vs *ad libitum* diet control without walnuts	↑ FMD (1.94 ± 3.76 vs control 1.54±4.31 from baseline, adjusted for EI)
Sauder *et al.*, 2015	Cross-over RCT	4 weeks	30 (15 females and 15 males)	Age 40-75 y (mean age 56.1 y), T2D patients	A moderate-fat diet (33% fat) containing pistachios (20% EI) vs isocaloric control (a LF control diet (27% fat) containing LF/HC snacks)	No difference in FMD
Orem *et al.*, 2013	Double control	4 weeks	21 (3 females	Mean age 44.6y, hypercholesterolaemia	18-20% EI hazelnut-enriched	↑ FMD by 56.6%

	sandwich model intervention		and 18 males)		diet vs control diet without hazelnut		
Almonds: Endothelial function							
Choudhury & Griffiths, 2014	Parallel RCT	4 weeks	60 males	Age ≥18y, healthy	50 g/d almonds vs control (no almond)	The vascular reactivity measured by FMD of venous significantly increased by 3.7% relative to control	
Chen *et al.*, 2015	Cross-over RCT	6 weeks	45 (27 females and 18 males)	Age 45–77 y (mean age 61.8 y), CAD patients	Incorporation of almonds (85 g/day) into the NCEP Step 1 diet vs NCEP Step 1 without almonds	No difference in FMD	Medications taken by the study subjects might have masked the effects of almonds
Tree nuts/Almonds: Liver fat							
Bowen *et al.*, 2019	Parallel RCT	8 weeks	76 (31 females, 45 males)	Age 20-70 y (mean age 60.6 y), at risk of developing T2D and T2D patients	56 g/d almond vs isocaloric snacks (higher carbohydrate biscuit snacks)	No difference in liver fat	
Tree nut: Heart rate variability							
Sauder *et al.*, 2014	Cross-over RCT	4 weeks	30 (15 females and 15 males)	Age 40-75 y (mean age 56.1 y), T2D patients	A moderate-fat diet (33% fat) containing pistachios (20% EI) vs isocaloric control (a LF control diet (27%	• ↓ Ambulatory SBP, with the greatest decrease during sleep • ↑ HRV during acute stress tasks	

		fat) containing LF/HC snacks)	(↑ rMSSD, HF and LF; these measures are explained in the **Table 2.8** in the Method chapter)

1.4 Research gap on almonds within the scope of current dietary challenge and cardiovascular disease prevention

Contemporary fast-paced daily routines prompt a snacking lifestyle that ultimately contributes 19-22% of TEI (Bellisle *et al.*, 2003; Garriguet, 2007; Murakami & Livingstone, 2016; Piernas & Popkin, 2010; Summerbell *et al.*, 1995; Vatanparast *et al.*, 2019). According to the snacking report in the UK and Ireland, the most consumed snacks are crisps and biscuits which contain high SFA, starch, free sugars and salts, the dietary determinants of CVD (Bord Bia – Irish Food Board, 2018). Reflecting on the consistently rising prevalence of CVD, dietary shifts to healthier food choices are of necessity. Snacking is a practical target for dietary modification taking into account its significant proportion of total daily energy intake. Tree nuts, including almonds, that are normally consumed as snacks in various forms, raw or processed, including roasted or blanched as whole seeds with skin or without, sliced or grounded, have been suggested to be cardioprotective due to high content of unsaturated FA and dietary fibre and low content of salts present as healthier options for snack replacement.

Due to the cardioprotective components, almond consumption and cardiovascular health has been a focus of many studies. Most literature majorly reported their effects on traditional intermediary risk factors of CVD, e.g. body composition, fasting blood glucose and lipids, insulin sensitivity, other circulatory biomarkers and BP. Furthermore, research investigating the impact of almond consumption on novel key drivers of cardiometabolic diseases due to atherosclerosis, such as ED and fatty liver, was limited in healthy adult population, the largest population category.

In the context of the UK adult population, the consumption trend of tree nuts, including almonds, and its association with CVD risk was still unknown. To help reduce CVD risk in the UK healthy adult population, it is also worth investigating the impacts of snacking with whole almonds for 20% daily estimated energy requirement (EER) as an isocaloric replacement of those typical snacks mentioned above on cardiometabolic disease risk factors, including the novel key drivers, endothelial function and liver fat, and other markers.

1.5 Hypotheses

In response to current suboptimal dietary challenges in CVD risk reduction scheme, it was hypothesised that substitution of typical snacks high in SFA, refined starch and free sugars with whole almonds would improve cardiometabolic disease risk measures, including improved endothelial function and decreased liver fat. This overall hypothesis was examined through some studies conducted and presented in specific chapters.

Chapter 3 and 4: Analysis of cross-sectional data was performed to explore the prevalence of tree nut and almond consumption in the UK adult population and test the hypothesis that tree nut and almond consumption is associated with better diet quality and lower CVD risk measures.

Chapter 5 and 6: A RCT was conducted to test the hypothesis that snacking on almonds (rich in unsaturated FA and dietary fibre and low in sodium) in displacement of typical snacks (high in SFA, starch, free sugars and salts) reduces cardiometabolic risk measures. Specifically; 1) improving endothelial function and lowering liver fat concentrations as novel key parameters; and 2) lowering BP, fasting blood glucose and lipids, other

circulatory biomarkers and pancreatic and muscle fats as well as increasing insulin sensitivity and HRV.

1.6 Aims

This doctoral programme aimed to address the current research gap and test the above hypothesis through observational and dietary interventional studies.

The first aim was to understand the trends of tree nut and specifically almond consumption in the UK adult population and examine the association between their consumption and overall health, including diet quality and CVD risk. For these reasons, observational studies were conducted, i.e. cross-sectional analysis using data from adult respondents (≥19 y) involved in the UK National Diet and Nutrition Survey (NDNS), the UK nationally representative database. These studies were detailed in Chapter 3 and 4.

The second aim was to investigate the effects of snacking on almonds in displacement of typical snacks high in SFA, starch and sugars and low in dietary fibre for 20% EER in the UK adults on cardiometabolic risk markers. A RCT dietary intervention study was undertaken, and it examined the impacts through 1) novel key markers, including endothelial function and liver fat accumulation; 2) other risk factors, such as body composition; fasting blood glucose and lipids, insulin sensitivity and other circulatory biomarkers; clinical and ambulatory BP; pancreatic and muscle fats; cardiac autonomic function via HRV for 24 h and during stress tasks; SCFA; and lipoprotein size via nuclear magnetic resonance (NMR). Chapter 5 and 6 detail this study. Chapter 5 covers the impact of snacking almonds on all measures, except HRV during stress tasks which is included in Chapter 6.

As a long-term goal, the results of these studies might suggest that a simple dietary swap between typically consumed snacks and tree nuts should be considered as a part of CVD risk reduction. Incorporating tree nuts in daily diets is regarded as practical and convenient dietary modification to lower CVD risk.

Chapter 2: Methods

This chapter covers some method parts of studies conducted in this doctoral programme that are not fully described in the published papers.

2.1Cross-sectional analysis

The statistical methods used in the cross-sectional analysis of the NDNS rolling programme (NDNS-RP) database is fully explained in the method section of Chapter 3 and 4.

2.2ATTIS study: a randomised controlled trial

The majority of ATTIS study methods are explained in the section "Subjects and Method", and Supplemental Method of Chapter 5, including recruitment of the sample population, design, dietary intervention, snack provision, outcomes analysis procedure, power calculation, statistical analysis and CONSORT diagram. Where the author had a major role in establishment of the method in the Department of Nutritional Sciences or method development, some aspects are only briefly described in Chapter 5, including: control snack development and acceptability testing/feasibility study; reproducibility of FMD measurements; liver and pancreatic fat quantification via MRI; IHL and extramyocellular lipid (EMCL) and intramyocellular lipid (IMCL) via MRS; and HRV measurement in response to acute stress procedure, thereby the further details are written here in Chapter 2.

2.2.1 Overview of the ATTIS study

The ATTIS study is a parallel RCT conducted between June 2017 and May 2019, aiming to assess cardiometabolic health effects of the typical snack displacement with whole almonds for 20% EER. The study was reviewed and approved by Health Research Authority, the London – Camberwell St Giles Research Ethics Committee (reference:

16/LO/1920) and also registered at ClinicalTrials.gov (identifier: NCT02907684). Prior to participation, all participants provided written, informed consent (**Appendix 5)**. The metabolic assessments were carried out at the Metabolic Research Unit (MRU), King's College London (KCL) and Clinical Research Facility (CRF) at St Thomas' Hospital, and the MRI visit was conducted at XMR, St Thomas' Hospital and Cancer Centre, Guy's Hospital. All the participant information sheets, questionnaires and clinical research form for measurement data records are attached in the appendices.

In this study, there were two treatment arms: control and almond group. The control group was designed to reflect typical snack consumption while the almond group consumed dry roasted whole almonds. Control snacks were formulated and developed to represent the nutrient profile of the average habitual snacks in the UK that were detailed in this Method section. The ATTIS study assessed the following parameters: endothelial function (FMD), regional fat distribution (SAT and VAT) and ectopic fat accumulation (IHL, intrapancreatic lipid (IPL) and IMCL), anthropometry measures, ambulatory blood pressure (ABP), ambulatory HRV, insulin sensitivity, blood glucose and lipid profiles, plasma fatty acids, SCFA from stool samples.

2.2.2 Control snacks – development and acceptability/feasibility study

As mentioned above, the control snacks of the ATTIS study reflected the UK average snack nutrient profile. Therefore, the control snack development was very crucial to ensure an appropriate control arm given the influence of nutrient displacement. Formulation of the control snack for ATTIS study included 1) the calculation of the average nutrient profile of snacks consumed by UK adults, conducted by MSc students, Lucy Francis and Monica Sharma, and the research assistant (RA)/dietitian, Leanne

Smith, 2) the formulation and development of the control snack with this profile for use in the main ATTIS trial, conducted by the RA/dietitian, and 3) the acceptability and feasibility study conducted by the RA/dietitian and the author to ensure that the control snacks were acceptable and had neutral effects to body composition and blood lipids.

2.2.2.1 Estimation of average nutrient profile of UK typical snacks

The first stage of the ATTIS control snack development was the estimation of UK average snack nutrient profile. There were several steps within this process. First, based on snack food categories classified by the UK and Irish Snacking Report 2014, snack food data including macro- and micronutrient profiles were extracted from the UK NDNS 2008-2012 database. The categories of snack foods according to the snacking report classification was as follows: confectionery (21%); crisps including popcorn and nuts (15%); dried and fresh fruits (12%); bread and rolls (11%); sweet bakery and buns (9%); fast food, biscuits and dairy (6%); health bars and hot/cold foods (4%) and cereals, smoothies, shakes and drinks (3%) (Bord Bia – Irish Food Board, 2014). There was a total of 577 snack foods identified from a list of 1000 food items in the UK NDNS 2008-2012 database based on snack categories from the snacking report. The list of snack food items identified from the database is presented in **Table 2.1** below.

Second, due to selection bias/error that might be induced by the consumption of main meal leftovers as snacks, such as breads and rolls, fast foods and cereals, the proportion of contribution from each snack category was calculated based on the share percentages of each snack category out of the total snack consumption. These data from the snacking report were generated from surveys involving 3000 respondents of about 12,244 snacking

occasions that was conducted online for ≥16 y adults aged and via completion of food diaries for mothers with 10-15 y kids.

Table 2.1. Snack food items identified in the UK NDNS 2008-2012 database based on snack categories classified by the UK and Irish snacking report 2014.

Snack category	Snack items identified in the UK NDNS 2008-2012 database
Confectionary	Milk chocolate, dark chocolate, jellies and hard sweets
Crisps	salted peanuts, crisps, healthier crisps, tortilla/doritoes, puffed crisps, popcorn, pork snacks, pretzels and Bombay mix
Bread and rolls	Brown sliced, white sliced, white roll/baguette, brown roll/baguette, toast, bagel/panini/wrap, plain crackers, oat cakes, cheese melts, savoury rice cakes, sweet rice cakes and breadsticks
Sweet bakery and buns	Fresh sponge/cup cake, cookies, tarts/pies/crumbles, muffins/danish/croissant and ambient bakery
Fast food	Chips, pizza, burger, variety, fish, kebabs, noodles, rice, chicken, beef and sausage/pork
Dairy	Yoghurt, cheese, egg, milk, ice cream, ice pop and sorbet
Non confectionery bars	Diet/low calorie bar, protein bar, natural ingredients bar, breakfast biscuits and breakfast bars
Hot and cold foods	Chicken, ham/bacon, beef, fish, pork, sausages, lamb/mutton, duck, soups, pasta/rice/noodles, baked beans, wedges, reheated meals, potatoes, potato chips, ready meals, creps
Breakfast cereal	Cold cereal, hot cereal, poptarts, museli/granola
Smoothies/shakes	Milk and others

Following the data extraction, the mean of percentage of energy contribution of each snack product was calculated, resulting in the nutrient profile of the average UK snack. **Table 2.2** shows the average nutrient profile (% energy) from snacks in the UK.

Table 2.2. The UK average snack nutrient profile.

Nutrients	% Energy
Total carbohydrate	55.30
Added sugar	23.78
Protein	9.84
Total fat	35.55
Saturated fat	13.47
MUFA	12.71
PUFA (n-3 and n-6)	5.27
n-3 PUFA	0.70
n-6 PUFA	1.62

2.2.2.2 Formulation and development of control snacks

Following the estimation of the UK average snack nutrient profile, control snacks were developed based on this nutrient profile using nutrition analysis software (Nutritics, version 4.3). Control snacks developed were sweet and savoury mini muffins that were designed to contain 80 kcal so that the participants would have several muffins that equalled to 20% EER to be eaten throughout the day, for example at snacking times in between mealtimes. For a person who had daily energy intake 2000 kcal, the 20% of EER would be 400 kcal, thereby this person would eat 5 mini muffins per day and would be asked to spread out the consumption of the mini muffins during the day, 2 mini muffins in between breakfast and lunch, another 2 in between lunch and dinner and the last one after dinner. There are some flavours of mini muffins developed in the beginning: vanilla, lemon, orange, banana and caramel for sweet flavours and garlic and parsley, chili and cheese for savoury flavours.

The nutrient composition of the developed mini muffins was verified through nutrition analyses conducted by a BSc student, Momena Rokib, at a lab at KCL. The nutrient

composition resulted from the analyses is presented in Supplemental Table 2 of Chapter 5 alongside the ingredients of each control mini muffin.

2.2.2.3 Feasibility study

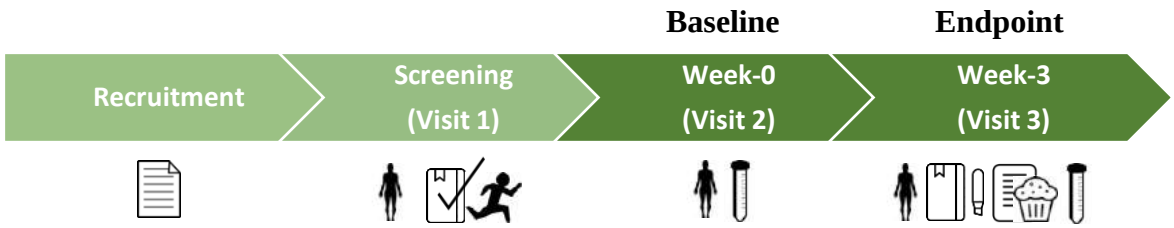
Feasibility study subjects, design, and procedure

After the control snacks were developed, a feasibility/acceptability study was conducted in September-November 2016 to verify the acceptability of the control mini-muffins and assess whether they had neutral effects on body composition and fasting blood lipids. **Figure 2.1** shows the flow of this feasibility study. Recruitment was conducted in September 2016 via electronic advertisement, such as Gumtree and Facebook. Healthy men and women (30-60 y) who were regular snack consumers (>2 snacks/d) were recruited. Exclusion criteria included gluten intolerance and other conditions described in Supplemental Table 1 of Chapter 5.

Each potential subject received participant information sheet and filled out pre-screening questionnaire to assess their eligibility for a screening visit. Prior to screening, participants were fasting (±12h) and abstain from high fat meals and strenuous activities. During screening visits, informed consent was obtained and eligibility for participation in the study was assessed from the results of measurements of anthropometry, including body weight (kg) and height (cm), BMI (kg/m^2), WC (cm) and percentage of body fat, as well as clinical seated blood pressure (cBP, mmHg). Eligible participants filled out 4-d estimated food and physical activity diaries.

All subjects consumed 20% of EER from control mini muffins for 3 weeks. During the study, participants were advised to replace their habitual snacks with these control mini muffins and avoid consumption of other snacks, nuts and food products containing nuts,

but maintain other aspects of their habitual diet as well as physical activity. The 20% of energy requirement was estimated using Henry equation (Henry, 2005) and PAL based on 4-d estimated food and physical activity diaries. At pre- (baseline) and post-intervention (endpoint), anthropometry and clinical blood pressure measurements and fasting blood sample collection via a single venepuncture to analyse and analysis of blood lipid analysis, such as TC (mmol/L), TAG (mmol/L), LDL (mmol/L), HDL (mmol/L) and TC:HDL were conducted. Participants collected control mini muffins at the end of their baseline visit. Snack provision protocol is described in Supplemental Method of Chapter 5. At the end of endpoint visit, participants filled out acceptability questionnaires, provided in **Appendix 2**. Another 4-d estimated food dairy was also completed by all subjects during the last week of intervention in which it was used to assess their dietary compliance.



Participant information sheet, pre-screening questionnaire

Informed consent taking, eating habit questionnaire, 4-d food and activity dairies

Anthropometry (body height, weight, BMI, % body fat and WC) and cBP measurements

Fasting blood sample collection

4-d food diary

Acceptability questionnaire

Location: Baseline and endpoint visits took place at MRU, KCL.

Figure 2.1. Feasibility study flow.

Measurements conducted in this feasibility/acceptability study are described below. The results of the study were also presented in this section later.

Anthropometry and blood pressure measurements

Body weight, % body fat and BMI by bioelectrical impedance analysis were measured using Tanita BC-418MA; Tanita Ltd., Middlesex, UK. WC was measured three times and the average value was taken. OMRON M2 Basic Intellisense monitors (OMRON Healthcare UK Ltd., Milton Keynes, UK) were used to measure cBP (clinical SBP (cSBP) and clinical DBP (cDBP)) according to British Hypertension Society guidelines. BP measurement was measured three times and the average value was taken from two measurements in which the readings were least different.

Blood sample analysis procedure

Fasting blood samples were collected at MRU, KCL, by trained phlebotomists using serum separator tubes. All the blood samples were centrifuged (3000 rpm, 4° C, 15 min; Eppendorf centrifuge 5702 R, Stevenage, UK). Serum samples from study days were stored at -80° C. Fasting blood lipids were analysed using an ILAB 650 auto-analyser (Werfen International, Milan, Italy) following standard biochemistry analysis procedure.

Statistical analysis

Statistical analysis was conducted using IBM SPSS 25. Baseline values of measurement and treatment effects were presented as mean ± standard deviation (SD). Paired t-test was used to examine whether there were differences between endpoint and baseline values. A two-sided p-value of <0.05 was considered to show statistical significance.

Feasibility study results

A total of 9 subjects (6 males and 3 females) completed the study and the baseline characteristics were shown in **Table 2.3**. The feasibility study demonstrated that there were no significant changes in plasma lipids and body composition. **Table 2.4** shows the measurement results.

Table 2.3. Baseline characteristics of the feasibility study participants from screening visits.

	Mean value ± SD
Age, y	39.1 ± 7.8
Weight, kg	76.3 ± 22.0
BMI, kg/m^2	25.9 ± 5.4
Body fat, %	24.2 ± 7.8
WC, cm	88.2 ± 0.8
cSBP, mmHg	123.2 ± 11.1
cDBP, mmHg	82.8 ± 10.9

Table 2.4. Comparison of blood lipid profile and body weight between pre- and post-intervention of ATTIS feasibility study.

	Mean value ± SD[1], n=9	
	Baseline	**Endpoint**
TC, mmol/L	5.32 ± 1.15	5.14 ± 0.93
TAG, mmol/L	1.26 ± 0.89	1.32 ± 1.06
HDL, mmol/L	1.54 ± 0.45	1.51 ± 0.40
LDL, mmol/L	3.20 ± 0.96	3.02 ± 0.79
TC:HDL	3.75 ± 1.50	3.65 ± 1.28
Body weight, kg	76.26 ± 22.04	75.91 ± 21.46

[1]No significant differences found between baseline and endpoint values

Acceptability assessment results

Lemon, orange, banana, caramel, garlic and parsley and cheese were the preferable sweet and savoury mini muffins according to the participants. The taste, texture and colour were acceptable. For the ATTIS study, these flavours were proceeded.

To summarise, the control mini-muffins developed did not alter body composition, BP and blood lipid profile. The organoleptic properties of these control mini-muffins were also acceptable. Therefore, it confirmed that these mini-muffins were used as control snacks in the ATTIS study.

2.2.3 Primary outcome: endothelium-dependent vasodilation (EDV) by flow-mediated dilation (FMD) measurement

As explained above, one of the ATTIS study's primary outcomes were EDV measured by FMD. There are various techniques to assess endothelial function, including both of invasive and non-invasive methods (see **Table 2.5**), but EDV is most widely measured via a reproducible and non-invasive technique that is correlated with the gold standard of coronary function, i.e. FMD of brachial artery, generated by shear-stress during the assessment, for example using blood pressure cuff inflation and release applied to the forearm to induce hyperaemia (Al-Qaisi *et al.*, 2008). FMD value is quantified as percentage of artery diameter change following shear-stress-induced hyperaemia relative to baseline diameter. In general, artery is dilated by 5-15% at hyperaemia (Walther *et al.*, 2004). Furthermore, the FMD value is predictive of CVD incidents in multi-ethnic adult populations (Yeboah *et al.*, 2009). Reduced EDV indicates impaired endothelial function mediated by decreased NO bioavailability.

Table 2.5. Endothelial function measurement techniques (Al-Qaisi et al.*, 2008).*

Non-invasive techniques	Attributes
Coronary epicardial vasoreactivity (QCA)	Acetylcholine infusion, exercise, pacing or cold pressor test as stimulus. Gold-standard, directly assesses epicardial coronary macrovascular function, but limited to patients undertaking angiography, invasive, expensive, time consuming and less reproducible.
Coronary microvascular function – Doppler wires	Acetylcholine, adenosine or papaverine infusion as stimulus. Directly assesses coronary microvascular function, but limited to patients undertaking angiography, invasive, expensive, time consuming and less reproducible.
FMD of brachial artery	Reactive hyperaemia as stimulus. Assesses brachial artery function, correlated with the gold standard – QCA, widely available, reproducible and inexpensive, but operator dependent and high inter-observer variability.
Flow-mediated magnetic resonance imaging	Reactive hyperaemia as stimulus. Assesses brachial artery function and less operator-dependent, but expensive and less accessible.
Venous occlusion plethysmography	Acetylcholine as stimulus. Assesses forearm microvascular function and widely available, but invasive and time consuming
Digital Peripheral Arterial Tonemetry (PAT) assessment	Reactive hyperaemia as stimulus. Assesses finger microvascular function, correlated with the invasive coronary microvascular function measurement (Bonetti *et al.*, 2004), widely available, non-invasive and automated but less endothelial-specific.

The FMD protocol of the ATTIS study is illustrated in **Figure 2.2**. In the following 15 minutes, endothelium-independent vasodilation was also measured via 25 µg of glyceryl trinitrate (GTN) administration to find out whether the intervention had specific effects on NO-mediated EDV only. This GTN concentration induces a vasodilation level close to FMD (Leeson *et al.*, 1997; Ghiadoni *et al.*, 2001). The protocol is shown by **Figure 2.3**.

These measurements were mentioned in Section Outcomes – Endothelial function, Chapter 5. During the measurement, the ultrasound probe was hold by a clamp, so it helped to retain the position of probe and brachial artery captured. The quality of the images obtained were checked by the FMD team based at the CRF, St. Thomas' Hospital, under the supervision of Prof Philip Chowienczyk from Department of Vascular Risk and Surgery, KCL. Prior to the start of ATTIS study, a reproducibility study of FMD measurement was conducted to ensure the validity of measurement technique and analysis that would be used in ATTIS study by the observer using the same ultrasound (Siemens Accuson CV70) that would be operated in the study.

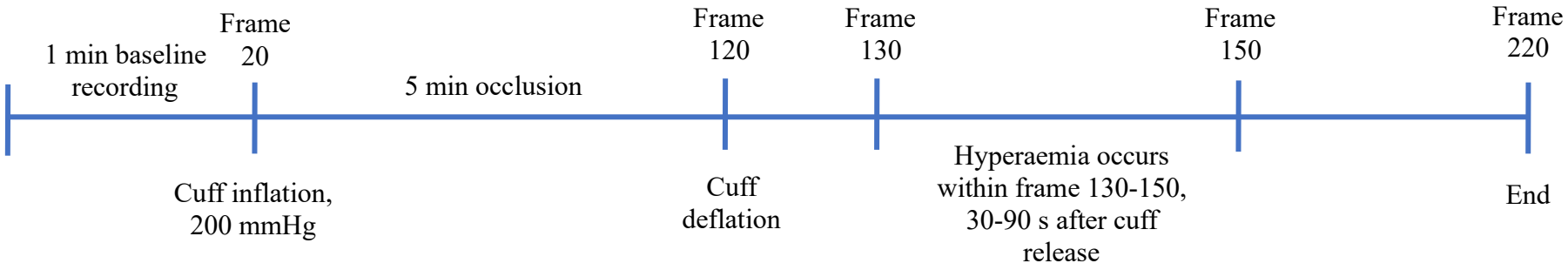


Figure 2.2. The protocol of FMD measurement.

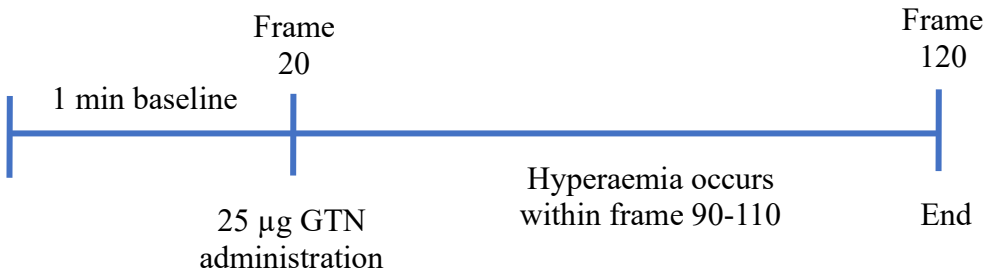


Figure 2.3. The protocol of GTN-mediated dilation measurement.

2.2.3.1 Reproducibility study

Prior to performing FMD measurement in the ATTIS study, the author was trained by Dr Benyu Jiang from Department of Vascular Risk and Surgery, KCL, for 4 h every week at the CRF, St. Thomas' Hospital, from October 2016 to January 2017. The author received a signed certificate of competency upon training completion.

After the training, a reproducibility study was conducted to investigate whether the FMD measurement learned was reproducible. A single observer (the author) measured FMD on 5 adult volunteers (mean age 30 y) in supine position at 4 occasions in total including intra- and inter-day measurements. Within one day (intra-day), two measurements with 30-minute interval were conducted. Inter-day measurements were carried out the following 3 days at similar time. The image quality obtained were checked by the trainer.

FMD was quantified blindly using Brachial Analyzer software with the following formula that was largely used by previous studies (Sanders *et al.*, 2011; Harris *et al.*, 2010): FMD = ((brachial artery diameter at hyperaemia – diameter at baseline) / diameter at baseline) x 100%. The study flow is shown in **Figure 2.4** and the FMD values and coefficient of covariance (CV) obtained are shown in **Table 2.6**. Coefficients of variance of intra- and inter-day measurements were 7.16% and 12.09% respectively indicating that the technique was considered reproducible.

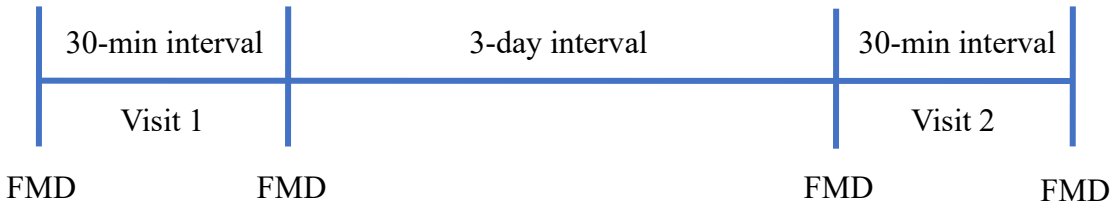


Figure 2.4. Reproducibility study flow.

Table 2.6. FMD values obtained from reproducibility study, n=5.

Subject	Visit	Baseline diameter, mm	After occlussion diameter, mm	% FMD	Intraday %FMD			Interday %FMD		
					Mean	SD	CV, %	Mean	SD	CV, %
1	1	3.79	4.19	10.55	11.71	1.64	13.97	10.99	1.03	9.34
		3.73	4.21	12.87						
	2	3.50	3.90	11.43	10.26	1.65	16.11			
		3.63	3.96	9.09						
2	1	2.52	2.74	8.73	8.53	0.28	3.29	9.24	1.00	10.79
		2.64	2.86	8.33						
	2	2.53	2.83	9.88	9.94	0.08	0.84			
		2.60	2.87	10.00						
3	1	2.58	2.65	5.04	5.87	1.17	19.94	5.84	0.04	0.62
		2.54	2.67	6.69						
	2	2.61	2.76	5.75	5.81	0.10	1.64			
		2.72	2.97	5.88						
4	1	3.02	3.26	7.95	8.13	0.25	3.12	9.56	2.02	21.15
		3.01	3.26	8.31						
	2	2.60	2.90	11.54	10.99	0.78	7.12			
		2.78	3.07	10.43						
5	1	2.55	2.77	8.63	8.44	0.27	3.22	9.71	1.80	18.55
		2.79	3.02	8.24						
	2	2.13	2.28	10.80	10.98	0.26	2.38			
		1.97	2.12	11.17						
					Average CV, %		7.16	Average CV, %		12.09

2.2.4 Primary outcome: fat quantification by magnetic resonance imaging (MRI) and proton magnetic resonance spectrometry (^{1}H-MRS)

Besides FMD, fat quantification using MRI/^{1}H-MRS was also the primary outcome of the ATTIS study. This section briefly discusses the principle of MRI/^{1}H-MRS, but it is of importance to emphasise that this section more focuses on its application as an emerging technique for ectopic fat measurement in the field of nutritional sciences. Specifically, in this doctoral programme, MRI/^{1}H-MRS was required to address the research gap outlined in the introduction and applied in the ATTIS study.

MRI is a medical imaging technique employing certain atoms, strong magnetic fields, magnetic field gradients and radio waves to produce images. As hydrogen atom presents in abundant amount in the human body, especially in water and fat, thereby for clinical purposes, proton MRI is mostly used generating a map of water and fat location in the body. The protons of hydrogen atoms are electrically charged particles which have a natural spin with associated very small-scale magnetic field. The spin of protons follows a natural orientation relying on the chemical structure and the molecule polarisation in the localized space. Naturally, hydrogen protons spins on their axes in the body with random alignment. When located in a magnetic field, protons take up and releases radio frequency (RF) energy. In an environment where there is strong external magnetic field, e.g. an MRI scanner that usually has magnetic strength 0.5-3 T, the hydrogen proton axes align with this external magnetic field, generating a net magnetisation in the same direction as this external magnetic field, known as the longitudinal magnetisation. The protons aligning with this longitudinal magnetisation undergo precession at a RF defined by the Larmor equation (Rigden, 1986). To quantify this magnetisation, an additionally external magnetic field is applied perpendicular to the longitudinal magnetisation at the

RF or Larmor frequency, altering axes' angle and producing a current in the coil. The sequential RF pulsing of magnetisation induces molecules to resonate resulted from their successive transition from being aligned with this longitudinal magnetisation to returned to their natural alignment. When the axes return to the natural alignment, referred as relaxation, the protons lose energy through emission of their own RF signals, known as free-induction decay (FID) response signals. Different molecules release different energy. The FID response signals are generated only when there is transverse magnetisation. These signals are measured by receiver coils surrounding the body of the subject being scanned. The proton density of the tissue determines the intensity; the higher the density, the higher the signal intensity. Frequency and relaxation time are the properties which are used to differentiate or separate molecules of water and fat on MRI (Berger, 2002).

These signals generated are transformed into images. Images from MR scanning are in the forms of a set of slices of a section of the body in which each slice is conceptualised into a matrix of individual tissue voxels with rows and columns. Especially in clinical purposes, multiple slices of images are required to enable the acquisition of specific anatomical region. The signals emitted from different slices and voxels are characterised by frequency, i.e. the number of oscillation cycle determined by spinning rate of the transverse magnetisation, as well as phase, i.e. the relationship between one voxel to the others related to the spinning direction; direction differences of one to another voxel are created by temporary changes on the spinning rate that occur naturally. Specific signal intensity is laid out in the corresponding image pixel through two stages: 1) signal encoding or addressing based on frequency and phase during the acquisition stage; and 2) signal intensity transfer to the specific image pixels according to the addresses during the reconstruction stage, as shown in **Figure 2.5**. The reconstruction stage, known as

Fourier transformation, employs mathematical process run by the computer. This plotting occurs on a grey scale and generates cross-sectional 3-dimentional (3D) images (Berger, 2002). In the case of multiple slices, phase-encoding is used to generate thin slices during the reconstruction stage (Sprawls, 2000).

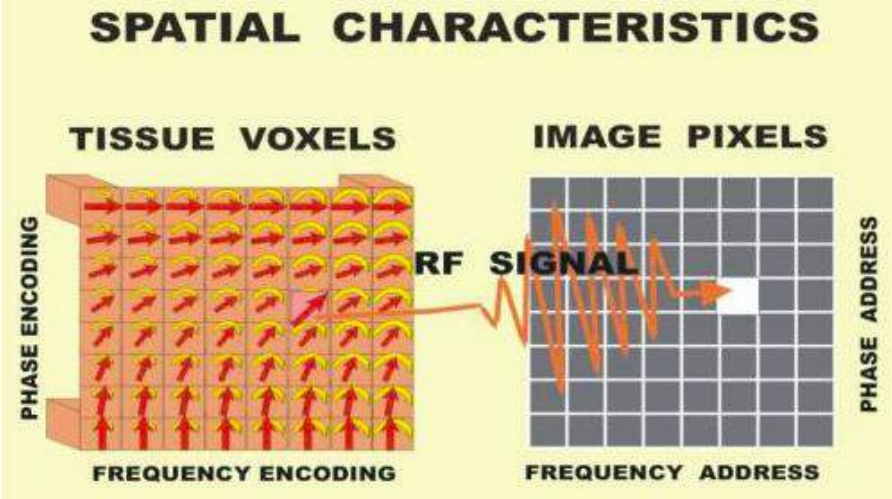


Figure 2.5. The reconstruction stage of radiofrequency signals from protons in body tissue voxels to image pixels. This figure is adapted from Sprawls (2009).

^{1}H-MRS employs the same fundamental principles of MRI in differentiation of molecules. It generates spectra while MRI results in images (Tognarelli *et al.*, 2015). The energy or RF pulse produces differing from one molecule to another is detected and acquired by the coil receiver and converted into a spectrum. The spectra consist of peaks representing different molecule components that are separated by chemical shift under a magnetic field (Boesch *et al.*, 1997).

2.2.3.2 The quantification of fat deposition: subcutaneous, visceral and ectopic fat via MRI and ^{1}H-MRS

The assessment of regional fat distribution including SAT, VAT and ectopic fat stores has been a focus in the investigations into the metabolic impacts of excess fat

deposition/adiposity (Wang *et al.*, 2014). There are a number of techniques for total and regional adipose tissue assessment including skinfold measurement, underwater weighing, air displacement, bioelectrical impedance, computerised tomography (CT), dual-energy x-ray absorptiometry (DEXA) and MRI (Thomas *et al*., 2013). MRI and CT employ advanced technology allowing comprehensive and thorough assessment of fat deposition (Borga *et al.*, 2018) and these techniques are non-invasive, accurate and require less time to conduct (Thomas *et al.*, 2013). In comparison to CT which uses ionising radiation, MRI is more preferable due to safety aspect (non-radiation) as well as the high resolution and more detailed images acquired, especially for soft tissues in the body (Hu, 2012). Therefore, MRI is the most widely used technique and also considered as the most accurate non-invasive technique for fat quantification. Furthermore, validity of MRI against invasive methods in human and animal cadavers has been assessed (Abate *et al.*, 1994; Fowler *et al.*, 1992).

Regarding ectopic fat, it was previously assessed via tissue biopsies, an invasive method. Furthermore, tissue biopsies are unable to be used for the assessment of heart and pancreas (Thomas *et al.*, 2013). MRI is non-invasive method that enables the assessment of these organs.

The advent of MRI as an advance non-invasive method is of utmost useful with its unique features enabling differentiation between fat and non-fat tissues. This separation results from the differences of natural chemical shift between the water molecules within fat and non-fat tissues (Springer *et al.*, 2010; Proctor & Yu, 1950). For clinical purposes, the Dixon technique, a type of MRI sequences, is widely used to produce two images, one that counts for water and fat signals in-phase (0°) and another image that counts for water

and fat signals out-of-phase (180°). The sum of these two images results in a water-only image and the subtraction generates fat-only image. Fat-only and water-only images result in more visual clarity of separation between fat tissues and other tissues (Dixon, 1984; Karstaedt *et al.*, 1983). Clear fat- and water-only images are generated through uniform fat suppression. In the Dixon technique, suppression of fat signals is applied to minimise the artefacts by hybrid chemical shifts because fat generates pulse that has a short relaxation time and results in bright fat signal that could produce aggravated motion-related artefacts and decrease discernibility of lesion. In addition, due to its chemical shift, fat can generate spatial misregistration artefacts that can arise along the direction of frequency-encoding and the slice selection (Ma, 2008).

Therefore, the MRI Dixon technique enables quantification of SAT, VAT and ectopic fat depots including, IHL and IPL using MRI images. **Figure 2.6** shows abdominal fat-only MRI images obtained from a lean (A) and obese (B) subject. In comparison to the lean subject, obese individual is shown to have clearly visible greater SAT and IHL (Springer *et al.*, 2010). Ectopic fat quantification using multi-echo Dixon ^{1}H-MRI has been shown to be correlated and in agreement with the results from ^{1}H-MRS (Zhao *et al.*, 2019).

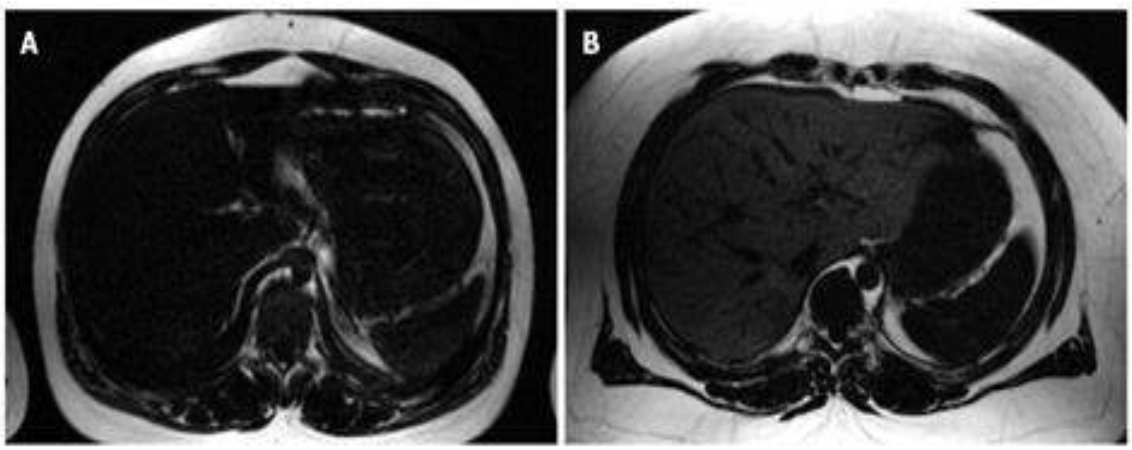


Figure 2.6. Abdominal fat-only MRI images. The Dixon-MRI sequences enable visual comparison between low and high fat deposits, for example in lean (A) and obese subject (B) respectively (Springer et al.*, 2010).*

MRI and ^{1}H-MRS have been emerging advanced techniques for fat deposition quantification in the field of nutritional sciences. Ryan *et al.* is one of the first research group who use these techniques to assess dietary intervention study outcomes. They reported that MD consumption for 6 weeks significantly decreased hepatic steatosis through assessment via ^{1}H-MRS in comparison to isocaloric LF/HC diet in 12 non-diabetic individuals with biopsy-proven NAFLD in a cross-over RCT (Ryan *et al.*, 2013). Ryberg *et al.* also used ^{1}H-MRS for assessment ectopic fat in an open label study, showing that palaeolithic-type diet (high-protein and unsaturated FA, mainly MUFA) consumption for 5 weeks significantly reduced IHL, but did not alter the IMCL concentrations in 10 healthy overweight postmenopausal women (Ryberg *et al.*, 2013).

2.2.3.3 MRI applied in the ATTIS study

The ATTIS study used MRI to assess truncal VAT and SAT, IHL and IPL. Participants underwent MRI/^{1}H-MRS scan (detailed below) at baseline and endpoint in the morning, between 7.30 am and 10.30 am, at the XMR, St Thomas' Hospital or Cancer Centre, Guy's Hospital, London. Prior to scanning, participants completed an MRI health safety screening form and changed into a hospital gown to ensure no metal objects were on the subjects clothing. Each participant was scanned, using a 6-point Dixon-based MRI sequence on a 1.5 Tesla Siemens Magnetom Aera scanner, from the neck to feet (excluding the arms) while lying in the supine position with surface coils placed around the scanned body area. The 6-point Dixon-based MRI scan protocol is described in Section Outcomes – Liver fat, Chapter 5. The combination of in-phase and out-of-phase images allowed the acquisition of water-only and fat-only MRI images. There were 56 abdominal image slices generated by the scanning which were used for liver fat quantification. During the abdominal image acquisition, participants were instructed by

the radiographer to hold their breath for four bouts of 15 seconds to reduce motion artefacts. A 2-point Dixon-based MRI sequence was also used to acquire additional abdominal images allowing clearer view for liver and pancreatic fat analysis.

Dr Geoffrey Charles-Edwards, the head of MR Physics, Guy's & St. Thomas' NHS Foundation Trust, set up the MRI/^{1}H-MRS scan protocols (**Appendix 19**). Haris Shuaib, a senior physicist in MR at Guy's and St. Thomas' NHS Foundation Trust, was responsible for downloading all the MRI/^{1}H-MRS data acquired from the MR data system. Only certain NHS staff obtain an access for the system. All MRI data were analysed using the open source image analysis software HOROS V 1.1.7 (www.horosproject.org) detailed below. Due to the subjective nature of the MRI analysis of IHL and IPL, the observer was blinded.

Analysis of truncal VAT and SAT

Truncal VAT and SAT quantification using MR images was conducted by Dr Brandon Whitcher and Dr Nicolas Basty from the University of Westminster using artificial intelligence-based image processing as further explained in Section Outcomes – Intrapancreatic lipid, intramyocellular lipids, and body composition, Chapter 5.

Analysis of intrahepatic lipids (IHL)

For IHL quantification using MRI, the author learnt what previous studies did for IHL analysis and improved and developed the analysis technique. Previously, Campo *et al.* applied some techniques of IHL analysis based on the sizes (1 cm^2, 4 cm^2 and largest fit while avoiding blood vessels, bile ducts and obvious artefacts in the liver), locations and numbers of circular regions of interest (ROI) (2 in which one is placed in each of left and right lobe; 4 in which one is placed in each of anterior, posterior, medial and lateral

segment; and 9 in which one is placed in each of Couinaud segment) (Campo *et al.*, 2017). For interobserver agreement, the technique including 4 largest fit ROIs placed in anterior, posterior, medial and lateral segment provided narrowest limits of agreement. However, the technique involving 9 largest fit ROIs place in each of Couinaud segment reported narrowest limits of intraobserver agreement. The author discussed with Dr Dimitra Christodoulou, a radiologist consultant at Guy's and St. Thomas' NHS Foundation Trust, about training on identification of Couinaud segment in the liver. It would take several months due to its complexity. Considering the time constraint of PhD, the author decided to proceed with the technique including 4 largest fit ROIs placed in anterior, posterior, medial and lateral segment (see **Figure 2.7**). The author also did more developments on the technique which were discussed with the supervisors. Instead of placing 4 largest fit ROIs in those 4 segments of the liver in one slice of image, the author included as many slices of image as possible wherein the liver was seen as the major organ in the abdominal image slices.

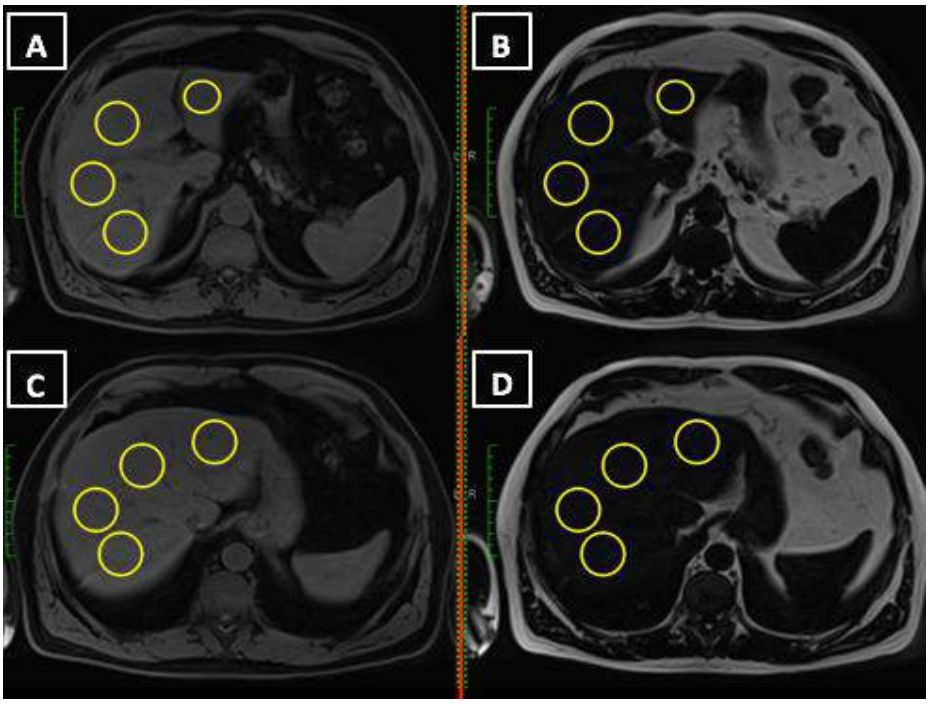


Figure 2.7. Pairs of water-only (A and C) and fat-only (B and D) images with four identical regions of interest located in identical positions in each pair of images.

Four circular ROIs were manually drawn in the liver tissue, by using the 'oval' tool, in water images of 2-point Dixon MRI sequence and these ROIs were copied and pasted to identical locations in the fat images of 6-point Dixon MRI sequence. ROIs were positioned with the purpose of including the anterior, posterior, medial and lateral segments of the liver. The ROI areas ranged from 10-20 cm^2 and covered as large an area of liver tissue as possible while avoiding blood vessels, bile ducts and obvious artefacts as previously recommended (Campo *et al.*, 2017). **Figure 2.8** shows an example of the ROI placement in two pairs of fat and water images that represent two axial locations in the abdomen. In each participant, a total of 24-36 ROIs were placed in the liver tissue on 6-9 abdominal images; IHL was quantified within each ROI and a mean of all 24-36 ROIs was taken.

For each ROI, a pixel signal intensity value is given in both of the fat-only and water-only images. To quantify liver fat, the signal intensity values obtained from ROIs placed in the liver on abdominal fat-only images of 6-point Dixon MRI sequence were used. Signal intensity of abdominal fat-only images generated by 6-point Dixon MRI sequence were programmed by Siemens to represent fat fraction, i.e. LiverLab. **Figure 2.8** shows a representation of the information provided for each ROI which includes the area of the ROI and the signal intensity of the pixels located within the ROI.

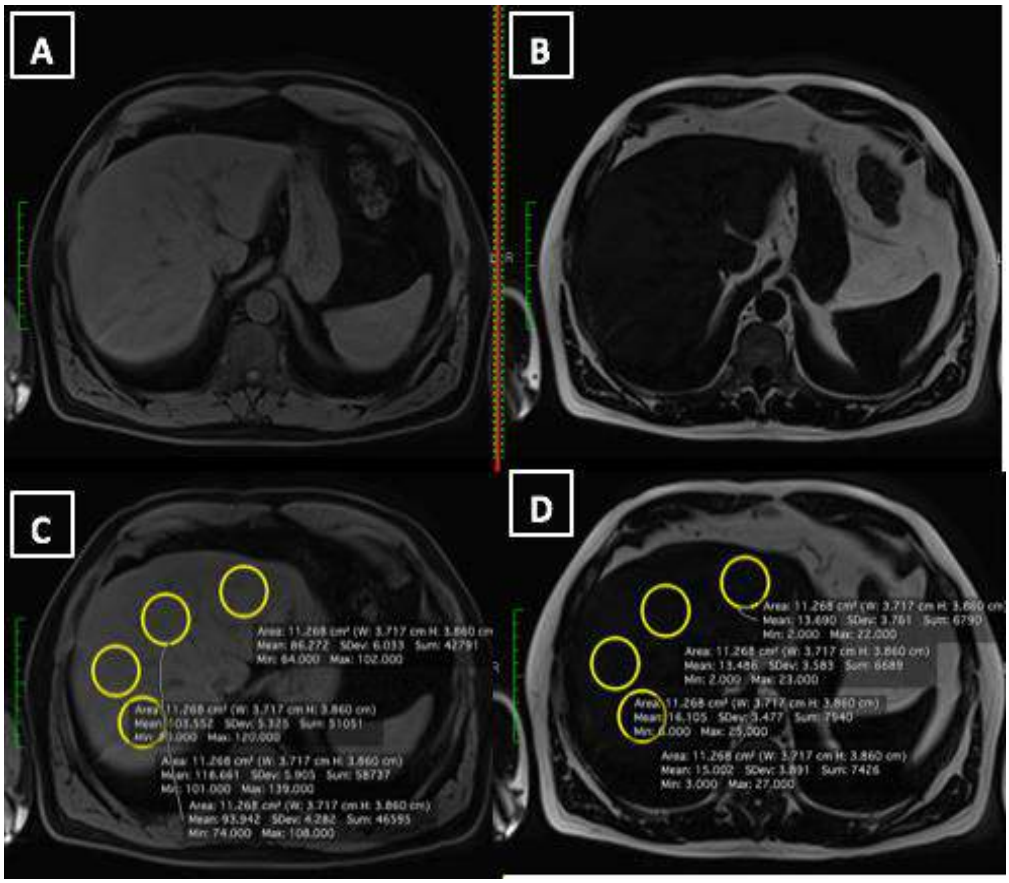


Figure 2.8. MRI images showing the information provided in the HOROS software for each region of interest from which the pixel signal intensities are used to determine percentage fat fraction of each ROI. Panels A and B represent images C and D respectively.

The Siemens Healthineers LiverLab provides in-depth liver fat quantification package via fat signal fraction which covers the entire liver with a single breath hold. The clinical package includes a fat and iron screening part (First look Dixon), and two evaluation methods: 1) HISTO sequences, voxel-based breath-hold spectroscopy, and 2) multi-echo Dixon VIBE, image-based. The ATTIS study specifically used the fat screening part. First look Dixon employs a 2-point Dixon technique, and is a single breath-hold 3D acquisition of out-of-phase and in-phase images and fat and water images that covers the entire liver. Following image acquisition, the system conducts liver segmentation in which the same segmentation is used in the next sequence, multi-echo Dixon. Using multi-echo Dixon

method, data are collected at multiple echo times (TE) generating multiple volumes with different magnitudes of contrast obtained for every RF pulse, thereby enabling sensitive detection of low concentration of fat with improved contrast. Another benefit on chemical shift fat suppression from using multi-echo Dixon method for water and fat separation is applying a single breath hold to obtain water and fat images circumventing misregistration and providing positive contrast to fat and less artefacts. Relative to 2-point Dixon approach, multi-echo 6-point Dixon acquisition allows robust fat and water separation involving correction for transverse relaxation of fat and water induced by magnetic field inhomogeneity. Inhomogeneities of magnetic field may disturb fat signal suppression so that it can results in incomplete water and fat separation and in more extreme situation can cause local fat and water signal exchange. More effective fat separation by multi-echo 6-point Dixon method provides a better signal to noise ratio (SNR), shorter acquisition time and maximum spatial resolution (Kellman *et al.*, 2009).

Therefore, multi-echo Dixon applying a single breath hold acquisition can cover the entire liver. It generates mean fat and water signal fractions so that the images produced are water and fat images and water and fat percentages images. Multi-echo 6-point Dixon approach is also apt to be applied in multispectral separation of fat and water signals. HISTO sequence is a spectroscopy sequence with 15 second breath-hold providing proton density fat fraction (PDFF) with also correction for transverse relaxation of fat and water (Sellers, 2016).

Hepatic fat quantification using multi-echo 6-point Dixon MRI has been shown to be accurate by Deng *et al*. The research group and Yurdaisik *et al.* revealed that the quantification was correlated with liver biopsy results in patients with NAFLD (Deng *et al.*, 2014; Yurdaisik *et al.*, 2020). Furthermore, the liver fat quantification using this

multi-echo Dixon technique was also correlated with the results from ^{1}H-MRS (Zhao *et al.*, 2019). The multi-echo 6-point Dixon has also been used to quantify muscle fat. Previous studies demonstrated that multi-echo 6-point Dixon method provided more reliable results on muscle fat quantification compared with 2-point Dixon (Grimm *et al.*, 2018; Yoo *et al.*, 2015). Grimm *et al.* also reported that the 6-point Dixon showed high reproducibility for muscle fat quantification (Grimm *et al.*, 2018).

Analysis of intrapancreatic lipids

Apart from IHL, IPL was also quantified using MRI. Prior to quantification, identification of pancreas including its head, body and tail in each participant's MR images was carried out. Due to the curved shape of the pancreas, different MRI slices were used with the greatest area of pancreatic tissue on the head, body and tail regions. To ensure the accurate positioning of the ROIs in each of these pancreatic, the identification of these regions was carried out alongside Dr Dimitra Christodoulou, a consultant radiologist who also has expertise in MRI pancreas anatomy.

Once the identification was done, one circular ROI of area 1 cm^2 was positioned on each of the head, body and tail regions in water images of 2-point Dixon MRI sequence and these ROIs were copied and pasted to identical locations in fat images. The relatively small areas of ROIs were used due to recommendation by a recent review of MR methods used to determine IPL; this ensured the ROIs were within the pancreatic borders and avoided contamination of the ROIs with VAT and the splenic vein (Al-Mrabeh *et al.*, 2017). An example of the positioning of 3 ROIs within the pancreas is shown in **Figure 2.9**.

Following the placing of ROIs in each of pancreatic regions, percentage IPL within each ROI using 2-point Dixon MRI sequence was applied using this below formula:

$$\text{Fat fraction (\%)} = \frac{F}{F + W} \times 100$$

Where F is the signal intensity of the ROI in the fat-only image and W is the pixel signal intensity of the ROI in the water-only image.

Using the pixel signal intensity data of each ROI, the pancreatic fat fraction was calculated in each region: IPL_{HEAD}, IPL_{BODY}, and IPL_{TAIL}. IPL_{MEAN} was calculated as the average of the head, body and tail regions.

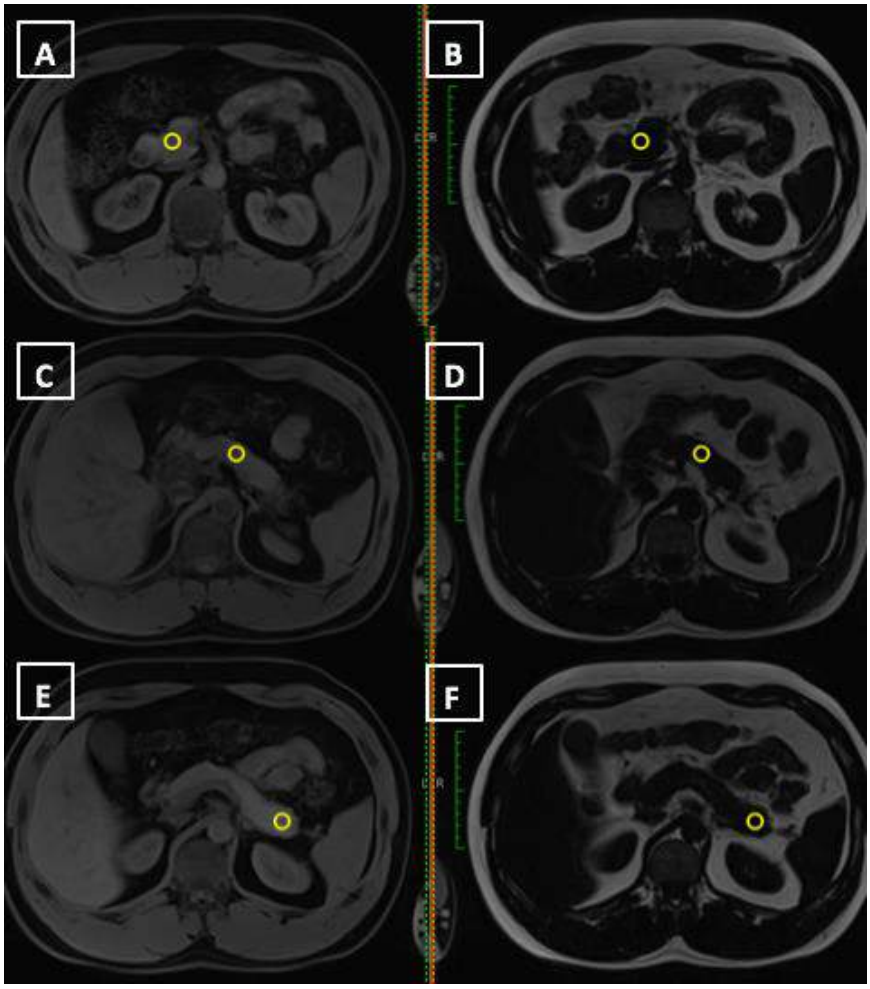


Figure 2.9. Positioning of regions of interest in corresponding fat and water images of the pancreas head, A and B, body, C and D, and tail, E and F, regions.

2.2.3.4 ^{1}H-MRS applied in the ATTIS study

Apart from MRI, the ATTIS study also used ^{1}H-MRS to quantify IHL, IMCL and EMCL. Dr Geoffrey Charles-Edwards and Dr Olah Hakim introduced the analysis software, Java-based Magnetic Resonance User Interface (jMRUI) version 5.0, for the quantifications IHL and IMCL/EMCL, respectively, to the author. The author further developed the technique for quantification of saturation and unsaturation degree of IHL.

To acquire the spectra from ^{1}H-MRS for IHL, IMCL and EMCL quantification, each participant underwent a ^{1}H-MRS scan of liver and soleus muscle (located in the posterior of the calf) on a 1.5 T Siemens system. While lying in the supine position, an extremity RF coil was placed on the right leg to obtain images of the soleus muscle. A radiographer used these images to identify a volume of interest and placed a voxel dimension 1.6 x 1.6 x 5.0 mm^3 in the liver and dimension 0.8 x 0.8 x 5.0 mm^3 in the soleus muscle while avoiding gross marbling of fat. Localise d proton spectra were acquired using a point resolved spectroscopy (PRESS) sequence (TR 2000 ms; TE 30 ms) to obtain two spectra: a water-suppressed spectrum and a non-water-suppressed spectrum that was used to determine the water peak resonance. The water resonance was set to 4.7 ppm, enabling the detections of IHL CH_2, IMCL CH_2 and EMCL CH_2 peaks. The IHL CH_2 and IMCL CH_2 resonances occur at frequencies of 1.3 ppm, while EMCL CH_2 at 1.5 ppm. Spectra obtained from the ^{1}H-MRS scans of all participants were analysed on jMRUI version 5.0.

Analysis of intrahepatic lipids

The liver fat spectra were obtained from abdominal scanning. During the abdominal scanning, volunteers were instructed to hold their breath for four times to acquire water-suppressed spectra and also another hold breath to acquire a non-water suppressed

spectrum. These four spectra were acquired and averaged during the analysis to enhance SNR. Prior to fitting metabolite peaks in the spectra, data preparation using jMRUI software was conducted including generating an average spectrum of these four spectra acquired and removing the remaining internal water-suppressed signal resonance in order to fit the peaks well via Hankel Lanczos squares singular values decomposition (HLSVD) filter tool in the software. The peaks shown in the original spectra are separated into the metabolite components by a deconvolution process with the use of prior knowledge of the peaks that is entered to the software to assist with identification of the individual peaks, including IHL CH_2 and other metabolite peaks allowing the determination of liver fat saturation and unsaturation indices as also mentioned in Section Outcomes – Liver fat, Chapter 5 and in **Table 2.7** below. IHL CH_2 represents liver fat analysed via ^{1}H-MRS. **Figure 2.10** shows a typical ^{1}H-MRS average spectrum of liver with the peaks obtained from a ^{1}H-MRS analysis.

Table 2.7. Functional groups of fatty acid peaks in ^{1}H-MRS (Johnson et al.*, 2008)*

Name	Functional group	Frequency (ppm)
Diallylic protons	=CH-C**H$_2$**-CH=	2.9
Methylenic protons in the α position relative to the carboxyl group	-COO-C**H$_2$**-CH$_2$-	2.3
Allylic protons	-C**H$_2$**-CH=CH-	2.0
IHL/methylene protons	-C**H$_2$**-	1.3
Methyl protons	-C**H$_3$**	0.9

The formulas used to determine the unsaturation index (UI), polyunsaturation index (PUI) and saturation index (SI) of the liver were as follows (Johnson *et al.*, 2008).

$UI = I_{diallylic} + I_{allylic} / I_{diallylic} + I_{allylic} + I_{methylene} + I_{methyl}$

$PUI = I_{diallylic} / I_{diallylic} + I_{allylic} + I_{methylene} + I_{methyl}$

$SI = 1-UI$

Wherein $I_{diallylic}$, $I_{allylic}$, $I_{diallylic}$, $I_{allylic}$, $I_{methylene}$ and I_{methyl} are the signal amplitudes of the diallylic, allylic, methylene and methyl repectively.

After deconvolution of the peaks, the amplitude of each peak is generated by the software which corresponds to the amount of the specific metabolite that the peak corresponds to. IHL were expressed in arbitrary units as the relative ratio of the methylene IHL peak's amplitude to internal water amplitude with the assumption that internal water amplitude is equivalent between subjects (Ingram *et al.*, 2011). The amplitude of the internal water peak was determined by deconvolution of the water peak (resonance frequency 4.7 ppm) from the unsuppressed water spectra.

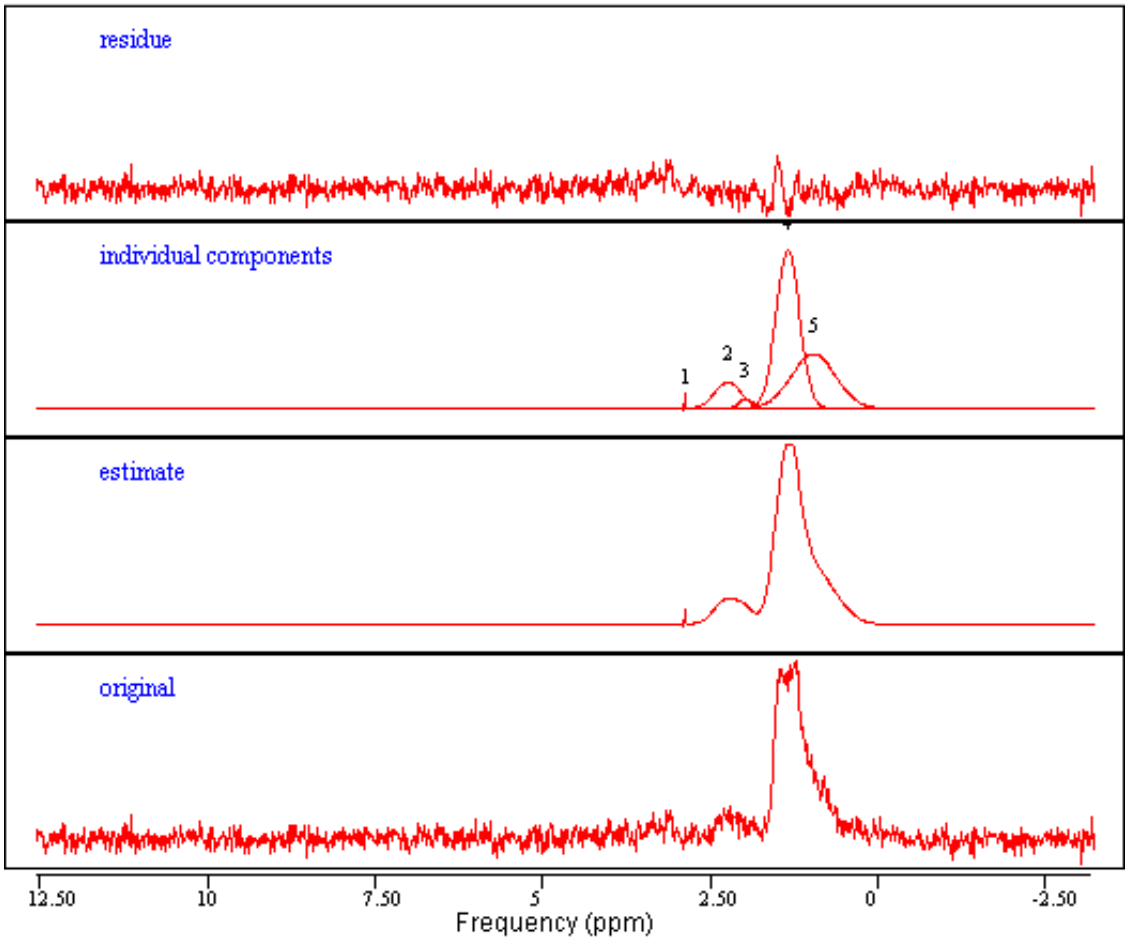


Figure 2.10. ^{1}H-MRS spectrum of the soleus muscle showing the deconvolution of the main lipid peaks. Individual components: 1. Diallylic protons, 2. Methylenic protons in the α position relative to the carboxyl group, 3. Allylic protons, 4. IHL/methylene protons, 5. Methyl protons

Analysis of intramyocellular lipids (IMCL)

Besides IHL, TAG in muscle cells, i.e. IMCL, was also assessed using ^{1}H-MRS. IMCL accumulation has been reported to disturb glucose uptake pathway in the muscle cells resulting in diminished insulin sensitivity (Bjornholm & Zierath, 2005). Literature shows that IMCL is directly associated with decrease insulin sensitivity in the muscles (Ingram *et al.*, 2011; Krssak *et al.*, 1999; Sinha *et al.*, 2002; Thamer *et al.*, 2003). In the T2D development, the whole-body IR is majorly induced by IR of the muscles (Bjornholm & Zierath, 2005), thereby increased IMCL is associated with IR and T2D (Standl *et al.*, 1980). On the other hand, EMCL are considerably metabolically inert, but EMCL accumulation leads to lost muscle strength associated with obesity (Velan *et al.*, 2008).

Lipid peaks obtained from ^{1}H-MRS spectra correspond to resonances of the hydrogen bonds from the methylene (CH_2) and methyl (CH_3) groups of IMCL and EMCL which are merged together within a range of 0.8-1.7 ppm. The large lipid peak in the original spectra is separated into the four lipid components by a deconvolution process using prior knowledge of the peaks entered to the software as detailed in Section Outcomes – Intrapancreatic lipid, intramyocellular lipids, and body composition, Chapter 5. Also, the line width (width of the peaks) of the EMCL CH_2 and IMCL CH_2 peaks and the EMCL CH_3 and IMCL CH_3 peaks were set to equal ratios. IMCL and EMCL were expressed in arbitrary units as the relative ratio of the methylene IMCL or EMCL peaks' amplitudes to internal water amplitude generated by the software. The amplitude of the internal water peak was determined by deconvolution of the water peak (resonance frequency 4.7 ppm) from the unsuppressed water spectra and assumed to be equivalent between subjects. **Figure 2.11** shows a typical ^{1}H-MRS spectrum of soleus muscle with the lipid peaks obtained from a ^{1}H-MRS analysis.

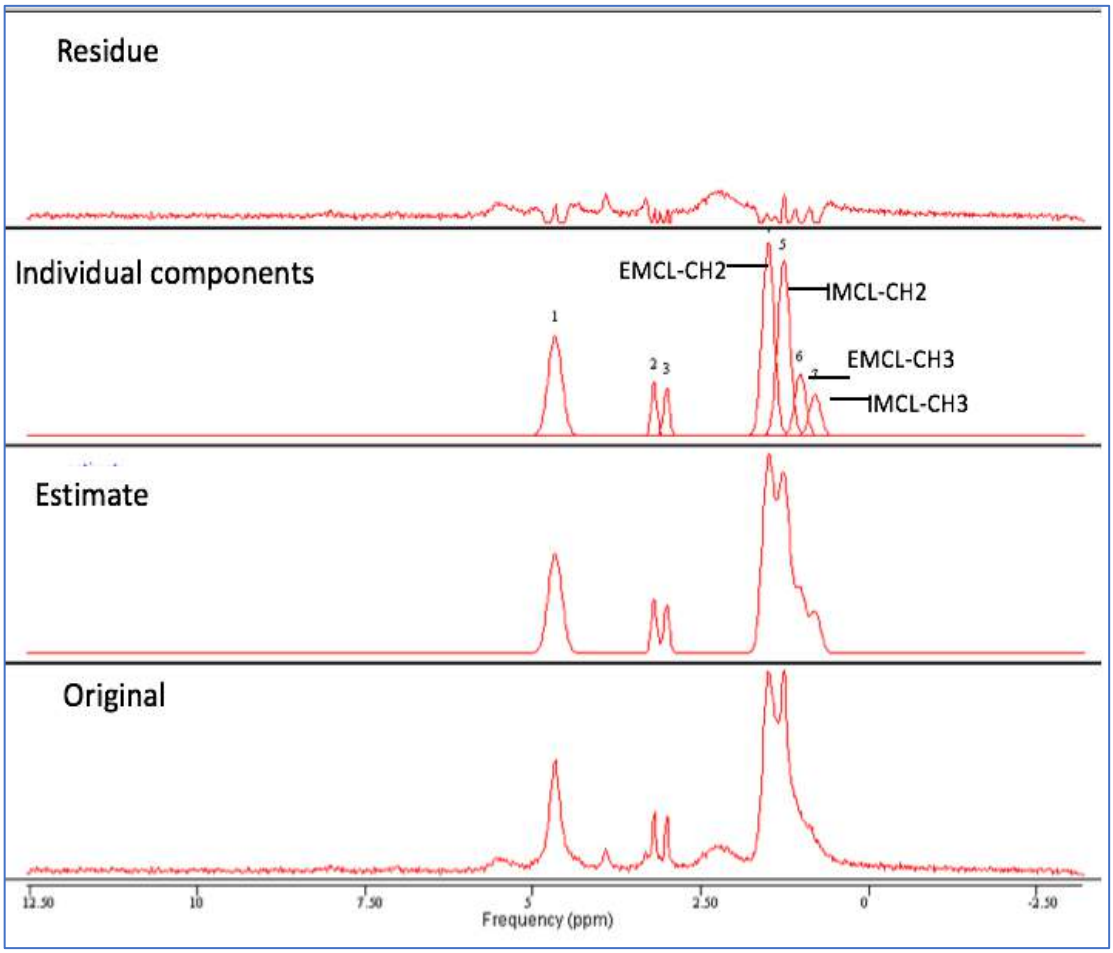


Figure 2.11. ^{1}H-MRS spectrum of the soleus muscle showing the deconvolution of the main lipid peaks

2.2.4 Secondary outcome measurement and analysis

2.2.4.1 Heart rate variability (HRV) measurement

Ambulatory HRV is 24 h HRV recording which is indirect measurement of ANS function reflecting the variability in interbeat intervals (IBI), the time intervals of QRS complex in electrocardiogram (ECG) (Task Force of the European Society of Cardiology the North American Society of Pacing Electrophysiology, 1996), see **Figure 2.12**. The parameters of HRV are described in **Table 2.8**.

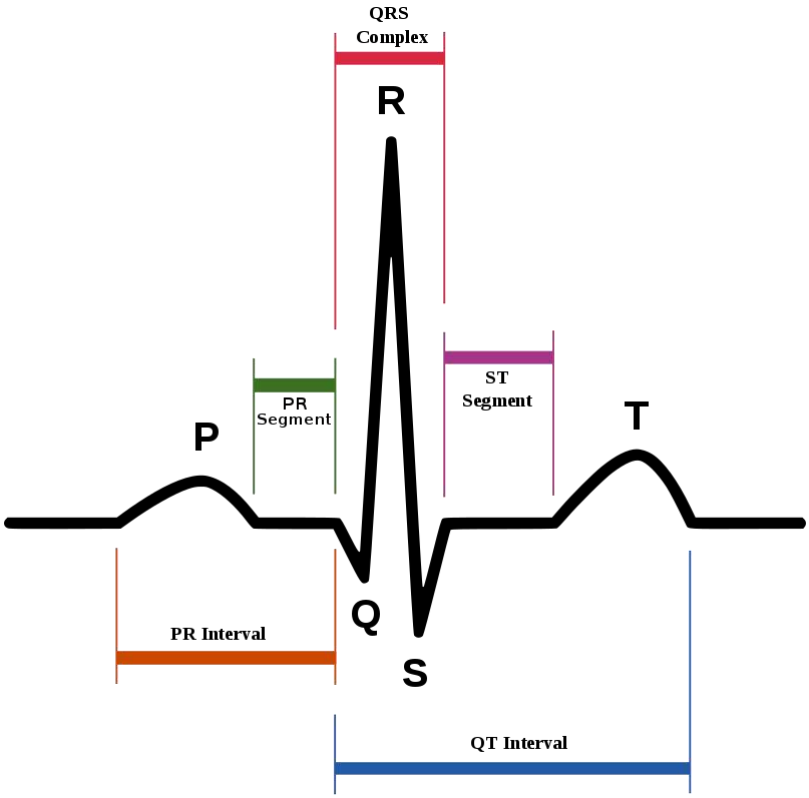


Figure 2.12. Normal ECG (Wikipedia, 2020)

HRV measurement has been established in our research group, Diet and Cardiovascular Health, at KCL. Software application, Cardiscope^{TM} ANALYTICS (SMART Medical), was used to set up ECG recording programme via eMotion Faros monitor and analyse data from ECG recorded generating HR, IBI and HRV values (see **Figure 2.13**). Some preparations were required to be done by male participants prior to fitting the eMotion Faros devices on their chests in order to capture good quality ECG signals. Male participants were requested to shave chest hair in the area where the monitor would be fitted (see **Figure 2.13)**. The monitor was attached to one end of an ECG electrode to be fitted in the chest and the other end was positioned under the last rib on the left side of the body forming an electrical axis of the heart. Both of the endings of the ECG electrode were clipped to electrode pads before attaching them to these two positions of the body.

Table 2.8. Heart rate variability (HRV) time- and frequency-domain measures (Task Force of the European Society of Cardiology the North American Society of Pacing Electrophysiology, 1996).

Parameter	Description
Linear measures: Time-domain measures (based off of beat-to-beat intervals)	
NN, ms	Normal beat-to-beat interval.
SDNN, ms	SD of NN intervals. Indicates the overall circadian oscillations in HRV.
rMSSD	Root mean square of successive RR interval differences. Indicates short-term oscillations in HRV and modulations in parasympathetic respiratory variation.
Linear measures: Frequency-domain measures (exerts power spectral analysis which gives information on power distribution (variance and amplitude) as frequency function)	
VLF, ms^2	Absolute power of the very-low-frequency band (0.0033-0.04 Hz). Indicated PNS and renin-aldosterone activities. Constitutes the majority of total power in HRV.
LF, ms^2	Absolute power of the low-frequency band (0.04-0.15 Hz). Modulated by baroreceptors. Indicates PNS and SNS activities.
HF, ms^2	Absolute power of the low-frequency band (0.15-0.4 Hz). Indicates parasympathetic respiratory alterations.
LF:HF	Ratio LF:HF. This has been used as a measure of autonomic balance, but it has been recently argued. Earlier studies suggested that LF mainly indicated sympathetic activity rendering LF:HF a parameter reflecting the balance between sympathetic and parasympathetic activities, but latest evidence suggests that LF indicates the activity of sympatho-vagal and factors.
Non-linear measures (reflect irregularity and randomness in measurement data)	
SD1:SD2	Ratio of the SD of beat-to-beat IBI variability (SD1) against the SD of long-term IBI variability (SD2) - Indicates normality of sinoatrial function.
ApEn	Approximate entropy - Indicates irregularity or randomness in measurement/recording data. Lower values indicate higher regularity. SampEn (sample entropy) reflects similar parameter, but for short measurements/recordings.

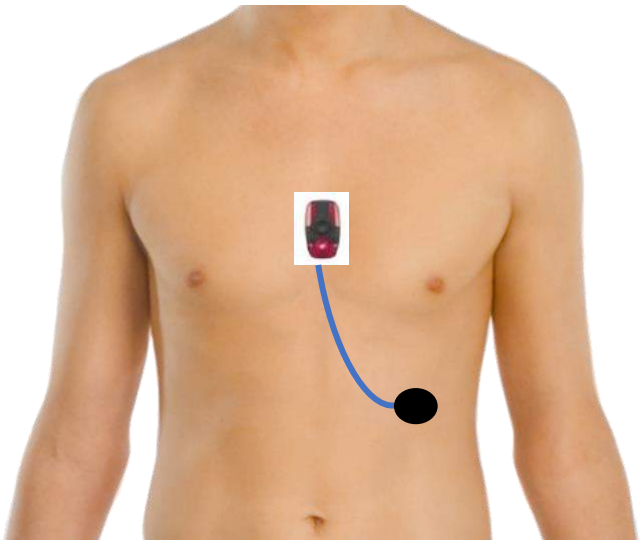


Figure 2.13. The eMotion Faros position in the chest.

ECG data can be recorded online and offline using this software. The online recording mode with real-time transmission utilising Bluetooth device was conducted during ATTIS study to measure HR, IBI and HRV during 5-min resting, 5-min physical (blood pressure cuff inflation) and 5-min mental stress (Stroop test) tasks in real-time. The offline recording mode utilising datalogger for 24 h HR, IBI and HRV measurements. Artefacts in the ECG recording data are automatically removed by the software application on beat-to-beat basis. Prior to both of online and offline recording, the monitor position on the study participants' chests and the signal were checked using the online real-time mode in order to ensure that the ECG recording was appropriate through visual examination on the software. When the signal shown were good, the real-time ECG recording for the resting, physical and mental stress tasks were performed.

Figure 2.14. ECG recording (top) and tachogram (bottom). Each green bar in the bottom indicates an IBI measured on the ECG recording on the top. Shaded green bars represent artefacts that are automatically removed by Cardiscope™ ANALYTICS software from the measurements.

Ambulatory HRV during day- and night-time was measured following the protocol detailed in Section Outcomes – Blood pressure and heart rate variability, Chapter 5. In addition to that, HRV was also measured in response to acute stress in supine position and the protocol is described in Chapter 6. The author contributed to adapt the measurement protocols to be used during acute stress tasks, including physical and mental stress tasks. We intended to observe whether physical and mental stress tasks could decrease beat-to-beat HRV. HRV measurement in response to acute physical stress was conducted during 5 min occlusion via blood pressure cuff 200 mmHg during FMD measurement; the protocol is shown in **Figure 2.15**. Mental stress was induced by 5 min Stroop colour-word test which was carried between FMD and GTN-mediated dilation measurements.

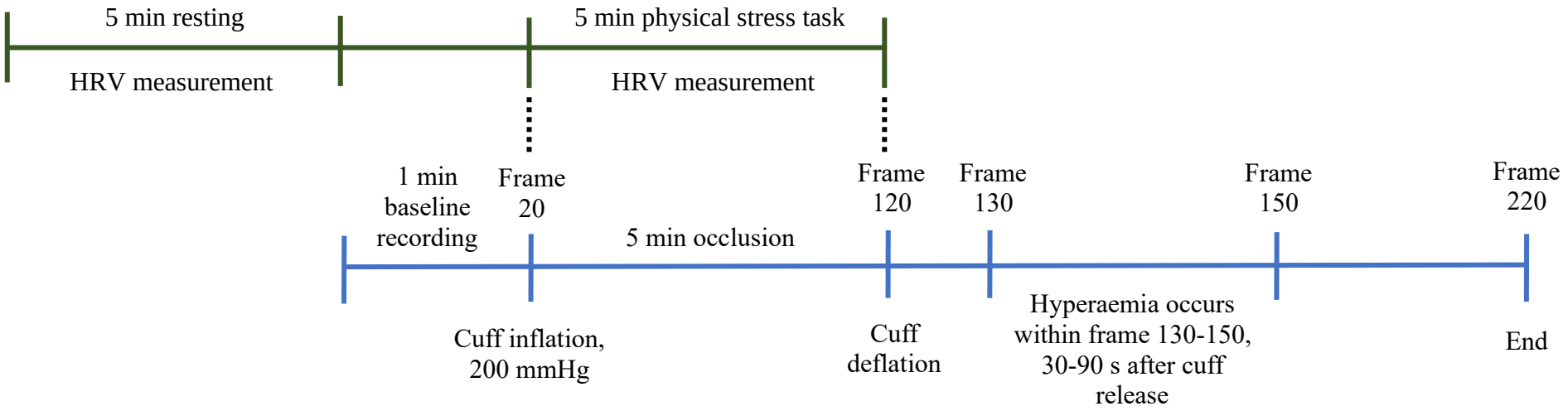


Figure 2.15. HRV measurement in response to acute physical stress induced by 5-min occlusion via blood pressure cuff 200 mmHg during FMD measurement.

Chapter 3: Tree nut snack consumption is associated with better diet quality and CVD risk in the UK adult population: National Diet and Nutrition Survey (NDNS) 2008-2014

This chapter of this thesis incorporating publications presents the published paper in the Public Health Nutrition.

(Dikariyanto *et al.*, 2020)

Public Health Nutrition: page 1 of 10 doi:10.1017/S1368980019003914

Tree nut snack consumption is associated with better diet quality and CVD risk in the UK adult population: National Diet and Nutrition Survey (NDNS) 2008–2014

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Submitted 13 July 2018: Final revision received 15 August 2019: Accepted 9 September 2019

Abstract

Objectives: To examine associations of tree nut snack (TNS) consumption with diet quality and cardiovascular disease (CVD) risk in UK adults from National Diet and Nutrition Survey (NDNS) 2008–2014.

Design: Cross-sectional analysis using data from 4-d food diaries, blood samples and physical measurements for CVD risk markers. To estimate diet quality, modified Mediterranean Diet Score (MDS) and modified Healthy Diet Score (HDS) were applied. Associations of TNS consumption with diet quality and markers of CVD risk were investigated using survey-adjusted multivariable linear regression adjusted for sex, age, ethnicity, socio-economic and smoking status, region of residency and total energy and alcohol intake.

Setting: UK free-living population.

Subjects: 4738 adults (≥19 years).

Results: TNS consumers had higher modified MDS and HDS relative to non-consumers. TNS consumers also had lower BMI, WC, SBP and DBP and higher HDL compared to non-consumers, although a dose-related fully adjusted significant association between increasing nut intake (g per 4184 kJ/1000 kcal energy intake) and lower marker of CVD risk was only observed for SBP. TNS consumption was also associated with higher intake of total fat, mono-, *n*-3 and *n*-6 polyunsaturated fatty acids, fibre, vitamin A, thiamin, folate, vitamin C, vitamin E, potassium, magnesium, phosphorus, selenium and iron; and lower intake of saturated fatty acids, *trans* fatty acids, total carbohydrate, starch, free sugar, sodium and chloride.

Conclusions: TNS consumers report better dietary quality and consumption was associated with lower CVD risk factors. Encouraging replacement of less healthy snacks with TNS should be encouraged as part of general dietary guidelines.

Keywords
Nuts
Cross-sectional analysis
Diet quality
CVD
Nutrients

An average of 2·55 snacks per day are consumed in the UK and Ireland, with over a third of these snacks being confectionary or crisps/popcorn/nuts[1]. Nuts are a popular snack as shown by the growing trend for consuming tree nuts over the past 10 years[2]. North America was the region with the highest production; however, it was Europe that was the largest consumer in the world. Almonds (*Prunusdulcis*), walnuts (*Juglansregia*), pecans (*Caryaillinoinensis*), pine nuts (*Pinuspinea*), cashews (*Anacardiumoccidentale*), macadamia nuts (*Macadamia*), hazelnuts (*Corylusavelana*), pistachios (*Pistaciavera*), Brazil nuts (*Bertholletiaexcelsa*) and chestnuts (*Castanea*) are examples of edible tree nuts that are produced commercially[2].

Almonds, walnuts, pecans, pine nuts, cashews, macadamia nuts, hazelnuts, pistachios and Brazil nuts differ to some extent in their nutrient profiles. However, tree nuts are generally energy-dense, with a high proportion of fat made up of unsaturated fatty acids; low in sodium; and rich in plant-based protein, dietary fibre, and micronutrients, including niacin, vitamin B_6, vitamin E, vitamin K, folic acid, calcium, magnesium, potassium, selenium, phosphorus and zinc. Tree nuts are also rich in phytosterols and (poly)phenols, which promote antioxidant and anti-inflammatory pathways[3–5]. Because of these properties, tree nuts and health outcomes have been the focus of many human clinical trials and observational studies.

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Previous dietary intervention studies showed that tree nut consumption resulted in lowered type-2 diabetes and cardiovascular disease (CVD) risk factors. Walnut, almond, pistachio, macadamia nut, cashew and hazelnut consumption favourably modified blood lipid profile[6–15], mixed nuts improved insulin sensitivity[14], and walnuts lowered inflammatory markers[16] and improved endothelium-dependent vasodilation[17], all of which would be predicted to reduce the risk of CVD. Furthermore, contrary to popular perception, nut-enriched diets are not linked with increased risk of weight gain[18] and tree nut consumption has been shown to assist weight loss as part of an energy-restricted diet in obese or overweight subjects[19].

Cross-sectional analysis of tree nut consumption and indicators of diet quality and cardiovascular health have also been undertaken. In the US adult population (≥19 years), using the National Health and Nutrition Examination Survey (NHANES) 2005–2010 database (*n* 14 386) based on 24-h dietary recalls, it was reported that tree nut consumption was linked to lower BMI, waist circumference (WC), systolic blood pressure (SBP), and insulin resistance index (HOMA-IR) and higher high-density lipoprotein (HDL-C) adjusted for age, sex, ethnicity, poverty index ratio, physical activity level, smoking status and alcohol intake[20]. O'Neil *et al.* (2015) also showed that tree nut consumers, compared to non-consumers, had significantly higher diet quality scores (HEI-2005, a diet quality score widely used in the USA) and greater nutrient adequacy for dietary fibre, vitamin A, vitamin E, vitamin C, folate, calcium, iron, magnesium, zinc and potassium[21].

The purpose of the current study was to examine associations between tree nut snack (TNS) consumption and diet quality, and CVD risk markers, in a nationally representative UK adult population, using data from the UK National Diet and Nutrition Survey (NDNS) rolling programme 2008–2014. Dietary data were derived from estimated 4-d food diaries in a population of 4738 adults (≥19 years)[22,23], which differs from the NHANES analysis 2005–2010, which was based on two multiple pass 24-h dietary recalls in a larger population of 14 386 adults[21]. The hypothesis of the current study was that greater TNS consumption would be associated with higher diet quality, greater nutrient adequacy, and lower prevalence of CVD risk markers in UK adults.

Materials and Methods

The National Diet and Nutrition Survey Rolling Programme (NDNS-RP) and study population

The NDNS-RP is a long-running government-funded scheme to assess diet, nutrient intake and nutritional status of the general population (>1·5 years) living in private households in the UK (England, Scotland, Wales and North Ireland)[22,23]. Random sampling was carried out on addresses throughout the UK. A single address could have multiple households and a household in an address was selected randomly. An adult in the household was also randomly selected. Selected participants were requested to complete a 4-d estimated food diary, interviewed to collect information, such as dietary habits, socio-demographic background and lifestyle as well as anthropometrically measured and blood sample taken[22,23].

The survey involves two stages: (i) interview visits to collect information on socio-demography, administer the 4-d food diaries, and carry out anthropometric measurements, and (ii) a nurse visit to do further physical measurements and collect blood and 24-h urine samples[22,23]. Following venepuncture, an EDTA and a serum gel monovette tube from each participant's sample set were sent by post, to the Immunology and Biochemistry Laboratory at Addenbrooke's Hospital in Cambridge for prompt analysis. The remaining samples (lithium heparin, serum or fluoride blood monovette tubes) were processed and stored below −40°C (or at a maximum of −20°C where −40°C facilities were not available), before being transported on dry ice to the Human Nutrition Research (HNR) facility for analysis. The cross-sectional analysis reported here included data from adult participants (≥19 years, *n* 4738), who completed a 4-d estimated food diary in the NDNS-RP 2008–2014 (years 1–6)[22,23].

CVD risk markers

Body mass index (BMI; kg/m^2), waist circumference (WC; cm), systolic blood pressure (SBP; mmHg), diastolic blood pressure (DBP; mmHg), total cholesterol (TC; mmol/l), triglycerides (TAG; mmol/l), HDL-cholesterol (HDL-C; mmol/l), LDL-cholesterol (LDL-C; mmol/l), TC:HDL-C (the ratio of TC and HDL-C) and C-reactive protein (CRP; mg/l) were CVD risk markers included in the analysis. Interviewer measurement protocols and procedures for blood sample collection, processing, analysis and quality controls are detailed elsewhere[22,23]. Body height and weight were measured using a portable stadiometer and a weight scale, and BMI was calculated by fieldworkers. WC measurement was taken using a tape measure. The discrepancy tolerances of repeat measurement readings were not detailed in the NDNS method protocols. Omron HEM907, an automated validated monitor, was used to measure blood pressure in a sitting position after a five-minute rest. Trained fieldworkers took blood pressure measurements three times and results were presented based on the mean value of second and third readings with one-minute intervals[22,23].

Diet quality indices

To estimate diet quality, two existing diet scores were used: the Mediterranean Diet Score (MDS)[24] and Healthy Diet Score (HDS)[25]. Maynard *et al.* (2004) developed HDS based on Healthy Diet Indicator (HDI) and the UK

guidelines at that point in time, as recommended by the Committee on Medical Aspects of Food Policy (COMA)[25]. Modifications were applied to HDS for the current study to reflect UK current recommendations[22,26–30], and nuts were removed from the MDS scoring system as appropriate for the current study on diet and health associations with nut consumption. The potential top score of the modified MDS remained the same: 9, but the modified HDS had a potential top score of 14 while the original HDS scoring range was 0–12 (see Table A2 in Appendices). Tables A1 and A2 in Appendices show original and modified items of MDS and HDS items, respectively.

Statistical analysis

Prior to statistical analysis, TNS intake was defined and determined. TNS consumption was defined as: (i) any amount of consumption or (ii) ≥7·08 g (¼ oz) of TNS. The ≥7·08 g (¼ oz) cut-off was adopted to facilitate comparisons with previous cross-sectional analysis of associations between tree nut consumption and dietary scores/nutrient adequacy in a US adult population[21]. Data on TNS consumption were isolated from the database prior to statistical analysis and total TNS intake was calculated. Tree nuts included were almonds, walnuts, pecans, pine nuts, cashews, macadamia nuts, hazelnuts, pistachios, Brazil nuts and chestnuts. Although the US Food and Drug Administration recognises coconuts as a tree nut, they were excluded since they are fruits of palm trees and not commonly consumed whole as a snack food. Peanuts were also excluded since they are classified as legumes.

Statistical analysis was carried out using SPSS IBM 23, and a two-sided *P*-value of 0·05 was considered statistically significant. Data are presented as adjusted means (95 % CI) for individual nutrient intakes, total diet quality scores as well as levels of CVD risk markers, and as medians (with IQRs) for the amount of TNS consumed and age. To examine whether there was a statistically significant association between tree nut consumption and alcohol and total energy intakes as well as demographic variables, that is, age, sex, ethnicity, socio-economic and smoking status and region of residency, survey-adjusted generalised linear model (GLM) with a binary logistic link function was used. Survey-adjusted GLM with a linear link function (predictors: age, sex, ethnicity, socio-economic and smoking status, region of residency, total energy and alcohol intake) was used to examine whether there were significant differences between TNS consumers and non-consumers in their diet quality scores, nutrient intakes and CVD risk markers. To investigate dose–response associations between TNS consumption (g/4184 kJ energy intake) and diet quality and CVD risk markers, survey-adjusted multivariable linear regression models were used adjusting for the same covariates mentioned above. Normal residual distributions were checked by visual inspection of histogram and Q–Q plots; data with non-normally distributed residuals were log transformed using $\log_{10}$ for analysis of survey-adjusted GLM and multivariable linear regression. The results of analysis were back transformed into the geometric mean values. Homoscedasticity was checked by plotting the standardised residuals of dependent variables and predictors.

During the analysis, the weight factor provided by the NDNS database resource was applied to adjust for non-response and known socio-economic differences in the survey to ensure that the data were nationally representative for the UK population and reducing selection bias and non-response bias [31,32]. The weight factor used is wti_Y14 (weight for individual and diary-all ages, combined Y1-4) and wti_Y56 (weight for individual and diary-all ages, combined Y5-6) for investigating differences in diet quality scores and nutrient intakes between TNS consumers and non-consumers, associations between tree nut consumption and demographic variables and multivariable linear regression including diet quality scores. Weight factors wtn_Y14 (weight for nurse-all ages, combined Y1-4) and wtn_Y56 (weight for nurse-all ages, combined Y5-6) were used for GLM and multivariable linear regression including variables BMI, WC and blood pressure; and wtb_Y14 (weight for blood-all ages, combined Y1-4) and wtb_Y56 (weight for blood-all ages, combined Y5-6) were used for GLM and multivariable linear regression for blood analyte variables including CRP and lipids[31,32].

Results

Demographic information

Table 1 shows background characteristics of TNS consumers and non-consumers. Median TNS-A (any amount of TNS intake) consumption (*n* 484) contributed 0·8 % of total energy intake while median consumption in the TNS-B group (including individuals who consumed ≥7·08 g TNS per day, equivalent to ¼ oz, *n* 224) was 2·3 % of total energy intake. On average, TNS consumers were significantly older than non-consumers and were more likely to be female and non-smokers. TNS-A consumption was significantly associated with the demographic factors included, such as sex, ethnicity, socio-economic status, smoking status and region of residency. TNS-B consumption was also significantly associated with these demographic variables, except region of residency.

Diet quality scores

Geometric estimated marginal mean total scores of modified MDS were significantly higher in TNS-A consumers (5·9; 95 % CI 5·2, 6·6) compared with non-consumers (4·9; 95 % CI 4·4, 5·4; $P < 0·001$). Similarly, geometric estimated marginal mean total scores for the modified HDS were significantly higher in TNS-A consumers (6·1; 95 % CI 5·5, 6·8) compared with non-consumers (5·4; 95 % CI 4·9, 6·0; $P < 0·001$). Results for TNS-B consumers were

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Table 1 Background characteristics of tree nut snack (TNS) consumers compared to non-consumers in the UK adult population (≥19 years) based on NDNS 2008–2014, *n* 4738

		TNS-A			TNS-B		
		Consumers, *n* 484	Non-consumers, *n* 4254	*P*-value	Consumers, *n* 224	Non-consumers, *n* 4514	*P*-value
Amount of tree nuts consumed	Gram						
	Median	6·5			14·0		
	IQR	10·8			10·6		
	% Total energy intake						
	Median	0·8			2·3		
	IQR	2·2			5·1		
Age				<0·001*			<0·001*
Median		51	48		53	48	
IQR		24	27		24	27	
Sex (%)	Male	31·1	41·6	<0·001*	32·8	40·8	<0·001*
	Female	68·9	58·4		67·2	59·2	
Ethnicity (%)	White	87·8	93·8	0·003*	88·9	93·4	0·016*
	Mixed ethnic group	1·7	0·9		0·9	1·0	
	Black or Black British	1·6	2·0		1·7	1·9	
	Asian or Asian British	5·9	2·2		5·4	2·4	
	Any other group	3·1	1·2		3·0	1·3	
Region (%)	England	68·1	54·8	0·003*	64·8	55·8	0·131
	Scotland	11·9	17·9		11·9	17·5	
	Wales	12·5	14·8		16·7	14·4	
	Northern Ireland	7·5	12·6		6·6	12·3	
Socio-economic status (%)	Higher managerial and professional occupations	27·2	13·7	<0·001*	25·9	14·6	<0·001*
	Lower managerial and professional occupations	31·2	23·1		26·4	23·9	
	Intermediate occupations	8·2	10·5		9·7	10·3	
	Small employers and own account workers	11·0	10·4		11·1	10·4	
	Lower supervisory and technical occupations	6·5	9·5		7·0	9·3	
	Semi-routine occupations	9·4	15·1		13·2	14·6	
	Routine occupations	3·4	12·9		4·5	12·2	
	Never worked	1·3	3·1		0·7	3·0	
	Others	1·9	1·7		1·5	1·8	
Smoking status (%)	Current smoker	11·7	25·0	<0·001*	12·5	24·2	<0·001*
	Ex-Regular smoker	25·7	23·6		27·3	23·7	
	Never regular smoker	62·6	51·4		60·2	52·2	
Alcohol intake (g/d)				0·012*			0·002*
Median		6·0	0·7		4·4	1·8	
IQR		18·4	16·7		17·0	16·9	
Energy intake (kJ/d)				<0·001*			<0·001*
Unadjusted mean		7950·9	7325·3		8168·8	7350·5	
SD		2023·8	2364·4		2087·0	2344·7	

This is a descriptive table. Survey-adjusted generalised linear model with a linear binary logistic function was used to investigate the association between TNS consumption and demographic variables.
**P* was <0·05 indicating a significant association.

almost identical (data not shown). To investigate dose–response associations between every gram increase in TNS consumption per 4184 kJ of adult's energy intake and diet quality scores, the survey-adjusted regression model was adjusted for age, sex, ethnicity, socio-economic and smoking status, alcohol and energy intakes. There was no dose response observed in the scores of modified MDS and modified HDS ($P = 0{\cdot}726$ and $P = 0{\cdot}971$, respectively).

Nutrient intake

TNS consumers had significantly higher total energy, food energy, fat, *cis*-monounsaturated fatty acids, *cis n*-6 fatty acids, *cis n*-3 fatty acids (TNS-A only), intrinsic milk sugars and fibre intakes, as shown in Table 2. Saturated fatty acids, *trans*-fatty acids, total carbohydrate, starch, non-milk extrinsic sugars, intrinsic milk sugar and starch and alcohol (TNS-B only) intakes were significantly lower in TNS consumers. For micronutrients, as shown in Table 2, fully adjusted analysis revealed that TNS consumers, relative to non-consumers, had significantly higher intakes of vitamin A (TNS-A only), vitamin E, thiamin, riboflavin (TNS-B only), folate, pantothenic acid, biotin, vitamin C, potassium, magnesium, phosphorus, iron, copper, zinc, manganese and selenium and lower intakes of sodium and chloride. However, there were no differences between groups for vitamins D, riboflavin (TNS-A only), niacin equivalents, vitamin B_6, vitamin B_{12}, calcium and iodine.

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Table 2 Energy, macro- and micronutrient intake of tree nut snack consumers defined by any amount of daily consumption or ≥7·08 gram consumption per day, in the UK adult population (≥19 years) based on National Diet and Nutrition Survey 2008–2014, *n* 4738

	TNS-A					TNS-B				
	Consumers		Non-consumers			Consumers		Non-consumers		
	n 484		*n* 4254			*n* 224		*n* 4514		
Nutrients	Estimated marginal mean	95 % CI	Estimated marginal mean	95 % CI	*P*-value	Estimated marginal mean	95 % CI	Estimated marginal mean	95 % CI	*P*-value
Macronutrients (diet only, % food energy)*										
Total energy (kJ)	7365·1†	6335·8, 8393·9	6548·0	5532·5, 7563·0	<0·001	7628·3†	6581·4, 8675·5	6782·7	5771·8, 7793·5	<0·001
Food energy (kJ)	7127.0†	6155·9, 8098·6	6373·9	5415·4, 7332·0	<0·001	7417·8†	6430·0, 8405·2	6580·2	5626·6, 7533·3	<0·001
Protein	17·4	15·5, 19·2	17·3	15·5, 19·2	0·827	17·5	15·7, 19·4	17·3	15·5, 19·1	0·384
Fat	37·1†	34·2, 40·0	35·1	32·2, 38·0	<0·001	37·6†	34·7, 40·5	35·2	32·3, 38·0	<0·001
Saturated fatty acids	12·2†	10·6, 13·7	12·5	11·0, 14·0	0·035	11·8†	10·2, 13·3	12·3	10·8, 13·9	0·008
cis-Monounsaturated fatty acids	14·3†	13·0, 15·6	13·0	11·8, 14·3	<0·001	15·0†	13·6, 16·3	13·1	11·8, 14·4	<0·001
cis n-6 fatty acids	6·2†	5·5, 6·9	5·3	4·6, 6·0	<0·001	6·6 †	5·8, 7·3	5·4	4·7, 6·1	<0·001
cis n-3 fatty acids‡	1·1†	0·9, 1·3	1·0	0·8, 1·2	<0·001	1·0	0·8, 1·1	1·0	0·9, 1·1	0·469
Trans fatty acids	0·5†	0·4, 0·7	0·6	0·5, 0·7	<0·001	0·5†	0·3, 0·6	0·6	0·4, 0·7	<0·001
Carbohydrate	45·6†	42·4, 48·8	47·6	44·4, 50·8	<0·001	44·9†	41·7, 48·2	47·6	44·4, 50·7	<0·001
Total sugars	17·7	14·5, 20·9	17·2	14·1, 20·4	0·139	18·1	14·9, 21·4	17·8	14·6, 20·9	0·399
Starch	27·9†	25·1, 30·6	30·3	27·6, 33·0	<0·001	26·7†	24·0, 29·5	29·8	27·1, 32·4	<0·001
Non-milk extrinsic sugars	7·7†	4·7, 10·7	8·7	5·8, 11·7	0·001	7·5†	4·4, 10·5	9·0	6·1, 11·9	<0·001
Intrinsic milk sugars and starch	37·8†	34·8, 40·9	38·8	35·8, 41·9	0·001	34·3†	32·5, 36·2	35·9	34·4, 37·6	0·003
Intrinsic milk sugars‡	10·0†	8·2, 11·8	8·5	6·8, 10·3	<0·001	9·9†	8·1, 12·1	7·9	6·5, 9·6	<0·001
Non-starch polysaccharides (Englyst Fibre, g)	15·1†	13·2, 16·9	13·3	11·4, 15·1	<0·001	15·8†	13·9, 17·7	13·7	11·9, 15·5	<0·001
Alcohol (g)‡§	12·1	8·8, 16·5	12·9	9·6, 17·5	0·290	10·0†	7·1, 14·0	12·7	9·6, 16·9	0·013

	TNS-A					TNS-B				
	Consumers		Non-consumers			Consumers		Non-consumers		
	n 484		*n* 4254			*n* 224		*n* 4514		
Nutrients	Estimated marginal mean	95 % CI	Estimated marginal mean	95 % CI	*P*-value	Estimated marginal mean	95 % CI	Estimated marginal mean	95 % CI	*P*-value
Micronutrients**										
Vitamin A (retinol equivalents) (μg)‡§	904·9†	753·4, 1086··9	828·9	696·5, 986·5	0·024	966·1	794·0, 1175·2	885·5	750·9, 1044·2	0·128
Vitamin D (μg)‡§	2·5	2·1, 3·0	2·4	2·0, 2·8	0·213	2·4	2·0, 2·9	2·4	2·0, 2·8	0·912
Vitamin E (mg)‡	11·6†	10·1, 13·0	9·8	8·4, 11·2	<0·001	11·6†	10·6, 12·7	9·3	8·6, 10·1	<0·001
Thiamin (mg)	1·4†	1·2, 1·6	1·3	1·1, 1·5	0·001	1·5†	1·3, 1·7	1·3	1·1, 1·5	<0·001
Riboflavin (mg)	1·5	1·2, 1·7	1·4	1·2, 1·7	0·196	1·5†	1·3, 1·8	1·4	1·2, 1·7	0·003
Niacin equivalent (mg)	33·5	28·7, 38·3	33·8	29·1, 38·6	0·486	34·4	29·5, 39·3	33·8	29·1, 38·5	0·382
Vitamin B_6 (mg)‡	1·9	1·5, 2·3	1·9	1·5, 2·3	0·992	1·9	1·7, 2·1	1·9	1·8, 2·1	0·530
Vitamin B_{12} (μg)‡§	4·6	3·6, 6·0	4·7	3·7, 6·0	0·777	4·5	3·9, 5·2	4·8	4·3, 5·5	0·154

Table 2 *Continued*

Nutrients	TNS-A					TNS-B				
	Consumers		Non-consumers			Consumers		Non-consumers		
	n 484		*n* 4254			*n* 224		*n* 4514		
	Estimated marginal mean	95 % CI	Estimated marginal mean	95 % CI	*P*-value	Estimated marginal mean	95 % CI	Estimated marginal mean	95 % CI	*P*-value
Folate (μg)	242·3†	201·9, 282·6	229·4	189·5, 269·2	0·001	255·7†	214·7, 296·6	237·0	197·5, 276·6	0·001
Pantothenic acid (mg)	5·6	4·6, 6·5	5·3	4·4, 6·3	0·007	5·9†	4·9, 6·8	5·3	4·4, 6·2	<0·001
Biotin (μg)	36·4†	30·1, 42·7	30·0	23·8, 36·3	<0·001	41·3†	34·9, 47·6	31·7	25·5, 37·9	<0·001
Vitamin C (mg)‡§	79·1†	58·4, 107·3	62·1	46·0, 83·8	<0·001	90·4†	75·3, 108·6	79·0	67·8, 92·3	0·012
Sodium (mg)	1854·8†	1596·0, 2113·6	2053·9	1798·5, 2309·3	<0·001	1732·2†	1469·6, 1994·8	2006·2	1752·6, 2259·8	<0·001
Potassium (mg)	2866·7†	2595·1, 3138·2	2645·3	2377·3, 2913·3	<0·001	3021·3†	2746·0, 3296·6	2694·8	2428·8, 2960·5	<0·001
Calcium (mg)	696·6	591·3, 801·8	702·8	599·0, 806·7	0·541	717·4	610·8, 824·0	709·6	606·7, 812·6	0·599
Magnesium (mg)	276·9†	251·0, 302·8	237·8	212·3, 263·4	<0·001	301·5†	275·3, 327·8	245·3	219·9, 270·6	<0·001
Phosphorus (mg)	1165·8†	1056·1, 1275·6	1125·4	1017·1, 1233·7	<0·001	1191·5†	1080·3, 1302·6	1126·2	1018·9, 1233·5	<0·001
Iron (mg)	11·1†	9·9, 12·4	10·4	9·1, 11·6	<0·001	11·2†	9·9, 12·4	10·5	9·3, 11·7	<0·001
Copper (mg)‡§	1·2†	1·0, 1·4	1·0	0·9, 1·2	<0·001	1·4†	1·3, 1·6	1·2	1·1, 1·3	<0·001
Zinc (mg)	9·0†	7·9, 10·0	8·8	7·7, 9·8	0·044	9·3†	8·2, 10·3	8·8	7·8, 9·8	0·002
Chloride (mg)	3017·1†	2631·3, 3402·9	3286·7	2906·0, 3667·5	<0·001	2877·8†	2486·4, 3269·1	3242·2	2864·3, 3620·1	<0·001
Manganese (mg)	3·3†	2·8, 3·8	2·8	2·3, 3·3	<0·001	3·5†	3·0, 4·0	2·9	2·4, 3·4	<0·001
Iodine (μg)	161·9	131·1, 192·7	158·7	128·3, 189·1	0·282	158·2	127·0, 189·4	158·6	128·5, 188·8	0·914
Selenium (μg)‡	56·9†	48·3, 65·4	51·9	43·4, 60·3	<0·001	54·9†	49·8, 60·5	50·5	46·6, 54·8	0·004

The actual sample size in the computation for vitamin A and vitamin D, for TNS-A consumers was 314 and for TNS-A non-consumers was 2172, whereas for TNS-B consumers was 138 and for TNS-B non-consumers was 2348. The actual sample size in the computation for alcohol, *cis*-*n*3 fatty acids, intrinsic milk sugars and starch, vitamin E, vitamin B_6, vitamin B_{12}, vitamin C, copper and selenium for TNS-B consumers was 138 and for TNS-B non-consumers was 2348. There were no missing values in the computation for other nutrients as outcomes.

*Survey-adjusted generalised linear model (GLM) with a linear link function and predictors such as age, sex, ethnicity, region of residency, socio-economic and smoking status was used for energy intake as an outcome for TNS-A; survey-adjusted GLM with a linear link function and predictors such as age, sex, ethnicity, region of residency, socio-economic and smoking status, alcohol and energy intakes was used for other macronutrient intake outcomes for TNS-A; survey-adjusted GLM with a linear link function and predictors such as age, sex, ethnicity, region of residency, socio-economic and smoking status, and energy intake was used for alcohol intake as an outcome for TNS-A. The same statistical analysis was conducted for TNS-B, but region of residency was excluded from predictors.

†$P < 0·05$ showed a significant difference.

‡Geometric marginal means were presented due to non-normally distributed residual data in TNS-B population.

§Geometric marginal means were presented due to non-normally distributed residual data in TNS-A population.

**Survey-adjusted GLM with a linear link function and predictors: age, sex, ethnicity, region of residency, socio-economic and smoking status, alcohol and energy intakes was used for TNS-A. The same statistical analysis was conducted for TNS-B but region of residency was excluded from predictors.

Public Health Nutrition

CVD risk markers

Blood samples were not available from all participants, and anthropometric and blood pressure data were also missing. Associations between TNS consumption and CVD risk markers were analysed for the remaining participants. The estimated marginal mean (95 % CI) values of CVD risk markers are shown in Table 3. For TNS-A consumers, BMI, WC, SBP and DBP were significantly lower, and HDL was significantly higher compared to non-consumers. For those consuming >7·08 g TNS/d (TNS-B), only WC, SBP and DBP were significantly lower compared to non-consumers (data shown in online supplementary material). Survey-adjusted regression analysis showed that for every gram increase in TNS consumption per 4184 kJ of adults' energy intake (Table 3), SBP was significantly lower demonstrating a dose–response relationship ($P = 0·028$).

Discussion

Interventional and observational evidence suggests that replacing refined carbohydrate-based snacks with tree nut snacks may improve blood lipid profiles, management of body weight[33,34] and nutrient intakes. However, TNS intakes in the general UK population have not been fully investigated. Previous studies have been conducted in the NHANES US adult population[20,21] using multiple 24-h dietary recalls to collect food intake data. This cross-sectional analysis using a representative UK adult population revealed that just 10 % of respondents reported consuming any amount of TNS during their 4-d food intake recording period, just less than 5 % reported consuming more than 7·08 g (¼ oz) per day on average (around a handful over the 4-d period), and only 0·34 % reported consuming the US Food and Drug Administration recommendation of 42·5 g per day[35]. The relatively small sub-population of TNS consumers was more likely to be female, white, older and living in England and less likely to be current smokers relative to non-consumers.

Increments in TNS consumption (g per 4184 kJ of energy intake) were not associated with significantly greater modified MDS and HDS in consumers. This lack of dose–response relationship could be due to the low consumption of TNS in the population (for TNS-A consumers, median 0·8 % of total energy intake and 6·5 g/d in terms of total weight intake; for TNS-B consumers, 2·3 % of total energy intake, and 14·0 g/d in terms of total weight intake). TNS consumption status may be an indicator of improved overall diet quality, but the actual amount consumed has very little practical impact.

Since TNS consumption status appears to act as a marker of healthy dietary patterns, it is not surprising that the overall nutrient intake profile of TNS consumers was more favourable compared to non-consumers. The contribution of non-milk extrinsic sugar intakes to energy was only marginally lower in TNS consumers (a difference of

Table 3 CVD risk marker values in UK adults (≥19 years) based on National Diet and Nutrition Survey 2008–2014, in consumers of any amount of tree nut snack (TNS-A) and non-consumers, and the association of tree nut snack consumption and risk markers

	Value†						Associations between tree nut consumption and CVD risk markers‡			
	Consumers *n* 484		Non-consumers *n* 4254							
CVD risk marker	Estimated marginal mean	95 % CI	Estimated marginal mean	95 % CI	*P*-value		*β*	95 % CI	*P*-value	R^2
BMI (kg/m²)§,‖	25·4	24·0, 26·8	26·3	25·0, 27·8	0·002*		1·035	0·991, 1·081	0·128	0·121
WC (cm)¶	91·5	88·5, 94·6	94·2	91·5, 97·0	<0·001*		0·094	−0·080, 0·268	0·289	0·293
SBP (mmHg)††	119·7	116·2, 123·2	124·0	120·8, 127·1	<0·001*		−0·242	−0·458, −0·026	0·028*	0·286
DBP (mmHg)††	69·2	66·8, 71·7	72·0	69·7, 74·2	<0·001*		−0·034	−0·196, 0·127	0·677	0·033
TC (mmol/l)‡‡	4·9	4·5, 5·3	4·9	4·5, 5·3	0·627		0·011	−0·007, 0·029	0·218	0·109
TAG (mmol/l)§,§§	1·1	0·9, 1·3	1·1	0·9, 1·4	0·220		0·972	0·813, 1·164	0·757	0·084
HDL-C (mmol/l)‡‡	1·5	1·4, 1·7	1·4	1·3, 1·6	0·008*		−0·001	−0·009, 0·007	0·754	0·277
LDL-C (mmol/l)‖‖	2·9	2·6, 3·3	2·9	2·6, 3·2	0·980		0·011	−0·006, 0·028	0·204	0·046
TC:HDL-C‡‡	3·5	3·1, 4·0	3·6	3·2, 4·0	0·412		0·016	−0·005, 0·037	0·143	0·163
CRP (mg/l)§,¶¶	1·9	1·3, 2·6	2·1	1·5, 2·9	0·062		1·194	0·933, 1·528	0·157	0·095

BMI, body mass index; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; TAG, triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; CRP, C-reactive protein

†Survey-adjusted generalised linear model with a linear link function and predictors, such as age, sex, ethnicity, region of residency, socio-economic and smoking status, alcohol and energy intakes was used.

‡Survey-adjusted multivariable linear regression was used, adjusted for age, sex, ethnicity, region of residency, socio-economic and smoking status and alcohol intake.

§Geometric marginal means and geometric *β* values were presented due to non-normally distributed residual data. Geometric *β* values were interpreted as ratios of geometric means.

Due to missing data, sample sizes were as follows: tree nut snack-A consumers ‖ 241, ¶ 384, †† 326, ‡‡ 274, §§,¶¶ 176 and ‖‖ 273; non-consumers ‖ 1,616, ¶ 3,110, †† 2,456, ‡‡ 2,132, §§ 1,161, ‖‖ 2,096 and ¶¶ 1,164.

*$P < 0·05$ showed a significant difference.

1·0 % of energy). Fibre (non-starch polysaccharides) intakes were 1·8 g higher in TNS consumers compared with non-consumers, but TNS intake is unlikely to contribute more than a third of this difference, with the remainder due to greater intakes of other fibre-rich foods.

The observations reported here and in the US population imply that TNS are usually eaten as a part of an overall healthier dietary pattern in industrialised countries[21], which would be predicted to translate to better cardiovascular health outcomes. UK TNS consumers had significantly reduced BMI, WC, SBP and DBP and significantly higher HDL-C, but the slightly lower mean CRP in TNS consumers did not reach statistical significance compared with non-consumers. O'Neil *et al.*(2015) previously reported that ≥7·08 g tree nut consumption was associated with lower BMI and WC, as well as SBP and higher HDL-C, in the US adult population adjusted for the same covariates as used in the present analysis, plus physical activity level[20]. Overall feeding trials have reported that higher tree nut consumption did not result in weight gain[18,19], which may be related to their satiating/satiety-inducing properties[36], as well as limited lipid bioaccessibility[37]. Since TNS consumers' median intake was low in the UK, observed differences in BMI and WC could be related to confounding factors such as physical activity levels, which was not considered in the present analysis due to lack of available data. Mean SBP was 4·3 mmHg lower and mean DBP was 2·8 mmHg lower in TNS consumers compared to non-consumers, a clinically meaningful difference that would be predicted to reduce risk of CVD. The SUN prospective cohort study reported that there was no association between tree nut consumption and blood pressure; the potential reasons could be an underestimated amount of nut consumption, no assessment on the change in nut consumption during follow-up, and no specific information on preparation method, for example, salted, roasted or raw[38]. The Physician's Health Study observed blood pressure reduction only in lean volunteers[39]. A recent meta-analysis of 21 randomised control trials reported that total nut consumption lowered SBP in participants without type 2 diabetes, and mixed nuts also lowered DBP[40]. Although plasma CRP concentrations were not significantly different in the NDNS cohort, a cross-sectional study using data from the Nurse's Health Study and Health Professional Follow-Up Study revealed that consumers eating tree nuts ≥5 times weekly based on FFQ had significantly lower CRP[41], suggesting larger differences in intake may be required to impact on systemic inflammatory markers. However, a meta-analysis of 20 randomised controlled trials suggested that tree nut consumption did not reduce CRP[42]. In the current study, the amount of nuts consumed by consumers in the current UK cohort was low, and therefore, the SBP and DBP differences observed are likely to be the sum effect of an overall healthier dietary pattern including TNS[43–46].

A significant difference was observed in HDL-C between TNS consumers and non-consumers. Cross-sectional analysis in the US adult population also reported higher HDL-C in TNS consumers[20]. There were no significant differences observed in other blood lipids. A recent meta-analysis of 61 interventional clinical trials revealed that tree nut intake reduced TC, TAG and LDL-C, and it was reported that the dosage of tree nut intake determined cholesterol lowering capacity rather than the nut types[47]. A pooled analysis of 25 feeding trials conducted in seven countries demonstrated the reduction of TC, LDL-C and the ratio of TC to HDL-C but failed to report the increase of HDL-C in response to tree nut intake[48]. These inconsistent associations of tree nut consumption and blood pressure, CRP and blood lipids between cross-sectional analysis and clinical trials could be due to different dosage and duration of consumption (duration of the study), residual confounding effects, characteristics such as baseline lipid profile, as well as study sample size relating to statistical power[41].

Strengths of the current study include using a relatively large, nationally representative UK population, and the close agreement with results reported in a nationally representative US population suggests that findings may be generalisable to other industrialised countries with similar dietary profiles. The availability of estimated portion size food diary data over a 4-d period is considered to be one of the more accurate dietary assessment methods in large populations, although underreporting of energy intake is a well-known problem with this methodology that limits the conclusions that can be drawn. Furthermore, the use of 4-d estimated food diaries means that significant nut intakes on other days may have been missed and a significant proportion of TNS consumers may have been wrongly classified as non-consumers; analysis based on frequency of tree nut consumption was not possible. Available information on physical activity was incomplete so statistical analysis models could not be adjusted for this potentially confounding factor. Different types of tree nuts have differing nutrient profiles and potentially nutrient bioaccessibility, and therefore it may be misleading to group them altogether in terms of associations with CVD risk factors. In addition to that, missing data for CVD risk factors resulted in lower sample sizes.

In conclusion, the prevalence of TNS consumers in the UK adult population is estimated to be approximately 10 %, and median intakes were low in the group classified as TNS consumers. Tree nut snack consumption was associated with higher diet quality scores and a more favourable nutrient intake profile. Tree nut snack consumption may be a marker of a healthy dietary pattern and is associated with lower adiposity and blood pressure. It is recommended that tree nuts should replace high refined carbohydrate-based snacks as part of a healthy diet. To determine the relative contribution of tree nuts to the sum impact of a healthier dietary pattern on risk of CVD, future randomised controlled trials should investigate the effect of replacing usual refined carbohydrate snacks with tree nuts on markers of cardiometabolic disease risk.

Acknowledgements

Acknowledgements: We thank the two anonymous reviewers for their valuable insights. *Financial support:* This work was supported in part by the Almond Board of California (grant number 15-BERRYS-01) and the Indonesia Endowment Fund for Education. The funders had no role in the design, analysis or writing of this article. *Conflict of interest:* None. *Authorship*: The authors' responsibilities were as follows: V.D., W.L.H., S.E.B. and G.K.P. designed the research; V.D. analysed the data; L.F. advised on analysis of the data; V.D. and W.L.H. wrote the manuscript; W.L.H. had primary responsibility for final content. All authors read, commented and approved the final manuscript. *Ethics of human subject participation:* The current study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were approved by the National Health Service Local Research Ethics Committee covering each of the postal sectors. Written informed consent was obtained from all participants.

Supplementary material

For supplementary material accompanying this paper visit https://doi.org/10.1017/S1368980019003914

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Appendix A: Diet quality scores

Table A1. Original and modified Mediterranean Diet Score (MDS) and its scoring system[24]

Food group		Scoring*	
Original MDS	Modified MDS		
Cereals	Cereals	>median	1 (else: 0)
Vegetables	Vegetables	>median	1 (else: 0)
Fruits and Nuts	**Fruits**	>median	1 (else: 0)
Legumes	Legumes	>median	1 (else: 0)
Fish	Fish	>median	1 (else: 0)
Meat	Meat	<median	1 (else: 0)
Dairy products	Dairy products	<median	1 (else: 0)
Ratio of unsaturated to saturated fats	Ratio of unsaturated to saturated fats	>median	1 (else: 0)
Alcohol	Alcohol	10-50 g/d for men	1 (else: 0)
		5-25 g/d for women	1 (else: 0)

*Except alcohol, scoring of each food group is based on population and sex-specific median.

Table A2. Original and modified Healthy Diet Score (HDS) and the cut-off values for scoring[25]

HDS			HDS – modified based on current UK recommendations		
Index item	Cut-off values		Index item	Cut-off values	
	Score 1	Score 0		Score 1	Score 0
Saturated fatty acids (% energy intake)	0-10	>10	Saturated fatty acids (% energy intake)	0-11[a]	>11
Polyunsaturated fatty acids (% energy intake)*	6-10	<6 or >10	Polyunsaturated fatty acids (% energy intake)	6[b]-10	<6 or >10
Protein (% energy intake)	10-15	<10 or >15	Protein (% energy intake)	9[c]-15	<9 or >15
Total carbohydrate (% energy intake)*	50-70	<50 or >70	Total carbohydrate (% energy intake)	50[d]-70	<50 or >70
Dietary fibre (g)*	18-32	<18 or >32	Dietary fibre (g)	18[d]-32	<18 or >32
Fruits and vegetables (g)	≥400	<400	Fruits and vegetables (g)	≥400[e]	<400

Pulses and nuts (g)*	≥30	<30	Pulses (g)	≥30	<30
Total non-milk extrinsic sugar (% total energy intake)*	0-10	>10	Total non-milk extrinsic sugar (% total energy intake)	0-5[d]	>5
Cholesterol (mg)*	0-245	>245	Trans-fatty acids (% energy intake)	≤2[e]	>2
Fish (g)*	≥32	<32	Fish (g)	≥40[e]	<40
Red meat and meat processed products (g)*	≤90	>90	Oily fish (g)	≥20[e]	<20
Calcium (mg)*	≥700	<700	Red meat and meat processed products (g)	≤70[i]	>70
			Calcium (mg)	≥700[c]	<700
			Sodium (mg)	≤2400[c]	>2400

* Items based on advice on healthy eating as recommended by the UK Committee on Medical Aspects of Food Policy (COMA)[25]

a Based on NDNS Results from Years 5 and 6 (combined) of the Rolling Programme (2012/2013 – 2013/2014)[22,23], UK Government Dietary Recommendations by Public Health England (2016)[26], Cardiovascular Disease Outcomes Strategy by UK Department of Health (2013)[27] and Draft report: Saturated fats and health by Scientific Advisory Committee on Nutrition (SACN) 2018[29]

b Based on UK Government Dietary Recommendations by Public Health England (2016)[26]

c Based on UK Government Dietary Recommendations by Public Health England (2016)[26]

d Based on UK Government Dietary Recommendations by Public Health England (2016)[26] and Scientific Advisory Committee on Nutrition (SACN) Report 2015 on Carbohydrates and Health[30]

e Based on NDNS Results from Years 5 and 6 (combined) of the Rolling Programme (2012/2013 – 2013/2014)[22,23] and The Eatwell Guide by Public Health England (2016)[28]

Appendix B: Associations between tree nut consumption (≥7.08 g) and CVD risk markers

Table B1. Cardiovascular disease risk marker values in UK adults (≥19 y) based on NDNS 2008-2014, n = 4,738, in consumers of ≥7.08 g of tree nut snack and non-consumers.

CVD risk marker	Value[a] (Estimated marginal mean (95% CI))		*P*-value
	Consumers, n=224	Non-consumer, n=4,514	
BMI (kg/m2)[b, ŧ]	26.0 (24.4, 27.6)	26.1 (24.8, 27.5)	0.705
WC (cm)[c]	91.6 (88.3, 94.9)	94.0 (91.2, 96.8)	0.015*
SBP (mmHg)[d]	118.3 (114.5, 122.2)	123.7 (120.5, 126.9)	<0.001*
DBP (mmHg)[d]	69.3 (66.5, 72.0)	71.7 (69.4, 74.0)	0.005*
TC (mmol/l)[e]	5.0 (4.6, 5.4)	4.9 (4.5, 5.3)	0.299
TAG (mmol/l)[f, ŧ]	1.1 (0.8, 1.4)	1.1 (0.9, 1.4)	0.424
HDL-C (mmol/l)[e]	1.5 (1.4, 1.7)	1.5 (1.3, 1.6)	0.053
LDL-C (mmol/l)[g]	2.9 (2.6, 3.3)	2.9 (2.6, 3.2)	0.531
TC:HDL-C[e]	3.6 (3.1, 4.0)	3.6 (3.2, 4.0)	0.924
CRP (mg/l)[h, ŧ]	2.0 (1.4, 2.8)	2.0 (1.5, 2.8)	0.685

a Survey-adjusted GLM with a linear link function and predictors: age, sex, ethnicity, socio-economic and smoking status, alcohol and energy intakes was used.

ŧGeometric marginal means were presented due to non-normally distributed residual data.

Due to missing data, sample sizes were as follows: TNS-B consumer 110[b], 186[c], 162[d], and 138[e,g], 86[f,h], non-consumers 1,747[a], 3,308[c], 2,620[d], 2,268[e], 1,251[f], 2,231[g], and 1,254[h].

Chapter 4: Whole almond consumption is associated with better diet quality and cardiovascular disease risk factors in the UK adult population: National Diet and Nutrition Survey (NDNS) 2008-2017

This chapter of this thesis incorporating publications presents the published paper in the European Journal of Nutrition.

(Dikariyanto *et al.*, 2020)

European Journal of Nutrition
https://doi.org/10.1007/s00394-020-02270-9

ORIGINAL CONTRIBUTION

Whole almond consumption is associated with better diet quality and cardiovascular disease risk factors in the UK adult population: National Diet and Nutrition Survey (NDNS) 2008–2017

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Received: 28 November 2019 / Accepted: 28 April 2020
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Abstract

Purpose This work aimed to estimate whole almond consumption in a nationally representative UK survey population and examine associations with diet quality and cardiovascular disease (CVD) risk.

Methods Four-day food record data from the National Diet and Nutrition Survey (NDNS) 2008–2017 ($n = 6802$, age ≥ 19 year) were analyzed to investigate associations between whole almond consumption and diet quality, measured by the modified Mediterranean Diet Score (MDS) and modified Healthy Diet Score (HDS), and CVD risk markers, using survey-adjusted multivariable linear regression.

Results Whole almond consumption was reported in 7.6% of the population. Median intake in whole almond consumers was 5.0 g/day (IQR 9.3). Consumers had higher diet quality scores relative to non-consumers; higher intakes of protein, total fat, monounsaturated, *n-3* and *n-6* polyunsaturated fats, fiber, folate, vitamin C, vitamin E, potassium, magnesium, phosphorus, and iron; and lower intakes of *trans*-fatty acids, total carbohydrate, sugar, and sodium. BMI and WC were lower in whole almond consumers compared to non-consumers: 25.5 kg/m^2 (95% CI 24.9, 26.2) vs 26.3 kg/m^2 (25.9, 26.7), and 88.0 cm (86.2, 89.8) vs 90.1 cm (89.1, 91.2), respectively. However, there were no dose-related fully adjusted significant associations between increasing almond intake (g per 1000 kcal energy intake) and lower CVD risk markers.

Conclusions Almond intake is low in the UK population, but consumption was associated with better dietary quality and lower CVD risk factors. Habitual consumption of whole almonds should be encouraged as part of a healthy diet.

Keywords Almonds · Cross-sectional analysis · Diet quality · Cardiovascular disease · Nutrients

Abbreviations

AKM	Almond kernel only plus almond kernel in mixed nuts
AKO	Almond kernel only
BMI	Body mass index
COMA	Committee on Medical Aspects of Food Policy
CRP	C-reactive protein
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
GLM	Generalized linear model
HDL-C	High-density lipoprotein
HDS	Healthy diet score
IQR	Interquartile range
LDL-C	Low-density lipoprotein
MDS	Mediterranean Diet Score
MUFA	Monounsaturated fatty acids
NDNS	National Diet and Nutrition Survey
NDNS-RP	National Diet and Nutrition Survey—Rolling Program
NHANES	National Health and Nutrition Examination Survey
PUFA	Polyunsaturated fatty acids
SBP	Systolic blood pressure

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00394-020-02270-9) contains supplementary material, which is available to authorized users.

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Published online: 16 May 2020

SD	Standard deviation
SFA	Saturated fats
TAG	Triglycerides
TC	Total cholesterol
WC	Waist circumference

Introduction

Urgent calls for a revolution in global food systems have been made to meet the United Nations (UN) Sustainable Development Goals (SDGs) and Paris Agreement to eradicate malnutrition and non-communicable diseases (NCDs) while conserving the environment and biodiversity [1–4]. The EAT Lancet report set dietary targets for healthy diets from sustainable food systems, including a doubling of consumption of fruits, vegetables, legumes and nuts. Almonds are the most commonly consumed tree nut in many countries, with global agricultural production of 2018/2019 having increased by 20% compared to a decade ago [5]. North America accounted for the world's highest production of tree nuts, but Europe was the largest consumer worldwide. Hence, many of the main importing countries were in Europe, including Spain, Germany, Italy, France, the Netherlands and the UK [5].

Almonds are characterized as nutrient-dense foods, being rich in protein, unsaturated fatty acids, dietary fiber, and micronutrients [6, 7], as well as having a low glycemic load attribute which have been linked to lower cardiometabolic disease risk [8–10]. Almonds are also a source of beneficial non-nutrient bioactives, such as (poly)phenolic compounds [11–13]. According to a qualified health claim issued by US Food and Drug Administration (FDA) in 2003, "scientific evidence suggests but does not prove that eating 1.5 oz (42.5 g) per day of most nuts, such as almonds, as part of a diet low in saturated fats and cholesterol, may reduce the risk of heart disease" [14]. Randomized controlled trials (RCTs) have provided evidence that almond consumption lowers blood LDL-cholesterol and maintains or increases HDL-cholesterol concentrations, lowers blood glucose levels, as well as some inflammatory markers [15–23]. Regarding weight management, high doses of almonds incorporated in a diet have been shown to cause a greater reduction of weight/body mass index (BMI), waist circumference (WC) and fat mass in overweight and obese subjects in comparison with a complex carbohydrate-enriched diet [24], although doses of < 42.5 g/day were not effective for weight loss in a meta-analysis [23].

Almonds can be consumed whole, chopped, sliced, ground, roasted, raw, blanched, salted, coated with chocolate or sweetened, or as an oil, butter or paste. Whole kernels, a convenient snack food, are the most efficient way of consuming quantities sufficient to modify LDL-cholesterol concentrations. Very little is known about population-level intakes of almonds. An observational study in USA adults (≥ 19 year) using data from the National Health and Nutrition Examination Survey (NHANES) 2001–2010 ($n = 24{,}808$) revealed that the prevalence of almond consumption (including whole almond kernels, with and without salt, almond butter, and almond paste) measured by 24 h dietary recalls was 1.6% [25]. This study also revealed that almond consumption (estimated usual intake 29.5 g/day) was associated with lower BMI and WC, and that consumers had better diet quality and greater nutrient adequacy than non-consumers [25]. Therefore, the current study aimed to investigate associations between whole almonds and diet quality, nutrient intakes, as well as CVD risk markers using 4 day food records from a nationally representative population of 6802 adults who participated in the UK National Diet and Nutrition Survey (NDNS) rolling program 2008–2017 [26]. It was hypothesized that whole almond consumption was linked to higher diet quality scores, better nutrient intakes, and improved profile of intermediary CVD risk factors.

Materials and methods

The National Diet and Nutrition Survey Rolling Programme (NDNS-RP) and study population

The NDNS-RP is a long-running government-funded scheme to assess diet, nutrient intake and nutritional status of the general population (> 1.5 year) living in private households in the UK (England, Scotland, Wales and North Ireland) [26–29]; the study is registered with the ISRTCN registry as ISRCTN17261407. Random sampling was carried out on addresses throughout the UK listed in Postcode Address File (PAF). Of all the addresses, Primary Sampling Units (PSUs) were created to make small clusters of geographical area based on postcode sectors to increase cost effectiveness. The randomly selected addresses were drawn from each PSU. An adult in each household was randomly selected, and where a single address had multiple households, a household was also selected randomly. Full details on the random selection procedure are available at the NDNS User Guide [30].

The cross-sectional analysis reported here included data from adult participants (≥ 19 year, $n = 6802$), who completed at least 3 days of 4 days estimated food diary in the NDNS-RP 2008–2017 (Year 1–9) [26–29]. Of 6802 adult respondents, 147 individuals completed only 3 days of 4 days estimated food diary and the remainder of the sample completed all 4 days. Participants were asked to record all food and drink consumed over 4 consecutive days comprising 3 week-days and a weekend day, including portion sizes, brand names, and recipes for home cooked foods. Food and

drink items were assigned a code and dietary analysis was conducted using the DINO (Diet in Nutrients Out) platform based on Public Health England's NDNS Nutrient Databank food composition data.

Ethics

For NDNS RP 2008–2013, ethical approval was obtained from the Oxfordshire A Research Ethics Committee (Ref. No. 07/H0604/113) and for NDNS RP 2014–2017, the approval was received from the Cambridge South NRES Committee (Ref. No. 13/EE/0016) [31]. Informed consent was obtained from every participant. The survey involved interview visits for questionnaires, 4 days food diaries, and a nurse visit for anthropometry and physical measurements and also blood and 24 h urine sample collections [26–29].

Definition of almond consumption

The intake of raw and roasted whole almonds was defined and determined both as a single nut product (almond kernel only), and also total almond kernel intake where also derived from mixed nut/fruit and nut products. Thus, whole almond consumption was defined as: (1) any amount of intake of whole almond kernels only (AKO), or (2) AKO in addition to any amount of intake of almond kernels from mixed nut products and mixed nut and fruit products (AKM). Data related to almond consumption were isolated from the NDNS Year 1–9 database, i.e. ALMONDS KERNEL ONLY, MIXED NUTS AND RAISINS UNSALTED, MIXED NUTS KERNELS ONLY SALTED, MIXED NUTS UNROASTED UNSALTED and TRAIL HAWAIIAN TROPICAL MIX MIXED NUTS DRIED FRUIT. It was necessary to estimate the amount of whole almond kernels in mixed nut products and mixed nut and fruit products by market sampling. Mixed nut products containing almond kernels from 19 brands were purchased from UK supermarkets, such as Tesco, Sainsbury's, Waitrose, M&S, ASDA, Coop and Lidl. Almonds contained in these mixed nut/fruit and nut products were weighed manually and the percentage of almond kernel portion in comparison with the total weight of the products was calculated in order to estimate total intakes of whole almond kernels from both mixed nut/fruit and nut products and almond kernel only products (see Supplementary material).

Diet quality indices

To estimate diet quality, two existing diet scores were adapted for the current study: the Mediterranean Diet Score (MDS) [32] and Healthy Diet Score (HDS) [33]. Maynard et al. (2004) developed the HDS based on Healthy Diet Indicator (HDI) and the UK guidelines at that point in time, as recommended by the Committee on Medical Aspects of Food Policy (COMA) [33]. Modifications were applied to HDS for this study to reflect UK current recommendations [27, 34–38], and nuts were removed from the MDS scoring system as appropriate for this study on diet and health associations with nut consumption. The potential top score of the modified MDS remained the same: 9, but the modified HDS had a potential top score of 14, while the original HDS scoring range was 0–12. Tables A1 and A2 in supplements show original and modified items of MDS and HDS items, respectively.

Cardiovascular disease risk markers

Body mass index (BMI; kg/m^2), waist circumference (WC; cm), systolic blood pressure (SBP; mmHg), diastolic blood pressure (DBP; mmHg), total cholesterol (TC; mmol/l), triglycerides (TAG; mmol/l), high-density lipoprotein (HDL-C; mmol/l), low-density lipoprotein (LDL-C; mmol/l), TC:HDL-C (the ratio of TC and HDL-C) and C-reactive protein (CRP; mg/l) were the CVD risk markers included in the analysis. Interviewer measurement protocols and procedures for blood sample collection, processing, analysis and quality controls are detailed elsewhere [26–29]. Body height and weight were measured using a portable stadiometer and a weight scale, and BMI was calculated by fieldworkers. Waist circumference measurement was taken using a tape measure. The discrepancy tolerances of repeat measurement readings were not detailed in the NDNS method protocols. Omron HEM907, an automated validated monitor, was used to measure blood pressure in a sitting position after a 5-min rest. Trained fieldworkers took blood pressure measurements three times and results were presented based on the mean value of second and third readings with one-minute intervals [26–29].

Statistical analysis

Statistical analysis was carried out using SPSS IBM 23 and a two-sided P value of 0.05 was considered statistically significant. Data are presented as adjusted means (95% CI) for individual nutrient intakes, total diet quality scores as well as levels of CVD risk markers, and as medians (with IQRs) for amount of whole almonds consumed and age. To examine whether there was a statistically significant association between almond consumption and alcohol and total energy intakes as well as demographic variables, i.e. age, sex, ethnicity, socio-economic and smoking status and region of residency, survey-adjusted generalized linear model (GLM) with a binary logistic link function was used. Survey-adjusted GLM with a linear link function

(predictors: age, sex, ethnicity, socio-economic and smoking status, region of residency, total energy and alcohol intake) was used to examine whether there were significant differences between whole almond consumers and non-consumers in their diet quality scores, nutrient intakes and CVD risk markers. These predictors were included due to their associations with CVD to determine whether differences in consumer groups were independent of these factors. Age, sex and ethnicity are known influencing factors in CVD risk development [39]. Socio-economic status has been reported to be associated with CVD risk [40] and may influence purchasing capacity for food. Smoking has proatherogenic effects via vascular dysfunction [41]. Energy and alcohol intake are dietary determinants of CVD; excess calorie is associated with obesity which is included in the pathophysiological pathway of CVD [42, 43]. Region of residency is considered to have influences on market access for almond and mixed nut or mixed nut and fruit products which further affect consumer access.

To investigate dose–response associations between whole almond consumption (g/1000 kcal energy intake) and diet quality and CVD risk markers, survey-adjusted multivariable linear regression models were used adjusting for the same covariates mentioned above. Normal residual distributions were checked by visual inspection of histograms and Q–Q plots; data with non-normally distributed residuals were log transformed using $\log_{10}$ for analysis of survey-adjusted GLM and multivariable linear regression. The results of analysis were back transformed into the geometric mean values. Homoscedasticity was checked by plotting the standardised residuals of dependent variables and predictors.

During the analysis, the weight factor provided by the NDNS database resource was applied to adjust for non-response and known socio-economic differences in the survey to ensure that the data were nationally representative for the UK population and reducing selection bias and non-response bias [30, 44]. The weight factor used was wti_Y19 (Weight for individual and diary-all ages, combined Year 1–9 (the UK NDNS-RP 2008–2017)) for investigating differences in diet quality scores and nutrient intakes between whole almond consumers and non-consumers, associations between almond consumption and demographic variables, and multivariable linear regression including diet quality scores. Weight factors wtn_Y19 (Weight for nurse-all ages, combined Year 1–9 (the UK NDNS-RP 2008–2017)) was used for GLM and multivariable linear regression including variables BMI, waist circumference and blood pressure; and wtb_Y19 (Weight for blood-all ages, combined Year 1–9 (the UK NDNS-RP 2008–2017)) was used for GLM and multivariable linear regression for blood analyte variables including C-reactive protein and lipids [30, 44].

Results

Sociodemographic and lifestyle characteristics

Mean and median intakes in the total study population (consumers and non-consumers combined) were 9.2 g/day (SD 12.4 g/day) and 5.0 g/day (IQR 9.3 g/day), respectively, ranging from < 0.01 to 109.9 g/day. Table 1 shows background characteristics of almond consumers and non-consumers. Median AKO (almond kernels only, $n = 317$, 4.7% of total adult population) and AKM (almond kernels plus almond kernels in mixed nut products and mixed nut and fruit products, $n = 481$, 7.1% of total adult population) consumption contributed 1.1% and 1.7% of total energy intake respectively. On average whole almond consumers were significantly 2 years older than non-consumers and were more likely to be female and non-smokers. A greater proportion of whole almond consumers identified as non-white and reported having lower or high managerial and professional occupations. Furthermore, a greater proportion of AKM consumers resided in England compared to non-consumers.

Diet quality scores

Modified MDS and modified HDS were significantly higher ($P < 0.001$) in AKO consumers (estimated marginal mean modified MDS 5.5; 95% CI 5.3, 5.7; estimated marginal mean modified HDS 6.4; 95% CI 6.2, 6.6) compared with non-consumers (estimated marginal mean MDS 4.7; 95% CI 4.6, 4.8; estimated marginal mean modified HDS 5.7; 95% CI 5.6, 5.8). Results for AKM consumers were almost identical (data not shown).

Nutrient intake

Almond consumers had significantly higher total energy and food energy intake (10% higher), as well as greater intakes of fat, cis-monounsaturated fatty acids, cis n-6 fatty acids, cis n-3 fatty acids, intrinsic milk sugars, and fiber intakes, as shown in Table 2. Trans-fatty acids, total carbohydrate, starch, non-milk extrinsic sugars, intrinsic milk sugar and starch intakes were significantly lower in consumers. For micronutrients, as shown in Table 2, fully adjusted analysis revealed that almond consumers, relative to non-consumers, had significantly higher intakes of vitamin E, thiamin, riboflavin, folate, pantothenic acid, biotin, vitamin C, potassium, magnesium, phosphorus, iron, copper, zinc, manganese and selenium, and lower intakes of sodium and chloride. However, there were no

Table 1 Background characteristics of whole almond consumers compared to non-consumers in the UK adult population (≥ 19 year) based on NDNS 2008–2017

	Total adult population	AKO			AKM		
		Consumer, $n=317$	Non-consumer, $n=6,485$	P value	Consumer, $n=481$	Non-consumer, $n=6,321$	P value
Amount of almonds consumed (Median (IQR))							
Gram		3.0 (5.9)			5.0 (9.3)		
% total energy intake		1.1 (1.9)			1.7 (3.1)		
Age (median (IQR))	49 (27)	50 (24)	49 (28)	0.298	51 (24)	49 (28)	0.001*
Sex							
Male (%)	41.3	28.7	41.9	< 0.001*	32.5	41.9	< 0.001*
Female (%)	58.7	71.3	58.1		67.5	58.1	
Ethnicity							
White (%)	92.7	86.4	93.0	< 0.001*	88.1	93.0	< 0.001*
Mixed ethnic group (%)	0.9	0.9	0.8		1.0	0.8	
Black or Black British (%)	2.1	1.9	2.1		1.7	2.1	
Asian or Asian British (%)	3.1	8.8	2.8		7.1	2.8	
Any other group (%)	1.2	1.9	1.2		2.1	1.2	
Region							
England (%)	57.4	68.1	56.9	0.130	65.5	56.9	0.033*
Scotland (%)	15.8	8.2	16.1		8.7	16.1	
Wales (%)	14.0	15.1	13.9		14.8	13.9	
Northern Ireland (%)	12.8	8.5	13.0		11.0	13.0	

differences between groups for vitamin A, vitamins D, riboflavin (AKO only), niacin equivalents, vitamin B12, calcium and iodine. Vitamin B6 was observed to be lower in only AKO consumers compared to non-consumers.

Cardiovascular disease risk markers

Blood samples were not available from all participants, and anthropometric and blood pressure data were also incomplete. Sample sizes and estimated marginal mean (95% CI) values of CVD risk markers for remaining participants are shown in Table 3. BMI was significantly lower for AKO by 0.8 kg/m^2 ($P=0.010$) and AKM consumers by 0.6 kg/m^2 ($P=0.019$) compared to non-consumers. WC was significantly lower for AKO consumers by 2.1 cm ($P=0.007$), but the difference between AKM consumers and non-consumers did not reach statistical significance. Survey-adjusted regression analysis showed that there was no dose–response relationship between almond consumption and CVD risk markers (data not shown).

Discussion

Inclusion of nuts in the diet is recommended as part of the emphasis on consuming more plant-based diets for the benefit of both human health and the environment [1, 4, 7, 22]. Almonds are the most consumed tree nut in high-income economies [5], and scientific evidence has demonstrated that consumption can lower LDL-cholesterol concentrations [22, 23], which could contribute to the prevention of coronary heart disease [45]. However, only 1.6% of the US adult population reported consuming whole and processed almonds using data collected by two 24 h dietary recalls [25]. According to 4 day food records, it is reported that 7% of a nationally representative sample of the UK population, surveyed between 2008 and 2017, consumed whole almond kernels (excluding other forms of almonds) during a 4 day period year. The NHANES and NDNS data are not directly comparable as different dietary assessment methods and timeframes were used, but it could indicate that almond consumption may be more prevalent in the UK compared to the US, especially since the NHANES estimate was not restricted to whole almond kernels.

Table 1 (continued)

	Total adult population	AKO			AKM		
		Consumer, $n=317$	Non-consumer, $n=6,485$	P value	Consumer, $n=481$	Non-consumer, $n=6,321$	P value
Socio-economic status							
Higher managerial and professional occupations (%)	15.2	26.4	14.7	< 0.001*	26.8	14.7	< 0.001*
Lower managerial and professional occupations (%)	24.1	31.2	23.7		29.5	23.7	
Intermediate occupations (%)	9.9	9.2	10.0		10.3	10..0	
Small employers and own account workers (%)	10.7	12.4	10.6		12.1	10.6	
Lower supervisory and technical occupations (%)	9.3	7.3	9.4		6.7	9.4	
Semi-routine occupations (%)	14.3	7.6	14.7		8.6	14.7	
Routine occupations (%)	12.1	2.9	12.5		3.6	12.5	
Never worked (%)	2.9	1.9	3.0		1.3	3.0	
Other (%)	1.4	1.0	1.4		1.3	1.4	
Smoking status							
Current smoker (%)	22.6	8.8	23.3	< 0.001*	10.2	23.3	< 0.001*
Ex-Regular smoker (%)	24.3	25.9	24.2		24.9	24.2	
Never regular smoker (%)	53.1	65.3	52.5		64.9	52.5	
Alcohol intake (g/day) (median (IQR))	0.8 (16.4)	4.9 (16.2)	0.5 (16.4)	0.078	4.9 (16.2)	0.5 (16.4)	0.092
Energy intake (kcal/day) (unadjusted mean ± SD))	1760.1 ± 564.7	1876.3 ± 520.8	1754.4 ± 566.2	< 0.001*	1876.3 ± 520.8	1754.4 ± 566.2	< 0.001*

This is a descriptive table. Survey-adjusted GLM with a linear binary logistic function was used to investigate the association between whole almond consumption and demographic variables

AKO almond kernel only, *AKM* almond kernel only plus almond kernel in mixed nuts

*P was < 0.05 indicating a significant association, $n=6,802$

Whole almond consumers were more likely to be female, white, non-smoking, older, and living in England. Scores of diet quality were significantly higher in almond consumers, agreeing with previous findings in the USA NHANES population [46]. These observations suggest that people who follow a healthier dietary pattern are more likely to include whole almonds. This association with better diet quality was reflected in the nutrient intake analysis: consumers had a higher intake of fiber and unsaturated fatty acids, and lower intakes of non-milk extrinsic sugars. Intakes of most

Table 2 Daily energy, macro- and micronutrient intake of whole almond consumers and non-consumers, in the UK adult population (≥ 19 year) based on NDNS 2008–2017

Macronutrient (diet only, % food energy)[a]	Estimated marginal mean (95% CI)					
	AKO			AKM		
	Consumers, $n=317$ (4.7% of total adult population)	Non-consumers, $n=6485$ (95.3% of total adult population)	*P* value	Consumers, $n=481$ (7.1% of total adult population)	Non-consumers, $n=6321$ (92.9% of total adult population)	*P* value
Total energy (kcal)	1851 (1786, 1915)*	1694 (1657, 1731)	< 0.001	1821 (1759, 1883)*	1653 (1609, 1698)	< 0.001
Food energy (kcal)	1794 (1733, 1855)*	1637 (1602, 1672)	< 0.001	1765 (1706, 1824)*	1603 (1561, 1646)	< 0.001
Protein	17.7 (17.3, 18.2)*	17.1 (16.8, 17.3)	0.001	17.7 (17.2, 18.1)*	17.2 (16.9, 17.5)	0.002
Fat	36.7 (35.9, 37.4)*	34.0 (33.6, 34.5)	< 0.001	36.4 (35.7, 37.1)*	34.0 (33.5, 34.6)	< 0.001
Saturated fatty acids	12.1 (11.7, 12.5)	12.0 (11.7, 12.2)	0.518	11.9 (11.5, 12.3)	12.1 (11.7, 12.4)	0.148
cis-Monounsaturated fatty acids	14.1 (13.7, 14.4)*	12.4 (12.2, 12.6)	< 0.001	14.0 (13.7, 14.4)*	12.4 (12.2, 12.6)	< 0.001
cis *n*-6 fatty acids	5.9 (5.7, 6.1)*	5.1 (5.0, 5.3)	< 0.001	5.9 (5.7, 6.1) *	5.1 (5.0, 5.2)	< 0.001
cis *n*-3 fatty acids	1.0 (1.0, 1.1)*	1.0 (0.9, 1.0)	0.002	1.0 (1.0, 1.1)*	1.0 (0.9, 1.0)	< 0.001
Trans fatty acids	0.4 (0.4, 0.5)*	0.5 (0.5, 0.5)	< 0.001	0.4 (0.4, 0.5)*	0.5 (0.5, 0.6)	< 0.001
Carbohydrate	45.2 (44.3, 46.0)*	48.6 (48.1, 49.0)	< 0.001	45.6 (44.8, 46.4)*	48.5 (48.0, 49.1)	< 0.001
Total sugars	17.9 (17.1, 18.6)	18.2 (17.8, 18.7)	0.283	17.6 (16.9, 18.4)	17.6 (17.1, 18.1)	0.838
Starch	26.1 (25.3, 26.8)*	28.6 (28.4, 29.3)	< 0.001	26.6 (25.9, 27.3)*	29.4 (28.9, 29.9)	< 0.001
Non-milk extrinsic sugars	7.6 (7.1, 8.2)*	9.6 (9.2, 10.0)	< 0.001	7.6 (7.0, 8.1)*	9.2 (8.7, 9.6)	< 0.001
Intrinsic milk sugars and starch	36.1 (35.3, 36.8)*	37.3 (36.9, 37.8)	< 0.001	36.5 (35.7, 37.2)*	37.7 (37.2, 38.3)	< 0.001
Intrinsic milk sugars	8.8 (8.3, 9.2)*	7.2 (6.9, 7.4)	< 0.001	8.7 (8.2, 9.1)*	7.0 (6.7, 7.3)	< 0.001
Non-starch polysaccharides (Englyst Fibre, g)	14.6 (14.1, 15.1)*	12.1 (11.9, 12.4)	< 0.001	14.0 (13.5, 14.5)*	11.9 (11.6, 12.2)	< 0.001
Alcohol (% total energy)	0.9 (0.7, 1.2)	1.0 (0.9, 1.2)	0.405	0.8 (0.6, 1.1)	0.9 (0.7, 1.0)	0.734
Micronutrients[b]						
Vitamin A (retinol equivalents) (µg)	638.7 (587.2, 695.0)	603.1 (574.7, 632.9)	0.124	598.5 (551.5, 649.6)	565.2 (533.2, 599.3)	0.069
Vitamin D (µg)	2.2 (2.1, 2.4)	2.2 (2.1, 2.3)	0.824	2.3 (2.2, 2.5)	2.2 (2.1, 2.3)	0.057
Vitamin E (mg)	10.7 (10.3, 11.1)*	8.6 (8.4, 8.8)	< 0.001	10.5 (10.1, 10.9)*	8.3 (8.2, 8.6)	< 0.001
Thiamin (mg)	1.4 (1.3, 1.4)*	1.3 (1.2, 1.3)	< 0.001	1.4 (1.3, 1.4)*	1.3 (1.2, 1.3)	< 0.001
Riboflavin (mg)	1.3 (1.3, 1.4)*	1.3 (1.2, 1.3)	0.047	1.3 (1.3, 1.4)*	1.3 (1.2, 1.3)	0.002
Niacin equivalent (mg)	32.5 (31.5, 33.5)	32.5 (31.9, 33.0)	0.983	32.9 (31.9, 33.9)	32.5 (31.8, 33.2)	0.277
Vitamin B6 (mg)	2.7 (2.6, 2.8)*	2.8 (2.7, 2.8)	0.028	1.7 (1.7, 1.8)	1.8 (1.7, 1.8)	0.152
Vitamin B12 (µg)	3.9 (3.8, 4.2)	4.1 (4.0, 4.3)	0.105	4.0 (3.6, 4.2)	4.0 (3.8, 4.2)	0.971
Folate (µg)	221.9 (213.1, 231.1)*	208.9 (204.1, 213.8)	0.001	218.8 (210.3, 227.6)*	202.5 (196.8, 208.2)	< 0.001
Pantothenic acid (mg)	5.1 (4.9, 5.2)*	4.9 (4.9, 5.0)	0.047	5.2 (5.0, 5.3)*	4.9 (4.8, 5.0)	< 0.001
Biotin (µg)	36.4 (34.9, 37.9)*	28.0 (27.3, 28.7)	< 0.001	35.6 (34.2, 37.1)*	27.0 (26.2, 27.8)	< 0.001
Vitamin C (mg)	83.9 (77.6, 90.8)*	63.2 (60.4, 66.1)	< 0.001	59.7 (56.5, 63.0)*	78.9 (73.1, 85.1)	< 0.001
Sodium (mg)	1614.8 (1562.1, 1668.9)*	1790.8 (1757.3, 1825.0)	< 0.001	1621.9 (1570.8, 1674.7)*	1819.1 (1778.1, 1861.1)	< 0.001
Potassium (mg)	2689.3 (2619.0, 2762.1)*	2423.9 (2387.4, 2461.1)	< 0.001	2880.8 (2811.2, 2950.5)*	2584.0 (2533.9, 2634.0)	< 0.001
Calcium (mg)	641.2 (619.7, 663.5)	624.2 (612.1, 636.5)	0.075	629.1 (608.5, 650.3)	617.3 (602.8, 632.1)	0.141
Magnesium (mg)	278.5 (271.3, 285.9)*	227.6 (224.2, 231.1)	< 0.001	270.9 (264.2, 277.9)*	223.2 (219.2, 227.3)	< 0.001
Phosphorus (mg)	1128.3 (1103.3, 1154.8)*	1060.0 (1046.6, 1073.7)	< 0.001	1130.1 (1105.6, 1154.8)*	1063.7 (1046.1, 1079.2)	< 0.001

Table 2 (continued)

Micronutrients[b]						
Iron (mg)	10.3 (10.0, 10.6)*	9.5 (9.3, 9.7)	< 0.001	10.1 (9.8, 10.4)*	9.4 (9.2, 9.6)	< 0.001
Copper (mg)	1.3 (1.2, 1.3)*	1.1 (1.0, 1.1)	< 0.001	1.2 (1.2, 1.3)*	1.0 (1.0, 1.0)	< 0.001
Zinc (mg)	8.1 (7.9, 8.4)*	7.8 (7.6, 7.9)	0.001	8.0 (7.8, 8.2)*	7.7 (7.6, 7.9)	0.001
Chloride (mg)	2690.5 (2611.2, 2772.3)*	2887.7 (2838.9, 2937.3)	< 0.001	2684.3 (2607.6, 2763.4)*	2912.4 (2852.6, 2973.4)	< 0.001
Manganese (mg)	3.4 (3.3, 3.6)*	2.7 (2.6, 2.8)	< 0.001	3.3 (3.2, 3.4)*	2.6 (2.6, 2.7)	< 0.001
Iodine (µg)	122.2 (116.2, 128.4)	123.5 (121.0, 128.1)	0.624	127.3 (121.3, 133.7)	122.7 (118.5, 127.1)	0.049
Selenium (µg)	50.0 (48.0, 52.1)*	46.2 (45.2, 47.4)	< 0.001	49.2 (48.2, 51.2)*	45.3 (44.0, 46.6)	< 0.001

All data are non-normally distributed, except total energy, food energy, fat, Saturated fatty acids, cis-Monounsaturated fatty acids, carbohydrate, starch and intrinsic milk sugars and starch. Presented values from non-normally distributed data are back log-transformed

AKO almond kernel only, *AKM* almond kernel only plus almond kernel in mixed nuts

*$P < 0.05$ showed a significant difference, $n = 6802$

[a]Survey-adjusted GLM with a linear link function and predictors: age, sex, ethnicity, socio-economic and smoking status was used for energy intake as an outcome for AKO; Survey-adjusted GLM with a linear link function and predictors: age, sex, ethnicity, socio-economic and smoking status, alcohol and energy intakes was used for other macronutrient intake outcomes for AKO; Survey-adjusted GLM with a linear link function and predictors: age, sex, ethnicity, socio-economic and smoking status, and energy intake was used for alcohol intake as an outcome for AKO. The same statistical analysis was conducted for AKM but region of residency was also included into predictors. Region of residence was not significantly correlated with AKO consumption; thus it was not included as a predictor for analysis related to AKO

[b]Survey-adjusted GLM with a linear link function and predictors: age, sex, ethnicity, socio-economic and smoking status, alcohol and energy intakes was used for AKO. The same statistical analysis was conducted for AKM but region of residency was also included into predictors. Region of residence was not significantly correlated with AKO consumption; thus it was not included as a predictor for analysis related to AKO

Table 3 Cardiovascular disease risk marker values of whole almond consumers and non-consumers, in the UK adult population (≥ 19 year) based on NDNS 2008–2017

Estimated marginal mean (95% CI)						
CVD risk marker	AKO[a]			AKM[b]		
	Consumers, $n = 317$ (4.7% of total adult population)	Non-consumer, $n = 6,495$ (95.3% of total adult population)	*P* value	Consumers, $n = 481$ (7.1% of total adult population)	Non-consumer, $n = 6321$ (92.9% of total adult population)	*P* value
BMI (kg/m^2)[c]	25.5 (24.9, 26.2)	26.3 (25.9, 26.7)	0.010*	25.8 (25.1, 26.5)	26.4 (25.9, 26.9)	0.019*
WC (cm)[d]	88.0 (86.2, 89.8)	90.1 (89.1, 91.2)	0.007*	90.1 (88.3, 91.9)	91.3 (90.0, 92.6)	0.065
SBP (mmHg)[e]	119.6 (117.3, 121.9)	121.2 (119.8, 122.6)	0.114	119.7 (117.4, 122.0)	121.3 (119.6, 123.0)	0.058
DBP (mmHg)[e]	71.3 (69.8, 73.0)	71.6 (70.6, 72.6)	0.720	71.2 (69.6, 72.8)	71.8 (70.6, 73.0)	0.316
TC (mmol/l)[f]	4.8 (4.6, 5.0)	4.8 (4.7, 4.9)	0.558	4.8 (4.6, 5.0)	4.8 (4.6, 4.9)	0.485
TAG (mmol/l)[g]	1.0 (0.9, 1.1)	1.1 (1.0, 1.2)	0.130	1.1 (1.0, 1.2)	1.1 (1.1, 1.2)	0.445
HDL-C (mmol/l)[f]	1.4 (1.4, 1.5)	1.4 (1.4, 1.4)	0.222	1.4 (1.3, 1.5)	1.4 (1.3, 1.4)	0.139
LDL-C (mmol/l)[h]	2.9 (2.7, 3.0)	2.8 (2.7, 2.9)	0.476	2.8 (2.7, 2.9)	2.8 (2.7, 3.0)	0.727
TC:HDL-C[f]	3.4 (3.2, 3.6)	3.5 (3.4, 3.6)	0.533	3.5 (3.3, 3.7)	3.5 (3.4, 3.6)	0.548
CRP (mg/l)[i]	2.2 (1.9, 2.6)	2.4 (2.2, 2.7)	0.158	2.3 (2.0, 2.7)	2.5 (2.3, 2.8)	0.146

All data are not normally distributed, thereby the presented values are back log-transformed. Due to missing data, sample sizes were as follows: AKO consumers 242[c], 247[d], 228[e], 184[f], 184 [g], 183 [h] and 184[i]; non-consumers 4466[c], 4645[d], 3784[e], 3183[f], 3174 [g], 3139 [h] and 3184[i]; AKM consumers 370[c], 380[d], 342[e], 274[f], 273 [g], 271 [h] and 274[i]; non-consumers 4338[c], 4512[d], 3670[e], 3093[f], 3084 [g], 3051 [h] and 3094[i]

AKO almond kernel only; *AKM* almond kernel only plus almond kernel in mixed nuts; *BMI* body mass index; *WC* waist circumference; *SBP* systolic blood pressure *DBP* diastolic blood pressure; *TC* total cholesterol; *TAG* triacylglycerol; *HDL-C* high density lipoprotein cholesterol; *LDL-C* low density lipoprotein cholesterol; *TC:HDL-C* total to HDL cholesterol ratio; *CRP* C-reactive protein

*$P < 0.05$ showed a significant difference, $n = 6802$

[a]Survey-adjusted GLM with a linear link function and predictors: age, sex, ethnicity, socio-economic and smoking status, alcohol and energy intakes was used. Region of residence was not significantly correlated with AKO consumption; thus it was not included as a predictor

[b]Survey-adjusted GLM with a linear link function and predictors: age, sex, ethnicity, region of residency, socio-economic and smoking status and alcohol intake was used

micronutrients, including vitamin E, thiamin, riboflavin, folate, pantothenic acid, biotin, vitamin C, phosphorus, iron, zinc, selenium, iodine, manganese, magnesium, potassium, and copper intakes were found to be higher in consumers following adjustment for energy intake, indicating that they were likely to consume a more nutrient-dense diet in general. Sodium and chloride intakes were lower in consumers indicating reduced salt consumption compared with non-consumers, in agreement with the higher scores for diet quality. Therefore, these data support the widely recommended approach that a healthy dietary pattern will include nuts as a plant-based source of protein and micronutrients. It is important to remember that the median almond intake by UK adult consumers was just 5 g/day (equivalent to four almonds), with the 75th percentile only reaching 12 g/day. This fact, alongside data from our previous study that reported the median intake of total tree nuts to be 7 g/day in UK adults [44], shows that daily intakes are far below what is considered to be one portion (28 g) of tree nuts [44], and are, therefore, unlikely to be eaten in quantities that could cause clinically meaningful LDL-cholesterol lowering effects in the majority of consumers.

Almond consumers had slightly lower BMI and WC. Similar body composition findings were observed in the US NHANES almond consumer population, using a statistical model adjusted for age, sex, ethnicity, poverty index ratio, physical activity level, current smoking status, alcohol consumption, and total energy intake [25]. Findings from randomized controlled trials have been mixed regarding the effects of almond consumption on body composition; a recent meta-analysis that pooled data from 15 trials concluded there was no difference in BMI between almond and control interventions in healthy and at risk subjects with a range of 25–100 g/day of almonds [23]. Physical activity levels were not available from the NDNS database and thus could be a confounding factor in the differences in body composition observed in this analysis.

There were no differences in blood pressure (SBP and DBP) according to almond consumption. Our previous analysis of the 2008–2014 NDNS sample showed that tree nut consumers had on average 4.3 mmHg significantly lower SBP than non-consumers, and that with every gram increase in tree nut consumption per 1000 kcal of energy intake, SBP was 0.2 mmHg lower [44]. The limited range of whole almond intakes is likely to explain the lack of dose–response relationship with SBP (and BMI and WC) in the current study. Other observational tree nut studies have reported conflicting findings for blood pressure, with reports that tree nut consumption is associated with lower SBP but not DBP in the USA NHANES database [47], but the SUN prospective cohort study found no associations at all with blood pressure [48]. No significant effect of almond intervention (dose 25–100 g/day) on SBP was reported in a meta-analysis of randomized controlled trials, but DBP was shown to be significantly decreased [23].

Whole almond consumption was not associated with a preferential lipid profile, such as higher HDL-cholesterol and lower total cholesterol, LDL-cholesterol, TAG, or the ratio of total to HDL-cholesterol. Again, the observational evidence is inconsistent with the interventional data; pooled analysis of 18 randomized controlled trials revealed HDL-cholesterol was not affected by almond consumption but there were significant reductions in total cholesterol, TAG and LDL-cholesterol [22]. These inconsistencies between observational and interventional studies may exist, because a higher dose (RCTs administered between 25 and 100 g almonds/day) is important for measurable differences in lipid profiles.

Previous literature on human clinical trials investigating the impact of almond consumption on CRP is inconsistent. Two randomized, controlled, crossover trials in adults with elevated LDL cholesterol found that 4 week and 6 week almond consumption at a dose of 50–75 g/day and 42.5 g/day, respectively, did not significantly modify CRP [15, 49], although other studies have reported reductions in CRP after 4 week almond consumption (as a replacement of 20% total energy) in subjects with type-2 diabetes [18], and after 4 weeks in healthy adults where 10% or 20% total energy was replaced by almonds [20]. A meta-analysis of 15 studies revealed that the overall difference between almond and control interventions did not reach statistical significance [23], but the number of studies is insufficient to determine whether baseline CRP status, dose and duration of intervention are important determinants.

Median almond intake was 5 g/day in the cohorts of UK consumers studied between 2008 and 2017, but the trend of whole almond consumption fluctuated across the period. The highest consumption level occurred in 2011–2012 (median 8.3 g/day), but consumption decreased to 3.9 g/day in the most recent cohort available, 2016–2017. Since intake of almonds was low in whole almond consumers, the superior diet quality of almond consumers is likely to reflect generally healthier dietary choices and patterns as shown by the higher diet quality scores observed in almond consumers versus non-consumers. If consumed in larger quantities by more individuals, whole almonds have the potential to directly improve the nutrient profile of the diet. Whole almonds are predominantly consumed as snacks and given that snacks account for 20–25% of estimated energy requirement in adults [50–55], snacking is a convenient food domain to target for improving diet quality. Almonds have higher unsaturated fat, fiber, magnesium, vitamin E and phenolics compared to typically consumed snacks in the UK [56, 57], and have been shown to improve other markers of cardiovascular health such as endothelial function (a measure of vascular health) [56] in addition to blood lipid

profiles [15], compared to typically consumed snacks. However, when encouraging almonds as a snack replacement to improve health we need to be mindful of potential barriers, such as affordability amongst low income groups and market accessibility, as well as the need for increased global production of almonds to meet greater demands, which would require careful consideration of long-term environmental sustainability in terms of cropland and water.

A strength of this study is that the diet data was generated from 4 day estimated diet diaries, which are considered to be more accurate relative to 24 h dietary recalls. On the other hand, the 4 day estimated diet diary might not record almond intake that occurred on other days, leading to underreporting and misclassification of non-consumers. A further strength of the study is that it is based on a large database that is considered to be nationally representative of the UK population. Furthermore, the survey is designed to facilitate representation of dietary intakes across all days of the week, avoiding potential bias arising from differences between week-days and weekend-days [58]. However, it must be noted that a weakness of the study was that the UK NDNS database does not provide data on the proportions of almonds within mixed nuts products. Despite this, our estimates of almond proportions in these products via systematic market sampling mitigates the risk of underestimating almond consumption.

Conclusion

UK almond consumers are characterized by overall healthier dietary patterns, which are likely to have been an important determinant of the more favorable markers of body composition observed in this group. It is unlikely that almond consumption independently determined lower adiposity in this population since intakes were very low. Encouraging snacking on nuts, including almonds, to replace snack foods high in saturated fatty acids, refined starches and free sugars may contribute to the sum effect of a healthy dietary pattern on reduced risk of cardiovascular diseases.

Acknowledgements We thank Dr Gerda K. Pot, a health and nutrition researcher at Louis Bolk Institute, the Netherlands, for her valuable input during the initial state of designing this cross-sectional study.

Author contributions Material preparation and data collection were performed by VD, LF and LS. Data analysis was conducted by VD. The first draft of manuscript was written by VD, but WLH had primary responsibility for the final content. WLH and SEB read and commented on the manuscript. All authors read and approved the final manuscript.

Funding This study was funded by the Almond Board of California and Indonesia Endowment Fund for Education (Grant number 15-BERRYS-01).

Compliance with ethical standards

Conflict of interest The authors have received research funding and reimbursement of travel expenses to attend conferences from the Almond Board of California.

Ethical approval For NDNS RP 2008–2013, ethics approval was obtained from the Oxfordshire A Research Ethics Committee (Ref. No. 07/H0604/113) and for NDNS RP 2014–2017, the approval was received from the Cambridge South NRES Committee (Ref. No. 13/EE/0016).

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Whole almond consumption is associated with better diet quality and intermediary cardiovascular disease risk factors in the UK adult population: National Diet and Nutrition Survey (NDNS) 2008-2017

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Supplement

Appendix A: Market analysis of almond snacks

Table 1. Whole almond kernel portion in mixed nut or mixed nut and fruit products in the UK supermarkets

Type	Supermarket name	Total weight of the product	Total weight of whole almond kernels	% of whole almond kernels in the product	Mean value of % whole almond kernels
Mixed roasted unsalted nuts	Waitrose	50.1	9.7	19.4	28.5
	M&S	173.9	41.7	24.0	
	Co-op	30.5	7.4	24.3	
	Sainsbury's	203.0	48.8	24.0	
	Tesco	201.0	56.6	28.2	
	Asda	201.0	102.2	50.8	
	Morrisons	224.9	80.2	35.7	
	Lidl	200.7	42.8	21.3	
Mixed roasted salted nuts	Waitrose	175.1	54.4	31.1	30.5
	M&S	177.0	46.0	26.0	
	Sainsbury's	201.2	59.3	29.5	
	Tesco	203.1	72.3	35.6	
Mixed nuts and fruits	Sainsbury's, Product 1	201.6	13.7	6.8	18.0
	Sainsbury's, Product 2	200.0	25.3	12.7	
	Tesco	251.5	25.4	10.1	
	Asda, Product 1	250.0	45.7	18.3	
	Asda, Product 2	200.0	62.6	31.3	
	Morrisons	200.3	35.3	17.6	
	Lidl	200.3	58.3	29.1	

Appendix B: Diet quality scores

Table 1. Original and modified Mediterranean Diet Score (MDS) and its scoring system [30]

Food group		Scoring*	
Original MDS	Modified MDS		
Cereals	Cereals	>median	1 (else: 0)
Vegetables	Vegetables	>median	1 (else: 0)
Fruits and Nuts	**Fruits**	>median	1 (else: 0)
Legumes	Legumes	>median	1 (else: 0)
Fish	Fish	>median	1 (else: 0)
Meat	Meat	<median	1 (else: 0)
Dairy products	Dairy products	<median	1 (else: 0)
Ratio of unsaturated to saturated fats	Ratio of unsaturated to saturated fats	>median	1 (else: 0)
Alcohol	Alcohol	10-50 g/d for men	1 (else: 0)
		5-25 g/d for women	1 (else: 0)

*Except alcohol, scoring of each food group is based on population and sex-specific median.

Table 2. Original and modified Healthy Diet Score (HDS) and the cut-off values for scoring [31]

HDS			HDS – modified based on current UK recommendations		
Index item	Cut-off values		Index item	Cut-off values	
	Score 1	Score 0		Score 1	Score 0
Saturated fatty acids (% energy intake)	0-10	>10	Saturated fatty acids (% energy intake)	0-11[a]	>11
Polyunsaturated fatty acids (% energy intake)*	6-10	<6 or >10	Polyunsaturated fatty acids (% energy intake)	6[b]-10	<6 or >10
Protein (% energy intake)	10-15	<10 or >15	Protein (% energy intake)	9[c]-15	<9 or >15
Total carbohydrate (% energy intake)*	50-70	<50 or >70	Total carbohydrate (% energy intake)	50[d]-70	<50 or >70
Dietary fiber (g)*	18-32	<18 or >32	Dietary fiber (g)	18[d]-32	<18 or >32
Fruits and vegetables (g)	≥400	<400	Fruits and vegetables (g)	≥400[e]	<400
Pulses and nuts (g)*	≥30	<30	Pulses (g)	≥30	<30
Total non-milk extrinsic sugar (% total energy intake)*	0-10	>10	Total non-milk extrinsic sugar (% total energy intake)	0-5[d]	>5
Cholesterol (mg)*	0-245	>245	Trans-fatty acids (% energy intake)	≤2[e]	>2
Fish (g)*	≥32	<32	Fish (g)	≥40[e]	<40

Red meat and meat processed products (g)*	≤90	>90	Oily fish (g)	≥20[e]	<20
Calcium (mg)*	≥700	<700	Red meat and meat processed products (g)	≤70[i]	>70
			Calcium (mg)	≥700[c]	<700
			Sodium (mg)	≤2400[c]	>2400

* Items based on advice on healthy eating as recommended by the UK Committee on Medical Aspects of Food Policy (COMA) [31]

a Based on NDNS Results from Years 7 and 8 (combined) of the Rolling Program (2014/2015 – 2015/2016) [29], UK Government Dietary Recommendations by Public Health England (2016) [32], Cardiovascular Disease Outcomes Strategy by UK Department of Health (2013) [33] and Draft report: Saturated fats and health by Scientific Advisory Committee on Nutrition (SACN) 2018 [35]

b Based on UK Government Dietary Recommendations by Public Health England (2016) [32]

c Based on UK Government Dietary Recommendations by Public Health England (2016) [32]

d Based on UK Government Dietary Recommendations by Public Health England (2016) [32] and Scientific Advisory Committee on Nutrition (SACN) Report 2015 on Carbohydrates and Health [36]

e Based on NDNS Results from Years 7 and 8 (combined) of the Rolling Program (2014/2015 – 2015/2016) [29] and The Eatwell Guide by Public Health England (2016) [34]

Chapter 5: Snacking on whole almonds for 6 weeks improves endothelial function and lowers LDL cholesterol but does not affect liver fat and other cardiometabolic risk factors in healthy adults: the ATTIS study, a randomized controlled trial

This chapter of this thesis incorporating publications presents the published paper in the American Journal of Clinical Nutrition.

(Dikariyanto *et al.*, 2020)

Snacking on whole almonds for 6 weeks improves endothelial function and lowers LDL cholesterol but does not affect liver fat and other cardiometabolic risk factors in healthy adults: the ATTIS study, a randomized controlled trial

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ABSTRACT

Background: There is convincing evidence that daily whole almond consumption lowers blood LDL cholesterol concentrations, but effects on other cardiometabolic risk factors such as endothelial function and liver fat are still to be determined.

Objectives: We aimed to investigate whether isoenergetic substitution of whole almonds for control snacks with the macronutrient profile of average snack intakes, had any impact on markers of cardiometabolic health in adults aged 30–70 y at above-average risk of cardiovascular disease (CVD).

Methods: The study was a 6-wk randomized controlled, parallel-arm trial. Following a 2-wk run-in period consuming control snacks (mini-muffins), participants consumed either whole roasted almonds ($n = 51$) or control snacks ($n = 56$), providing 20% of daily estimated energy requirements. Endothelial function (flow-mediated dilation), liver fat (MRI/magnetic resonance spectroscopy), and secondary outcomes as markers of cardiometabolic disease risk were assessed at baseline and end point.

Results: Almonds, compared with control, increased endothelium-dependent vasodilation (mean difference 4.1%-units of measurement; 95% CI: 2.2, 5.9), but there were no differences in liver fat between groups. Plasma LDL cholesterol concentrations decreased in the almond group relative to control (mean difference −0.25 mmol/L; 95% CI: −0.45, −0.04), but there were no group differences in triglycerides, HDL cholesterol, glucose, insulin, insulin resistance, leptin, adiponectin, resistin, liver function enzymes, fetuin-A, body composition, pancreatic fat, intramyocellular lipids, fecal SCFAs, blood pressure, or 24-h heart rate variability. However, the long-phase heart rate variability parameter, very-low-frequency power, was increased during nighttime following the almond treatment compared with control (mean difference 337 ms^2; 95% CI: 12, 661), indicating greater parasympathetic regulation.

Conclusions: Whole almonds consumed as snacks markedly improve endothelial function, in addition to lowering LDL cholesterol, in adults with above-average risk of CVD. This trial was registered at clinicaltrials.gov as NCT02907684. *Am J Clin Nutr* 2020;111:1178–1189.

Keywords: almonds, endothelial function, liver fat, dietary intervention, cardiovascular disease, cardiometabolic disease

Introduction

Cardiovascular disease (CVD) continues to be the leading cause of global mortality. The development of CVD is preceded by cumulative interrelated hemodynamic and metabolic disturbances that develop over the life course and also feature in the pathophysiological progression to type 2 diabetes (T2D) (1). Dietary guidelines have been formulated partly to mitigate the progression of cardiometabolic risk phenotypes, such as raised blood pressure, dyslipidemia, and central adiposity, by encouraging a healthy eating pattern, limiting intake of added sugars, SFAs, and sodium, and choosing nutrient-dense foods in all food groups (2, 3). Most people in the United States and United Kingdom consume ≥2 snacks/d, contributing ∼20–25% of energy intake on average (4, 5). Data derived from respondents to the UK National Diet and Nutrition Survey 2008–2012 revealed that the average snack nutrient profile had 14% of energy as saturated fats and 23% of energy as sugars

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(predominantly added sugars) (6), exceeding dietary reference values for upper limits of their intake. Therefore, snacks present an easily modifiable target for improving overall diet quality.

Whole nuts, for example, almonds, which are mainly eaten as snacks, are encouraged as part of recommended healthy eating patterns because they are rich in protein, dietary fiber, unsaturated fatty acids, and micronutrients (vitamin E, riboflavin, niacin, and magnesium) (7, 8), and could displace other snack foods that are rich in SFAs, refined starch, and added sugar, and low in fiber. Inclusion of almonds in the diet is associated with reduced risk of CVD and T2D, and higher intakes can lower plasma LDL cholesterol and fasting blood glucose concentrations without leading to any increase in body weight (9, 10). Almond skin is a source of nonnutrient bioactives, for example, (poly)phenolic compounds, that can play a role in the mechanism of CVD prevention (11, 12). Almonds also contain significant amounts of L-arginine, the biological precursor of the potent vasodilator, nitric oxide, and they are a natural source of phytosterols, which can contribute to the LDL cholesterol–lowering properties of almonds to a limited extent (13). However, the impact of regular whole almond consumption on endothelial function, a key factor in the initiation, progression, and disease manifestation of atherosclerosis, is not yet known. Endothelial function is adversely affected by chronic low-grade inflammation and increased oxidative stress, which are pathological features associated with obesity, fatty liver, and insulin resistance. Displacement of SFAs and refined carbohydrates from typical snack products consumed in industrialized countries with unsaturated fats, protein, and fiber from whole almonds, could potentially reduce liver fat, which can subsequently impact cardiometabolic risk.

Funding was received from the Almond Board of California and the Indonesia Endowment Fund for Education. Almonds for the dietary intervention were supplied by the Almond Board of California.

Data described in the manuscript will be made available upon request.

Supplemental Methods, Supplemental Tables 1–5, and Supplemental Figure 1 are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/ajcn/.

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Abbreviations used: ABP, ambulatory blood pressure; ALT, alanine aminotransferase; ATTIS, Almonds Trial Targeting Dietary Intervention with Snacks; BP, blood pressure; CAIPIRINHA, controlled aliasing in parallel imaging results in higher acceleration; CVD, cardiovascular disease; DBP, diastolic blood pressure; EDV, endothelium-dependent vasodilation; EER, estimated energy requirement; EMCL, extramyocellular lipid; FMD, flow-mediated dilation; FoV, field of view; GGT, γ-glutamyltransferase; GTN, glyceryl trinitrate; HF, high-frequency power; HRV, heart rate variability; IHL, intrahepatic lipid; IMCL, intramyocellular lipid; IPL, intrapancreatic lipid; MR, magnetic resonance; MRS, magnetic resonance spectroscopy; NAFLD, nonalcoholic fatty liver disease; NN, normal-to-normal; RMSSD, root mean square of successive differences of NN intervals; ROI, region of interest; SAT, subcutaneous adipose tissue; SBP, systolic blood pressure; SDNN, standard deviations of the NN intervals; TA, acquisition time; TC, total cholesterol; T2D, type 2 diabetes; VAT, visceral adipose tissue; VLF, very-low-frequency power; WC, waist circumference; 1H-MRS, proton magnetic resonance spectroscopy.

Received January 14, 2020. Accepted for publication April 16, 2020.

First published online May 15, 2020; doi: https://doi.org/10.1093/ajcn/nqaa100.

This dietary intervention trial (Almonds Trial Targeting Dietary Intervention with Snacks, or ATTIS) was designed to compare the effects of replacing habitual daily snacks with either whole almonds or control snacks with a nutritional profile matching the average UK macronutrient intakes from snacks (excluding fruit) on endothelial function and liver fat, the primary outcomes. Secondary outcomes included blood glucose, insulin, lipid profile, adipokines, markers of fatty liver, body composition, blood pressure, heart rate variability, pancreatic and skeletal muscle fat, fecal SCFAs, plasma fatty acid profiles, and metabolomic profiles. It was hypothesized that substituting whole almonds for typically consumed snacks would increase endothelium-dependent vasodilation and decrease liver fat.

Methods

Study population

Study participants were adult men and women (aged 30–70 y), with above-average risk of developing CVD, and self-reported regular snack consumers (≥2 snacks/d) recruited from London, United Kingdom, and the surrounding area between March 2017 and January 2019. Further details are given in **Supplemental Methods**. A CVD risk score system adapted from the Framingham risk score system was used to identify volunteers (scoring ≥2) who were above-average risk (14). Inclusion and exclusion criteria are detailed in **Supplemental Table 1**. All participants gave written informed consent before enrollment.

Study design

The ATTIS study, conducted between March 2017 and May 2019, was approved by the UK National Research Ethics Service (REC 16/LO/1910) and registered with clinicaltrials.gov (NCT02907684). The trial was run in accordance with the Declaration of Helsinki and the principles of Good Clinical Practice.

The study was a randomized, parallel-arm design with 2 intervention groups, almonds and control, in a free-living cohort. Treatment was randomly allocated by the lead researcher using minimization software (MinimPy 0.3; Mahmoud Saghaei; http://minimpy.sourceforge.net), with age, sex, ethnicity, cardiometabolic score, and willingness to undergo MRI/magnetic resonance spectroscopy (MRS) scanning as minimization variables. A 2-wk run-in period consuming control snacks preceded random allocation to ensure that the study protocol was tolerable to individual participants prior to starting the intervention phase, and to collect baseline diet and physical activity data. Body composition, clinic blood pressure (BP), and 24-h ambulatory blood pressure (ABP) were measured; 4-d food and activity diaries were collected. Participants abstained from alcohol and strenuous activity for 24 h before baseline and end-point clinic visits, and consumed low-fat meals and no alcohol in the evening before. **Figure 1** shows the timeline of study visits and measurements. In brief, during the 6-wk intervention period, participants attended 4 visits. Baseline and end-point visits (separated by 6 wk) involved measurements of flow-mediated dilation (FMD) to assess endothelial function; MRI/MRS scan to assess liver, pancreas, and abdominal fat and myocellular lipids; ABP; heart rate variability (HRV); blood samples for glucose,

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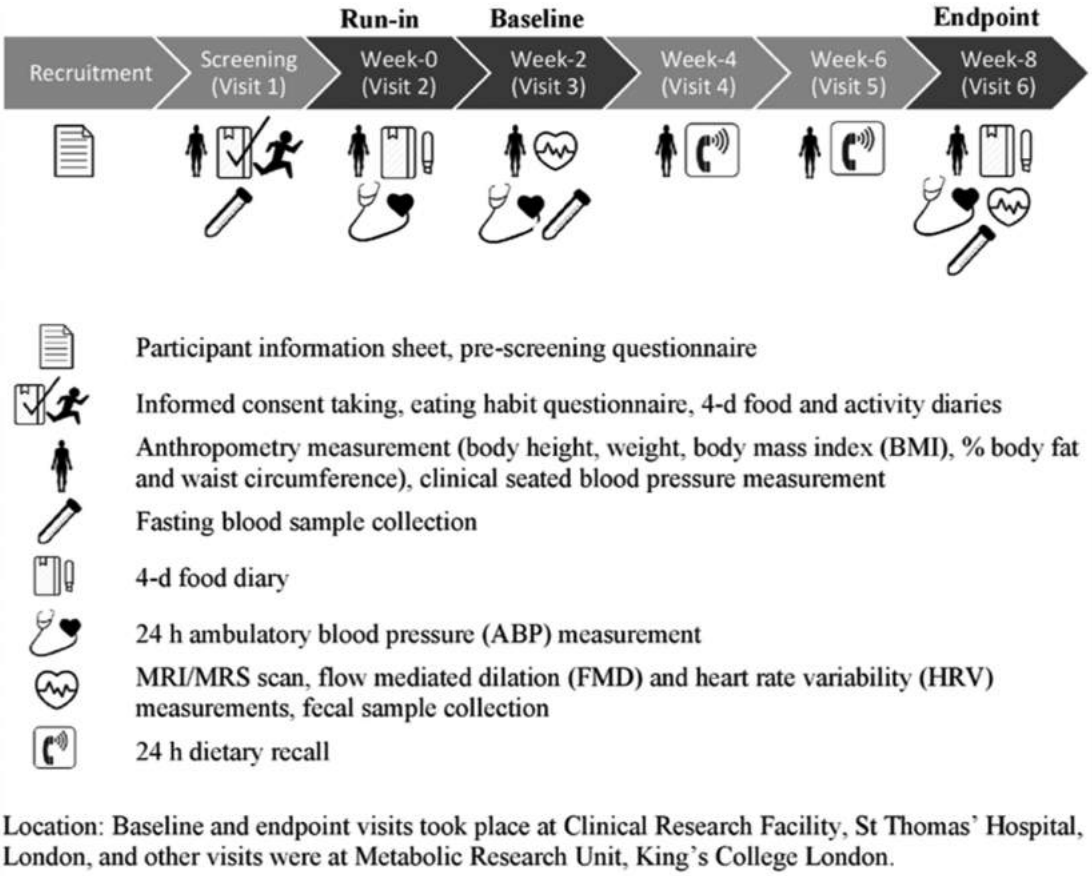

FIGURE 1 Almonds Trial Targeting Dietary Intervention with Snacks (ATTIS) study design flowchart. MRS, magnetic resonance spectroscopy.

insulin, lipids, and adipokines; and stool samples for fecal SCFAs. Intermediate visits (weeks 2 and 4 of the randomized intervention period) included measurements of body composition and clinic BP, and completion of a 24-h dietary recall to monitor compliance to intervention.

Dietary intervention

The dietary intervention included a 2-wk run-in period, during which participants consumed control snacks providing 20% of estimated energy requirement (EER), followed by random allocation to either control or almond snacks at 20% of EER. Henry equations and physical activity level estimated from 4-d activity diaries were used to calculate EER (15). Sweet and savory mini-muffins were baked at the study center as the control snacks; these were formulated to provide a macronutrient profile that was representative of the average macronutrient intakes from snacks (excluding fruit) in the UK National Diet and Nutrition Survey population (6), as detailed in Supplemental Methods and **Supplemental Table 2**. Almond snacks were dry-roasted whole nonsalted almonds of the Nonpareil variety, supplied by the Almond Board of California (US grade extra no. 1). The whole almonds were weighed and packed in a daily portion for each subject. For example, 63 g (2.2 oz, or $\frac{1}{4}$ cup) of almonds were provided daily for an individual estimated to require 2000 kcal (8.37 MJ) (Supplemental Methods and **Supplemental Table 3**). During run-in and intervention periods, participants were provided with snack information sheets and dietary advice from the research dietitian and instructed to only consume study snacks between meals and to maintain their habitual mealtime eating habits and fruit consumption. All subjects were asked to avoid the consumption of additional nuts or nut products for the duration of the study.

Outcomes

Required sample sizes were calculated for specified primary outcomes based on a 1.25% unit difference in a similar study of walnuts (SD: 1.9) in FMD (16), a measure of endothelium-dependent vasodilation (EDV), and a 2.2% unit difference in liver fat (intrahepatic lipid, IHL) between groups as being clinically important. A sample size of 50 subjects per treatment group had 90% power to detect a 1.25% unit effect of treatment on FMD with a 2-tailed α of 0.05. For liver fat, 20 subjects per group was sufficient to detect a 2.2% unit change (SD: 2.1) (17) with 90% power and a 2-tailed α of 0.05.

Endothelial function

Following 15 min of supine rest, endothelial function was assessed as EDV of the brachial artery using the FMD technique by ultrasound (Siemens Accuson CV70), as previously reported

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(18). FMD was also measured post glycerol trinitrate (GTN) administration to determine endothelium-independent vasodilation. Scans were evaluated using Brachial Analyzer software (Medical Imaging Applications LLC) by a single researcher who was blinded to the identity of the participant and treatment allocation.

Liver fat

Fifty subjects, 25 in each treatment group, agreed at screening to undergo MRI scanning. For quantification of IHL by MRI, participants were scanned from neck to foot in the supine position following an overnight fast on a 1.5-T Siemens Magnetom Aera scanner at baseline and end-point visits. The magnetic resonance protocol from which fat and water images were produced as part of the 6-point Dixon sequence was as follows (19): 8 contiguous stations covering neck-foot, acquisition time (TA) 16 s; 56 slices, field of view (FoV) 500 × 72.3%, acquired voxel size 2.2 × 2.2 × 3.5 mm, 7/8 phase and slice partial Fourier, 28.6% slice oversampling, acceleration factor 2 (CAIPIRINHA, controlled aliasing in parallel imaging results in higher acceleration; a data acquisition technique that facilitates high-resolution images for breath-hold examinations), flip angle 4°, and slice thickness 3.5 mm. To reduce motion artifacts, participants were instructed to hold their breath for 15 s while abdominal images were acquired. Liver fat was quantified using HOROS V 1.1.7 software (available at: www.horosproject.org) by a single analyst who was blinded to the participant identification code and treatment allocation of clinical data. Four regions of interest (ROIs) were placed on each slice of abdominal cavity images where the liver was visually the major sized organ in the images, avoiding blood vessels, bile ducts, and obvious artifacts (20). The ROIs were drawn in 2-point Dixon to have details of liver images visualized more clearly including vessels and ducts, and then copied and pasted into 6-point Dixon sequence. The 2-point Dixon protocol was as follows: TA 13 s; 56 slices, FoV 500 × 72.3%, acquired voxel size 1.1 × 1.1 × 3.5 mm, 7/8 phase and slice partial Fourier, 28.6% slice oversampling, acceleration factor 2 (CAIPIRINHA), flip angle 12°, and slice thickness 3.5 mm. Hepatic fat fraction was calculated in each ROI by using the mean of pixel signal intensity, and IHL was calculated as the mean of all ROIs in each slice.

IHL, alongside lipid saturation/unsaturation, was also quantified by proton magnetic resonance spectroscopy (^{1}H-MRS) according to previously reported methods (21). MRS spectra were analyzed on the Java-based Magnetic Resonance User Interface (jMRUI) software with the inclusion of prior knowledge to assist in identification of the peaks of interest. Prior knowledge of a 5-resonance model was applied to fit the lipid peaks: diallylic protons [-(CH_2)n, ~2.9 ppm], methylenic protons (-CH_2, ~2.3 ppm) in the α position relative to the carboxyl group, allylic protons [-(CH_2)n, ~2.0 ppm], IHL/methylene protons [-(CH_2)n, ~1.3 ppm], and methyl protons (-CH_3, ~0.9 ppm). The formulas were used to determine the unsaturation index, polyunsaturation index, and saturation index of the liver fat, as previously explained by Johnson et al. (21).

Intrapancreatic lipid, intramyocellular lipids, and body composition

Intrapancreatic lipid (IPL) quantification was measured by 2-point Dixon sequence MRI scanning. One circular 1-cm^2 ROI was drawn on the head, body, and tail regions of the pancreas. IPL was quantified from each ROI using the formula: %IPL = [F/(F + W)] × 100, where F and W are the pixel signal intensities of the fat and water images, respectively. Mean IPL was calculated as the mean of the head, body, and tail IPL. A consultant radiologist checked and confirmed the position of ROIs. Intramyocellular lipid (IMCL) and extramyocellular lipid (EMCL) of the soleus muscle of the calf were quantified by ^{1}H-MRS according to previously reported methods (22). To fit the lipid peaks for IMCL and EMCL quantification, the model used was as follows (22): water resonance 4.7 ppm, choline 3.2 ppm, creatine 3.0 ppm, EMCL-CH_2 1.5 ppm, IMCL-CH_2 1.3 ppm, EMCL-CH_3 1.1 ppm, and IMCL-CH_3 0.9 ppm. IMCL and EMCL were quantified as the ratio of the methylene IMCL or EMCL peaks (IMCL-CH_2 or EMCL-CH_2) to internal water.

Images for the estimation of truncal visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) volumes were obtained from the top of the acquisition (neck) to the last slice of the abdominopelvic cavity by the same 6-point Dixon MRI scanning protocol described for liver fat, and quantification was performed using artificial intelligence–based image processing software developed at the University of Westminster, London, United Kingdom. Body weight and percentage body fat by bioelectrical impedance analysis were also measured (Tanita BC-418MA; Tanita Ltd).

Blood pressure and heart rate variability

Ambulatory blood pressure (ABP) was measured using TM-2430 ABP monitors (A&D Inc) worn for 24 h. Readings were obtained every 30 min during daytime and every 60 min at night (22:00 to 07:00). Participants kept a record of their physical activity and sleep time in a 24-h activity diary, with self-reported sleep cutoffs being used in the analysis of daytime and nighttime BP. A&D Professional Analysis software was used to analyze mean 24-h, daytime, and nighttime SBP, DBP, and pulse. OMRON M2 Basic Intellisense monitors (OMRON Healthcare UK Ltd) were used to measure clinical blood pressure according to British Hypertension Society guidelines (23).

A small, lightweight, chest-worn, wireless 2-lead ambulatory heart rate/ECG monitor (eMotion Faros 180, Mega Electronics Ltd) was fitted to measure 24-h ambulatory HRV (24 h, daytime and nighttime). Time-domain HRV parameters included the mean of the SDs of the normal-to-normal (NN) intervals (SDNN) and root mean square of successive differences of NN intervals (RMSSD). Frequency-domain HRV parameters included high-frequency (HF) power and very-low-frequency (VLF) power. Real-time HRV was recorded during a 5-min rest, a 5-min physical stressor (inflation of blood pressure monitor cuff to 200 mmHg), and a mental stressor [the Stroop color-word test (24)]. Cardiscope Analytics software (HASIBA Medical GmbH) was used to generate HRV parameters as previously described (25).

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Blood biomarkers and fecal SCFAs

Blood and fecal sample collection procedures and analysis are explained in Supplemental Methods. Fasting insulin, glucose, nonesterified fatty acids, plasma lipids [total cholesterol (TC), HDL cholesterol, LDL cholesterol, triglyceride, and calculated TC:HDL cholesterol ratio], fatty liver indicators [fetuin-A, γ-glutamyltransferase (GGT), and alanine transaminase (ALT)], clinically relevant biomarkers of metabolic dysregulation/insulin resistance (adiponectin, resistin, and leptin), plasma fatty acids, and metabolomics were analyzed using venous blood samples taken at baseline and end point. Metabolomic profiling was performed using a high-throughput serum NMR metabolomics platform (Nightingale Health), as described by Soininen et al. (26). All blood samples were processed by laboratory technicians blinded to participant identification and treatment allocation. Fecal samples were collected at baseline and end point in a subset of participants ($n = 18$ almond group, $n = 17$ control group) and processed within 2 h before analysis by gas chromatography, as described in Supplemental Methods.

Compliance and dietary assessment

As outlined in Figure 1, compliance with the dietary advice was verified by telephone 24-h recalls and 4-d estimated portion size food records, which were analyzed using Nutritics software (v5.026; Nutritics Ltd) and inspection of stool samples for almond particles. Compliance scores included: *1*) complete consumption of study snacks; *2*) avoidance of consumption of other snacks; and *3*) avoidance of consumption of nuts other than almonds, as assessed by 4-d food diaries and 24-h dietary recall. The scores were as follows: score 0—did not comply; score 1—partial compliance; score 2—moderate compliance; score 3—full compliance. For participants who did not complete 4-d food diaries, the compliance was checked based solely on 24-h dietary recalls. Explanations for missing data are provided in **Supplemental Figure 1.**

Statistical analysis

Statistical analysis was performed using IBM SPSS 25. Normality of data was assessed visually using histogram and Q-Q plot of residuals. Baseline data are shown as mean value and SD, or, if not normally distributed, as median (IQR). Treatment effects are presented as adjusted marginal mean differences of change between end-point and baseline values of the 2 groups and 95% CI. A chi-square test was conducted to investigate whether there were differences in sex and ethnicity between control and almond groups at baseline. To examine whether there were differences in other baseline characteristics and also in baseline data between the two treatment groups, independent *t* tests were used for normally distributed data, and a Mann–Whitney U test was used for nonnormally distributed data. To investigate the significance of treatment effects between the 2 groups, ANCOVA was used, with change from baseline as the dependent variable, adjusting for baseline value and baseline BMI; analysis results are presented as change values from baseline generated from estimated marginal means with CI. In addition to normality checks of residuals distribution of the changes, homogeneity of variance was examined by plotting standardized residual values compared with predicted values, and also by using Levene tests. A 2-sided P value <0.05 was considered to show statistical significance.

Results

The CONSORT flow diagram, showing the flow of participants through the study, is shown in Supplemental Figure 1. Of 294 potential participants interested in taking part in the study, 109 were eligible and 107 were randomly allocated to control or almond treatments. One hundred and five participants completed the trial: 51 in the control group and 54 in the almond group. Reasons for noncompletion included gastrointestinal intolerance of almonds ($n = 2$). The baseline characteristics of study participants randomly allocated to treatment were not different between the control and almond groups (shown in **Table 1**). Approximately 30% of participants were male and 70% female, and ~37% identified as belonging to an ethnic group other than white.

Compliance with dietary intervention

Compliance with the intervention was high as evidenced by mean (±SD) compliance scores of 2.8 ± 0.3 in the almond group and 2.7 ± 0.4 in the control group, out of a possible maximum of 3. Only 80 participants completed both sets of food diaries as per instructions with satisfactory data quality, but compliance scores based on 24-h dietary recalls did not differ significantly between those who completed both food diaries adequately and those who did not [mean compliance for food diary completers in almond group 2.9 ± 0.3 ($n = 40$), in control group 2.7 ± 0.4 ($n = 40$); for food diary noncompleters in almond group 2.9 ± 0.3 ($n = 14$) and control group 2.5 ± 0.6 ($n = 11$)].

Table 2 shows the nutrient intake of the study population as measured from 4-d diet diaries at baseline (week 0) and end of intervention from the 40 participants of each group who completed diaries at both time points. At baseline, SFAs provided 12.3% and 12.5% of energy intake, and free sugars provided 5.9% and 5.5% of energy intake for the control and almond groups, respectively. There was no difference in the change from baseline in energy intake between control and almond groups during the intervention. Relative to the control group, the almond group reduced intakes of total carbohydrates, starch, and free sugars as a percentage of energy intake by 9.3%, 7.0%, and 3.0%, respectively, and increased dietary fiber intake by 7.4%. Total fat intake as a percentage of energy intake and the ratio of unsaturated to saturated fatty acids were significantly increased in the almond group, by 10.8% and 1.3%, respectively, compared with the control group. The almond group also increased intakes of potassium, magnesium, vitamin E, and riboflavin, and lowered intake of sodium relative to the control group.

To determine whether pre–post intervention changes were significant in the almond group but not the control group, repeated measures ANCOVA (with time as a repeated measure and BMI as covariate) showed that the decreases in carbohydrate, starch, free sugars, and sodium intakes in the almond group were significant (time × intervention group interactions are presented in **Supplemental Table 4**), whereas there were no significant changes in the control group for these nutrients. Furthermore,

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TABLE 1 Participant characteristics at screening for those randomized to treatment[1]

	Control, $n = 51$	Almond, $n = 56$
Age, y	56.0 ± 10.7	56.3 ± 10.3
Sex, M/F, *n*	15/36	17/39
Ethnicity (black/South Asian, Southeast Asian, and Middle Eastern/Far East/white/other), *n*	2/6/2/34/7	9/7/3/34/3
Cardiometabolic score	4.2 ± 2.1	4.5 ± 2.0
BMI, kg/m^2	26.7 ± 4.5	27.3 ± 4.4
Waist circumference, cm	93.3 ± 12.5	93.6 ± 12.5
Body fat, %	32.7 ± 8.5	34.4 ± 8.4
cSBP, mmHg	124.4 ± 15.1	126.2 ± 17.6
cDBP, mmHg	80.6 ± 7.7	83.8 ± 10.8
Glucose, mmol/L	5.1 ± 0.6	5.1 ± 0.5
TC, mmol/L	5.6 ± 1.2	5.6 ± 1.0
TAG, mmol/L	1.2 ± 0.5	1.2 ± 0.6
LDL, mmol/L	3.5 ± 1.0	3.4 ± 0.9
HDL, mmol/L	1.6 ± 0.5	1.6 ± 0.5
TC:HDL	3.6 ± 0.9	3.7 ± 1.1

[1] Values are means ± SD or categorical totals. Ethnicity was determined by self-reporting. Chi-square test was used to examine whether there were differences in sex and ethnicity. Independent *t* test was used to examine whether there were differences in other characteristics. There were no differences between groups for any of the parameters included in this table. cDBP, clinical diastolic blood pressure; cSBP, clinical systolic blood pressure; TAG, triglyceride; TC, total cholesterol.

the increases in intakes of dietary fiber, fat, MUFAs, PUFAs, the ratio of unsaturated to saturated fatty acids, and vitamin E in the almond group were significant, but there were no significant changes in the control group. Potassium, magnesium, and riboflavin significantly increased in the almond group but also significantly decreased in the control group, although the magnitude of the changes was smaller in the control group for magnesium and riboflavin. There were no significant changes in total energy, SFA, protein, and niacin intake within either group.

Vascular health

Vascular-related measures are shown in **Table 3**. There was a significant treatment difference in the change from baseline values for FMD. Almond consumption significantly increased FMD by 4.1% units relative to the change following control snacks (95% CI: 2.2, 5.9% units; $P < 0.00005$). There were no differential changes in baseline brachial artery diameter or in endothelium-independent vasodilation (following GTN) between groups. VLF power, a frequency domain parameter of

TABLE 2 Nutrient intakes estimated from 4-d food diaries at baseline (prior to run-in) and the final week of the dietary intervention[1]

	Control,[2] $n_{max} = 40$		Almond,[2] $n_{max} = 40$		Mean comparison between groups	*P* value
	Baseline	Change	Baseline	Change		
Energy intake,[3] kcal/d	2088.9 ± 538.5	−5.8 (−124.7, 113.2)	1769.4 ± 475.0	−85.3 (−204.3, 33.7)	−79.5 (−251.8, 92.8)	0.361
Protein, %E	15.4 ± 3.8	0.5 (−0.5, 1.5)	15.9 ± 3.6	1.0 (0.0, 2.0)	0.5 (−0.9, 1.9)	0.466
Total carbohydrate, %E	43.3 ± 7.1	1.7 (−0.1, 3.5)	41.8 ± 6.6	−7.6 (−9.4, −5.8)	−9.3 (−11.9, −6.8)	<0.001
Starch, %E	23.9 ± 5.1	2.5 (0.9, 4.1)	23.5 ± 5.3	−4.5 (−6.1, −2.9)	−7.0 (−9.3, −4.8)	<0.001
Free sugars, %E	5.9 ± 3.8	0.4 (−0.5, 1.2)	5.5 ± 2.8	−2.6 (−3.5, −1.8)	−3.0 (−4.2, −1.8)	<0.001
Dietary fiber,[3] g/d	23.8 ± 6.2	−1.9 (−4.5, 0.6)	20.7 ± 7.7	5.5 (3.0, 8.1)	7.4 (3.8, 11.1)	<0.001
Fat, %E	36.5 ± 6.5	−2.6 (−4.3, −0.8)	37.1 ± 6.2	8.3 (6.5, 10.0)	10.8 (8.4, 13.3)	<0.001
SFA, %E	12.3 ± 3.6	−0.6 (−1.3, 0.1)	12.5 ± 3.7	−1.4 (−2.1, −0.6)	−0.7 (−1.8, 0.3)	0.153
MUFA, %E	11.5 ± 3.4	−1.1 (−2.4, 0.0)	12.4 ± 3.7	8.6 (7.4, 9.8)	9.8 (8.1, 11.5)	<0.001
PUFA, %E	5.9 ± 2.5	−0.8 (−1.4, −0.1)	5.9 ± 1.7	2.0 (1.4, 2.6)	2.8 (1.9, 3.7)	<0.001
Unsaturated:saturated fatty acid ratio	−0.1 ± 0.6	−0.1 (−0.3, 0.1)	1.1 ± 1.1	1.1 (0.9, 1.3)	1.3 (1.0, 1.5)	<0.001
Sodium, mg	2151.2 ± 766.3	179.7 (−15.8, 375.3)	1926.1 ± 866.1	−490.8 (−686.4, −295.3)	−670.6 (−948.6, −392.6)	<0.001
Potassium,[3] mg	3028.9 ± 936.2	−352.5 (−590.4, −114.5)	2534.7 ± 854.5	221.3 (−16.7, 459.3)	573.8 (231.0, 916.6)	0.001
Calcium,[3] mg	868.4 ± 455.8	24.2 (−57.6, 106.0)	703.8 ± 242.5	57.3 (−24.5, 139.0)	33.1 (−84.0, 150.2)	0.575
Magnesium,[3] mg	368.7 ± 180.9	−36.0 (−68.9, −3.0)	278.5 ± 92.1	112.6 (79.7, 145.5)	148.6 (100.9, 196.3)	<0.001
Vitamin E, mg	10.7 ± 3.7	−1.9 (−3.5, −0.4)	8.9 ± 4.0	13.5 (11.9, 15.0)	15.4 (13.2, 17.6)	<0.001
Riboflavin, mg	1.8 ± 1.6	−0.1 (−0.3, 0.0)	1.5 ± 0.8	0.4 (0.2, 0.6)	0.5 (0.3, 0.8)	<0.001
Niacin, mg	16.1 ± 8.9	−1.6 (−3.3, 0.1)	14.7 ± 9.4	−0.4 (−2.1, 1.3)	1.2 (−1.3, 3.6)	0.339

[1] Baseline data are mean ± SD. Values of change and main comparisons of changes between groups are presented as mean (95% CI) generated from estimated marginal means from ANCOVA, adjusted for baseline value and baseline BMI. ANCOVA assumptions were met. $P < 0.05$ indicates a significant difference. .

[2] Data were analyzed using 40 diaries collected from each group. Missing data are due to poor quality of diet diaries or failure to complete by participant.

[3] Baseline value was different between control and almond group. %E, % of energy intake.

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TABLE 3 Changes in indices of vascular function, blood pressure, and heart rate variability following randomization to almond and control snacks[1]

	Control,[2] $n_{max} = 51$		Almonds,[2] $n_{max} = 54$		Main comparison between groups[4]
	Baseline[3]	Change	Baseline[3]	Change	
Endothelium-dependent vasodilation					
FMD,[5] %	7.0 ± 4.8	−0.8 (−2.1, 0.5)	3.6 ± 3.9	3.3 (2.0, 4.5)	4.1 (2.2, 5.9)[6]
Prehyperemia brachial artery diameter, mm	3.49 ± 0.58	0.00 (−0.09, 0.09)	3.56 ± 0.48	−0.01 (−0.09, 0.08)	−0.01 (−0.14, 0.11)
GTN, %	14.8 ± 6.5	−0.2 (−1.6, 1.3)	12.6 ± 4.9	0.7 (−0.6, 2.0)	0.9 (−1.1, 2.9)
Clinic blood pressure					
cSBP, mmHg	127.8 ± 12.9	−5.2 (−7.9, −2.6)	127.3 ± 19.3	−6.3 (−8.9, −3.7)	−1.0 (−4.7, 2.6)
cDBP, mmHg	84.6 ± 7.9	−3.2 (−5.1, −1.3)	85.5 ± 10.6	−3.5 (−5.3, −1.6)	0.2 (−2.9, 2.4)
Ambulatory blood pressure					
24-h SBP,[5] mmHg	122.7 ± 9.7	0.4 (−2.0, 2.8)	128.0 ± 15.0	−1.0 (−3.3, 1.3)	−1.4 (−4.8, 1.9)
24-h DBP,[5] mmHg	74.1 ± 6.2	−0.2 (−1.9, 1.5)	77.6 ± 9.3	−0.6 (−2.3, 1.0)	−0.4 (−2.8, 2.0)
24-h pulse, beats/min	71.9 ± 8.4	−0.4 (−2.2, 1.4)	72.6 ± 9.3	−1.0 (−2.8, 0.7)	−0.7 (−3.2, 1.9)
Daytime SBP,[5] mmHg	126.1 ± 9.8	−0.1 (−2.7, 2.6)	132.6 ± 16.0	−0.4 (−2.9, 2.2)	−0.3 (−4.1, 3.5)
Daytime DBP,[5] mmHg	76.2 ± 6.5	0.1 (−1.8, 2.1)	80.9 ± 9.9	−0.2 (−2.0, 1.7)	−0.3 (−3.1, 2.4)
Daytime pulse, beats/min	74.5 ± 8.5	0.1 (−1.9, 2.1)	75.3 ± 9.5	−1.3 (−3.3, 0.6)	−1.1 (−4.2, 1.3)
Nighttime SBP, mmHg	109.3 ± 11.9	1.2 (−1.9, 4.9)	111.6 ± 15.3	−1.0 (−4.3, 2.4)	−3.0 (−7.2, 1.3)
Nighttime DBP, mmHg	64.6 ± 7.6	−0.1 (−2.3, 2.0)	65.6 ± 8.5	−1.4 (−3.4, 0.6)	−1.3 (−4.2, 1.6)
Nighttime pulse, beats/min	61.5 ± 8.1	−0.8 (−2.7, 1.0)	63.0 ± 9.7	−0.2 (−1.9, 1.5)	0.7(−1.8, 3.2)
Heart rate variability (24 h and nighttime)					
24-h SDNN, ms	148.8 ± 36.5	−8.2 (−17.3, −0.9)	142.2 ± 35.0	−5.2 (−14.9, 4.5)	3.0 (−10.3, 16.4)
24-h rMSSD, ms	28.5 ± 8.9	1.9 (−0.6, 4.3)	30.5 ± 9.8	0.6 (−2.1, 3.2)	−1.3 (−4.9, 2.3)
Nighttime SDANN, ms	64.1 ± 22.3	−0.8 (−8.3, 6.8)	66.5 ± 33.0	1.0 (−6.4, 8.4)	1.8 (−8.8, 12.3)
Nighttime rMSSD, ms	31.3 (20.9)	1.9 (−1.3, 5.0)	33.9 (17.4)	−1.1 (−4.1, 1.9)	−3.0 (−7.3, 1.4)
Nighttime VLF, ms^2	1664 (2720)	−293 (−523, −62)	1595 (1387)	44 (−182, 270)	337 (12, 661)[7]
Nighttime HF, ms^2	311.5 (545.3)	0.7 (−84.1, 85.6)	356 (331)	−0.2 (−83.3, 82.8)	−10.0 (−120.0, 118.1)

[1] Values of change and main comparisons of changes between groups are presented as mean (95% CI) generated from estimated marginal means from ANCOVA.

[2] Not all data were analyzed due to poor-quality read-outs. FMD and prehyperemia brachial artery diameter: $n = 42$ (control) and 47 (almond). GTN: $n = 32$ (control) and 41 (almond). 24-h SBP, 24-h DBP, 24-h pulse, daytime SBP, DBP, and pulse: $n = 45$ (control) and 49 (almond). Nighttime SBP, DBP, and pulse: $n = 40$ (control) and 46 (almond). 24-h SDNN and 24-h rMSSD: $n = 33$ (control) and 29 (almond). Nighttime SDANN: $n = 45$ (control) and 47 (almond). Nighttime rMSSD: $n = 45$ (control) and 50 (almond). Nighttime VLF and sleep-time HF: $n = 45$ (control) and 47 (almond). Nighttime SDANN: $n = 45$ (control) and 47 (almond).

[3] Median (IQR) for nighttime rMSSD, VLF, and HF data because they are nonnormally distributed. Mean ± SD for other data that are normally distributed.

[4] ANCOVA, adjusted for baseline outcome value and baseline BMI (mean difference in the change from baseline, almonds minus control). ANCOVA assumptions were met.

[5] Baseline value was different between control and almond group; independent *t* test was used, $P < 0.05$ indicated a significant difference.

[6] $P < 0.00005$.

[7] $P < 0.05$. cDBP, clinic diastolic blood pressure; cSBP, clinic systolic blood pressure; DBP, diastolic blood pressure; FMD, flow-mediated dilation; GTN, glycerol trinitrate; HF, absolute power of the high-frequency band (0.15–0.04 Hz); rMSSD, root mean square of successive R-R interval differences; SBP, systolic blood pressure; SDANN, standard deviation of the average NN intervals for each 5-min segment of heart rate variability recording; SDNN, standard deviation of normal-to-normal (NN) intervals; VLF, absolute power of the very-low-frequency band (0.0033–0.04 Hz).

HRV, was increased during sleep-time by 337 ms^2 (95% CI: 12, 661 ms^2; $P < 0.05$) following almond consumption. There were no significant group differences observed in changes in blood pressure or in 24-h HRV.

Other cardiometabolic outcomes

As shown in **Table 4**, there were no treatment effects of diet on BMI, waist circumference, percentage body fat (measured by bioelectrical impedance), or truncal VAT or SAT volumes (measured by MRI). Liver fat was unaffected by treatment, as was pancreatic and skeletal muscle fat (measured by MRI and MRS). Fasting plasma non-HDL and LDL cholesterol concentrations were significantly reduced by almond snacks relative to control by −0.22 mmol/L (95% CI: −0.42, −0.01 mmol/L; $P = 0.037$) and −0.25 mmol/L (95% CI: −0.45, −0.04 mmol/L; $P = 0.017$), respectively (**Table 5**), but there were no significant treatment effects on HDL cholesterol, triacylglycerol, glucose, insulin, HOMA-IR, adipokines, and markers of fatty liver (adiponectin, leptin, resistin, fetuin-A, ALT, and GGT). **Table 6** presents fasting plasma fatty acid profiles in both groups. Plasma concentrations of oleic acid, which made up 66% of total fatty acid content of the almonds used in the study, were increased after almond consumption relative to control by 228 μmol/L (95% CI: 7, 449 μmol/L; $P = 0.043$), but no significant differences in plasma concentrations of the other main fatty acid, linoleic acid (22% of total fatty acid content of these almonds), nor any other plasma fatty acids were observed. Furthermore, there were no effects of treatment on fecal SCFA composition or total SCFA concentrations (**Table 7**). There were no clear treatment effects on metabolomic profiles (measured by NMR), although serum total ω-3 concentrations decreased by −0.04 mmol/L ($P = 0.031$) and citrate concentrations increased by 0.01 mmol/L ($P = 0.018$) in the almond group relative to the control group (see **Supplemental Table 5**).

Discussion

This study set out to test the hypothesis that substituting whole almonds for typically consumed snacks would increase EDV, decrease liver fat, and improve other markers of cardiometabolic health. Replacing usual snacks with whole almonds, relative to a neutral control snack, caused a 4% unit increase in EDV (FMD) in healthy adults at above-average risk of CVD. According to meta-analyses of clinical studies reporting the predictive value of FMD for cardiovascular events in non-CVD patients, this

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TABLE 4 Body composition and measures of ectopic fat, following randomization to almond and control snacks[1]

	Control,[2] $n_{max} = 51$		Almonds,[2] $n_{max} = 54$		Main comparison between groups[4]
	Baseline[3]	Change	Baseline[3]	Change	
Physical activity by accelerometry,[5] cpm	74.2 ± 17.1	1.5 (−4.0, 7.1)	74.6 ± 21.8	− 2.4 (−7.7, 2.8)	− 4.0 (−11.6, 3.6)
BMI, kg/m^2	27.1 ± 4.4	− 0.2 (−0.4, 0.0)	27.2 ± 4.5	0.1 (−0.1, 0.3)	0.2 (−0.1, 0.5)
WC, cm	93.3 ± 11.7	0.1 (−0.9, 1.2)	94.1 ± 12.2	− 0.6 (−1.6, 0.5)	− 0.7 (−2.2, 0.8)
Body fat, %	31.1 ± 7.7	− 0.5 (−1.1, 0.0)	32.2 ± 7.7	0.3 (−0.3, 0.8)	0.8 (−0.0, 1.6)
MRI and ^{1}H-MRS					
Liver fat %	2.7 (2.5)	0.4 (−0.5, 1.3)	1.7 (1.7)	1.1 (0.1, 2.0)	0.7 (−0.6, 2.0)
IHL %	2.9 (4.0)	0.1 (−1.3, 1.6)	1.7 (2.3)	− 0.6 (−2.1, 0.8)	− 0.8 (−2.8, 1.3)
UI	0.21 ± 0.12	0.03 (−0.06, 0.12)	0.32 ± 0.20	0.01 (−0.08, 0.10)	− 0.02 (−0.15, 0.10)
PUI	0.03 (0.11)	0.02 (−0.05, 0.10)	0.04 (0.21)	0.03 (−0.04, 0.10)	0.01 (−0.09, 0.11)
SI	0.77 (0.13)	− 0.03 (−0.12, 0.05)	0.70 (0.35)	− 0.01 (−0.10, 0.08)	0.02 (−0.10, 0.15)
Pancreatic fat, %	10.7 (3.8)	0.1 (−1.1, 1.3)	10.7 (3.2)	0.1 (−1.1, 1.4)	0.0 (−1.7, 1.8)
SAT, mL	13,703 ± 5290	678 (−41, 1397)	12,981 ± 5264	− 20 (−771, 732)	− 697 (−1741, 347)
VAT, mL	3492 ± 1930	− 13 (−95, 69)	3009 ± 1889	34 (−50, 118)	47 (−71, 165)
IMCL	0.037 ± 0.027	0.002 (−0.005, 0.010)	0.030 ± 0.014	− 0.005 (−0.012, 0.003)	− 0.007 (−0.018, 0.003)
EMCL	0.035 (0.027)	0.001 (−0.009, 0.010)	0.020 (0.015)	− 0.001 (−0.011, 0.009)	− 0.001 (−0.015, 0.013)

[1] Values of change and main comparison of the changes between groups are presented as mean (95% CI) generated from estimated marginal means from ANCOVA.

[2] MRI/MRS scanning was planned on a subset of study participants: $n = 50$ (25 per group). Not all data were analyzed due to technical problems. Physical activity by accelerometery: $n = 45$ (control) and 50 (almond). BMI: $n = 45$ (control) and 50 (almond). WC: $n = 49$ (control) and 51 (almond). Body fat: $n = 49$ (control) and 52 (almond). Liver fat and pancreatic fat: $n = 26$ (control) and 24 (almond). IHL, UI, PUI, and SI: $n = 22$ (control) and 23 (almond). SAT: $n = 24$ (control) and 22 (almond). VAT: $n = 23$ (control) and 22 (almond). IMCL and EMCL: $n = 23$ (control) and 22 (almond).

[3] Median (IQR) for liver fat, IHL, PUI, SI, pancreatic fat, and EMCL data because they are nonnormally distributed. Mean ± SD for other data that are normally distributed. Baseline biomarker values were not different between the 2 groups.

[4] ANCOVA, adjusted for baseline outcome value and baseline BMI (mean difference in change from baseline, almonds minus control); there were no significant differences between groups. ANCOVA assumptions were met.

[5] Physical activity by accelerometery data were generated from heart rate variability monitoring. Cpm, counts per minute; EMCL, extramyocellular lipid; IHL, intrahepatic lipid; IMCL, intramyocellular lipid; PUI, polyunsaturation index; SAT, subcutaneous fat; SI, saturation index; UI, unsaturation index; VAT, visceral fat; WC, waist circumference; ^{1}H-MRS, proton magnetic resonance spectroscopy.

would be equivalent to an adjusted RR reduction of 32% (pooled RR: 0.92; 95% CI: 0.89, 0.96) per 1% increase in FMD) (27). Thus, simply targeting the quality of snacks eaten between meals can have a measurable beneficial impact on vascular health and would be predicted to significantly reduce risk of CVD. Studies are lacking on the effect of almonds on FMD in healthy individuals (28), although previous meta-analyses of randomized controlled trials of nut consumption (mainly walnuts, but also 2 studies on pistachios, 1 on almonds, and 1 on hazelnuts) in participants with T2D or other health issues have reported smaller effect sizes (0.4–0.8% unit differences) compared with the large difference in the current study (29, 30). The improvement in FMD observed in our current study was not accompanied by an increase in prehyperemia diameter nor endothelium-independent dilation (post-GTN administration), demonstrating that almond consumption had a specific impact on nitric oxide–mediated EDV.

Whole almonds have been shown to reduce postprandial glycemia (31) and lipemia (32) compared with almond products with a high lipid bioaccessibility where lipid has been mechanically released from the plant cell walls. This can attenuate acute increases in oxidative stress and inflammation postprandially (33, 34). Almonds also contain cardioprotective chemicals, including L-arginine [essential in the process of nitric oxide synthesis (33)], phenolic compounds, vitamin E, and folate. Whereas concentrations of intracellular L-arginine already exceed that required for nitric oxide synthesis (33), the increased intake of (poly)phenolics, vitamin E, and folate could reduce oxidative stress and inflammation, thus potentially improving nitric oxide bioavailability (35–39).

Liver fat was a main outcome due to its central role in metabolic regulation and as a predictive factor of CVD and T2D risk (40). No effect of almond consumption was observed on liver fat measured with MRI or ^{1}H-MRS (both total percentage and degree of saturation), nor inferred from biochemical markers of liver health (fetuin-A or ALT/GGT). There were relatively low amounts of liver fat in our study population at baseline, with only 10% classified as having fatty liver (liver fat ≥5.5%). Despite participants having higher concentrations of liver fat at baseline (8–10%) in another recent trial, no impact of almond consumption on liver fat was observed (56 g/d almonds compared with isocaloric biscuits over 8 wk; $n = 36$) in participants who were overweight/obese (41). These results demonstrate that almond consumption does not modify liver fat in either direction in a healthy population, despite almonds being a high-fat food. The fact that liver fat did not increase could be due to the fact that on average there was no shift to a positive energy balance and also the low lipid bioaccessibility of almonds (42, 43).

Indicators of insulin sensitivity remained unaffected by the almond intervention. These findings are consistent with previous reports of almonds intakes of 15% of energy or 42–56 g/d in study populations of individuals who were overweight/obese and/or at increased risk of T2D (41, 44–46). The fact that insulin sensitivity was unaffected is perhaps unsurprising considering there were no

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TABLE 5 Circulating biomarkers of cardiometabolic risk following randomization to almond and control snacks[1]

	Control,[2] $n_{max} = 51$		Almonds,[2] $n_{max} = 54$		Main comparison between groups[4]
	Baseline[3]	Change	Baseline[3]	Change	
HOMA-IR	1.78 ± 1.38	0.21 (−0.17, 0.60)	1.64 ± 0.96	0.05 (−0.32, 0.41)	−0.16 (−0.70, 0.37)
Glucose, mmol/L	5.3 ± 0.6	0.02 (−0.14, 0.17)	5.3 ± 0.7	0.03 (−0.12, 0.18)	0.01 (−0.20, 0.23)
Insulin, mIU/L	7.1 ± 4.3	0.70 (−0.68, 2.07)	6.9 ± 3.5	0.08 (−1.24, 1.39)	−0.62 (−2.52, 1.28)
NEFA, mmol/L	0.66 ± 0.24	−0.03 (−0.09, 0.04)	0.64 ± 0.24	−0.00 (−0.06, 0.07)	−0.03 (−0.07, 0.12)
TC, mmol/L	5.26 ± 1.13	0.03 (−0.15, 0.20)	5.40 ± 0.93	−0.18 (−0.35, −0.02)	−0.21 (−0.45, 0.03)
TAG, mmol/L	1.17 (0.69)	−0.11 (−0.20, 0.01)	1.07 (0.73)	−0.08 (−0.17, 0.02)	0.03 (−0.11, 0.16)
Non-HDL-C, mmol/L	3.92 ± 1.16	0.11 (−0.04, 0.26)	4.00 ± 0.98	−0.11 (−0.25, 0.03	−0.22 (−0.42, −0.01)[5]
LDL-C, mmol/L	3.63 ± 1.16	0.15 (0.01, 0.30)	3.74 ± 0.91	−0.09 (−0.23, 0.05)	−0.25 (−0.45, −0.04)[5]
HDL-C, mmol/L	1.61 ± 0.45	0.04 (−0.04, 0.11)	1.66 ± 0.51	−0.04 (−0.11, −0.03)	−0.08 (−0.18, 0.03)
TC:HDL-C	3.45 ± 0.91	−0.04 (−0.15, 0.07)	3.47 ± 1.01	−0.03 (−0.14, 0.07)	0.00 (−0.15, 0.16)
Leptin, μg/L	12.50 (13.41)	−0.34 (−2.08, 1.41)	17.49 (20.24)	0.25 (−1.44, 1.94)	0.59 (−1.86, 3.03)
Adiponectin, mg/L	7.64 (5.96)	−0.16 (−0.76, 0.44)	7.92 (5.86)	−0.14 (−0.72, 0.44)	0.02 (−0.82, 0.85)
ALT, IU/L	22.00 (9.8)	−0.46 (−3.52, 2.59)	22.10 (10.10)	1.31 (−1.63, 4.25)	1.77 (−2.47, 6.01)
GGT, IU/L	12.60 (11.80)	−0.77 (−3.58, 2.05)	15.20 (11.00)	0.92 (−1.79, 3.63)	1.69 (−2.22, 5.59)
Fetuin A, mg/L	698.60 ± 132.86	−2.70 (−37.71, 32.32)	665.56 ± 136.83	15.91 (−18.08, 49.89)	18.60 (−30.33, 67.53)
Resistin, μg/L	5.25 ± 2.29	0.12 (−0.20, 0.44)	5.04 ± 1.79	−0.01 (−0.32, 0.30)	−0.13 (−0.57, 0.31)

[1] Values of change and main comparisons of the changes between groups are presented as mean (95% CI) generated from estimated marginal means from ANCOVA.

[2] Not all data were analyzed due to technical problems and sample loss. HOMA-IR and glucose: $n = 48$ (control) and 53 (almond). Insulin, NEFA, TC, TAG, non-HDL-C, LDL-C, HDL-C, TC:HDL-C, ALT, GGT: $n = 49$ (control) and 53 (almond). Leptin, adiponectin, fetuin-A, and resistin: $n = 49$ (control) and 52 (almond).

[3] Median (IQR) for TAG, leptin, and GGT data because they are nonnormally distributed. Mean ± SD for other data that are normally distributed. Baseline biomarker values were not different between the 2 groups.

[4] ANCOVA, adjusted for baseline outcome value and baseline BMI (mean difference in change from baseline, almonds minus control); $P < 0.05$ indicated a significant difference. ANCOVA assumptions were met.

[5] $P < 0.05$ indicated a significant difference for values of mean difference between 2 groups. ALT, alanine aminotransferase; GGT, γ-glutamyltransferase; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; NEFA, nonesterified fatty acid; TAG, triglyceride; TC, total cholesterol.

differences between groups in parameters of ectopic fat, assessed by MRI imaging of visceral, pancreatic, and hepatic fat, and MRS analysis of muscle lipids, nor in anthropometric assessments of body fat. An important aim of the current study was to ensure that both intervention arms were isoenergetic, and the consistency in body weight over time and between treatments demonstrates that this was achieved. Although observational studies report inverse associations between tree nut and almond consumption with BMI and WC (47, 48), average almond intakes are low and therefore these associations are likely to indicate that almond consumers have overall healthier diets leading to lower risk of excess body fat (48). Bowen et al. (41) also reported no significant differences in SAT and VAT measured by MRI/MRS. In contrast, displacement of snacks with 42 g/d almonds for 6 wk in human

TABLE 6 Plasma fatty acid profile following randomization to almond and control snacks[1]

	Control, $n = 48$		Almonds, $n = 53$		Main comparison between groups[3]
FA, μmol/L	Baseline[2]	Change	Baseline[2]	Change	
Palmitic (16:0)	2334.3 ± 992.4	−105.6 (−322.3, 111.1)	2296.8 ± 933.1	53.6 (−152.6, 259.9)	159.2 (−140.1, 458.6)
Palmitoleic (16:1)	300.8 ± 175.8	−18.2 (−42.1, 5.7)	282.9 ± 156.7	−25.1 (−47.9, −2.4)	−7.0 (−40.0, 26.0)
Stearic (18:0)	690.4 ± 268.1	−24.9 (−76.4, 26.6)	674.6 ± 241.2	5.2 (−43.8, 54.2)	30.1 (−41.0, 101.2)
Oleic (18:1n–9)	2300.4 ± 1032.1	−111.3 (−271.1, 48.5)	2232.9 ± 915.3	116.6 (−35.5, 268.7)	227.9 (7.2, 448.7)[4]
Linoleic (18:2n–6)	2873.4 ± 1004.6	−23.2 (−247.9, 201.6)	2853.6 ± 810.4	107.7 (−124.1, 303.6)	112.9 (−197.5, 423.3)
α-Linolenic (18:3n–3)	155.9 ± 81.6	4.3 (−16.5, 25.1)	134.6 ± 67.5	−20.3 (−40.0, −0.6)	−24.6 (−53.4, 4.2)
γ-Linolenic (18:3n–6)	76.1 (76.5)	−9.5 (−21.2, 2.2)	70.8 (83.3)	−9.5 (−20.6, 1.7)	0.0 (−16.1, 16.2)
Homo-γ-linoleic (20:3n–6)	141.8 ± 65.3	−2.8 (−17.1, 11.4)	136.5 ± 60.0	0.9 (−12.7, 14.4)	3.7 (−16.0, 23.4)
Arachidonic (20:4n–6)	601.5 ± 302.3	−26.8 (−72.9, 19.4)	623.3 ± 243.4	15.0 (−28.9, 59.0)	41.8 (−22.0, 105.6)
Eicosapentaenoic (20:5n–3)	101.2 (77.3)	8.6 (−9.7, 26.9)	96.1 (81.1)	−11.6 (−29.0, 5.8)	−20.2 (−45.4, 5.1)
Docosatetraenoic (22:4n–6)	31.9 (25.3)	−1.1 (−3.5, 1.3)	29.0 (28.2)	−2.4 (−4.6, −0.1)	−1.3 (−4.6, 2.0)
Docosapentaenoic (22:5n–3)	47.9 ± 18.9	−0.4 (−4.3, 3.6)	47.0 ± 19.9	−3.6 (−7.4, 0.2)	−3.2 (−8.7, 2.2)
Docosapentaenoic (22:5n–6)	47.2 (59.8)	−0.2 (−4.9, 4.6)	49.0 (49.4)	1.3 (−3.3, 5.8)	1.5 (−5.1, 8.0)
Docosahexaenoic (22:6n–3)	184.9 ± 77.3	5.0 (−10.9, 20.9)	176.8 ± 81.0	−5.9 (−21.0, 9.2)	−10.9 (−32.8, 11.1)
Total plasma FA	9913.6 ± 3685.5	−303.4 (−1014.5, 407.7)	9741.3 ± 3275.5	201.4 (−475.2, 878.1)	504.8 (−477.4, 1487.0)

[1] Values of change and main comparisons of changes between groups are presented as mean (95% CI) generated from estimated marginal means from ANCOVA.

[2] Median (IQR) for γ-linolenic (18:3n–6), eicosapentaenoic (20:5n–3), docosatetraenoic (22:4n–6), and docosapentaenoic (22:5n–6) acids data because they are nonnormally distributed. Mean ± SD for other data that are normally distributed. Baseline biomarker values were not different between the 2 groups.

[3] ANCOVA, adjusted for baseline outcome value and baseline BMI (mean difference in change from baseline, almonds minus control); $P < 0.05$ indicated a significant difference. ANCOVA assumptions were met.

[4] $P < 0.05$ indicated a significant difference for values of mean difference between 2 groups. FA, fatty acid.

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TABLE 7 Fecal SCFAs from a subset of study population following random allocation to almond and control snacks[1]

SCFA, μmol/g	Control, $n = 17$		Almonds, $n = 18$		Main comparison between groups[3]
	Baseline[2]	Change	Baseline[2]	Change	
Acetic acid	50.1 (32.3)	−0.56 (−8.78, 9.90)	49.4 (31.8)	4.61 (−4.47, 13.68)	4.05 (−9.05, 17.15)
Propionic acid	12.8 (7.3)	2.26 (−2.14, 6.66)	13.8 (10.5)	−0.68 (−4.96, 3.59)	−2.94 (−9.13, 3.24)
Isobutyric acid	1.91(0.5)	0.04 (−0.41, 0.50)	1.6 (1.1)	0.27 (−0.18, 0.71)	0.22 (−0.42, 0.87)
Butyric acid	13.6 (8.7)	0.31 (−3.97, 4.59)	13.7 (12.9)	3.06 (−1.10, 7.21)	2.74 (−3.28, 8.77)
Isovaleric acid	2.2 (0.4)	0.10 (−0.43, 0.63)	1.9 (1.4)	0.30 (−0.22, 0.81)	0.20 (−0.55, 0.95)
Valeric acid	1.7 (1.2)	0.11 (−0.22, 0.45)	2.0 (0.8)	0.06 (−0.27, 0.38)	−0.06 (−0.52, 0.41)
Total SCFA	90.1 (52.0)	3.31 (−13.37, 20.0)	85.4 (68.0)	7.66 (−8.55, 23.87)	4.35 (−19.05, 27.75)

[1]Values of change and main comparisons of changes between groups are presented as mean (95% CI) generated from estimated marginal means from ANCOVA.
[2]Median (IQR) because data are nonnormally distributed. Baseline biomarker values were not different between the 2 groups.
[3]ANCOVA, adjusted for baseline outcome value and baseline BMI (mean difference in change from baseline, almonds minus control). ANCOVA assumptions were met. SCFA, short chain fatty acid.

adults with raised blood concentrations of LDL cholesterol, but who were otherwise healthy (9), lowered abdominal and leg fat measured by DXA despite no differences in body weight. One factor that could influence regional fat distribution is the timing of the snack intake. Evidence for this is provided by results of a 16-wk trial in young Korean adults who consumed 56 g almonds/d immediately before a meal, showing a reduction in visceral fat without changes in body weight, but the same effect was not demonstrated when almonds were consumed >2 h before or after a meal (49). This could relate to a lowering of glycemic and insulinemic responses induced by consumption of almonds immediately before a meal.

Consistent with previous research, we observed a reduction in LDL cholesterol, which is likely to be due to displacement of snacks high in saturated fats with almonds that are rich in unsaturated fats, phytosterols, and fiber. The average reduction in the LDL fraction of 0.25 mmol/L reported here, relative to control, is greater than that reported in a recent meta-analysis of 15 previous almond intervention studies, that is, 0.14 mmol/L (50).

In addition to endothelial function, other measures of cardiovascular function were measured, including BP and HRV. We observed no difference in BP, in contrast to a recent meta-analysis of 15 RCTs (50), which reported a significant reduction in DBP in studies where >42 g almonds (for ≥3 wk) were consumed. A unique finding of the study is the relative increase in the longer-phase HRV parameter VLF during sleep, representing greater parasympathetic regulation at night. Low VLF is predictive of mortality (51) and associated with high biomarkers of systemic inflammation (52). Over 24 h, however, there were no effects on any parameter of HRV. During waking hours, parasympathetically driven longer-phase fluctuations in heart rate (e.g., VLF and SD of the average NN intervals for each 5-min segment of a 24-h HRV recording) in response to neurohormonal and circadian physiological changes are likely to be largely overwhelmed by larger sympathetically driven oscillations generated by physical activities and emotional/psychological influences, unless measured under highly controlled conditions, for example, during supine rest. It was previously reported that 4-wk consumption of pistachios at 20% of EER increased HRV in the resting state in adults with T2D compared with low-fat/high-carbohydrate snacks (53).

A key strength of the current study was the considered design of the control dietary intervention to ensure that treatment effects on cardiometabolic risk factors were not the result of a deterioration in diet quality in the control group. Although the size of the decrease in potassium intake in the control group was larger than the increase in potassium intake in the almond group, all other differences in the changes from baseline for macronutrients and micronutrients were attributable to significant changes in dietary intake in the almond group. Combining measures of liver fat ensured regional variability in hepatic fat distribution was accounted for (by MRI of the whole liver) but small changes could be detected using a more sensitive method (MRS) (54). However, future studies could also benefit from recruiting people with diagnosed fatty liver to enable greater scope for diet-mediated change. Limitations of the study were the fact that there were some differences between groups in cardiometabolic disease risk factors at baseline, despite the minimization of groups for age, sex, ethnicity, and cardiometabolic risk score, and the similarity between groups at baseline for screening variables. The difference in baseline FMD values was unexpected, although the statistical analysis adjusted for differences in baseline values. The imbalance in recruitment by sex could mean that the results might not be as applicable to men because they made up just 30% of the randomized sample. Due to difficulties in obtaining fecal samples, it is likely that SCFA analysis was statistically underpowered. The lack of treatment effect contrasts with results from an in vitro digestion model experiment, where butyrate significantly increased after almond treatment (55). Lastly, there were 25 participants who did not complete usable 4-d food diaries and therefore accurate dietary intake data are missing for nearly one-quarter of the sample population, although inspection of 24-h dietary recalls indicated that compliance did not differ in these participants.

In conclusion, the results of this trial show that replacing typical snacks with almonds can have a meaningful impact on daily nutrient intakes and can improve endothelial function, cardiac autonomic function, and lower LDL cholesterol. However, isoenergetic snack substitution in this trial did not modify regional fat deposition and therefore markers of insulin sensitivity were unaffected. The degree of improvement in endothelial function and LDL cholesterol concentrations suggests that incorporating almonds in the diet in place of typically consumed

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snacks has the potential to reduce CVD risk by ≤30% and therefore could play a powerful role in enhancing cardiovascular health.

We thank Jo Bruce, the R&D manager of ADM Europe, for the expertise in oil formulation and blending. We also thank Clinical Research Facility (CRF) staff and the clinical imaging team at St Thomas' Hospital and Cancer Centre, Guy's Hospital, for their assistance in MRI/MRS scanning; and staff at King's College London: Anne-Catherine Perz for assistance in the laboratory and FMD training; Timothy Wingham for assistance in phlebotomy; and Robert Gray for assistance in running gas liquid chromatography-flame ionizable detector for quantification of SCFAs from stool samples and plasma fatty acids from fasting blood samples.

The authors' responsibilities were as follows—WLH, SEEB: designed the research; PRE, PJC: contributed to the early design of the research protocol; VD, LS, LF, CP, MR, EK, MO'C-L: conducted the research including the recruitment of study volunteers and the collection of samples and data; GC-E, HS: acquired MRI and MRS data; VD, MD'A: analyzed and interpreted data; BW, NB: specifically analyzed the whole-body MRI data to estimate visceral and subcutaneous fat volumes; DC: assisted VD in MRI pancreas image analysis; GC-E: assisted VD in analyzing MRI liver and MRS data; VD, WLH: performed statistical analysis and interpreted data; VD, WLH, SEEB: wrote the paper; PRE: contributed to subsequent versions; WLH, SEEB: had primary responsibility for final content; and all authors: read and approved the final manuscript.

WLH and SEEB report grants and nonfinancial support from the Almond Board of California; LS received salary for her post as research assistant on the ATTIS study from the Almond Board of California; and VD reports financial support in the form of a PhD studentship from the Indonesia Endowment Fund for Education. All other authors report no conflicts of interest.

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Online Supplementary Material

Snacking on whole almonds for 6 weeks improves endothelial function and lowers LDL cholesterol but does not affect liver fat and other cardiometabolic risk factors in healthy adults: the ATTIS study, a randomized controlled trial.

Authors: Dikariyanto V. et al.

Supplemental Methods

Study population

Recruitment was conducted via advertisements in newspapers (London Metro, London Evening Standard, Camden New Journal, Islington Tribune, Westminster Extra), letters to patients registered at two General Practices (GP) in London, study posters at local libraries, study flyers at community centres and leisure centres throughout London and electronic advertisements (social media, e-mail, GumTree). The recruitment process comprised the dissemination of participant information sheet; the completion of a pre-screening questionnaire, and a screening visit.

Potential participants who were eligible from the pre-screening questionnaire, conducted over the phone or completed on-line, attended a screening visit at King's College London (KCL) metabolic research unit (MRU) in the fasted state (12h). During the screening visit potential participants completed an eating habit questionnaire and informed consent was taken. Measurements of anthropometry (body height and weight, waist circumference (WC), body mass index (BMI), body fat composition) and clinical blood pressure (cBP) were made. Blood was collected by venepuncture for analysis of blood glucose, full lipids, liver function and full blood count, by Affinity Biomarker Labs (London, UK) using standard clinical chemistry

techniques. At the end of the visit, participants were given a 4-d food and activity diary to be completed once they were informed that they were eligible for the study based on the screening results. The inclusion and exclusion criteria are outlined in **Supplemental Table 1.**

Supplemental Table 1. Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
• Men and women • 30-70 y • Regular snack consumer (consume 2 or more snacks per day) • Cardiovascular disease risk score of 2 or more according to Framingham risk score system. To be included individuals had to score a total of 2 or more points in one or more of the following criteria: (1) total plasma cholesterol (TC) 6.2–7.2 mmol/L (2 points), 5.2–6.1 mmol/L (1 point); (2) high-density lipoprotein (HDL) cholesterol in men ≤0.9 mmol/L (2 points), 1.0–1.1 (1 point) and women ≤1.1 mmol/L (2 points), 1.2–1.3 mmol/L (1 points); (3) blood pressure (BP) either systolic BP (SBP) ≥140 mmHg (2 points), 130–139 mmHg (1 point) or diastolic BP (DBP) ≥90 mmHg (2 points); (4) obesity/adiposity either body mass index (BMI) >30 kg/m$_2$ (2 points), 25–30 kg/m$_2$ (1 point) for Asian populations [27.5 kg/m$_2$ (2 points), 23–27.5 kg/m$_2$] (1 point) or waist circumference in men [102 cm (2 points), 94–101 cm (1 point) and women [88 cm (2 points), 80–87 cm (1 point).	• Pre-diabetic or diabetic condition; • Reported history of heart attack (myocardial infarction) or stroke, cancer (excluding basal cell carcinoma) in the last five years, epilepsy or regular fainting, cholestatic liver diseases, pancreatitis, alcohol or drug abuse; • Diagnosis of cardiovascular problems, angina, thrombosis, pacemaker, gastrointestinal disorders, renal or bowel diseases; • Use of a drug likely to alter gastrointestinal motility or nutrient absorption; • Presence of metal inside the body; • Allergy or intolerance to nuts; • Currently pregnant, planning pregnancy, breastfeeding or having given birth in the preceding 12-months; • Unwillingness to follow the protocol and/or give informed consent; • Weight change of >3 kg in preceding 2-months; • BMI of <18 kg/m$_2$ or >40 kg/m$_2$; • Current smokers or individuals who quit smoking within the last 6-months; • Participation in other research trials involving dietary or drug intervention and/or blood collection in the past 3-months; • Unable or unwilling to comply with study protocol.

Formulation of control test meals

Sweet and savory mini muffin snacks were developed to replicate the average UK snack nutrient profile (**Supplemental Table 2)**, which was calculated from snack foods identified in

the UK National Diet and Nutrition Survey (NDNS) database (1) (55% energy from available carbohydrate, 36% total fat (14% saturated fat), and 10% protein (2). Prior to commencing the study, a 3-wk feasibility study was conducted and the study verified that the snacks had a neutral effect on lipids, blood pressure and body weight/composition and ensured acceptability of the dietary intervention (2). A blend of plant oils (provided by ADM Oils & Fats Ltd, Erith, UK) was blended with butter to replicate the estimated average fatty acid profile of UK snack-derived fat intake.

Supplemental Table 2. Recipe and Nutrient composition of control muffins

	Sweet Muffins[1] (each muffin 21 g and 80 kcal)	Savory muffins[2] (each muffin 28 g and 80 kcal)
Blend of plant oils	3.5	3.3
Butter	0.3	0.3
White plain flour	8.0	16.7
White granulated sugar	7.3	-
Flavouring	0.3	-
Egg white powder	1.2	0.3
Egg white (liquid)	5.3	3.3
Baking Powder	0.2	0.2
Salt	-	0.2
Seasoning	-	0.3
Water	-	9.3
Nutrient composition		
Energy, *kcal (kJ) per 100 g*	380 (1582)	383 (1600)
Fat, *g per 100 g (% Energy)*	14.8 (35.1%)	15.8 (37.1%)
Carbohydrate, *g per 100 g (% Energy)*	52 (51.3%)	50 (49.0%)
of which sugars, *g per 100 g (% Energy)*	29.6 (29.2%)	0.4 (0.4%)
Protein, *g per 100 g (% Energy)*	8.7 (9.2%)	8.7 (9.1%)
Fatty acid composition		
MUFA, *g per 100 g (% Energy)*	6.5 (15.4%)	6.8 (16.0%)
PUFA, *g per 100 g (% Energy)*	2.2 (5.2%)	2.5 (5.9%)
Linoleic, g per 100 g	*0.2*	*0.3*
Alpha-linolenic, g per 100 g	*1.9*	*2.2*
SFA (g) (palmitic and stearic acid), *g per 100 g (% Energy)*	5.2 (12.3%)	5.4 (12.7%)

[1]Sweet flavours: lemon, orange, banana and caramel
[2]Savory flavours: cheese, garlic and parsley

Snack provision

Participants in the control group collected muffins in 2 week intervals and were requested to store them in their freezer immediately. Each morning, participants defrosted their daily portion of control muffins using a microwave or allowed them to get to room temperature throughout the day depending on time of consumption. Almonds were provided as pre-portioned daily packs at 2 week intervals. Participants were provided with 20% estimated energy requirements (EER) from the muffins or almonds which for a 2000 kcal EER, equated to five control muffins daily (400 kcal in (80kcal/muffin)), or 63g/d almonds (100g almonds contains 634 kcal). Comparison of nutritional composition between almonds and control muffins is shown in **Supplemental Table 3**. The ratio of sweet: savory muffins consumed was 70:30, with a mean of 20% of energy from free sugars in total muffins.

Supplemental Table 3. Nutritional composition of almonds and control snacks per 400 kcal isocaloric portions. Values given as 20% of the estimated average requirement for energy (2000 kcal) for adult women.

		Average of sweet and	Control snacks	
Nutrient	Almonds	savory control snacks	Sweet	Savory
Energy, kcal	400	400	400	400
Protein, %E	13.4	18.0	18.0	18.0
Carbohydrate, %E	13.2	54.1	54.3	53.8
Starch, %E	-	38.7	24.5	52.9
Sugars, %E	2.3	15.1	29.7	0.4
Fibre, g	9.3	2.1	1.3	2.8
Fat, %E	73.6	36.5	35.8	37.1
SFA, g	5.6	12.8	12.6	12.8
MUFA, g	47.9	16.0	15.8	16.0
PUFA, g	16.0	5.6	5.4	5.9
Sodium, mg	<2.5	452.1	187.4	716.8
Potassium, mg	463.7	162.8	145.9	179.6
Calcium, mg	164.7	130.1	46.1	214.1
Magnesium, mg	181.7	19.0	15.4	22.6
Vitamin E, mg	14.3	1.3	1.1	1.4

Blood collection and handling procedures

Blood was collected by trained phlebotomists. At screening, fasting blood was collected by venepuncture using the vacutainer technique for analysis of glucose, lipids, liver function and full blood count. For baseline and endpoint visits, venous cannulation was performed and three samples were collected at 5 minute intervals (0 min, 5 min and 10 min) for analysis of insulin, glucose and non-esterified fatty acid (NEFA) to calculate insulin sensitivity using the Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) method. Plasma lipids (total, HDL-, and LDL-cholesterol and TAG, calculated TC:HDL-C ratio and lipoprotein), fatty liver indicators (fetuin-A and alanine transaminase (ALT)), clinically relevant biomarkers of metabolic dysregulation/insulin resistance (adiponectin, resistin and leptin), and plasma fatty acids, were also obtained from cannulation samples at timepoint 0 min only at baseline and endpoint.

Blood samples were collected using serum, EDTA and sodium fluoride containing tubes. All the blood samples were centrifuged (3000 rpm, 4° C, 15 min; Eppendorf centrifuge 5702 R, Stevenage, UK), except the EDTA tubes from screening (which were kept at room temperature for analysis of full blood count). Screening samples were analysed by the Biochemistry Department, King's College Hospital on the day of collection. Serum and plasma samples from study days were stored at -80° C and analysed in batches. Fasting blood glucose and non-esterified fatty acids (NEFA) were analysed at King's College London using an ILAB 650 auto-analyser (Werfen International, Milan, Italy). Insulin, plasma lipids, fetuin-A, ALT, adiponectin, resistin and leptin were analyzed by Affinity Biomarker Labs in London, UK. Metabolomics was measured using high-throughput proton NMR metabolomics (Nightingale Health Ltd, Helsinki, Finland) to quantify routine lipid concentrations within 14 subclasses, fatty acid composition, amino acids, ketone bodies, and other metabolites associated with gluconeogenesis. Details of analysis have been described elsewhere (3).

Plasma fatty acids were analysed by gas liquid chromatography-flame ionization detector (GLC-FID) using a modification of the method detailed by Lepage & Roy (1986) (4). Inside Teflon lined screw capped tubes, 0.1 ml internal standard (1 mg/ml pentadecanoic acid in methanol) and 0.1 ml plasma were added. Into this tube, 2 ml of a prepared methanol/toluene 4:1 volume mixture acidified at 10% volume with acetyl chloride was also added. Following the addition of the fatty acid methyl esters (FAME) reagent, tubes were capped tightly and heated in a water bath for 2 h at 60°C to catalyze transesterification. The mixture was neutralized with 5 ml of 6% w/v aqueous potassium carbonate during the cooling process. Tubes were vortex mixed and centrifuged at 2,500 rpm for 10 min. Supernatant containing the concentrated FAMEs was withdrawn for 0.05 ml into a 250 µl GLC tri-spring glass insert, and this solution was injected into the GLC-FID. FAMEs were separated on an Agilent 6890 Gas Chromatograph fitted with a flame ionisation detector and a 25 m x 220 µm x 0.25 µm BP70 capillary column (SGE). Peaks for the FAMEs were identified by comparison with standard of known composition (fish oil and hemp seed oil).

Quantification of fecal short chain fatty acids (SCFA) by GLC-FID

Stool samples were homogenized and stored at -80 °C. SCFA were extracted after the addition of the extraction buffer containing 2,2-dimethyl-butyric acid (internal standard), mercury chloride and phosphoric acid in a ¼ (w/v) dilution (3 g, 12 ml). The fecal slurry was centrifuged at 5000 g for 45 min at 4 °C. The top layer was aspirated by a 5-ml syringe and approximately 1 ml was filtered through a sterile 0.2 µm filter into a GLC vial and stored at -20 °C until analysis.

Calibration was conducted with a 6-component blend of pure SCFA solutions at 4 different concentrations to produce gas-chromatography peak area ratio with respect to internal

standard versus SCFA amount plots. Using linear regression analysis, the best equation for the best fit straight line for the calibration curves was obtained.

Extracted SCFA were injected into a 6890 series GLC-FID system (Agilent Technologies, US) equipped with a 220 μm internal diameter, 25 m fused silica capillary column with a film thickness of 0.25 μm (BP21, SGE, AUS). Peaks for each of the SCFA were identified by comparing the equations of the standard curves with the calculated area ratios. Samples were run in duplicate and a 1.2 % formic acid cleaning solution was injected to minimize carry-over from the previous samples. Samples with a variation of more than 5 % were re-analyzed.

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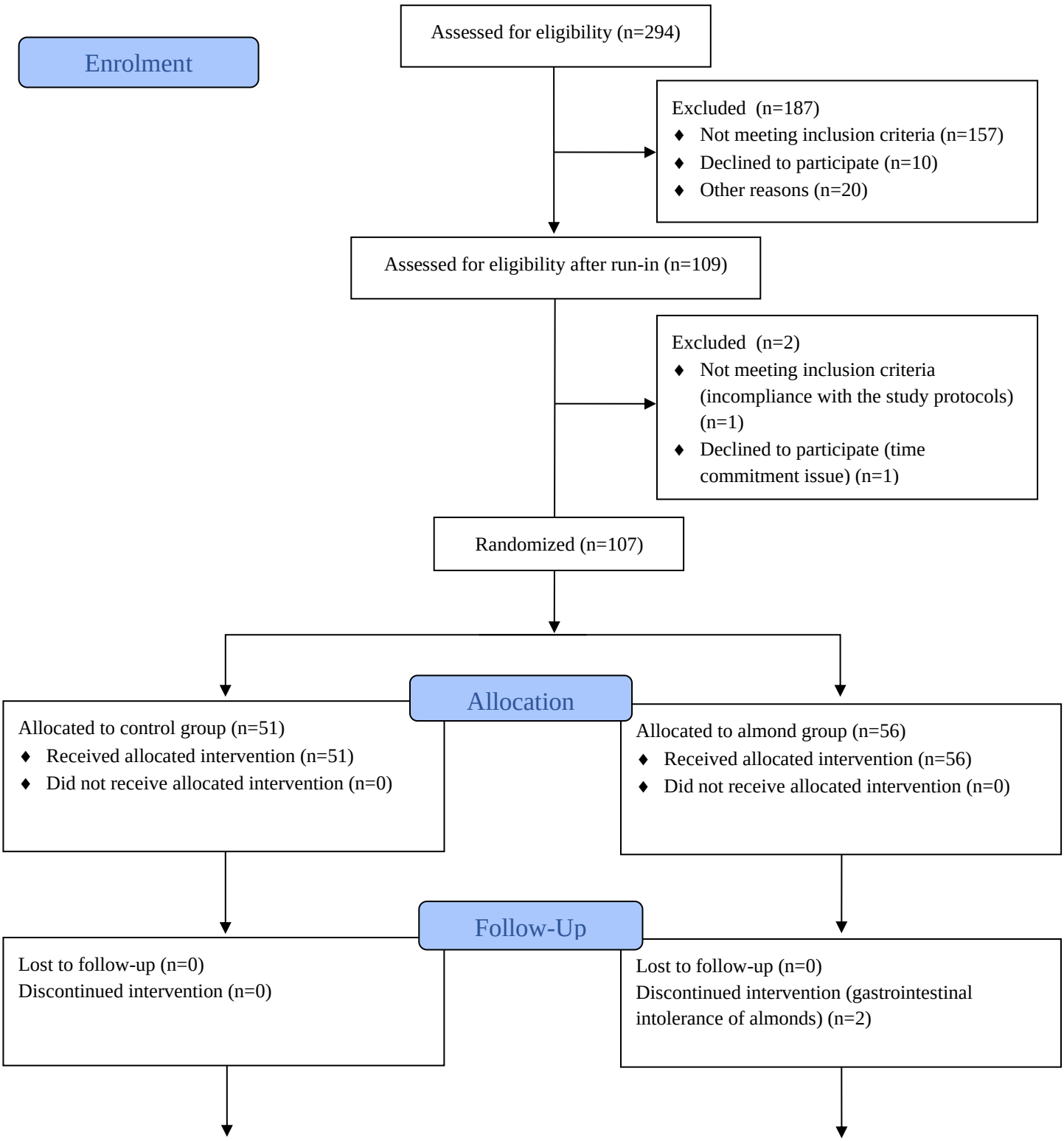
Supplemental Figure 1. CONSORT 2010 Flow Diagram



Analysis

Analyzed (n_{max}=51)

- Included during analysis:
  - Nutrient intakes: n=40
    (missing data are due to poor quality diet diaries or failure to complete by participants)
  - *Vascular function, blood pressure, heart rate variability*
    (missing data are due to poor quality read-outs and technological problems)
    - Endothelial function via flow-mediated dilation: n=42
    - Clinical blood pressure: n=51
    - 24-h and day-time blood pressures: n=45
    - Night-time blood pressures: n=40
    - 24-h SDNN and rMSSD: n=33
    - Night-time rMSSD: n=45
    - Night-time SDANN: n=45
    - Night-time VLF and HF: n=45
  - *Body composition and measures of ectopic fat*
    (MRI/MRS scanning was planned on a subset of study participants: n = 50 (25 per group. Missing data are due to technical problems)
    - BMI: n=45, WC: n=49, Body fat: n=49
    - Liver fat and pancreatic fat via MRI: n=26
    - Intrahepatic fat; unsaturation, polyunsaturation and saturation indices, via H-MRS: n=22
    - Subcutaneous fat via MRI: n=24
    - Visceral fat via MRI: n=23
    - Intra- and extramyocellular lipid: n=23
  - *Circulating biomarkers, plasma fatty acid and metabolomic profiles, fecal short chain fatty acids*
    (*Fecal* sample collection was planned on a subset of study participants: n = 30 (15 per group.)
    - HOMA-IR, blood glucose, plasma fatty acids and metabolomic profiles: n=48
    - Insulin, NEFA, blood lipids, ALT and GGT: n=49
    - Leptin, adiponectin, fetuin-A and resistin, n=49
    - *Fecal* Short chain fatty acids: n=17

Analyzed (n_{max}=54)

- Included during analysis:
  - Nutrient intakes: n=40
    (missing data are due to poor quality diet diaries or failure to complete by participants)
  - *Vascular function, blood pressure, heart rate variability*
    (missing data are due to poor quality read-outs and technological issue)
    - Endothelial function via flow-mediated dilation: n=47
    - Clinical blood pressure: n=54
    - 24-h and day-time blood pressures: n=49
    - Night-time blood pressures: n=46
    - 24-h SDNN and rMSSD: n=29
    - Night-time rMSSD: n=50
    - Night-time SDANN: n=47
    - Night-time VLF and HF: n=47
  - *Body composition and measures of ectopic fat*
    (MRI/MRS scanning was planned on a subset of study participants: n = 50 (25 per group. Missing data are due to technical problems)
    - BMI: n=50, WC: n=51, Body fat: n=52
    - Liver fat and pancreatic fat via MRI: n=24
    - Intrahepatic fat; unsaturation, polyunsaturation and saturation indices via H-MRS: n=23
    - Subcutaneous fat via MRI: n=22
    - Visceral fat via MRI: n=22
    - Intra- and extramyocellular lipid: n=22
  - *Circulating biomarkers, plasma fatty acid and metabolomic profiles, fecal short chain fatty acids*
    (*Fecal* sample collection was planned on a subset of study participants: n = 30 (15 per group.)
    - HOMA-IR, blood glucose, plasma fatty acids and metabolomic profiles: n=53
    - Insulin, NEFA, blood lipids, ALT, GGT: n=53
    - Leptin, adiponectin, fetuin-A and resistin: n=52
    - *Fecal* short chain fatty acids: n=18

Supplemental Table 4. Dietary intake

	Control, n_{max}=40[1]		Almond, n_{max}=40[1]		*P* value
	Baseline	Endpoint	Baseline	Endpoint	(time*intervention group)
Energy intake, kcal/d	2088.0 (1927.2, 2248,7)	1999.8 (1860.9, 2138.6)	1770.2 (1609.5, 1931.0)	1767.5 (1628.6, 1906.3)	0.401
Protein, %E	15.4 (14.2, 16.5)	16.0 (-0.5, 1.5)	15.9 (14.7, 17.1)	16.8 (15.6, 18.0)	0.731
Total carbohydrate, %E	43.3 (41.1, 45.5)	44.7 (42.5, 46.9)	41.8 (39.6, 43.9)	34.5 (32.3, 36.7)	<0.001
Starch, %E	23.9 (22.3, 25.6)	26.3 (24.5, 28.1)	23.5 (21.9, 25.2)	19.1 (17.3, 20.9)	<0.001
Free sugars, %E	5.9 (4.9, 7.0)	6.1 (5.2, 7.0)	5.5 (4.4, 6.5)	3.0 (2.1, 3.9)	0.002
Dietary fibre[1], g/d	23.8 (21.6, 25.9)	21.1 (18.4, 23.8)	20.8 (18.7, 23.0)	27.2 (24.5, 29.9)	<0.001
Fat, %E	36.5 (34.5, 38.5)	34.1 (32.3, 36.0)	37.1 (35.1, 39.1)	45.2 (43.3, 47.0)	<0.001
SFA, %E	12.3 (11.1, 13.4)	11.8 (11.0, 12.5)	12.5 (11.4, 13.7)	11.1 (10.3, 11.9)	0.231
MUFA, %E	11.5 (10.4, 12.6)	10.8 (9.6, 12.0)	12.4 (11.3, 13.6)	20.6 (19.4, 21.8)	<0.001
PUFA, %E	5.9 (5.2, 6.6)	5.2 (4.5, 5.8)	5.9 (5.1, 6.5)	7.9 (7.2, 8.6)	<0.001
Unsaturated: saturated fatty acid ratio	1.5 (1.3, 1.7)	1.4 (1.2, 1.6)	1.6 (1.4, 1.8)	2.7 (2.5, 2.9)	<0.001
Sodium, mg	2151.8 (1892.6, 2410, 9)	2245.7 (2042.5, 2448.9)	1925.5 (1666.4, 2184.7)	1520.5 (1317.3, 1723.7)	0.013

Potassium, mg	3025.4 (2743.0, 3307.9)	2540.9 (2275.3, 2806.4)	2538.2 (2255.7, 2820.7)	2891.6 (2626.0, 3157.2)	<0.001
Calcium, mg	867.0 (751.9, 982.0)	832.6 (746.3, 918.8)	705.2 (590.2, 820.3)	821.1 (734.8, 907.3)	0.071
Magnesium, mg	368.0 (322.9, 413.1)	297.3 (263.9, 330.7)	279.2 (234.0, 324.3)	426.5 (393.1, 459.9)	<0.001
Vitamin E, mg	10.6 (9.4, 11.9)	7.9 (6.4, 9.4)	9.0 (7.7, 10.2)	23.3 (21.7, 24.8)	<0.001
Riboflavin, mg	1.8 (1.4, 2.2)	1.5 (1.3, 1.8)	1.5 (1.1, 1.9)	2.0 (1.8, 2.2)	0.002
Niacin, mg	16.2 (13.3, 19.0)	14.1 (12.1, 16.2)	14.6 (11.7, 17.5)	14.7 (12.7, 16.7)	0.227

ANCOVA repeated measures was used, adjusted for baseline BMI. ANCOVA assumptions were met.
P<0.05 indicating a significant treatment * time interaction.
[1]Data were analyzed using 40 diaries collected from each group. Missing data are due to poor quality diet diaries or failure to complete by participant.

Supplemental Table 5. Metabolomics data from NMR analysis

	Control, n_{max} = 51[1]		Almond, n_{max} = 54[1]		Mean comparison between groups[3]
	Baseline[2]	Change	Baseline[2]	Change	
Total-C, mmol/L	4.81 ± 1.08	-0.01 (-0.25, 0.11)	4.93 ± 0.89	-0.15 (-0.33, -0.02)	-0.08 (-0.33, 0.17)
VLDL-C, mmol/L	0.69 ± 0.22	-0.03 (-0.07, 0.01)	0.66 ± 0.25	-0.03 (-0.07, 0.01)	-0.00 (-0.06, 0.05)
Remnant-C, mmol/L	1.43 ± 0.40	-0.04 (-0.10, 0.03)	1.41 ± 0.37	-0.05 (-0.12, 0.01)	-0.02 (-0.11, 0.08)
LDL-C, mmol/L	1.83 ± 0.57	-0.02 (-0.11, 0.08)	1.88 ± 0.42	-0.05 (-0.14, 0.04)	-0.04 (-0.17, 1.00)
HDL-C, mmol/L	1.54 ± 0.39	-0.02 (-0.08, 0.05)	1.64 ± 0.44	-0.04 (-0.10, -0.02)	-0.03 (-0.12, 0.06)
HDL2-C, mmol/L	1.04 ± 0.36	-0.02 (-0.08, 0.04)	1.13 ± 0.41	-0.04 (-0.10, -0.01)	-0.03 (-0.11, 0.06)
HDL3-C, mmol/L	0.50 ± 0.04	0.00 (-0.00, 0.01)	0.51 ± 0.04	-0.00 (-0.01, 0.00)	-0.01 (-0.01, 0.00)
Esterified-C, mmol/L	3.39 ± 0.78	-0.05 (-0.19, 0.08)	3.48 ± 0.64	-0.10 (-0.23, -0.03)	-0.04 (-0.23, 0.14)
Free-C, mmol/L	1.41 ± 0.30	-0.02 (-0.07, 0.03)	1.45 ± 0.25	-0.04 (-0.08, -0.01)	-0.02 (-0.09, 0.04)
Total-TG, mmol/L	1.14 ± 0.44	-0.03 (-0.12, 0.06)	1.09 ± 0.56	-0.06 (-0.15, 0.02)	-0.03 (-0.15, 0.10)
VLDL-TG, mmol/L	0.71 ± 0.36	-0.02 (-0.10, 0.05)	0.68 ± 0.47	-0.05 (-0.12, 0.03)	-0.02 (-0.13, 0.08)
LDL-TG, mmol/L	0.18 ± 0.05	-0.00 (-0.01, 0.00)	0.17 ± 0.47	-0.01 (-0.01, 0.00)	-0.00 (-0.01, 0.01)
HDL-TG, mmol/L	0.14 ± 0.04	-0.00 (-0.01, 0.00)	0.13 ± 0.04	-0.01 (-0.01, -0.00)	-0.00 (-0.01, 0.01)
Phosphoglycerides, mmol/L	2.16 ± 0.42	-0.05 (-0.15, 0.04)	2.21 ± 0.43	-0.09 (-0.18, 0.00)	-0.03 (-0.16, 0.09)
TG:PG	0.54 ± 0.21	-0.02 (-0.06, 0.01)	0.49 ± 0.23	-0.01 (-0.05, 0.03)	0.01 (-0.04, 0.07)
Cholines, mmol/L	2.51 ± 0.46	-0.03 (-0.13, 0.07)	2.58 ± 0.48	-0.09 (-0.18, 0.01)	-0.05 (-0.19, 0.09)
Phosphatidylcholines, mmol/L	2.17 ± 0.41	-0.05 (-0.14, 0.04)	2.21 ± 0.43	-0.09 (-0.17, 0.00)	-0.04 (-0.16, 0.09)

	Control, n_{max} = 51[1]		Almond, n_{max} = 54[1]		Mean comparison between groups[3]
	Baseline[2]	Change	Baseline[2]	Change	
Sphingomyelins, mmol/L	0.45 ± 0.10	-0.00 (-0.02, 0.02)	0.48 ± 0.08	-0.02 (-0.04, -0.00)	-0.02 (-0.05, 0.00)
Apolipoprotein B, g/L	0.89 ± 0.20	-0.01 (-0.04, 0.02)	0.89 ± 0.18	-0.02 (-0.06, 0.01)	-0.01 (-0.06, 0.03)
Apolipoprotein A1, g/L	1.60 ± 0.23	-0.02 (-0.06, 0.02)	1.65 ± 0.26	-0.04 (-0.08, 0.00)	-0.02 (-0.08, 0.04)
Apolipoprotein B:Apolipoprotein A1	0.56 ± 0.13	-0.00 (-0.02, 0.02)	0.55 ± 0.14	-0.00 (-0.02, 0.02)	0.00 (-0.03, 0.03)
Total fatty acids, mmol/L	12.25 ± 2.15	-0.32 (-0.80, 0.18)	12.31 ± 2.54	-0.53 (-1.00, -0.07)	-0.22 (-0.89, 0.46)
Unsaturation degree	1.23 (0.09)	0.00 (-0.01, 0.02)	1.24 (0.10)	-0.01 (-0.01, 0.02)	0.01 (-0.02, 0.03)
Omega-3, mmol/L	0.52 ± 0.11	-0.00 (-0.03, 0.03)	0.52 ± 0.17	-0.05 (-0.07, -0.02)	-0.04 (-0.08, -0.00)[4]
Omega-6, mmol/L	4.17 ± 0.74	-0.10 (-0.25, 0.06)	4.21 ± 0.72	-0.14 (-0.29, 0.01)	-0.04 (-0.26, 0.17)
Polyunsaturated fatty acids, mmol/L	4.69 ± 0.83	-0.10 (-0.28, 0.08)	4.74 ± 0.85	-0.19 (-0.36, -0.02)	-0.09 (-0.33, 0.16)
Monounsaturated fatty acids, mmol/L	3.21 ± 0.67	-0.11 (-0.28, 0.05)	3.20 ± 0.85	-0.14 (-0.30, 0.02)	-0.03 (-0.25, 0.20)
Saturated fatty acids, mmol/L	4.34 ± 0.77	-0.11 (-0.28, 0.07)	4.38 ± 0.96	-0.21 (-0.37, -0.04)	-0.10 (-0.39, 0.14)
Linoleic acid, mmol/L	3.62 ± 0.75	-0.10 (-0.27, 0.07)	3.64 ± 0.73	-0.12 (-0.28, 0.04)	-0.02 (-0.25, 0.22)
Docosahexaenoic acid, mmol/L	0.17 ± 0.04	-0.00 (-0.01, 0.01)	0.17 ± 0.06	-0.02 (-0.03, -0.01)	-0.02 (-0.03, -0.00)
Alanine, mmol/L	0.39 ± 0.05	-0.00 (-0.02, 0.01)	0.39 ± 0.05	-0.01 (-0.02, 0.01)	0.00 (-0.02, 0.02)
Glutamine, mmol/L	0.52 ± 0.06	-0.02 (-0.03, -0.01)	0.051 ± 0.05	-0.01 (-0.02, 0.01)	0.01 (-0.01, 0.03)
Glycine, mmol/L	0.26 ± 0.05	-0.00 (-0.01, 0.00)	0.27 ± 0.06	-0.01 (-0.02, 0.00)	-0.00 (-0.02, 0.01)

	Control, n_{max} = 51[1]		Almond, n_{max} = 54[1]		Mean comparison between groups[3]
	Baseline[2]	Change	Baseline[2]	Change	
Histidine, mmol/L	0.07 ± 0.01	0.20 (0.18, 0.22)	0.07 ± 0.01	0.19 (0.17, 0.21)	-0.01 (-0.04, 0.02)
Isoleucine, mmol/L	0.05 ± 0.01	0.00 (-0.00, 0.00)	0.05 ± 0.01	-0.00 (-0.01, 0.00)	-0.00 (-0.01, 0.00)
Leucine, mmol/L	0.07 ± 0.01	0.00 (-0.00, 0.00)	0.07 ± 0.01	-0.00 (-0.00, 0.00)	-0.00 (-0.01, 0.00)
Valine, mmol/L	0.16 ± 0.03	0.01 (-0.00, 0.01)	0.15 ± 0.04	-0.00 (-0.01, 0.01)	-0.01 (-0.02, 0.00)
Phenylalanine, mmol/L	0.07 ± 0.01	0.00 (-0.00, 0.00)	0.07 ± 0.01	-0.00 (-0.00, 0.00)	-0.00 (-0.00, 0.00)
Tyrosine, mmol/L	0.05 ± 0.01	0.00 (-0.00, 0.00)	0.05 ± 0.01	-0.00 (-0.00, 0.00)	-0.00 (-0.00, 0.00)
Glucose, mmol/L	3.96 ± 0.39	-0.04 (-0.14, 0.05)	3.89 ± 0.40	-0.00 (-0.09, 0.09)	0.04 (-0.09, 0.17)
Lactate, mmol/L	1.28 ± 0.33	0.04 (-0.06, 0.14)	1.37 ± 0.39	-0.02 (-0.11, 0.08)	-0.06 (-0.20, 0.09)
Pyruvate, mmol/L	0.07 ± 0.02	0.00 (-0.00, 0.01)	0.07 ± 0.02	0.00 (-0.01, 0.01)	-0.00 (-0.01, 0.01)
Citrate, mmol/L	0.12 ± 0.02	-0.01 (-0.01, 0.00)	0.12 ± 0.02	0.00 (-0.00, 0.01)	0.01 (-0.00, 0.02)[4]
Glycerol, mmol/L	0.08 ± 0.03	-0.01 (-0.02, -0.00)	0.09 ± 0.03	-0.00 (-0.01, 0.00)	0.01 (-0.00, 0.02)
Acetate, mmol/L	0.04 ± 0.01	-0.00 (-0.01, 0.00)	0.05 ± 0.02	0.00 (-0.00, 0.01)	0.00 (-0.00, 0.01)
Acetoacetate, mmol/L	0.04 (0.02)	0.00 (-0.01, 0.01)	0.03 (0.02)	0.00 (-0.00, 0.01)	0.00 (-0.01, 0.01)
Beta-hydroxybutyrate, mmol/L	0.16 ± 0.08	-0.01 (-0.03, 0.02)	0.16 ± 0.10	0.01 (-0.01, 0.03)	0.02 (-0.01, 0.05)
Creatinine, mmol/L	0.06 ± 0.01	0.00 (-0.00, 0.00)	0.06 ± 0.01	0.00 (-0.00, 0.00)	0.00 (-0.00, 0.00)
Albumin, signal area	0.10 ± 0.01	-0.00 (-0.01, 0.00)	0.10 ± 0.01	-0.00 (-0.01, -0.00)	-0.00 (-0.00, 0.00)
Glycoprotein acetyls, mmol/L	1.33 ± 0.17	-0.05 (-0.09, 0.01)	1.31 ± 0.20	-0.03 (-0.07, 0.00)	0.01 (-0.04, 0.07)
XXL-VLDL-P, µmol/L	0.00 (0.00)	-0.00 (-0.00, 0.00)	0.00 (0.00)	-0.00 (-0.00, 0.00)	-0.00 (-0.00, 0.00)
XXL-VLDL-L, µmol/L	12.60 (18.15)	-0.42 (-4.00, 3.17)	8.04 (20.09)	-1.70 (-5.12, 1.71)	-1.29 (-6.24, 3.67)

	Control, n_{max} = 51[1]		Almond, n_{max} = 54[1]		Mean comparison between groups[3]
	Baseline[2]	Change	Baseline[2]	Change	
XXL-VLDL-PL, µmol/L	1.41 (2.32)	-0.06 (-0.51, 0.39)	0.87 (2.37)	-0.22 (-0.66, 0.21)	-0.16 (-0.79, 0.46)
XXL-VLDL-C, µmol/L	2.27 (3.77)	0.04 (-0.58, 0.66)	1.15 (3.53)	-0.35 (-0.94, 0.24)	-0.39 (-1.24, 0.47)
XXL-VLDL-CE, µmol/L	1.58 (2.90)	0.04 (-0.33, 0.41)	1.08 (2.24)	-0.20 (-0.56, 0.15)	-0.24 (-0.76, 0.27)
XXL-VLDL-FC, µmol/L	0.67 (1.22)	-0.00 (-0.27, 0.26)	0.19 (1.32)	-0.15 (-0.39, 0.10)	-0.14 (-0.50, 0.22)
XXL-VLDL-TG, µmol/L	9.03 (12.14)	-0.40 (-2.94, 2.15)	6.50 (13.85)	-1.13 (-3.55, 1.30)	-0.73 (-4.24, 2.78)
XL-VLDL-P, µmol/L	0.00 (0.00)	0.00 (-0.00, 0.00)	0.00 (0.00)	0.00 (0.00, 0.00)	0.00 (0.00, 0.00)
XL-VLDL-L, µmol/L	31.48 (49.16)	-1.50 (-10.43, 7.43)	13.25 (53.94)	-5.39 (-13.89, 3.11)	-3.89 (-16.22, 8.44)
XL-VLDL-PL, µmol/L	4.75 (8.25)	-0.18 (-1.67, 1.31)	2.58 (9.78)	-0.91 (-2.33, 0.51)	-0.73 (-2.79, 1.33)
XL-VLDL-C, µmol/L	6.06 (7.67)	-0.04 (-1.74, 1.67)	3.18 (10.11)	-0.89 (-2.52, 0.73)	-0.86 (-3.22, 1.50)
XL-VLDL-CE, µmol/L	3.43 (4.55)	-0.09 (-1.02, 0.84)	1.89 (5.54)	-0.51 (-1.40, 0.37)	-0.43 (-1.71, 0.86)
XL-VLDL-FC, µmol/L	2.21 (3.83)	0.05 (-0.74, 0.84)	1.21 (4.42)	-0.38 (-1.13, 0.37)	-0.43 (-1.52, 0.66)
XL-VLDL-TG, µmol/L	19.37 (31.96)	-1.29 (-7.10, 4.53)	9.60 (33.90)	-3.59 (-9.12, 1.94)	-2.30 (-10.33, 5.72)
L-VLDL-P, µmol/L	0.00 ± 0.00	0.00 (-0.00, 0.00)	0.00 ± 0.00	-0.00 (-0.00, 0.00)	0.00 (-0.00, 0.00)
L-VLDL-L, µmol/L	194.63 ± 147.08	-8.02 (-39.91, 23.87)	182.98 ± 199.46	-20.23 (-50.58, 10.12)	-12.21 (-56.24, 31.81)
L-VLDL-PL, µmol/L	36.56 ± 26.85	-1.45 (-7.25, 4.36)	34.40 ± 36.70	-3.81 (-9.33, 1.71)	-2.36 (-10.37, 5.64)
L-VLDL-C, µmol/L	46.38 ± 34.71	-2.55 (-9.87, 4.77)	42.63 ± 46.13	-5.11 (-12.07, 1.86)	-2.56 (-12.67, 7.55)
L-VLDL-CE, µmol/L	28.08 ± 18.94	-1.92 (-5.84, 2.01)	25.23 ± 24.41	-2.74 (-6.48, 0.99)	-0.82 (-6.25, 4.60)
L-VLDL-FC, µmol/L	18.30 ± 16.06	-0.64 (-4.15, 2.86)	17.40 ± 22.06	-2.35 (-5.69, 0.98)	-1.71 (-6.55, 3.13)

	Control, n_{max} = 51[1]		Almond, n_{max} = 54[1]		Mean comparison between groups[3]
	Baseline[2]	Change	Baseline[2]	Change	
L-VLDL-TG, µmol/L	111.68 ± 86.02	-4.03 (-22.87, 14.81)	105.95 ± 116.83	-11.31 (-29.24, 6.61)	-7.29 (-33.29, 18.72)
M-VLDL-P, µmol/L	0.01 ± 0.01	-0.00 (-0.00, 0.00)	0.01 ± 0.01	-0.00 (-0.00, 0.00)	0.00 (-0.00, 0.00)
M-VLDL-L, µmol/L	498.84 ± 241.84	-20.92 (-70.71, 28.87)	466.69 ± 313.19	-30.77 (-78.15, 16.61)	-9.85 (-78.62, 58.92)
M-VLDL-PL, µmol/L	103.89 ± 47.55	-4.50 (-14.20, 5.20)	97.06 ± 61.42	-6.06 (-15.29, 3.16)	-1.56 (-14.96, 11.83)
M-VLDL-C, µmol/L	150.28 ± 66.03	-8.66 (-21.92, 4.60)	139.01 ± 84.02	-10.04 (-22.66, 2.58)	-1.38 (-19.70, 16.94)
M-VLDL-CE, µmol/L	95.03 ± 37.24	-6.31 (-13.59, 0.98)	88.08 ± 45.92	-5.76 (-12.69, 1.18)	0.55 (-9.53, 10.62)
M-VLDL-FC, µmol/L	55.25 ± 30.99	-2.36 (-8.66, 3.94)	50.92 ± 39.27	-4.27 (-10.26, 1.73)	-1.91 (-10.61, 6.80)
M-VLDL-TG, µmol/L	244.67 ± 132.86	-7.83 (-35.21, 19.54)	230.62 ± 169.70	-14.60 (-40.65, 11.44)	-6.77 (-44.57, 31.03)
S-VLDL-P, µmol/L	0.03 ± 0.01	-0.00 (-0.00, 0.00)	0.03 ± 0.01	-0.00 (-0.00, 0.00)	-0.00 (-0.00, 0.00)
S-VLDL-L, µmol/L	580.88 ± 191.1	-27.60 (-64.01, 8.81)	548.19 ± 222.80	-28.79 (-63.43, 5.86)	-1.19 (-51.51, 49.14)
S-VLDL-PL, µmol/L	138.07 ± 41.15	-6.75 (-14.87, 1.38)	131.3 ± 48.10	-6.55 (-14.28, 1.18)	0.20 (-11.03, 11.43)
S-VLDL-C, µmol/L	230.10 ± 74.32	-12.11 (-25.21, 0.99)	219.37 ± 74.03	-11.98 (-24.44, 0.49)	0.14 (-17.97, 18.24)
S-VLDL-CE, µmol/L	148.56 ± 50.20	-7.67 (-16.27, 0.94)	142.47 ± 45.04	-7.60 (-15.78, 0.59)	0.07 (-11.82, 11.95)
S-VLDL-FC, µmol/L	81.54 ± 27.15	-4.35 (-9.57, 0.87)	76.90 ± 30.98	-4.46 (-9.43, 0.51)	-0.11 (-7.32, 7.11)
S-VLDL-TG, µmol/L	212.71 ± 88.79	-8.60 (-26.09, 8.89)	197.51 ± 106.88	-10.39 (-27.03, 6.26)	-1.79 (-25.96, 22.39)
XS-VLDL-P, µmol/L	0.04 ± 0.01	-0.00 (-0.00, 0.00)	0.04 ± 0.01	-0.00 (-0.00, 0.00)	0.00 (-0.00, 0.00)
XS-VLDL-L, µmol/L	580.88 ± 191.10	-27.60 (-64.01, 8.81)	548.19 ± 222.80	-28.79 (-63.43, 5.86)	-1.19 (-51.51, 49.14)
XS-VLDL-PL, µmol/L	168.71 ± 44.70	-3.49 (-10.03, 3.04)	169.42 ± 36.26	-4.61 (-10.83, 1.60)	-1.12 (-10.14, 7.90)

	Control, n_{max} = 51[1]		Almond, n_{max} = 54[1]		Mean comparison between groups[3]
	Baseline[2]	Change	Baseline[2]	Change	
XS-VLDL-C, µmol/L	252.46 ± 70.37	-6.10 (-17.02, 4.83)	249.80 ± 55.10	-6.64 (-17.04, 3.76)	-0.54 (-15.63, 14.54)
XS-VLDL-CE, µmol/L	164.11 ± 48.72	-3.81 (-12.16, 4.55)	161.92 ± 38.38	-4.16 (-12.11, 3.79)	-0.35 (-11.89, 11.18)
XS-VLDL-FC, µmol/L	88.35 ± 22.58	-2.26 (-5.63, 1.11)	87.88 ± 18.90	-2.51 (-5.72, 0.70)	-0.25 (-4.90, 4.41)
XS-VLDL-TG, µmol/L	106.78 ± 32.40	-3.75 (-9.98, 2.48)	101.59 ± 38.10	-3.91 (-9.84, 2.02)	-0.16 (-8.76, 8.45)
IDL-P, µmol/L	0.11 ± 0.03	-0.00 (-0.01, 0.00)	0.12 ± 0.02	-0.00 (-0.01, 0.00)	-0.00 (-0.01, 0.01)
IDL-L, µmol/L	1167.57 ± 321.88	-12.08 (-61.54, 37.37)	1181.56 ± 237.80	-32.55 (-79.61, 14.51)	-20.46 (-88.74, 47.81)
IDL-PL, µmol/L	317.71 ± 82.31	-320.65 (-320.65, -320.65)	322.24 ± 59.41	-320.65 (-320.65, -320.65)	-0.00 (-0.00, 0.00)
IDL-C, µmol/L	737.57 ± 219.79	-7.01 (-42.56, 28.54)	749.88 ± 158.88	-21.09 (-54.93, 12.74)	-14.09 (-63.17, 35.00)
IDL-CE, µmol/L	522.63 ± 156.19	-5.17 (-31.28, 20.95)	529.62 ± 113.91	-14.94 (-39.79, 9.91)	-9.77 (-45.82, 26.28)
IDL-FC, µmol/L	214.94 ± 64.42	-1.88 (-11.54, 7.78)	220.26 ± 46.52	-6.12 (-15.31, 3.07)	-4.24 (-17.58, 9.09)
IDL-TG, µmol/L	112.29 ± 29.54	-2.20 (-7.20, 2.80)	109.44 ± 31.53	-3.46 (-8.22, 1.30)	-1.26 (-8.17, 5.65)
L-LDL-P, µmol/L	0.19 ± 0.05	-0.00 (-0.01, 0.01)	0.20 ± 0.04	-0.01 (-0.01, 0.00)	-0.00 (-0.02, 0.01)
L-LDL-L, µmol/L	1389.83 ± 390.38	-15.25 (-78.15, 47.66)	1413.76 ± 290.57	-41.03 (-100.89, 18.83)	-25.79 (-112.63, 61.06)
L-LDL-PL, µmol/L	346.55 ± 81.45	-3.61 (-17.07, 9.84)	352.32 ± 60.57	-8.67 (-21.48, 4.14)	-5.06 (-23.64, 13.52)
L-LDL-C, µmol/L	945.01 ± 288.55	-9.99 (-57.42, 37.45)	964.84 ± 211.22	-29.03 (-74.17, 16.11)	-19.04 (-84.53, 46.44)

	Control, n_{max} = 51[1]		Almond, n_{max} = 54[1]		Mean comparison between groups[3]
	Baseline[2]	Change	Baseline[2]	Change	
L-LDL-CE, µmol/L	679.47 ± 217.08	-8.22 (-44.28, 27.84)	693.23 ± 160.83	-22.48 (-56.79, 11.83)	-14.26 (-64.04, 35.52)
L-LDL-FC, µmol/L	265.53 ± 72.05	-1.78 (-13.29, 9.73)	271.61 ± 51.50	-6.54 (-17.50, 4.41)	-4.76 (-20.66, 11.14)
L-LDL-TG, µmol/L	98.28 ± 26.13	-1.70 (-5.73, 2.34)	96.61 ± 25.36	-3.29 (-7.12, 0.55)	-1.59 (-7.16, 3.98)
M-LDL-P, µmol/L	0.16 ± 0.05	-0.00 (-0.01, 0.01)	0.16 ± 0.03	-0.00 (-0.01, 0.00)	-0.00 (-0.01, 0.01)
M-LDL-L, µmol/L	813.94 ± 231.79	-9.44 (-47.50, 28.62)	829.79 ± 174.64	-22.79 (-59.01, 13.43)	-13.35 (-65.89, 39.20)
M-LDL-PL, µmol/L	210.96 ± 44.65	-2.68 (-10.31, 4.95)	214.04 ± 35.36	-5.06 (-12.32, 2.20)	-2.38 (-12.91, 8.15)
M-LDL-C, µmol/L	553.86 ± 177.62	-6.02 (-35.58, 23.54)	567.16 ± 130.66	-15.98 (-44.11, 12.15)	-9.96 (-50.77, 30.85)
M-LDL-CE, µmol/L	402.73 ± 145.10	-4.68 (-28.74, 19.39)	412.95 ± 106.87	-13.01 (-35.92, 9.89)	-8.34 (-41.56, 24.89)
M-LDL-FC, µmol/L	151.13 ± 32.81	-1.35 (-6.93, 4.23)	154.21 ± 24.31	-2.96 (-8.27, 2.35)	-1.61 (-9.32, 6.10)
M-LDL-TG, µmol/L	49.12 ± 13.00	-0.78 (-2.87, 1.32)	48.59 ± 12.76	-1.72 (-3.72, 0.27)	-0.95 (-3.84, 1.95)
S-LDL-P, µmol/L	0.18 ± 0.05	-0.00 (-0.01, 0.01)	0.19 ± 0.04	-0.01 (-0.01, 0.00)	-0.00 (-0.01, 0.01)
S-LDL-L, µmol/L	518.43 ± 139.83	-6.61 (-29.51, 16.29)	531.48 ± 107.35	-14.51 (-36.30, 7.29)	-7.90 (-39.52, 23.73)
S-LDL-PL, µmol/L	152.63 ± 28.38	-2.19 (-7.27, 2.89)	155.84 ± 23.68	-3.71 (-8.55, 1.12)	-1.52 (-8.54, 5.49)
S-LDL-C, µmol/L	335.88 ± 106.68	-3.65 (-21.03, 13.73)	346.06 ± 78.45	-9.26 (-25.80, 7.28)	-5.61 (-29.61, 18.39)
S-LDL-CE, µmol/L	245.39 ± 87.55	-2.70 (-16.76, 11.36)	253.25 ± 64.01	-7.42 (-20.81, 5.96)	-4.72 (-24.14, 14.69)
S-LDL-FC, µmol/L	90.49 ± 19.54	-0.96 (-4.38, 2.45)	92.82 ± 14.93	-1.83 (-5.08, 1.42)	-0.87 (-5.59, 3.85)
S-LDL-TG, µmol/L	29.92 ± 8.35	-0.82 (-2.38, 0.75)	29.57 ± 9.60	-1.49 (-2.98, 0.00)	-0.67 (-2.83, 1.49)

	Control, n_{max} = 51[1]		Almond, n_{max} = 54[1]		Mean comparison between groups[3]
	Baseline[2]	Change	Baseline[2]	Change	
XL-HDL-P, µmol/L	0.41 ± 0.25	-0.00 (-0.05, 0.04)	0.49 ± 0.27	-0.03 (-0.08, 0.01)	-0.03 (-0.09, 0.04)
XL-HDL-L, µmol/L	413.02 ± 257.10	-2.67 (-49.96, 44.62)	494.59 ± 276.00	-31.38 (-76.36, 13.60)	-28.71 (-94.39, 36.97)
XL-HDL-PL, µmol/L	225.98 ± 140.62	-6.19 (-31.18, 18.80)	266.78 ± 151.56	-18.81 (-42.58, 4.95)	-12.62 (-47.29, 22.05)
XL-HDL-C, µmol/L	174.66 ± 115.95	2.98 (-20.27, 26.24)	215.50 ± 124.71	-10.33 (-32.44, 11.78)	-13.31 (-45.64, 19.02)
XL-HDL-CE, µmol/L	125.45 ± 81.85	2.37 (-14.83, 19.58)	155.38 ± 87.79	-6.61 (-22.97, 9.75)	-8.98 (-32.91, 14.95)
XL-HDL-FC, µmol/L	49.21 ± 34.66	0.42 (-0.01, 0.01)	60.12 ± 37.62	-0.00 (-0.01, 0.00)	-0.00 (-0.01, 0.01)
XL-HDL-TG, µmol/L	12.39 ± 7.63	0.00 (-5.90, 6.75)	12.31 ± 7.69	-3.55 (-9.57, 2.47)	-3.97 (-12.75, 4.81)
L-HDL-P, µmol/L	1.26 ± 0.58	-0.03 (-0.12, 0.06)	1.37 ± 0.66	-0.07 (-0.16, 0.01)	-0.04 (-0.16, 0.08)
L-HDL-L, µmol/L	790.73 ± 374.02	-19.63 (-76.59, 37.33)	864.08 ± 426.66	-45.53 (-99.73, 8.66)	-25.90 (-104.69, 52.89)
L-HDL-PL, µmol/L	393.74 ± 165.70	-12.86 (-39.86, 14.13)	425.37 ± 188.47	-21.12 (-46.80, 4.57)	-8.25 (-45.58, 29.08)
L-HDL-C, µmol/L	367.09 ± 199.49	-5.81 (-34.97, 23.35)	410.20 ± 228.83	-21.90 (-49.64, 5.85)	-16.09 (-56.44, 24.27)
L-HDL-CE, µmol/L	286.67 ± 152.02	-4.73 (-27.22, 17.75)	318.90 ± 174.22	-16.92 (-38.31, 4.47)	-12.19 (-43.30, 18.92)
L-HDL-FC, µmol/L	80.42 ± 47.61	-1.09 (-7.82, 5.65)	91.30 ± 54.70	-4.96 (-11.37, 1.44)	-3.88 (-13.20, 5.44)
L-HDL-TG, µmol/L	29.89 ± 15.46	-0.94 (-3.17, 1.30)	28.51 ± 13.56	-2.54 (-4.67, -0.42)	-1.61 (-4.69, 1.47)
M-HDL-P, µmol/L	2.23 ± 0.42	-0.07 (-0.16, 0.02)	2.27 ± 0.43	-0.06 (-0.15, 0.02)	0.00 (-0.11, 0.13)

	Control, n_{max} = 51[1]		Almond, n_{max} = 54[1]		Mean comparison between groups[3]
	Baseline[2]	Change	Baseline[2]	Change	
M-HDL-L, µmol/L	947.62 ± 182.64	-29.32 (-66.70, 8.07)	965.17 ± 190.69	-26.62 (-62.19, 8.96)	2.70 (-48.92, 54.32)
M-HDL-PL, µmol/L	427.40 ± 77.79	-11.68 (-27.93, 4.57)	433.73 ± 79.36	-12.26 (-27.73, 3.20)	-0.58 (-23.02, 21.86)
M-HDL-C, µmol/L	473.24 ± 104.61	-15.27 (-35.85, 5.32)	487.75 ± 113.80	-12.18 (-31.77, 7.41)	3.09 (-25.35, 31.52)
M-HDL-CE, µmol/L	385.83 ± 83.54	-11.97 (-28.22, 4.29)	397.98 ± 91.43	-9.30 (-24.77, 6.17)	2.67 (-19.79, 25.12)
M-HDL-FC µmol/L	87.41 ± 21.48	-3.29 (-7.68, 1.11)	89.77 ± 22.72	-2.89 (-7.07, 1.29)	0.40 (-5.67, 6.47)
M-HDL-TG, µmol/L	46.98 ± 13.61	-2.28 (-4.91, 0.35)	43.69 ± 14.12	-2.25 (-4.91, 0.35)	0.03 (-3.61, 3.67)
S-HDL-P, µmol/L	5.22 ± 0.54	-0.09 (-0.21, 0.03)	5.17 ± 0.52	-0.06 (-0.17, 0.05)	0.03 (-0.14, 0.19)
S-HDL-L, µmol/L	1161.61 ± 119.48	-19.61 (-46.43, 7.21)	1152.75 ± 115.44	-13.27 (-38.78, 12.25)	6.34 (-30.71, 43.40)
S-HDL-PL, µmol/L	589.38 ± 79.88	-10.79 (-24.87, 3.29)	582.77 ± 68.98	-8.59 (-21.99, 4.81)	2.20 (-17.25, 21.65)
S-HDL-C, µmol/L	524.87 ± 68.68	-8.23 (-25.36, 8.91)	526.60 ± 70.77	-3.45 (-19.76, 12.86)	4.78 (-18.88, 28.44)
S-HDL-CE, µmol/L	411.51 ± 64.73	-5.93 (-21.57, 9.70)	414.29 ± 64.61	-1.65 (-16.52, 13.23)	4.29 (-17.30, 25.87)
S-HDL-FC, µmol/L	113.37 ± 14.55	-2.36 (-5.11, 0.39)	112.31 ± 13.08	-1.74 (-4.36, 0.89)	0.62 (-3.18, 4.42)
S-HDL-TG, µmol/L	47.36 ± 14.47	-1.11 (-3.47, 1.26)	43.38 ± 16.12	-0.77 (-3.01, 1.48)	0.34 (-2.93, 3.61)
VLDL size, nm	36.10 ± 1.25	-0.16 (-0.39, 0.06)	35.84 ± 1.32	-0.08 (-0.30, 0.14)	0.08 (-0.23, 0.40)
LDL size, nm	23.51 ± 0.10	0.01 (-0.01, 0.02)	23.50 ± 0.06	-0.00 (-0.02, 0.01)	-0.01 (-0.03, 0.02)
HDL size, nm	9.94 ± 0.29	0.01 (-0.03, 0.05)	10.00 ± 0.30	-0.03 (-0.07, 0.01)	-0.04 (-0.10, 0.01)

Values of change and main comparison between groups are presented as mean (95% CI)
[1]Not all data were analysed due to technical problems and sample loss.

VLDL-C, esterified-C, free-C, phosphoglycerides, TG:PG, cholines, phosphatidylcholines, sphingomyelins, total fatty acids, unsaturation, omega-3, omega-6, polyunsaturated fatty acids, monounsaturated fatty acids, saturated fatty acids, linoleic acid, docosahexaenoic acid: n = 48 (control) and 52 (almond)
Glutamine: n = 48 (control) and 50 (almond)
Histidine: n = 25 (control) and 33 (almond)
Other biomarkers: n = 48 (control) and 53 (almond)
[2]Mean ± SD for normal distributed data or median (IQR) for non-normally distributed data. Baseline biomarker values were not different between the two groups.
[3]ANCOVA, adjusted for baseline outcome value and baseline BMI (mean difference almonds – control); $P < 0.05$ indicating a significant difference.
[4]$P < 0.05$ indicating a significant difference for values of mean difference between two groups.
C, cholesterol; Remnant-C, TG, triglycerides; PG, phosphoglycerides; VLDL, very low-density lipoprotein; LDL, low-density lipoprotein; HDL, high-density lipoprotein; IDL, intermediate density-lipoprotein; remnant cholesterol (non-HDL, non-LDL -cholesterol); sizes of lipoprotein: XXL – chylomicrons and extremely large, XL – very large, L – large, M – medium, S – small, XS – very small; P, particles; L, total lipids; PL, phospholipids; CE, cholesteryl esters; FC, free cholesterol; XXL-VLDL-P, concentration of chylomicrons and extremely large VLDL particles; XXL-VLDL-L, total lipids in chylomicrons and extremely large VLDL; XXL-VLDL-PL, phospholipids in chylomicrons and extremely large VLDL; XXL-VLDL-C, cholesterol in chylomicrons and extremely large VLDL; XXL-VLDL-CE, cholesteryl esters in chylomicrons and extremely large VLDL; XXL-VLDL-FC, free cholesterol in chylomicrons and extremely large VLDL; XXL-VLDL-TG; triglycerides in chylomicrons and extremely large VLDL; and so on according to size and density of lipoproteins.

Chapter 6: Snacking on whole almonds for six weeks increases heart rate variability during mental stress in healthy adults: a randomized controlled trial

This chapter of this thesis incorporating publications presents the published paper in the Nutrient.

(Dikariyanto *et al.*, 2020)

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Snacking on Whole Almonds for Six Weeks Increases Heart Rate Variability during Mental Stress in Healthy Adults: A Randomized Controlled Trial

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Received: 29 May 2020; Accepted: 17 June 2020; Published: 19 June 2020

Abstract: Cardiac autonomic regulation can be indirectly measured by heart rate variability (HRV). Low HRV, which can be induced by mental stress, is a predictor of risk of sudden cardiac death. Few studies have investigated cause-and-effect relationships between diet and HRV. Nut consumption is associated with CVD risk reduction, but the impact on HRV, particularly in response to stress, is unclear. Men and women (30–70 y) with above average risk of developing CVD were randomly assigned in a 6-week randomized, controlled, parallel arm trial to consume either whole almond or isocaloric control snacks (20% of daily estimated energy requirement). Control snacks contained the average nutrient profile of UK snacks. Five-minute periods of supine heart rate (HR) and HRV were measured at resting and during mental stress (Stroop color-word test) at baseline and six weeks. High frequency (HF) power, which reflects parasympathetic regulation of HR, was increased following almonds during the mental stress task relative to control (mean difference between groups 124 ms2; 95% CI 11, 237; $p = 0.031$, $n = 105$), but other indices were unaffected. Snacking on whole almonds instead of typical snacks may reduce risk of CVD partly by ameliorating the suppression of HRV during periods of mental stress.

Keywords: almonds; randomized controlled trial; heart rate variability; cardiovascular disease; snacking; stress

1. Introduction

Mental stress, increasingly a feature of modern fast-paced lifestyles, is recognized as contributing to the risk of developing cardiovascular disease [1,2]. Mental stressors stimulate cardiovascular responses which can be indirectly assessed via heart rate variability (HRV), a measure of fluctuation in the length of interbeat interval (IBI), modulated by the dynamic regulation of autonomic nervous system (ANS). The ANS consists of two branches that control variations in heart rate (HR), the sympathetic nervous system (SNS) which is activated by conditions of stress and increases HR, and the parasympathetic nervous system (PNS), specifically the vagus nerve, which counteracts the SNS and is dominant under conditions of rest, decreasing HR. Mental stress also attenuates baroreflex sensitivity [3] and increases endothelial dysfunction [4], which may be followed by slower recovery of HRV leading to the development of CVD risk [5,6]. Evidence from neuroimaging studies shows that HRV is associated with

parts of the brain that regulate emotional responses [7], and greater cardiovascular reactivity in response to mental stress and slower recovery from stress are longitudinally linked to impaired cardiovascular risk status, including higher BP, hypertension and greater carotid intima-media thickness (subclinical atherosclerosis) [8].

HRV can be reported using the analysis of time- and frequency-domain parameters [9], as well as non-linear parameters, which are able to assess complexity of patterns and randomness in the HRV signal. Low HRV indicates a dominance of sympathetic activity and suppressed parasympathetic control; prolonged low HRV indicates impaired regulation of HR in response to dynamic demands and is associated with increased risk of cardiovascular morbidity and mortality in both the general healthy population [10,11] and patients with coronary heart disease (CHD) [12–15]. Conversely, high HRV suggests resilience of the cardiac ANS in adapting to stress and is inversely associated with cardiovascular disease (CVD) [16].

Lifestyle factors, such as diet and levels of physical activity, may influence HRV [17]. Scientific understanding of the impact and mechanism of dietary modifications on HRV is still very limited [17], and mainly relates to the effects of omega-3 supplementation [18,19]. During times of stress, people are more inclined to seek reward in the shape of snack foods with a less favorable nutrient profile, for example, higher in saturated fats, refined starch, free sugars, and sodium [20,21]. Substituting typical snacks with healthier snacks may ameliorate the reduction in HRV following a stress stimulus by both these pathways. Improvements in diet quality may enhance HRV both by improving neurological function and by directly influencing the responsivity of the target organ (e.g., the heart) to parasympathetic efferent input. Diet may also indirectly enhance HRV through improving mental health and reducing psychological stress, thereby reducing excess sympathetic activity [17].

In this study, almonds are utilized as a model healthy snack, since they have previously been shown to have cardioprotective effects using intermediary risk factors. Almonds, the most consumed tree nut globally [22], mostly as a snack food, are rich in unsaturated fats, dietary fiber, potassium, magnesium and vitamin E, and low in saturated fatty acids, sugar and sodium [23]. Almond consumption can improve intermediary cardiovascular risk factors [24–26], such as low-density lipoprotein (LDL), apolipoprotein B (apo-B), blood pressure and adiposity [27]. Using a randomized, controlled, parallel 6-week arm study design [28], we aimed to investigate the impact of replacing usual snacks with whole almonds on the HRV response to stress in a healthy, free-living adult population sample who were at above average risk of cardiovascular disease. It was hypothesized that snacking on almonds, relative to control snacks, would result in higher HRV during a laboratory mental stress test.

2. Materials and Methods

2.1. Study Subjects

The ATTIS (Almond Trial Targeting dietary Intervention with Snacks) study was specifically designed to include male and female adults aged 30–70 y with moderate risk of developing CVD that were self-reported regular snack consumers (consumed 2 or more snacks per day) around London. Identification of the risk of developing CVD was conducted following the Framingham risk score system, based on total plasma cholesterol (TC), high-density lipoprotein (HDL) cholesterol, blood pressure, and body mass index (BMI)/waist circumference (WC); moderate risk was defined as having score 2 or more [29].

Exclusion criteria included self-reported prediabetic or diabetic condition; history of heart attack (myocardial infarction) or stroke, cancer (excluding basal cell carcinoma) in the last five years, epilepsy or regular fainting, cholestatic liver disease, pancreatitis, alcohol or drug abuse; diagnosis of cardiovascular problems, angina, thrombosis, pacemaker, gastrointestinal disorders, renal or bowel disease; use of a drug likely to alter gastrointestinal motility or nutrient absorption; presence of metal inside the body; allergy or intolerance to nuts; currently pregnant, planning pregnancy, breastfeeding or having given birth in the preceding 12 months; unwillingness to follow the protocol and/or give informed consent; weight change of >3 kg in preceding 2 months; body mass index (BMI) of <18 kg/m^2 or >40 kg/m^2; current smokers or individuals who quit smoking within the last 6 months; and participation in other research trials involving dietary or drug intervention and/or blood collection in the past 3 months.

Participants were recruited from the general population in the London area. Eligible potential participants were invited to a screening visit at King's College London (KCL). Anthropometry (body height and weight, WC, body fat composition by bioelectrical impedance) and clinic blood pressure (cBP) were measured, and fasted blood samples were collected for analysis of blood glucose, full lipids, liver function and full blood count.

2.2. Study Design

The ATTIS study [28] was approved by the UK National Research Ethics Service (REC 16/LO/1910, approved 24 January 2017), registered with ClinicalTrials.gov (NCT02907684), and run in accordance with the Declaration of Helsinki 1975, revised in 2013, and the principles of Good Clinical Practice. Data collection was carried out between June 2017 and May 2019. All subjects gave their informed consent for inclusion before they participated in the study. The study was primarily designed to investigate the effects of almond snack consumption on endothelial function and liver fat, with HRV as a secondary outcome.

The trial used a randomized, controlled, parallel design in a free-living cohort. Participants were randomly assigned to one of two intervention arms, almonds or control. The research coordinator conducted treatment allocation using minimization software (MinimPy 0.3, Copyright (c) 2011 Mahmoud Saghaei, http://minimpy.sourceforge.net/), with age, sex, ethnicity, cardiometabolic score and willingness to undergo MRI/MRS scan as minimization variables (base probability 0.7, 1:1 allocation ratio). A 2-week run-in period consuming control snacks preceded randomization to ensure study subjects were able to tolerate the study protocol prior to starting the intervention period and to collect 4-day food diary and physical activity data. Following run-in, participants attended baseline visits at the Clinical Research Facility, St Thomas' Hospital, London, for measurements of HRV in the resting state and during a mental stress test, as well as providing a fasted blood sample, having anthropometric measurements, and other procedures to assess changes in cardiometabolic risk factors. At 2 and 4 weeks of intervention, participants attended the Metabolic Research Unit at KCL for measurements of body composition and they also completed 24 h dietary recalls to monitor compliance. Following 6 weeks of dietary intervention, participants attended the endpoint visit at the Clinical Research Facility, St Thomas' Hospital, and underwent the same measurements and procedures as at the baseline visit. A further 4-day food diary was completed. Before baseline and endpoint clinic visits, subjects were requested to avoid alcohol and strenuous activity for 24 h, and to consume low-fat meals and no alcohol in the evening before. Due to the nature of the intervention, participants and research staff administering interventions and conducting physiological and anthropometric measurements on study days were not blinded, but blood biomarker analysis and statistical analysis were conducted by analysts blinded to treatment allocation.

2.3. Intervention

Snacks usually contribute to 20–25% of energy intake [20,30,31]. Thus, the dietary intervention included a 2-week run-in period, wherein participants consumed control snacks equaling 20% of estimated energy requirements (EER), followed by random allocation to either control or almond snacks at 20% of EER for 6 weeks. EER was calculated using Henry equations and physical activity level (PAL) was estimated from 4-d activity diaries [32]. Control snacks were sweet and savory mini-muffins, baked at KCL, and were formulated and developed to be representative of the average macronutrient intakes from snacks (excluding fruit) in the UK National Diet and Nutrition Survey population [20], as detailed elsewhere [28]. Almond snacks were dry-roasted whole non-salted almonds of the nonpareil variety, supplied by the Almond Board of California (US grade extra no. 1). Participants were provided with snack information sheets and dietary advice from the research dietitian including instructions to only consume study snacks in between meals, maintain their habitual mealtime, eating habits and fruit consumption as well as avoid the consumption of additional nuts or nut products. To verify participants' compliance with the dietary advice, telephone 24 h recalls and four-day estimated portion size food records were conducted.

Participants were provided with 20% EER from the muffins or almonds, which for a 2000 kcal EER, equated to five control muffins daily (400 kcal in (80 kcal/muffin)), or 63 g/d almonds (100 g almonds contains 634 kcal). Comparison of nutritional composition between almonds and control muffins is detailed in Supplementary Table S1. The ratio of sweet: savory muffins consumed was 70:30, with a mean of 20% of energy from free sugars in total muffins.

2.4. Anthropometry, Blood Pressure Measurements, and Blood Sampling

WC was measured using a measuring tape and body weight using electronic scales (Tanita BC-418MA, Tanita Ltd., Middlesex, UK). OMRON M2 Basic Intellisense monitors (OMRON Healthcare UK Ltd., Milton Keynes, UK) were used to measure seated blood pressure according to British Hypertension Society guidelines [33]. At screening, fasting blood was collected for analysis of glucose, lipids, liver function and full blood count. For baseline and endpoint visits, fasting blood samples were taken for analysis of plasma lipids, such as total, HDL-, and LDL-cholesterol and TAG and calculated TC:HDL-C ratio, as reported previously [28]

2.5. HRV Measurement

HRV assessments encompass acute fluctuations in IBIs (beat-to-beat HRV, parasympathetically regulated), as well as longer-phase oscillations (also reflecting other rhythms related to thermoregulation, hormonal fluctuation and circadian patterns). To measure real-time HRV in the supine position during mental stress, a small, light-weight, chest-worn wireless 2-lead ambulatory heart rate/ECG monitor (eMotion Faros 180, Mega Electronics Ltd., Kuopio, Finland) was fitted. Mental stress was induced by requiring the participant to complete the Stroop test—a test with colored words that has been used previously for this purpose during cardiovascular measurements [34,35]. The 5-min Stroop test was conducted 15 min after a 5-min resting HRV recording.

Cardiscope™ Analytics software (HASIBA Medical GmbH, Graz, Austria) was used to analyze the ECG data. Linear time-domain HRV parameters included the mean of the standard deviations of the normal-to-normal (NN) intervals (SDNN; indicating overall HRV) and root mean square of successive differences of NN intervals (RMSSD; indicating beat-to-beat, respiration-driven variability, and representing parasympathetic regulation) and linear frequency-domain HRV parameters included high-frequency (HF) power (reflects parasympathetic respiratory modulation) and LF/HF (previously assumed to reflect relative sympathetic to parasympathetic activity, but due to conflicting evidence for the role of the LF component in signifying sympathetic activity, this has been challenged) [9,36]. HF is expressed both as absolute unit (ms^2) and normalized unit (nu), the latter being an adjustment for changes in total spectral power (except VLF). The short duration of stress test in our study precluded the inclusion of VLF power (0.003–0.04 Hz), a parameter of longer-phase oscillations in autonomic regulation and the renin-angiotensin system [9]. The non-linear parameter, the Poincaré ratio (SD1/SD2; the ratio of the SD of beat-to-beat IBI variability (SD1) against the SD of long-term IBI variability (SD2), indicating normality of sinoatrial firing patterns), is also measured as a non-linear measure analysis parameter [9].

2.6. Statistical Analysis

The sample size required per treatment arm was 50 subjects with 90% power and a two-tailed alpha set at 0.05 to detect significant between-treatment differences in flow-mediated dilation (FMD) of 1.25% unit difference (SD 1.9) [37], a primary outcome for the study. Allowing for potential drop-outs, the researchers enrolled 109 subjects to commence the run-in phase. HRV measures were secondary outcomes of the ATTIS study. Due to poor quality food diaries, a per protocol analysis was performed on nutrient intake data; other data were missing due to attrition, human error or technical failure. IBM SPSS 25 was used for statistical analysis. Normality of data was assessed visually using histogram and Q–Q plot of residuals. Baseline data are shown as mean value and standard deviation (SD), unless not normally distributed, and then, shown as median (IQR). Treatment effects are presented as the adjusted mean differences between groups at endpoint, with 95% CI. Chi-square test was conducted to investigate whether there were differences in sex and ethnicity between the control and almond groups at baseline. To examine whether there were differences in other baseline characteristics and also in baseline data between the two treatment groups, an independent t-test was used for normally distributed data and Mann–Whitney U test was used for non-normally distributed data. To investigate the significance of treatment effects between the two groups, analysis of covariance (ANCOVA) was used, adjusting for baseline value and baseline BMI. A two-sided p-value of <0.05 was considered to show statistical significance.

3. Results

3.1. Participant Characteristics

There were 109 participants recruited, 107 randomized and 105 completed the study. Drop-outs were due to non-compliance, time commitment issues and gastrointestinal intolerance of almonds. Table 1 shows characteristics at screening of participants who were randomized to treatment. Of 107 participants randomized, 75 were females and 32 were males, and the average age was 56 y. The CONSORT diagram in Supplementary Figure S1 shows the full stream of participant enrolment, allocation to treatment and disposition.

Table 1. Subject characteristics at screening for those randomized to treatment (mean ± SD).

	Control, *n* = 51	**Almond, *n* = 56**
Age, y	56.0 ± 10.7	56.3 ± 10.3
Sex, M/F, *n*	15/36	17/39
Ethnicity (Black/South Asian, Southeast Asian and Middle Eastern/Far East/White/Other), *n*	2/6/2/34/7	9/7/3/34/3
Cardiometabolic score	4.2 ± 2.1	4.5 ± 2.0
BMI, kg/m^2	26.7 ± 4.5	27.3 ± 4.4
WC, cm	93.3 ± 12.5	93.6 ± 12.5
% body fat	32.7 ± 8.5	34.4 ± 8.4
cSBP, mmHg	124.4 ± 15.1	126.2 ± 17.6
cDBP, mmHg	80.6 ± 7.7	83.8 ± 10.8
Glucose, mmol/L	5.1 ± 0.6	5.1 ± 0.5
TC, mmol/L	5.6 ± 1.2	5.6 ± 1.0
TAG, mmol/L	1.2 ± 0.5	1.2 ± 0.6
LDL-C, mmol/L	3.5 ± 1.0	3.4 ± 0.9
HDL-C, mmol/L	1.6 ± 0.5	1.6 ± 0.5
TC:HDL	3.6 ± 0.9	3.7 ± 1.1

Ethnicity was determined by self-reporting. Blood measures taken in a fasting state. BMI, body mass index; WC, waist circumference; cSBP, clinic systolic blood pressure; cDBP, clinic diastolic blood pressure; TC, total cholesterol; TAG, triacylglycerol; LDL-C, low-density cholesterol; HDL-C, high-density cholesterol; TC:HDL, total cholesterol:high-density cholesterol.

3.2. Nutrient Intakes and Blood Markers

Total energy intake of both treatment groups was not different, as shown in Table 2. The almond group had significantly higher intakes of dietary fiber, fat, mono- and polyunsaturated fatty acids, the ratio of unsaturated to saturated fatty acids, potassium, magnesium, vitamin E and riboflavin. Intake of total carbohydrate, starch, free sugar and sodium was shown to be significantly lower in the almond group.

There were no significant differences in changes in body composition (Supplementary Table S2), nor markers of insulin sensitivity (data reported elsewhere [28]). Plasma non-HDL and LDL were significantly lowered by 0.22 mmol/L (95% CI −0.42, −0.01) and 0.25 mmol/L (95% CI −0.45, −0.04) respectively following almonds relative to control, as previously reported [28].

3.3. Heart Rate Variability at Rest and during Mental Stress

Compared to resting values, mean 5-min NN decreased during mental stress in both treatment groups (1030.3 ± 156.4 vs. 921.1 ± 148.6, paired t-test $p \leq 0.001$). As shown in Table 3, in the resting state, there were no significant differences between treatment groups in the change in HRV indices following intervention. However, during mental stress, HF power was higher following almond treatment by 124 ms2 (95% CI 11, 237), relative to control. Moreover, LF/HF was lower by −1.0 (95% CI −1.9, −0.1) relative to control. No differences were found in other indices during mental stress.

Table 2. Nutrient intakes estimated from 4-d food diaries at baseline (prior to run-in) and the final week of the dietary intervention.

	Control, $n_{max} = 40$ [2]		Almond, $n_{max} = 40$ [2]		Mean Comparison between Groups	*p*-Value
	Baseline	Change	Baseline	Change		
Energy intake [1], kcal/d	2088.9 ± 538.5	−5.8 (−124.7, 113.2)	1769.4 ± 475.0	−85.3 (−204.3, 33.7)	−79.5 (−251.8, 92.8)	0.361
Protein, %E	15.4 ± 3.8	0.5 (−0.5, 1.5)	15.9 ± 3.6	1.0 (0.0, 2.0)	0.5 (−0.9, 1.9)	0.466
Carbohydrate, %E	43.3 ± 7.1	1.7 (−0.1, 3.5)	41.8 ± 6.6	−7.6 (−9.4, −5.8)	−9.3 (−11.9, −6.8) *	<0.001
Starch, %E	23.9 ± 5.1	2.5 (0.9, 4.1)	23.5 ± 5.3	−4.5 (−6.1, −2.9)	−7.0 (−9.3, −4.8) *	<0.001
Free sugars, %E	5.9 ± 3.8	0.4 (−0.5, 1.2)	5.5 ± 2.8	−2.6 (−3.5, −1.8)	−3.0 (−4.2, −1.8) *	<0.001
Dietary fibre [1], g/d	23.8 ± 6.2	−1.9 (−4.5, 0.6)	20.7 ± 7.7	5.5 (3.0, 8.1)	7.4 (3.8, 11.1) *	<0.001
Fat, %E	36.5 ± 6.5	−2.6 (−4.3, −0.8)	37.1 ± 6.2	8.3 (6.5, 10.0)	10.8 (8.4, 13.3) *	<0.001
SFA, %E	12.3 ± 3.6	−0.6 (−1.3, 0.1)	12.5 ± 3.7	−1.4 (−2.1, −0.6)	−0.7 (−1.8, 0.3)	0.153
MUFA, %E	11.5 ± 3.4	−1.1 (−2.4, 0.0)	12.4 ± 3.7	8.6 (7.4, 9.8)	9.8 (8.1, 11.5) *	<0.001
PUFA, %E	5.9 ± 2.5	−0.8 (−1.4, −0.1)	5.9 ± 1.7	2.0 (1.4, 2.6)	2.8 (1.9, 3.7) *	<0.001
Unsaturated: saturated fatty acid ratio	1.5 ± 0.5	−0.1 (−0.3, 0.1)	1.6 ± 0.7	1.1 (0.9, 1.3)	1.3 (1.0, 1.5) *	<0.001
Sodium, mg	2151.2 ± 766.3	179.7 (−15.8, 375.3)	1926.1 ± 866.1	−490.8 (−686.4, −295.3)	−670.6 (−948.6, −392.6) *	<0.001
Potassium [1], mg	3028.9 ± 936.2	−352.5 (−590.4, −114.5)	2534.7 ± 854.5	221.3 (−16.7, 459.3)	573.8 (231.0, 916.6) *	0.001
Calcium [1], mg	868.4 ± 455.8	24.2 (−57.6, 106.0)	703.8 ± 242.5	57.3 (−24.5, 139.0)	33.1 (−84.0, 150.2)	0.575
Magnesium [1], mg	368.7 ± 180.9	−36.0 (−68.9, −3.0)	278.5 ± 92.1	112.6 (79.7, 145.5)	148.6 (100.9, 196.3) *	<0.001
Vitamin E, mg	10.7 ± 3.7	−1.9 (−3.5, −0.4)	8.9 ± 4.0	13.5 (11.9, 15.0)	15.4 (13.2, 17.6) *	<0.001
Riboflavin, mg	1.8 ± 1.6	−0.1 (−0.3, 0.0)	1.5 ± 0.8	0.4 (0.2, 0.6)	0.5 (0.3, 0.8)	<0.001
Niacin, mg	16.1 ± 8.9	−1.6 (−3.3, 0.1)	14.7 ± 9.4	−0.4 (−2.1, 1.3)	1.2 (−1.3, 3.6)	0.339

Values of change and main comparison between groups are presented as mean (95% CI). ANCOVA was used, adjusted for baseline value and baseline BMI. * $p < 0.05$ indicating a significant difference. [1] Baseline value was different between control and almond group. [2] Data were analyzed using 40 diaries collected from each group. Missing data are due to poor quality diet diaries or failure to complete by participant.

Table 3. Heart rate variability values measured during 5-min periods of rest and mental stress (Stroop test), following randomization to almond and control snacks.

	Control, n_{max} = 51 [1]		Almond, n_{max} = 54 [1]		Main Comparison between Groups at Endpoint [4]	*p*-Value between Groups at Endpoint
	Baseline [2]	Endpoint	Baseline [2]	Endpoint		
Resting						
NN, ms	1009 ± 166	1006 (977, 1035)	1050 ± 146	1012 (984, 1040)	6 (−34, 47)	0.760
HR	60.1 ± 10.7	60.5 (58.7, 62.3)	58.0 ± 7.6	61.0 (59.3, 62.7)	0.5 (−2.0, 3.0)	0.704
rMSSD, ms	32.6 (24.6)	37.7 (34.0, 41.5)	31.9 (25.7)	37.2 (33.5, 40.8)	−0.6 (−5.8, 4.7)	0.831
SDNN, ms	54 ± 27	49 (44, 544)	47 ± 21	50 (45, 55)	1 (−6, 8)	0.740
SD1/SD2	0.5 ± 0.3	0.4 (0.4, 0.5)	0.4 ± 0.1	0.4 (0.4, 0.5)	0 (−0.1, 0)	0.651
HF, ms [2]	373 (631)	599 (468, 730)	322 (541)	514 (391, 636)	−85 (−265, 95)	0.348
HFnu	0.40 ± 0.17	0.41 (0.36, 0.46)	0.41 ± 0.14	0.42 (0.37, 0.46)	0.01 (−0.06, 0.07)	0.871
LF:HF	1.5 (1.7)	1.9 (1.5, 2.2)	1.4 (1.3)	1.9 (1.5, 2.2)	0 (−0.5, 0.5)	0.924
Mental stress (Stroop test)						
NN [3], ms	888 ± 166	935 (907, 963)	950 ± 127	924 (899, 950)	−11 (−49, 28)	0.310
HR[3]	68 ± 10	65.4 (63.5, 67.3)	64 ± 8	66.2 (64.5, 67.9)	0.8 (−1.8, 3.4)	0.543
rMSSD, ms	38.2 ± 22.4	31.8 (27.1, 36.5)	33.8 ± 14.0	34.0 (29.8, 38.2)	2.2 (−4.1, 8.5)	0.494
SDNN, ms	54 (32)	44 (41, 47)	45 (22)	48 (44, 51)	4 (−1, 8)	0.137
SD1/SD2	0.4 (0.2)	0.4 (0.3, 0.4)	0.4 (0.1)	0.4 (0.4, 0.4)	0 (0, 0.1)	0.420
HF, ms [2]	394 (473)	281 (197, 364)	264 (495)	405 (331, 480)	124 (11, 237)	0.031 *
HFnu	0.31 ± 0.10	0.26 (0.22, 0.30)	0.32 ± 0.12	0.32 (0.28, 0.35)	0.06 (0, 0.11)	0.040 *
LF:HF	2.3 (1.5)	3.6 (3.0, 4.3)	2.0 (1.7)	2.6 (2.0, 3.2)	−1.0 (−1.9, −0.1)	0.023 *

Endpoint values and main comparison between groups at endpoint are presented as mean (95% CI). [1] Not all data were analyzed due to technical problems. Resting NN, HR, rMSSD, SDNN and SD1/SD2: n = 40 (control) and 43 (almond). Resting HF, HFnu, and LF:HF: n = 35 (control) and 40 (almond). Mental stress (Stroop test) NN and HR: n = 35 (control) and 44 (almond). Mental stress (Stroop test) rMSSD, SDNN and SD1/SD2: n = 36 (control) and 44 (almond). Mental stress (Stroop test) HF, HFnu and LF:HF: n = 28 (control) and 35 (almond). [2] Mean ± SD for baseline data that are normally distributed. Median (IQR) for other data as they are non-normally distributed. [3] Baseline value was different between control and almond group; independent *t*-test was used for normally distributed data while Mann–Whitney U test was used for non-normally distributed data; $p < 0.05$ indicating a significant difference. [4] ANCOVA, adjusted for baseline outcome value and baseline BMI (mean difference almonds—control at endpoint). * $p < 0.05$. NN, normal-to-normal intervals; HR, heart rate; rMSSD, root mean square of successive R-R interval differences; SDNN, standard deviation of normal-to-normal (NN) intervals; HF, absolute power of the high-frequency band (0.15–0.04 Hz); HFnu, normalized HF (HFnu = HF/(HF + LF)).

4. Discussion

Mental stress can lower HRV, which is associated with increased risk of developing CVD [7,8]. Improvements in diet quality have the potential to increase HRV, but evidence for a direct causal relationship is limited. We report the novel finding that snacking on whole almonds for six weeks, compared with isocaloric snacks with a more typical nutrient profile (high in saturated fats, starch and free sugars and low in dietary fiber), increased HRV parameters of parasympathetic activity during acute mental stress. This could be due to lower levels of background daily stress in the almond group, improvements in neurological autonomic function, or an increase in cardiac tissue responsivity to ANS neurotransmission and/or hormonal modulation. Although the underlying mechanism of effect is unclear, the results of the study suggest that a simple dietary modification resulting in increased intake of micronutrients, dietary fiber and unsaturated fatty acids and reduced intake of free sugars and sodium improved vagal tone during mental stress.

Variability in beat-to-beat intervals, assessed by HF power in the frequency domain, is associated with respiration, known as respiratory sinus arrhythmia (RSA). RSA is mediated by the parasympathetic nervous system modulated by vagal motor neurons linked with the lung inflation reflex [38–40]. Only a very limited amount of research has been done previously on the effects of tree nuts on HRV. In agreement with our findings, higher HF power was previously observed by Sauder et al. during two acute stress tests, i.e., mental arithmetic and hand cold pressor, following 4-week pistachio nut consumption at 20% of energy intake, as well as increased rMSSD and LF power [41]. In our study, there were no treatment effects observed for rMSSD and LF, possibly due to variability across studies in baseline stress levels and methodological differences such as type of mental stressor and measurements being made in the seated position in contrast to our study where measurements were made in the supine position. In the current study, the ratio of LF to HF power was shown to be decreased in almond group, which could suggest that replacing typical snacks with almonds might tip the balance of sympathetic to parasympathetic nervous system activity to a more favorable one. However, the interpretation of LF/HF ratio is controversial; the original belief that LF power is related to sympathetic modulation has been widely discounted as results of experiments inducing pharmacological sympathetic blockade and other manipulations of sympathetic activity [42]. Thus, the difference between treatments in LF/HF ratio is likely to be largely attributable to increased vagal tone rather than any reduction in sympathetic outflow [43,44].

Although weight loss is known to increase parasympathetic activity [45], there were no differences between groups in the change from baseline in body weight and adiposity, nor energy intake [28]. The established effects of almond consumption in lowering plasma low-density lipoprotein (LDL) cholesterol concentrations may have some bearing on HRV responses, as the literature reports that plasma TC and LDL are inversely associated with HR and HRV [46,47]. Statin treatment was associated with improved HRV in 40 hypercholesterolemic patients with or without CAD [48] and healthy individuals with 48 h sleep deprivation [49]. Hypercholesterolemia elevates reactive oxygen species (ROS) and oxidative stress in vessel walls and induces inflammation, causing dysfunctional nitric oxide synthase (eNOS) activity, an enzyme catalyzing nitric oxide (NO) production and greater degradation of NO [50], resulting in dysregulation of vascular tone [51]. Baroreceptors sense systemic arterial pressures via stretching, and impaired vascular tone is likely to disrupt baroreceptor sensitivity, resulting in reduced baroreflex control of HR, potentially attenuating HRV [52]. Therefore, reductions in LDL cholesterol concentrations following almond intervention may have indirectly contributed to enhancement of baroreceptor sensitivity and maintenance of vagal tone. This proposed cardiovascular mechanism is strengthened by our finding reported previously that NO-mediated vasodilation was increased following the almond intervention in the same study [28].

Lower dietary glycemic load as a result of displacing typical snack nutrient intakes with almonds may be an important factor in the improved HRV observed under mental stress, as previous studies have observed increased LF/HF and reductions in total power from spectral analysis following oral glucose loads [53–56]. Insulin secretion is implicated in this effect, since hyperinsulinemia

can reduce the functioning of the sinoatrial node and alter ANS activity [53,54]. Furthermore, the literature also demonstrated that glucose intake and hyperinsulinemia are dose-dependently associated with the level of circulating NE, a sympathetic-induced neurotransmitter [57]. Sodium and potassium levels may also play a part. Potassium is inversely associated with aldosterone, a hormone regulating sodium reabsorption which is involved in the renin-angiotensin-aldosterone system (RAAS). Lower sodium leads to reduced water retention in the kidney, smaller blood volume and lower blood pressure [58]. Blood pressure maintenance could promote baroreflex sensitivity and influence sympathetic activity [52].

Almonds are a good source of magnesium and in this study, the almond group had higher magnesium intakes relative to the control group. Animal experiments demonstrated that magnesium has anti-arrhythmic effects involving improvement in sinus rhythm [59], but human data are inconsistent in treatment of arrhythmia [60–62] and in associations with HRV [63–65]. Almonds are rich in vitamin E, and 4-month supplementation in a double-blind RCT improved HRV by increasing RR interval, TP and HF and reducing LF and LF:HF in T2D patients, possibly due to a reduction in oxidative stress [66]. Although it is not possible to pinpoint the components of almonds that may be responsible for the greater cardiac resilience to mental stress observed in the current study, it is possible that the relative augmentation in vagal tone during the Stroop test by almond consumption was a combined result of all or many of the mechanisms discussed.

The main strength of our study was the randomized, controlled, single-blinded study design involving control test snacks that represented typical snacks consumed in the adult population. Almond consumption did not affect either body weight, central adiposity (waist circumference) or overall adiposity (body fat percentage), and therefore, fat mass loss was not a confounding factor [67–70]. Although we recruited healthy adults at risk of developing CVD, recording of self-reported mental and mood conditions before the mental stress task and HRV measurements were not included and may be a limitation. The Stroop test was conducted following 10 min recovery after blood-pressure cuff inflation applied within a flow-mediated dilation (FMD) measurement that could cause physical stress, which might affect baroreceptor-derived autonomic outflow, and therefore, influence the resting measurements. Furthermore, it would be of interest to determine the response in recovery HRV after the Stroop test. Recruiting patients with obesity or type-2 diabetes, determinants of impaired sympathovagal balance, should be a priority in future research to understand more the effects of whole almonds on HRV in at risk populations.

5. Conclusions

Snacking on whole almonds in place of typical snacks can increase HRV parameters of parasympathetic activity in response to mental stress, indicating improved cardiac autonomic function. Incorporating tree nuts as daily snacks is encouraged as a positive lifestyle change and enhances cardiovascular health, not only by lowering cholesterol, but also potentially by increasing resilience to mental stress.

Supplementary Materials: The following are available online at http://www.mdpi.com/2072-6643/12/6/1828/s1, Table S1: Nutritional composition of almonds and control snacks per 400 kcal isocaloric portions, Figure S1: CONSORT Flow Diagram, Table S2: Body composition, clinical blood pressure and circulating biomarkers of cardiometabolic risk following randomization to almond and control snacks.

Author Contributions: Conceptualization, W.L.H., P.J.C. and S.E.B.; methodology, W.L.H., V.D. and L.S.; investigation, V.D. and L.S.; data curation, V.D.; writing—original draft preparation, V.D.; writing—review and editing, W.L.H., S.E.B., L.S. and P.J.C.; supervision, W.L.H. and S.E.B.; funding acquisition, W.L.H. and S.E.B. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Almond Board of California and Indonesia Endowment Fund for Education. The almonds were provided by the Almond Board of California.

Acknowledgments: We thank Molly O'Callaghan-Latham for assistance in collecting data and all ATTIS study participants.

Conflicts of Interest: L.S. received salary from the Almond Board of California for her post as a research assistant on the study. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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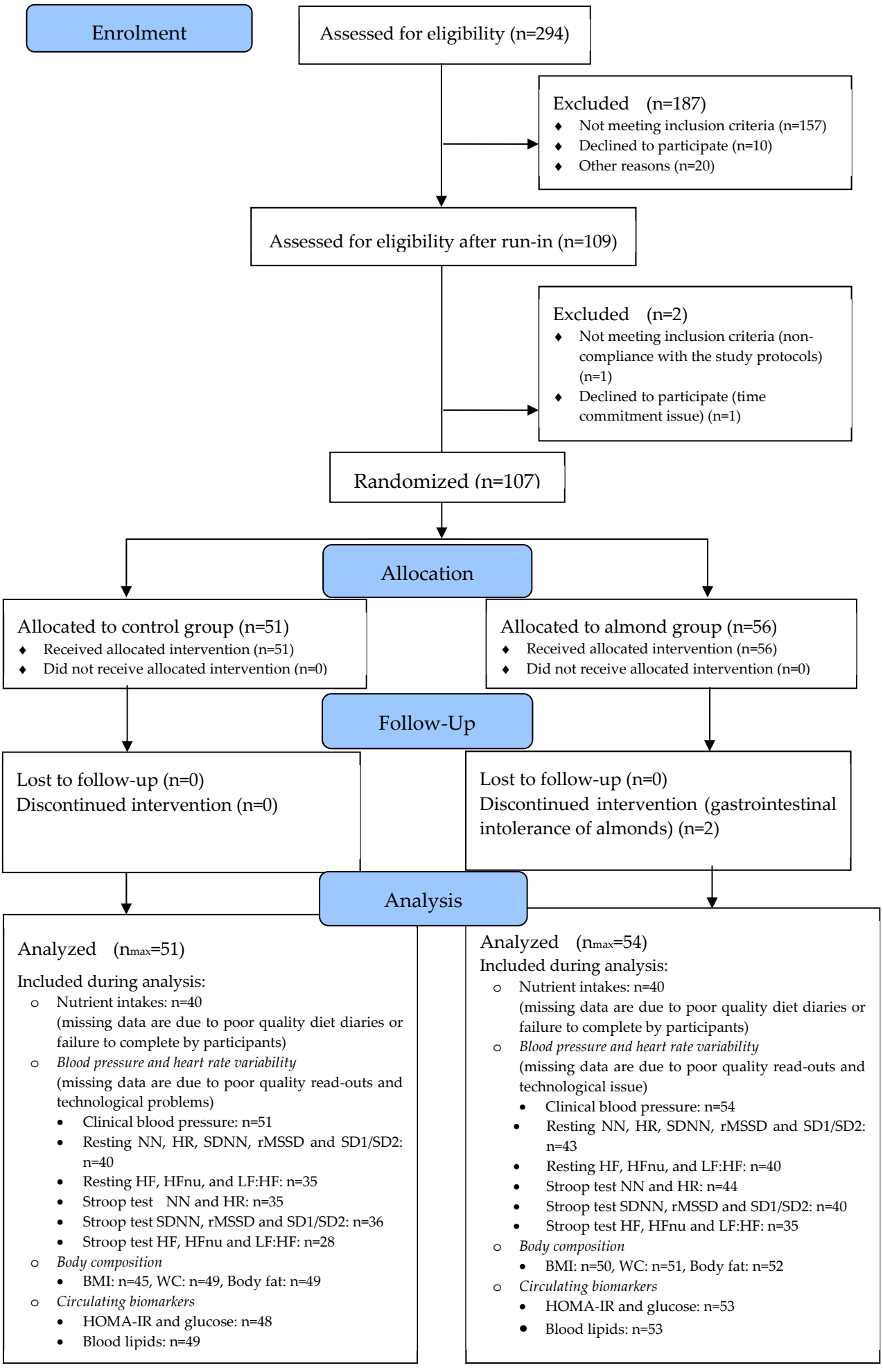
Supplementary Materials

Supplemental Table S1. Nutritional composition of almonds and control snacks per 400 kcal isocaloric portions. Values given as 20% of the estimated average requirement for energy (2000 kcal) for adult women.

Nutrient	Almonds	Average of sweet and savory control snacks	Control snacks	
			Sweet	Savory
Energy, kcal	400	400	400	400
Protein, %E	13.4	9.8	9.8	9.8
Carbohydrate, %E	13.2	54.1	54.3	53.8
Starch, %E	-	38.7	24.5	52.9
Sugars, %E	2.3	15.1	29.7	0.4
Fibre, g	9.3	2.1	1.3	2.8
Fat, %E	73.6	36.5	35.8	37.1
SFA, g	5.6	12.8	12.6	12.8
MUFA, g	47.9	16.0	15.8	16.0
PUFA, g	16.0	5.6	5.4	5.9
Sodium, mg	<2.5	452.1	187.4	716.8
Potassium, mg	463.7	162.8	145.9	179.6
Calcium, mg	164.7	130.1	46.1	214.1
Magnesium, mg	181.7	19.0	15.4	22.6
Vitamin E, mg	14.3	1.3	1.1	1.4

Supplemental Figure S1. CONSORT Flow Diagram



Supplemental Table S2. Body composition, clinical blood pressure and circulating biomarkers of cardiometabolic risk following randomization to almond and control snacks.

	Control, n_{max} = 51[1]		Almonds, n_{max} = 54[1]		Main comparison between
	Baseline[2]	Change	Baseline[2]	Change	groups[3]
BMI, kg/m^2	27.1 ± 4.4	-0.2 (-0.4, 0.0)	27.2 ± 4.5	0.1 (-0.1, 0.3)	0.2 (-0.1, 0.5)
WC, cm	93.3 ± 11.7	0.1 (-0.9, 1.2)	94.1 ± 12.2	-0.6 (-1.6, 0.5)	-0.7 (-2.2, 0.8)
Body fat, %	31.1 ± 7.7	-0.5 (-1.1, 0.0)	32.2 ± 7.7	0.3 (-0.3, 0.8)	0.8 (-0.0, 1.6)
cSBP, mmHg	127.8 ± 12.9	-5.2 (-7.9, -2.6)	127.3 ± 19.3	-6.3 (-8.9, -3.7)	-1.0 (-4.7, 2.6)
cDBP, mmHg	84.6 ± 7.9	-3.2 (-5.1, -1.3)	85.5 ± 10.6	-3.5 (-5.3, -1.6)	0.2 (-2.9, 2.4)
TC, mmol/L	5.26 ± 1.13	0.03 (-0.15, 0.20)	5.40 ± 0.93	-0.18 (-0.35, -0.02)	-0.21 (-0.45, 0.03)
TAG, mmol/L	1.17 (0.69)	-0.11 (-0.20, 0.01)	1.07 (0.73)	-0.08 (-0.17, 0.02)	0.03 (-0.11, 0.16)
Non-HDL, mmol/L	3.92 ± 1.16	0.11 (-0.04, 0.26)	4.00 ± 0.98	-0.11 (-0.25, 0.03	-0.22 (-0.42, -0.01)[4]
LDL, mmol/L	3.63 ± 1.16	0.15 (0.01, 0.30)	3.74 ± 0.91	-0.09 (-0.23, 0.05)	-0.25 (-0.45, -0.04)[4]
HDL, mmol/L	1.61 ± 0.45	0.04 (-0.04, 0.11)	1.66 ± 0.51	-0.04 (-0.11, -0.03)	-0.08 (-0.18, 0.03)
TC:HDL	3.45 ± 0.91	-0.04 (-0.15, 0.07)	3.47 ± 1.01	-0.03 (-0.14, 0.07)	0.00 (-0.15, 0.16)

Values of change and main comparison of the changes between groups are presented as mean (95% CI) generated from estimated marginal means from ANCOVA.

[1]Not all data were analysed due to technical problems and sample loss.

BMI: n = 45 (control) and 50 (almond).

WC: n = 49 (control) and 51 (almond).

Body fat: n = 49 (control) and 52 (almond).

TC, TAG, Non-HDL, LDL, HDL and TC:HDL: n = 49 (control) and 53 (almond).

[2]Median (IQR) for TAG data as they are non-normally distributed. Mean ± SD for other data that are normally distributed. Baseline biomarker values were not different between the two groups.

[3]ANCOVA, adjusted for baseline outcome value and baseline BMI (mean difference in change from baseline, almonds minus control); $P < 0.05$ indicating a significant difference.

[4]$p<0.05$ indicating a significant difference for values of mean difference between two groups.

BMI, body mass index; WC, waist circumference; cSBP, clinical systolic blood pressure; cDBP, clinical diastolic blood pressure; TC, total cholesterol; TAG, triglycerides; LDL, low-density lipoprotein cholesterol; HDL, high-density lipoprotein cholesterol.

Chapter 7: Discussion and conclusion

This chapter discusses strengths and limitations of the research conducted and suggests directions for future research.

7.1 General discussion

This thesis aimed to investigate:

1) The pattern of tree nut and almond consumption in the UK adult population and their association with CVD risk measures and diet quality.
2) The cardiometabolic effects of consuming 20% energy from almonds in replacement for typical UK snacks high in SFA, starch and sugars and low in dietary fibre, in adults.

The key findings from this body of research were:

1) The prevalence of tree nut, including almond, consumption in the UK adult population was low (median 6.5 g/d for tree nuts and 5 g/d for almonds), but tree nut and almond consumers had a better diet quality and more favourable CVD risk measures, indicating that nut consumption was a marker of a healthier overall eating pattern (*Chapter 3 and 4*).
2) The ATTIS study, a RCT, was conducted to determine causality. Snacking with whole almonds for 20% daily EI as a replacement of typical snacks for 6 weeks improved endothelial function, increased HRV and lowered LDL. Therefore, a simple dietary swap could be effective in reducing CVD risk (*Chapter 5 and 6*).

Having shown that tree nut and almond consumption is associated with better diet quality and reduced CVD risk, swapping typical snacks with whole almonds is a simple dietary strategy to lower cardiometabolic risk but the UK adult population does not consume

enough tree nuts or almonds, relative to the dose suggested by the US FDA to gain the cardiovascular health benefits (Taylor, 2003). What are the barriers to increased consumption of tree nuts and almonds? And how can consumers be encouraged to purchase more, to gain the cardiometabolic benefits? These following sections discuss the potential barriers and strategies to improve nut consumption with focuses on food preference, environmental impacts and affordability.

7.1.1 Research implications

Following industrial revolutions, since the 20th century, people have had better purchasing power, enabling them to nourish and expect longer and more productive lifespan. However, it has also created environmental pollution, sedentary lifestyles and radical dietary shifts wherein traditional eating patterns are superseded by a Western diet high in animal products and refined carbohydrates and low in whole grains, fruits, and vegetables (Hu, 2008). Globalisation also aggravates workplace effort-reward imbalances, job insecurity, and long working hours, inducing psychosocial stress (Hu, 2008), resulting in a greater likelihood of seeking reward by consuming more high-sugary and fatty foods. These combined diet and lifestyle changes have contributed to increment in diet related ill-health arising from increased rate of chronic diseases that are developed over ageing, including overweight, obesity, T2D and CVD.

Food-based dietary guidelines provide dietary strategies to prevent the diseases (Health Canada, 2019; National Health and Medical Research Council, 2013; Public Health England, 2016; U.S. Department of Health and Human Services and U.S. Department of Agriculture, 2015) focusing on the consumption of more plant-based foods rich in unsaturated fats and dietary fibre. Replacement of SFA with unsaturated fats and

consumption of more dietary fibre and less free sugars are encouraged. However, the adherence to dietary guidelines is still low across the globe (Springmann *et al.*, 2020), indicating that modifying diet and lifestyle is challenging. Therefore, simple dietary strategies are required to improve diet-related diseases.

Tackling poor adherence to dietary guidelines by changing snack foods: a simple strategy for better cardiometabolic health.

The average diet consumed by the current population still comprises a higher level of free sugars, SFA and sodium (Bates *et al.*, 2019), relative to values recommended by the dietary guidelines (Springmann *et al.*, 2020). As diets high in refined starch, sugars and SFA and low in dietary fibre are linked to increased risk of many chronic diseases, it is important to develop simple dietary strategies to modify the diet to a healthier profile. Snacking, accounting for a large proportion of EI (19-22% TEI), is considered to be a convenient target of dietary modification.

Tree nuts, commonly eaten as snacks, are a nutrient-dense food high in unsaturated fats, dietary fibre, micronutrients, phytochemicals and phytosterols that have been shown to be cardioprotective. Literature has demonstrated that tree nuts have capacity in lowering blood glucose, TC and LDL concentrations and helping with body weight management (Del Gobbo *et al.*, 2015; Viguiliouk *et al.*, 2014; Wien *et al.*, 2003). Nuts are also included as plant-based protein source option in healthy eating patterns suggested by dietary guidelines (Public Health England, 2016; U.S. Department of Health and Human Services and U.S. Department of Agriculture, 2015; WHO, 2018). Out of all types of tree nuts, almonds have the highest market volume (International Nut & Dried Fruit, 2019), thereby

investigating further into the impacts of almond consumption on cardiovascular health is considered to be of importance.

The cross-sectional analysis within this doctoral programme revealed that tree nut and almond consumption was associated with lowered CVD risk markers, such as BMI, central adiposity measures, and BP, and better dietary determinants of CVD risk, i.e. lower free sugars, SFA and sodium, as well as higher MUFA, PUFA and fibre in the UK adult population, although the prevalence of consumption was low. Tree nut snack consumers only made up 11% of the population with an average intake 6.5 g/d, and whole almond snack consumers constituted an even smaller proportion of the total adult population, i.e. 5%; this consumer group consumed almonds 5 g/d which equalled to 5 seeds of whole almonds. This indicates that the health benefits observed in these observational studies are related to an overall healthy eating pattern of the nut consumers. Indeed, nut consumers had better diet quality and more favourable overall nutrient intakes relative to non-consumers.

The RCT conducted in this doctoral programme, the ATTIS study, as well as other previous studies demonstrated that increasing amount of whole almond consumed daily could reduce LDL-cholesterol, a risk marker of CVD. The ATTIS study has additionally showed increment in endothelial function and HRV, other markers of CVD, in healthy UK adults with moderate risk of developing CVD through snacking on whole almonds for 6 weeks for 20% EER as displacement of isocaloric typical snack that are high in saturated fats and added sugars and low in fibre. Furthermore, another considerably meaningful finding of the ATTIS study is that snacking on whole almonds may increase the resilience of cardiac autonomic function during acute stress, which has a particular

resonance for the current modern globalised society wherein chronic psychosocial stress is likely to have a major impact on risk of CVD.

All in all, the results of this series of studies strengthens existing evidence of tree nuts being recommended as a healthier snack food choice and provide insights for public health practitioner.

Tree nuts and almonds are plant-based foods beneficial for cardiovascular health, but are there any other factors influencing consumers' preferences related to the consumption?

Considering that tree nuts are one of the components of healthy eating patterns based on dietary guidelines and the increased consumption beyond current intake has showed beneficial health effects, what barriers prevent higher intake?

Tree nuts are a component of plant-based diet which is aligned with the EAT-Lancet advocacy on healthy diet (reducing chronic disease risks and optimising human wellbeing) within planetary boundaries (improved sustainability of agricultural practices) (EAT, 2019). Recently, plant-based diets are rising in popularity, particularly in industrialised countries. As revealed by Mintel through a survey of 1040 British adults, this phenomenon is driven by the concern on health, body weight, animal welfare and the environment (see **Figure 7.1**) (Jones, 2020). Therefore, the prevalence of tree nut and almond consumption may be influenced by these factors.

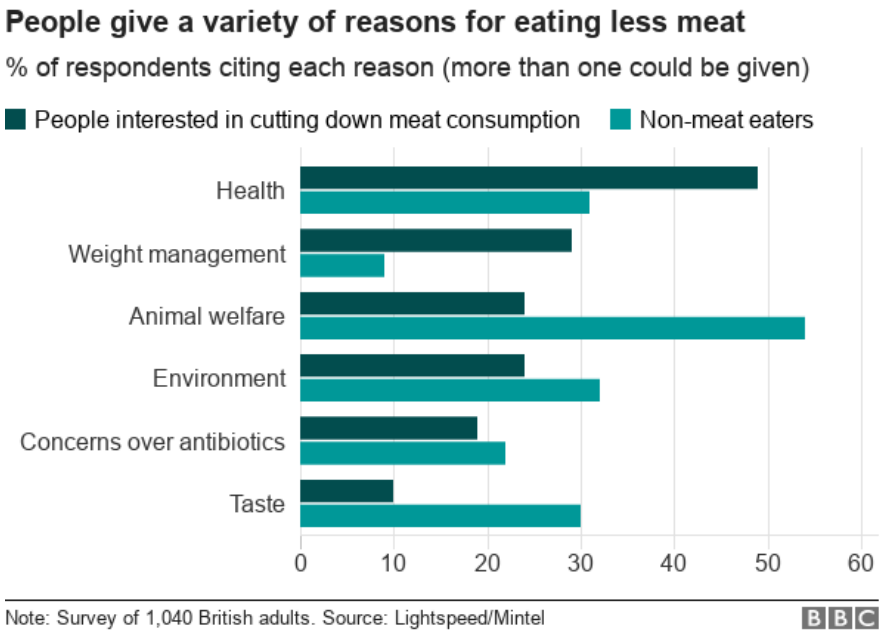


Figure 7.1. Survey results on public reasoning regarding eating less meat (Jones, 2020).

Almonds suit the context of health, weight management and environment. As already discussed, almond consumption has been demonstrated to positively impact cardiovascular health. Evidence also suggests that almonds may support weight management (Wien, 2003). Furthermore, plant-based food has been reported as more environmentally friendly compared to animal-sourced food. The carbon and water footprint produced by plant-based food is lower relative to animal-based food, especially livestock (Audsley *et al.*, 2009; Baroni *et al.*, 2007; Carlsson-Kanyama A & González AD, 2009; Fulton *et al.*, 2019; Poore & Nemecek, 2018). However, there may be issues with land use related to the world supply and as pollinators of almonds, the welfare of honeybees. Some approaches have been undertaken to mitigate this issue as discussed later below. In addition to these four top factors, tree nuts in particular are expensive in terms of affordability/price at individual consumer level.

Regarding the environmental impacts, snacking with whole almonds produces lower carbon footprint by more than 50% relative to the most typical snacks consumed in the UK adult populations, i.e. potato crisps and biscuits (Bord Bia – Irish Food Board, 2018) which are high in saturated fats and added sugars and low in fibre (see **Table 7.1**). Almonds have also been reported to have lower water print relative to livestock (Fulton *et al.*, 2019).

Although almonds have been reported to have lower carbon emission value and water use, a large amount of land is required to grow almonds. Furthermore, pesticides applied to almond orchards may cause malformed pollinating bee broods or kill the adult bees (Wade *et al.*, 2019).

Table 7.1. Comparison of carbon footprint generated by whole almonds, potato crisps and biscuits

	Energy/100g, kcal[1]	g/400 kcal[2]	kg CO_2-eq/kg	kg CO_2-eq/400 kcal
Nonpareil California dry-roasted whole almonds	634.0	63.00	0.88	0.05
Walkers ready salted crisps (one of the UK's largest crisps brand (BBC, 2017))	526.2	76.02	2.24	0.17
McVities milk chocolate digestive biscuits (the UK's most consumed biscuit (Statista, 2019)	497.0	80.48	1.39	0.11

[1]Based on the list of nutrient composition published by Almond Board of California (Almond Board of California, 2017), Walkers (Walkers, 2020) and McVitie's (Tesco, 2020).
[2]Calculated values based on an individual with 2000 kcal daily EER who snacks 20% EER (400 kcal).

As for the land use issue, tree nuts are low-yielding crops cultivated in specific climate in a few key regions, thereby in a long term as the world's population continues growing,

larger areas of orchards are required to produce more nuts and meet the demands, in the condition wherein people who are allergic to nuts are excluded. If 0.5% of the world's adult population is assumed to have this allergy (Stiefel, 2017), in the future scenario in 2050 where the world's total population would approximately consist of 10 billions of people as projected by EAT-Lancet, there would be 9.95 billions of people are to be fed. To cover recommended nut intake (25 g/d) of projected future adult population, i.e. 6.50 billions of people, within a condition where the global growth of adult proportion is constant (currently the proportion makes up 65.3% (World Bank, 2019)), an annual production would require 59.3 million tonnes. The total worldwide nut production was 4.4 million tonnes in 2018/2019 from a total global harvested area of 12.7 million hectares and compared to last year the production increased for 5% (International Nut & Dried Fruit, 2019). Assuming the annual production growth would be the same, 5%, in 2050, the total global production of tree nuts would be 20 million tonnes, leading to a gap of 39.3 million tonnes of nuts in meeting the scenario global demand of adult population, and 300% farming land expansion would be required.

This climatic dependence and land-use context are suggested to partly cause high market price of almonds. The production cannot keep up with rising demands as the population is growing creating a supply-demand imbalance and a higher equilibrium price. Compared with typical snacks, almonds are expensive (Bowes, 2014).

Taking into account these health and environmental impacts and affordability, how can we increase the overall consumption of tree nuts to bring the impacts to the real population scale as an attempt to lower the prevalence of CVD risk?

Tree nuts as a component of plant-based diet are beneficial for cardiovascular health and are environmentally 'friendly'. However, when consumers make buying decision on food, they need to balance the nutritional benefits and environmental impacts alongside affordability.

Some efforts have been made to address the issues on the water issue and bee welfare that might give more positive perceptions to consumer. Researchers and almond growers for the past 20 years have put efforts to improve the efficiency of water usage by 33% with advanced water-saving micro-irrigation (Almond Board of California, 2014). Honey Bee Best Management Practices have also been developed (Almond Board of California, 2018). Livestock farming in key producing regions might be targeted to be reduced in the future to increase almond supply since livestock products have a lot more carbon and water footprint and land use (Poore & Nemecek, 2018). For example, in California – the world's largest almond producing area, livestock and dairy farming is also a great-sized business. However, this solution might induce a trade-off related to nutritional, environmental and socio-economic values for the government. This land conversion between farming types might be alignment with the EAT-Lancet dietary shift scheme to achieve healthy diet transformation by 2050 in which global consumption of fruits, vegetables, nuts and legumes will have to double and consumption of red meat and sugar will have to be reduced by more than 50% (EAT, 2019), contingent upon more efficient water use and more effective management of pesticide use related to honey bees.

Another potential barrier for greater tree nut consumption is the high price at the markets. Expanding taxation on sugar reduction that is not limited to sugary beverages only and imposing taxation on other unhealthy foods such as fast foods notably high in SFA can provide more income to the country and this budget can then be used to subsidise healthy foods, thereby helping narrow down the price gap between tree nuts and typical snacks (Blakely *et al.*, 2020; Carter *et al.*, 2019; Cobiac *et al.*, 2017; Lee *et al.*, 2019; Peñalvo *et al.*, 2017; Schönbach *et al*., 2019)

Despite its long-term benefits in encouraging people to eat more healthy foods, the whole process of policy making from formulation to implementation has to consider a lot of factors and therefore is a slow-moving process. Ultimately, consumers decision-making at present will also require the involvement of other values, such as needs, other relevant information related to the product, and alternatives.

Consumers decision-making behaviour is driven by a combination of Systems 1 (fast, automatic, frequent, emotional, stereotypic, unconscious) and Systems 2 (slow, effortful, infrequent, logical, calculating, conscious) thinking based on psychology and behavioural economics research (Kahneman, 2011). Given the high fat content, tree nuts were considered unhealthy (Ferdman, 2016; Pawlak *et al.*, 2017). However, this perception has altered over the last decade due to the rising popularity of plant-based diet (Ferdman, 2016) and more studies revealing the health benefits of tree nuts. These positive impacts on health have also been largely informed. Since tree nuts are now still expensive, decision-making on purchasing tree nuts is likely to be built through rationale (Systems 2) that they are nutritious, healthier and less damaging to the environment. To substantially increase tree nut consumption, there should be more health-conscious

consumers who have fast and automatic decision-making (System 1). Therefore, more effective methods to communicate the cause-and-effect of CVD, the importance of healthy diet as well as nutritional and environmental values of almonds are required to increase the consumption. Inclusion of technological based public engagement in this digital era can create health awareness culture, reduce the prevalence of CVD risk in the population and national health burden as well as boost productivity and national economy.

In summary, the prevalence of tree nut or almond consumption in the UK adult population is low. Higher daily intake of almonds shows CVD risk reduction through decreased LDL concentrations and improved endothelial function and HRV. However, there are still barriers preventing consumers from having higher intake of nuts, including affordability and the impacts on ecosystem. Some efforts have been made in the context of environmental impacts, but there should be policies that can help reduce the price. Furthermore, consumers are encouraged to have higher level of health consciousness so that they will choose healthy food options, including almonds as daily snacks.

7.2 Strengths and limitations

This thesis has several strengths worth noting with some limitations to consider. As for cross-sectional analysis, the strengths and limitations have been fully discussed in Chapters 3 and 4. However, it is important to add that there was no variable for physical activity level (PAL) in the NDNS database and therefore analyses were not adjusted for any confounding effects of habitual physical activity. Previous studies reported that physical activity was associated with better dietary pattern (Charreire *et al.*, 2011; Jezewska-Zychowicz *et al.*, 2018). Mediterranean Diet which includes nuts as one of the

components was also shown to be associated with more physical activity relative to Western-style dietary pattern (Bibiloni *et al.*, 2017).

Regarding the RCT (Chapters 5 and 6), subjects were at moderate risk of developing CVD allowing scope for improvements in markers of cardiometabolic health. The RCT was designed specifically to study the impact of snack displacement leading to the development of specific control snacks that reflected the nutrient profile of average snacks consumed in the UK adult population based on the NDNS data. This control snack design thereby did not cause any exaggeration of the treatment effect due to worsening outcomes for the control group. Furthermore, novel key physiological determinants of CVD, endothelial function and liver fat concentrations were measured using the most accurate methodology currently available. However, a limitation of the study was that subjects included in the subset of MRI/^{1}H-MRS analysis had low levels of liver fat at baseline. This did not allow much room for improvement following the study treatments. The quantifications of pancreatic fat could also be conducted using ^{1}H-MRS as an additional method for considerably more accuracy (Hu, *et al.*, 2010). Furthermore, there is an imbalance in number between male and female subjects that might render treatment effects less representative of all genders.

7.3 Future directions

Almonds have shown to be cardioprotective and help reduce the development of CVD in adults at moderate risk of developing CVD. Future cross-sectional analyses should include investigations to determine whether associations are independent of PAL. Using the NDNS database, PAL can be calculated and interpreted through other variables that

were obtained from questionnaires during the survey although the process of generating PAL can take significant amount of time.

Future RCTs should recruit participants with different subject characteristics, such as healthy individuals, T2D or NAFLD patients to understand the health impacts of almond consumption in a wide range of population. Recruiting participants with fatty liver (steatosis; ≥5% baseline liver fat) in order to show proof of principle effect is also suggested for future research. Furthermore, more stool samples should be collected to give more meaningful findings on SCFA production. It would also enable the examination of gut microbiota composition, allowing more investigations on the effects of almond consumption on gut health.

7.4 Conclusion and final remarks

Tree nuts including almonds, widely eaten as snacks, contain dietary components which have been shown to be cardioprotective. Tree nut and almond consumption in the cross-sectional analysis UK adult population was low but associated with better diet quality and more favourable levels of traditional CVD risk markers (lower BMI, WC and BP, and higher HDL in tree nut including almond consumers; lower BMI and WC in specifically almond consumers, relative to non-consumers). To examine the effects of replacing typically consumed snacks with almonds, the ATTIS study was conducted. It revealed that snacking whole almonds for 20% EER for 6 weeks in UK adults at moderate risk of developing CVD as replacement of typical snacks high in SFA, starch and free sugars and low in dietary fibres, improved endothelial function, a novel determinant of atherosclerosis, lowered LDL, and augmented parasympathetic activity during night-time and mental stress task, benefitting the prevention of cardiac arrythmia.

In summary, these findings show that a simple dietary swap of typically consumed UK snacks for tree nuts can help reduce CVD risk. Promoting this dietary substitution would also address the issue of plant-based diets on ensuring good health and wellbeing while protecting the environment.

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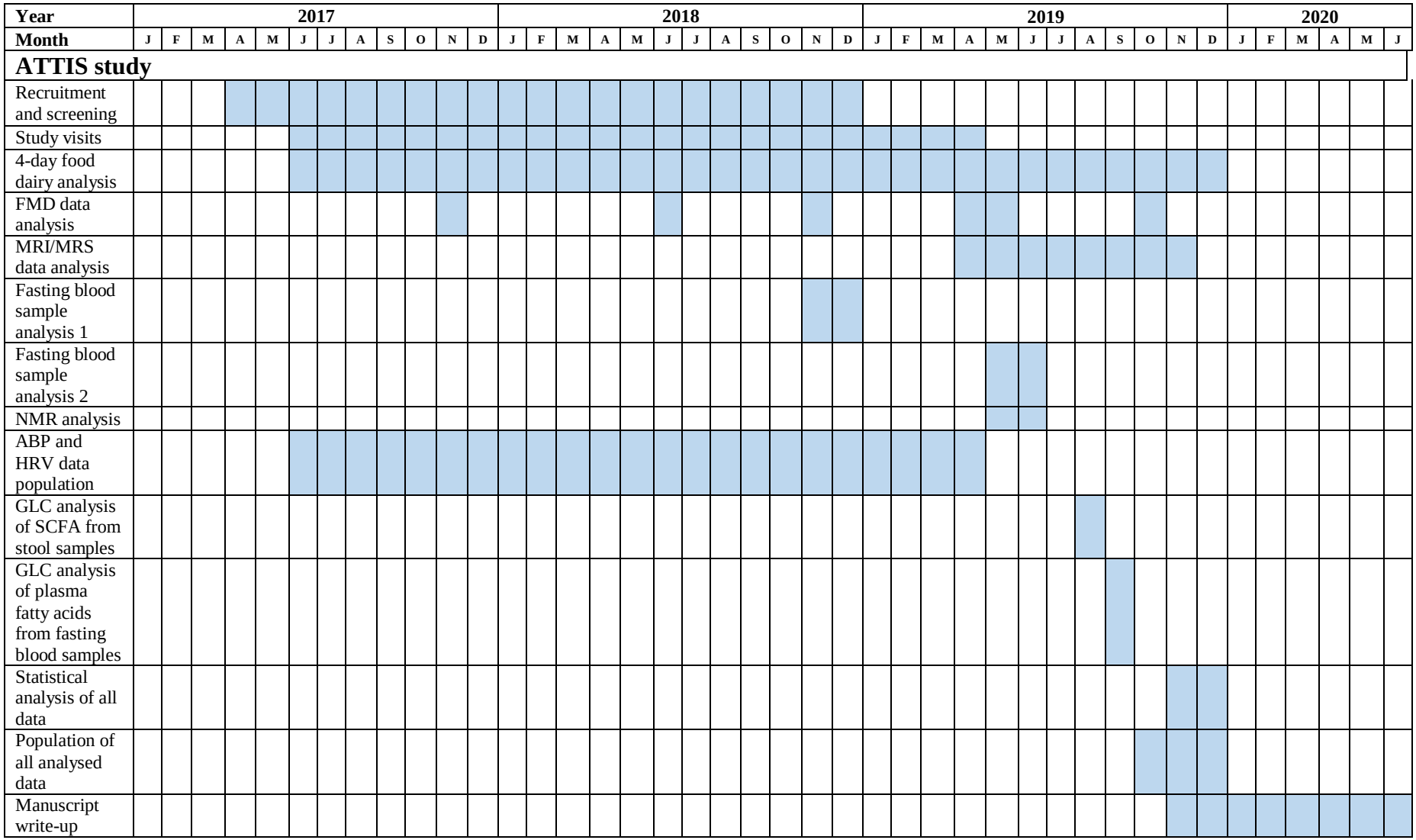
Appendices

Appendix 1: Timelines

Year	2016						2017												2018												2019												2020					
Month	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J
Observational study: Tree nuts																																																
Understanding the design of cross-sectional analysis																																																
Cross-sectional data isolation & preparation from the UK NDNS 2008-2014																																																
Statistical training and advice from statisticians																																																
Statistical analysis																																																
Result data population																																																
Manuscript write-up, submission, peer-review and publication																																																

Year	2016						2017												2018												2019												2020					
Month	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J
Observational study: Almonds																																																
Market sampling of almond proportion in mixed nut products/mixed nut and fruit products																																															
Data isolation and preparation for cross-sectional analysis from the UK NDNS 2008-2014 and 2008-2017																																															
Statistical analysis																																															
Result data population																																															
Manuscript write-up, submission, peer-review and publication																																															

Year	2016						2017												2018												2019											
Month	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D
ATTIS study (preparation)																																										
Prepare and obtain ethical approval and NHS R&D approval																																									
Produce study protocol and SOPs																																									
Control meal development																																									
Control meal production																																									
Preparation of feasibility & acceptability study of control meals																																									
Recruitment & screening of feasibility and acceptability study participants																																									
Feasibility & acceptability study visits																																									
Fasting blood sample analysis from feasibility & acceptability study																																									
Training on FMD																																									


Year
2017
2018
2019
2020
Month
J F M A M J J A S O N D J F M A M J J A S O N D J F M A M J J A S O N D J F M A M J
ATTIS study
Recruitment and screening
Study visits
4-day food dairy analysis
FMD data analysis
MRI/MRS data analysis
Fasting blood sample analysis 1
Fasting blood sample analysis 2
NMR analysis
ABP and HRV data population
GLC analysis of SCFA from stool samples
GLC analysis of plasma fatty acids from fasting blood samples
Statistical analysis of all data
Population of all analysed data
Manuscript write-up

Appendix 2: Acceptability questionnaire for participants of feasibility/acceptability study

Participant code ☐

Date form completed ☐

Acceptability and Use Questionnaire

Purpose: This interviewer-administered form is used to collect participant's acceptability and the product (mini muffins) use feedback.

General information/Instructions: Please select one option for each question.

We would like to ask you some questions about your attitudes and experiences consuming the mini muffins as a daily snack. Your honest answers will help us to investigate 'The impact of muffin snacks versus almond nut consumption on emerging markers of cardiovascular and metabolic disease' for future studies.

All the information will be kept confidential and will not be shared with anyone else besides the research study staff.

1. Rate how well you liked the overall flavour of the mini muffins?

☐ 1 I like it

☐ 2 Neither like nor dislike

☐ 3 Dislike

2. How much did you like the following features of the mini muffins?

	Like 1	**Neither like nor dislike 2**	**Dislike 3**
a. Colour			
b. Shape			
c. Texture			
d. Portion size			
e. Mouth feel			

3. **Did you like the packaging of the mini muffins?**

☐ 1 Yes
☐ 2 No
☐ 3 Somewhat

4. **Rate the portion size of the muffins as an everyday snack?**

☐ 1 Too little
☐ 2 Enough
☐ 3 More than enough

5. **How often did you have problems storing the bag of mini muffins at home?**

☐ 1 Never
☐ 2 Sometimes
☐ 3 Often

6. **How difficult or easy was it to incorporate these mini muffins in your diet?**

☐ 1 Very difficult
☐ 2 Difficult
☐ 3 Easy
☐ 4 Very easy

7. **Did you have any of the following problems after eating the mini muffins?**

	Yes	**A little**	**None**
a. Reflux/ nausea			
b. Burps/belching/bloating			
c. Severe indigestion			

8. **Rate your willingness to continue eating these mini muffins as a snack?**

☐ 1 Not at all willing
☐ 2 Unsure
☐ 3 Extremely willing

9. How often would you purchase these muffins as an alternative to your regular snacks if available in the market?

☐ 1 Never

☐ 2 Sometimes

☐ 3 Often

10. Any specific feature you like (specify)

_____

_____

_____

_____

_____

_____

11. Any specific feature you dislike (specify)

_____

_____

_____

_____

_____

12. Do you have any suggestions for enhancement or improvement of any feature of the mini muffins?

_____

_____

_____

_____

_____

_____

_____

Appendix 3: Participant information sheet of ATTIS study

Guy's and St Thomas' NHS
NHS Foundation Trust

KING'S College LONDON

Version Number 4.0: 21/04/2017
REC: 16/LO/1910
IRAS Project number: 212398

Appendix 12 – Participant information sheet

INFORMATION SHEET FOR PARTICIPANTS

REC Reference Number:16/LO/1910

YOU WILL BE GIVEN A COPY OF THIS INFORMATION SHEET

Short project title
The impact of almond nut consumption on markers of cardiovascular and metabolic disease

Full project title
A randomised, controlled parallel dietary intervention study to investigate the effect of almond snack consumption on cardio-metabolic disease risk markers compared with isocaloric snacks, in adults at moderate risk of cardiovascular disease.

Project background
The ill-health from heart disease and diabetes is increasing year by year. These diseases are extremely costly for the country and negatively influence the quality of life and national economies. Certain risk factors such as an increase in fat in the liver, heart and organs (known as ectopic fat) and poor blood sugar control (known as insulin resistance) can increase the chances of developing heart disease and diabetes.

Snack choice can improve diet quality and is one area of diet and lifestyle modification which can potentially reduce these risk factors and therefore reduce the chances of developing heart disease and diabetes.

Previous research has shown that almonds have reduced some risk factors for heart disease when consumed at 50-60g per day. Almonds are higher in protein, fat and vitamins and minerals but lower in carbohydrates than typical UK snacks. This research will help the researchers understand the effects of eating almond nuts as daily snack and will form part of undergraduate and postgraduate degrees.

What's involved?

This following study aims to investigate the health effects of consuming almond nuts as a replacement of regular snack products in adults at moderate risk of developing heart disease.

This project will be a dietary intervention study that will take place at King's College London (KCL) Waterloo Campus as well as Guys and St Thomas' hospitals. The participation in this study is through a series of visits. There will be an initial health screening questionnaire to complete via email or over the phone, consent will be taken prior to pre-screening processes. If this shows that you are eligible and you are willing to participate, you will be invited to a screening assessment (visit 1) and asked to sign a consent form. If based on the screening assessment results you are a suitable subject, you will be invited to attend the research facility at the start of the 2-week run-in (visit 2). In total, there will be six visits, including the screening session, separated by two-week intervals. Below you will find the details of the visits. Measurements will be taken at each visit. The total study duration will be 8 weeks, comprising 6-week intervention arms with a 2-week run-in period.

You will be asked to consume 20% of your energy requirements as snacks for 6 weeks. You will be randomly allocated (i.e. have no control) to eat either almonds (provided by a wholesaler recommended by our sponsors) or muffins and crackers (developed for the study by the study Dietitian and research team and baked within the KCL dietetic kitchen) for the 6 weeks of the study. The snacks that you receive will be energy (calorie) matched/ of equal energy value to other participants and allows us to compare the difference between the snacks.

The main outcome we will measure are major risk factors for heart disease and type 2 diabetes, this includes blood vessel function (measured by ultrasound of the blood vessels in the arm using a technique called flow-mediated dilatation (FMD)) and liver fat (measured by magnetic resonance imaging (MRI)). The other measures we will look at are body weight/composition, pancreatic, muscle and visceral fat, blood lipids, parameters of glucose control, signalling messengers produced by the fat stores in the body, faecal fats, 24 h heart rate variability (HRV; measured from a small device stuck onto the skin of the chest which takes constant measures of the heart rate over a 24 hr period) and 24 h ambulatory blood pressure (ABP; blood pressure measured at regular intervals over a 24 hr period from a small portable machine fitted to the upper arm).

Am I eligible? Participant criteria:

To participate in this study, you must:

- be between 30-70 years who are at above average risk for developing CVD and regularly consume ≥2 snack products a day
- be willing to follow the protocol and/or give informed consent
- have a stable body weight (weight gain or loss <3 kg in the past two months) and 18 kg/m^2< BMI (Body Mass Index) < 40 kg/m^2
- have no allergy to nuts and seeds
- have no presence of glucosuria (glucose in urine), proteinuria (protein in urine) or anaemia (low red blood cell count)
- not be taking medication or a prescribed diet known to affect lipid or glucose metabolism
- not have smoked cigarettes, cigars, or pipes within the last 6 months
- have no history of myocardial infarction or cancer, black-outs/epilepsy, diabetes mellitus, on-going coronary, kidney, liver, pancreatic, bowel and gastrointestinal disease
- have no history of abuse of drugs and/or alcohol
- not be pregnant or breast-feeding nor planning pregnancy within the study period
- have no presence of metal inside the body (implants, devices, shrapnel, metal particles in eyes from welding etc.)

During the study participation, you will be advised to not make any lifestyle changes, including not changing your daily calorie intake or diet. If you become pregnant or start smoking, or your health deteriorates in any way, you have to let us know, as it may not be appropriate for you participate.

If you are willing to participate, you will receive this Participant Information Sheet to ensure you fully understand what will be required of you so that you can ask any questions regarding the study. We will send you a pre-screening health and eating habits questionnaire to assess your initial eligibility into the study, prior to the screening visit.On these forms we will ask for your contact details and contact details for your GP. If you are initially eligible, we will invite you to attend a screening session where the study is explained to you and you can ask question freely so that you are sure you can provide fully informed consent.

Preparations for every study visit

The day prior to each visit and on the study day, you will be asked to refrain from any vigorous physical exercise or sporting activities (e.g. a race, cycling or the gym) and avoid eating food high in fat (e.g. Pizza or ice-cream) and drinking alcohol (e.g. wine or beer) and caffeine-containing drinks (tea, coffee, cola). To stabilize your health markers before each study visit, it is important that you try to eat at the same time and approximately the same type of low fat dinner on the day before the study day.

You will also be required to fast overnight (you will not eat and drink anything, except water, after 10 pm the evening before the first study day.)

The study visits will take place in the following places:

The Metabolic Research Unit (MRU) on the 4th Floor, Corridor A, Franklin-Wilkins Building - KCL, 150 Stamford Street, London, SE1 9NH (close to Waterloo Station).

The Clinical research Facility (CRF) on the 4th floor, North Wing, St Thomas' Hospital, Westminster Bridge Road, London, SE1 7EH (close to Westminster Station)

And on some very occasional instances: Guy's hospital MRI on the 2nd Floor, Tower Wing, Guys Hospital, Great Maze Pond, London SE1 9RT (close to London Bridge station).

Please see study visits below for details of where each visit will occur and what will happen at each of the visits:

First visit (Screening visit at MRU, near Waterloo)

You will be given the opportunity to ask questions before being guided through the consent form. The consent form asks you to give permission for the measurements of the study. This study includes the analysis of stool samples from some of participants. Therefore, when you are about to sign the consent form, we will also discuss the stool collections (2 samples) and **if you are willing** to make stool collection the relevant box on the consent form will be ticked. If you would prefer not to, you will still be able to participate the study anyway.

Following the signing of the consent form, we will measure your blood pressure (BP) and body composition your weight, height, waist circumference and % body fat (measured by bioelectrical impedance which involves standing bare foot on some scales and holding some 'handles' The scales will pass a very small, harmless electrical current through your body to analyse your body fat and body water. It takes just 2 minutes to complete and you will not feel anything). We will also take a fasting blood samples to check your biochemistry is within the normal ranges (approx..16.5ml/3 teaspoons)

A clinician will assess you against the inclusion/exclusion criteria and 'fitness'.

If you are suitable based on the assessment, you will be informed by telephone or email to attend the second visit (week 0 of study). You will also receive a 4-day food diaryand a physical activity questionnaire that you will fill out each day for 4 days in a row before your second visit as well as individualized dietary advice and a dietary handbook detailing the study outline and dietary modifications.

Visit 2 (Week 0 – run-in, MRU, Waterloo)
We will collect your filled 4-day food diary and physical questionnaire. Then, we will measure your BP and body composition (weight, waist circumference and % body fat). We will also give you snack products that you will consume every day for two weeks until the second visit. When giving you the products, you will receive verbal and written dietary advice on how to incorporate them into your diet from us.

Before you leave, you will receive a biohazard bag and equipment to collect your stool sample (to be collected at home on the morning of the next study visit (if you are willing) or during your visit to the CRF. You will be provided with verbal and written instructions if you have consented to stool sample collection. You will also receive a 4-day food diary that you will fill out each day for 4 days in a row before your visit and snack products to be consumed every day until your next visit.

Visit 3 (Week 2 – baseline measurements, CRF St Thomas' hospital)
Your general well-being will be checked and then we will measure your BP and body composition (weight, waist circumference and % body fat). Your fasting blood samples will be taken (27ml/ 5 teaspoons).

We will also measure your 24 h HRV and 24 hour ABP. You will be fitted with these non-invasive monitors and asked to wear them for 24 hours whilst carrying on your normal daily activities. They will record your blood pressure and heart rate variability over a 24 hour period. You will be given verbal instructions on the use of these monitors and will be asked to turn off the ABP and HRV monitors after a 24 hour period. You will be asked to complete a 'diary card' during the time that the measurements are being made so we can monitor your activities during this time. We will ask that monitors be returned the next day by courier which we will arrange (City Sprint, pre paid) or that you bring them to the next study visit.

You will also get a flow mediated dilation (FMD) test which will involve being fitted with an arm cuff which is inflated (like a blood pressure cuff) and the use of a non-invasive ultrasound scan. You will also be asked to have a substance called 'glycerol trinitrate' which opens your blood vessels and helps with the scan. This is safe to use and will be given under medical supervision. Your ultrasound videos will be digitised and stored under your participant ID number. This scan will take approx. 25 minutes. The results will be analysed by the researchers later in the study.

We will also measure you heart rate variability (HRV) while you are having the FMD tests in addition to your HRV during a short, mental stress test called 'Stroop'. This is a simple test widely used by psychological researchers. You will be asked to read a list of words for colours, but the words are printed in a colour different to the word itself. For example, the word "orange" would be listed as text, but printed in green. Your reading time of the words on the list is then recorded. Next, you will repeat the test with a new list of words, but should name the colours that the words are printed in. So, when the word "orange" is printed in green, you should say "green" and move on to the next word. While you are completing this test, we will also scan your artery as before but without inflating the cuff. We ask that you remain still during the procedure. It will last approx. 10 minutes.

Additionally, if you are willing and deemed eligible (based on a score calculated from a blood marker of liver function, blood fats, and waist circumference, measured during your screening visit) you will also have a MRI scan at this visit. MRI is a type of scan that uses strong magnetic fields and radio waves to produce detailed images of the inside of the body. For this study we will be using the scans to look at your liver, muscle and pancreatic fat. The MRI scanner is a large tube that contains powerful magnets and you will be asked to change

into a gown (to ensure you are not wearing any metal) and then asked to lie inside the MRI tube during the scan. As it can be very noisy in the scanner, you will be given some ear defenders to wear which can play music throughout the scan. The MRI scan will take place at St Thomas' hospital in the first instance but if there is nil scanner availability, you will travel via pre paid taxi, with one of the researchers to Guy's hospital. The MRI scan will take approximately 30 minutes to complete. The results will be stored under your participant code and analysed later in the study by the research team

Also, throughout the duration of this visit, participants (who have given consent to do so) will be asked to use the bathroom facilities at their leisure so that they can collect a stool sample (if they have not already collected a stool sample at home in the morning). Full verbal and written instructions on this will be given. You will be given disposable gloves and equipment to aid stool sample collection.

Before you leave the CRF (or Guy's hospital), we will provide you with breakfast. You will also receive snack products to be consumed every day until your fourth visit, as well as a 4-day food diary to complete.

Visit 4 (Week 4) MRU, Waterloo
We will interview you on 24 h dietary recalls via phone during this week.

On the study day, we will measure your BP and body composition (weight, waist circumference and % body fat).

You will receive snack products to be consumed every day until your fifth visit as well as a 4-day food diary.

Visit 5 (Week 6) MRU Waterloo
You will be interviewed on 24 h dietary recalls. We will collect your filled 4-day food diary and then measure your BP and body composition (weight, waist circumference and % body fat).We will also ask you to fill out a consumer acceptability questionnaire.

Before you leave, you will receive a biohazard bag to collect your stool sample (at home on morning of the next study visit (if you are willing), verbal and written instructions and equipment will be given). You will also receive a 4-day food diary that you will fill out each day for 4 days in a row before your last visit and snack products to be consumed every day until your last visit.

Visit 6/Last visit (Week 8 – end point) CRF, St Thomas'
You will complete the same measurements as visit 3.

Reimbursement
For your time and effort participating in the study, you will receive compensation of £100, in addition to up to £10 reimbursement per visit for your travel costs. These compensations will be given following attendance at the study visits. Reimbursement will be via direct bank transfer which is arranged by the King's College accounts. You will be asked to complete a single claim at the end of each study and provide receipts for travel costs. We will only be able to compensate you if you complete this form

Do I have to take part?

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Your participation in the study is entirely voluntary and you are free to withdraw from the study at any time without giving a reason. This will not affect the standard of care you receive in the future.

If you have already given informed consent, but you lose capacity during this study then you will be withdrawn from the study. Identifiable data collected with consent would be retained and used in the study. No further data would be collected or any other research procedures carried out on or in relation to the participant.

What are the possible benefits of taking part?
The potential health benefits of participation in this study are comprehensive biochemical screening (full blood count, full lipid count, liver fat, glucose and insulin) and blood pressure assessment. You will be provided with copies of your screening results upon completion of the study. If any of your results are found to be abnormal we will discuss these with you and send a copy of the results to your GP. Following completion of the study you can contact the study organisers to access the results of the study.

In addition to travel reimbursement and payment of £100, you will be provided with breakfast on screening and on the baseline and endpoint study days. Participants undergoing MRI scans will receive an additional £20 per MRI scan.

What are the possible disadvantages and risks of taking part?
The risk associated with the work is minimal but blood collection does include a very small risk of bruising. The MRI scan is safe as it is painless, non-invasive and does not involve radiation. However, the subject must remain still in an enclosed machine, which may be a problem for claustrophobic subjects. We underline the point that in the inclusion criteria, the subjects must not have metal inside the body (implants, devices, shrapnel, metal particles in eyes from welding etc.) as metal implants may be affected by the strong magnet of the MRI unit. FMD is also safe as it is non-invasive, faster and safer compared to other methods. There is no risk associated with the consumption of the snack products to be served, however participants must not have nut allergies and/or allergies to the snack product ingredients.

Data Protection
Subject confidentiality and anonymity will be observed throughout the study by use of subject codes in place of names, and the storage of subject details in a secure place, in accordance with the Data Protection Act (2018) and General Data Protection Regulation (GDPR) (EU) 2016/679. Your personal details will be kept in a locked filing unit with a further lock to access the room. Only the researchers will have access to this room and cupboard. GP letters (if abnormal results were found) will be kept on password protected computer documents. The researchers will not share your personal information with other research groups or individuals. Your information will not be kept for any longer than necessary (duration of the study and study analysis period) after which it will be securely discarded/destroyed.

What if there is a problem?
If you have a concern about any aspect of this study, you should ask to speak to the researchers who will do their best to answer your questions (Leanne Smith, Research Assistant for the study. Email: Leanne.smith@kcl.ac.uk Tel 20 7848 4301)

If you remain unhappy and wish to complain formally, you can do this through the Guy's and St Thomas' Patients Advice and Liaison Service (PALS) on 020 7188 8801,

pals@gstt.nhs.uk. The PALS team are based in the main entrance on the ground floor at St Thomas' Hospital and on the ground floor at Guy's Hospital in the Tower Wing.

In the event that something does go wrong and you are harmed during the research you may have grounds for legal action for compensation against Guy's and St Thomas' NHS Foundation Trust and/or King's College London but you may have to pay your legal costs. The normal National Health Service complaints mechanisms will still be available to you (if appropriate).

How will my information be kept confidential?
Subject confidentiality and anonymity will be observed throughout the study by use of ID numbers in place of names, and the storage of your personal details in a secure place. Only the investigators at KCL will have access to this data. Should you wish to find out the results of this study you are welcome to contact us (details below) for a copy of the final report once the study is finished.

Data cannot be withdrawn once the study has been submitted as a study report, which will be on the 1st November 2018

Who is organising and funding this study?
The researchers who are organising this study are Dr. Sarah Berry and Dr. Wendy Hall from King's College London. This study is funded by Almond Board of California.

What will happen if new information becomes available during the study?
The researchers are unaware of any new information that may emerge during the study, which might affect your participation, but should any relevant information be introduced during the study it will be conveyed directly to the research team, and if appropriate it will be explained to you.

Who has reviewed this study?
This study has been reviewed by the NHS Research Ethics Committee, Reference 16/LO/1910 to protect your safety, rights, wellbeing and dignity.

What will happen to the samples I give?
Blood samples: The blood samples will be used for analysis of glucose/blood sugar control, blood lipids (blood fats such as cholesterol) and body fat tissue chemical messengers. The samples will be stored at KCL in individually ID labelled tubes. They will be stored in a -70◦C freezer within the Franklin Wilkins Building at KCL until they are required for batch analysis. The analysis will be carried out under the supervision of investigators at KCL, in the laboratories at KCL and Kings College Hospital (KCH). All samples will be anonymised. Spare blood samples will be taken for all participants, to ensure that in the unlikely event of sample loss/damage or need for repeat assays that we have spare samples to allow us to have a complete set of results. This conforms to the good practice we use in all our studies. The spare blood samples will be stored in the -70◦C freezer (in the Franklin-Wilkins building at KCL) and may be used for additional analysis related to this study in the future. The samples will not be used for other studies.

Stool sample: The stool samples will be self-collected. You will collect these at home 1 hour prior to study visits 3 and 6 and bring them with you to the CRF or you may choose to collect the stool sample during your visit 3 and 6 and therefore you will use the toilet facilities at the CRF (stool samples need to be processed and frozen within 1-2 hours so need to be fresh).

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The stool sample will be initially processed in a dedicated laboratory in the Franklin Wilkins building at KCL and then stored under your participant ID in a dedicated -70◦C freezer until they are batch analysed in the laboratory at KCL.
The stool samples will be used for analysis of short chain fats and gut bacteria composition. Spare samples will be kept in the -70◦C freezer and used if further analysis is required related to this study.

The results from MRI scan
Liver fat content will be analysed based on the results from your MRI scan. The results will also be used for analysis of pancreatic, muscle and abdominal fat.

The results from vascular measurements: FMD, a marker of vascular health, will be analysed to assess changes in the function of the arteries.

Further information and contact details

Research Assistant
Leanne Smith
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Faculty of Life Sciences & Medicine
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150 Stamford Street, London, SE1 9NH
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Email: almondstudy@kcl.ac.uk

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150 Stamford Street, London, SE1 9NH
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Appendix 4: Pre-screening questionnaire of ATTIS study

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KING'S College LONDON

Guy's and St Thomas' NHS
NHS Foundation Trust

Appendix 3 – Pre-screening health questionnaire

Date form completed ☐

Recruitment/pre-screening questionnaire

Study title: The impact of almond nut consumption on markers of cardiovascular and metabolic disease
To be completed prior to attending screening session. To complete by potential participant via return email or over the phone.

Ask before starting the pre-screening questionnaire: "Do you consume 2 or more snacks between meals every day?"(If answer is no – EXCLUDE)

To be filled in by the investigator

Date .....

Name .....

Gender: M ☐
F ☐

Females: Have you been through the menopause? Y/N
When was your last period?
Researcher to categorise females: Pre ☐ Peri ☐ Post ☐
Address
.....
.....
.....
.....

Date of Birth .....

Age
.....
.....

Ethnicity
.....
.....

Phone Number: Day ..... Evening
.....

Best time to phone
.....

E-mail address
.....

Preferred way to be contacted: Phone ☐ E-mail☐

General Practitioner
.....
Of (medical practice)
.....

.....
.....

Health

We would now like to ask you some Health questions. If there are any questions you would prefer not to answer please let us know.

Are you pregnant, planning to become pregnant or breastfeeding?	YES ☐	NO ☐
Have you had a weight change of more than 3 kg (7lbs) in the past 2 months?	YES ☐	NO ☐
Do you smoke? Have you recently given up smoking? Yes/No	YES ☐	NO ☐
If you have given up smoking, how long ago was this?		
Do you drink alcohol?	YES ☐	NO ☐
If yes, how many units of alcohol would you consume in a typical week? ..... (1 unit = 1 measure of spirits / 1 small glass of wine / 1 half pint of beer)		
Do you currently take any vitamin, mineral or oil supplements?	YES ☐	NO ☐
If **Yes**, please give details.		
Are you willing to discontinue and pro/prebiotics, fibre/ oil/ vitamin supplements for duration of study?	YES ☐	NO ☐
If no, participant to be excluded.		
Do you know your Body Mass Index? BMI = ...		

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Preferred way to be contacted: Phone ☐ E-mail ☐

General Practitioner
.....
Of (medical practice)
.....

.....
.....

Health
We would now like to ask you some Health questions. If there are any questions you would prefer not to answer please let us know.

Are you pregnant, planning to become pregnant or breastfeeding?	YES ☐	NO ☐
Have you had a weight change of more than 3 kg (7lbs) in the past 2 months?	YES ☐	NO ☐
Do you smoke? Have you recently given up smoking? Yes/No	YES ☐	NO ☐
If you have given up smoking, how long ago was this?		
Do you drink alcohol?	YES ☐	NO ☐
If yes, how many units of alcohol would you consume in a typical week? ..... (1 unit = 1 measure of spirits / 1 small glass of wine / 1 half pint of beer)		
Do you currently take any vitamin, mineral or oil supplements?	YES ☐	NO ☐
If **Yes**, please give details.		
Are you willing to discontinue and pro/prebiotics, fibre/ oil/ vitamin supplements for duration of study?	YES ☐	NO ☐
If no, participant to be excluded.		
Do you know your Body Mass Index? BMI = ...		

Do you suffer from any food allergies? If **yes**, please give details	YES ☐	NO ☐
Do you have any nut allergies? Or ever suffered any allergic reaction to nuts? If **yes**, please give details	YES ☐	NO ☐
Have you taken part in any other research in the past 6 months? If **yes**, please give details. Please state amount of blood donated if applicable.	YES ☐	NO ☐

Investigator Signature.....
Date.....

Version 1.0, Dated 07/07/15

BDM/xx/xx-xx

Please return to:
Leanne Smith (Research Dietitian)
Room 4.46, Diabetes and Nutritional Sciences Division

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King's College London, Franklin Wilkins Building, 150 Stamford Street, London SE1 9NH
Email: almondstudy@kcl.ac.uk Tel: 020 7848 4301

Appendix 5: Consent form for participants of ATTIS study

Version Number 3 21/04/2017
REC 16/LO/1910
IRAS Project number: 212398

KING'S College LONDON Guy's and St Thomas' NHS NHS Foundation Trust

CONSENT FORM FOR PARTICIPANTS

Please complete this form after you have read the Information Sheet and/or listened to an explanation about the research.

Centre Number	King's College London
Study Number	
King's College Research Ethics Committee Ref	**16/LO/1910**
Participant Identification Number for this trial	
Title of Project	The impact of almond nut consumption on markers of cardiovascular and metabolic disease
Name of Researcher	Dr Sarah Berry and Dr Wendy Hall

Please initial box

1. I confirm that I have read the information sheet dated..... (version...) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected. ☐
3. I confirm that I meet all the inclusion criteria as specified in the Participant Information Sheet. ☐
4. 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 Data Protection Act (2018) and General Data Protection Regulation (EU) 2016/679. ☐

When completed: 1 for participant; 1 for researcher site file; 1 (original) to be kept in medical notes.

5. I agree to donate blood samples for the purpose of this study. ☐

6. I agree to donate faecal samples for the purpose of the study. ☐

7. I agree for my blood pressure, body composition and heart rate to be analysed for the purpose of this study. ☐

8. I agree to have FMD tests and a mental stress test 'Stroop' as described in the PIS ☐

9. I agree (if eligible) to undergo MRI scans for the purpose of the study. ☐

10. I understand that the information collected about me may be used to support other research in the future, and may be shared anonymously with other researchers. ☐

11. I understand that confidentiality and anonymity will be maintained and it will not be possible to identify me in any publications. ☐

12. I agree that if health concerns arise as a result of any measures taken in this study, they will be discussed with me and the results will be provided to my General Practitioner and myself. ☐

13. 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. ☐

14. I agree to be contacted in the future by King's College London researchers who may wish to invite me to participate in follow up studies to this project, or in future studies of a similar nature. ☐

15. I agree to take part in the above study. ☐

When completed: 1 for participant; 1 for researcher site file; 1 (original) to be kept in medical notes.

16. I understand that if I lose capacity during the study, data and samples already collected will be retained and used in the study. But, no more data or samples would be collected. ☐

17. I agree to my stool sample being stored for future additional analysis related to this study only. ☐

18. I agree to blood samples being stored for future additional analysis related to this study only. ☐

Name of Participant	Date	Signature
Name of Person taking consent	Date	Signature

When completed: 1 for participant; 1 for researcher site file; 1 (original) to be kept in medical notes.

Appendix 6: Pre-screening dietary habit questionnaire

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Appendix 4 – Dietary habits questionnaire

KING'S College LONDON

Participant code ☐☐☐☐☐☐

Date form completed ☐☐/☐☐/☐☐☐☐

Eating Habits

Study title: The impact of almond nut consumption on markers of cardiovascular and metabolic disease

Please read this carefully

We would now like to ask you about some foods which you may eat.
Please answer ALL the questions by ticking the box which you think most applies to you.

MILK

1. What kind of milk do you **usually** use for drinks, in tea or coffee and on cereals? Is it ...
If you usually use soya, rice or other non-dairy milk substitutes please tick "do not drink milk" and record details in space below:

21

Tick ONE box

Whole milk	☐ 1	
Semi-skimmed milk, including dried semi-skimmed	☐ 2	
Skimmed milk, including dried skimmed	☐ 3	☐☐ **GO TO Q2**
Do not have a usual type	☐ 4	
Do not drink milk	☐ 5	☐☐ **GO TO Q3**

Details of non-dairy milk substitutes: _____

MILKQUA

2. About how much milk do you yourself use each day, on average (for drinks, in tea and coffee, on cereals etc.). Is it …

22

Tick ONE box

Less than a quarter of a pint	1
About a quarter of a pint	2
About half a pint	3
One pint or more	4

CHEESE

3. How often, on average, do you eat a serving of any type of cheese, except cottage cheese?

23

Tick ONE box				
6 or more times a week	**3 to 5 times a week**	**1 to 2 times a week**	**Less than once a week**	**Rarely or never**
1	2	3	4	5

WHITMEAT

4. How often, on average, do you eat a serving of chicken or turkey?

24

Tick ONE box				
6 or more times a week	**3 to 5 times a week**	**1 to 2 times a week**	**Less than once a week**	**Rarely or never**
1	2	3	4	5

INCLUDE: processed chicken or turkey, chicken roll, chicken nuggets, turkey burgers

REDMEATB

5. How often, on average, do you eat a serving of beef, pork or lamb?

INCLUDE: burgers, sausages, bacon, cold meats, ham, corned beef, luncheon meat, spam, meat pies, meat curries, casseroles.

Tick ONE box 25

6 or more times a week	3 to 5 times a week	1 to 2 times a week	Less than once a week	Rarely or never
☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

FRIEDFDB

6. How often, on average, do you eat a serving of any fried food?

INCLUDE: Fried fish or chicken, chips (including oven chips), cooked breakfast, samosas.

Tick ONE box 26

6 or more times a week	3 to 5 times a week	1 to 2 times a week	Less than once a week	Rarely or never
☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

FISH

7. Apart from fried fish, how often, on average, do you eat a serving of fish?

INCLUDE: Prawns, tinned fish such as tuna.

Tick ONE box 27

6 or more times a week	3 to 5 times a week	1 to 2 times a week	Less than once a week	Rarely or never
☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

SNACK

8. How often, on average, do you eat sweet or savoury snacks such as chocolates, crisps, nuts or biscuits?

INCLUDE: savoury biscuits such as cream crackers.

Tick ONE box 28

6 or more times a week	3 to 5 times a week	1 to 2 times a week	Less than once a week	Rarely or never
☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

CAKESC

9. How often, on average, do you eat a serving of cakes, pies, puddings or pastries?

Tick ONE box 29

6 or more times a week	3 to 5 times a week	1 to 2 times a week	Less than once a week	Rarely or never
☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

Appendix 7: Case study form of ATTIS study

ATTIS STUDY PARTICIPANT CASE REPORT FORM

A randomised, controlled parallel dietary intervention study to investigate the effect of almond snack consumption on cardio-metabolic disease risk markers compared with isocaloric snacks, in adults at moderate risk of cardiovascular disease

Study Number:

PARTICIPANT ID:

Principal Investigator: Sarah Berry, Ph.D. and Wendy Hall, Ph.D.
Sponsor: Almond Board of California
Name of site: King's College London, St. Thomas' Hospital and Guy's Hospital
CRF Version Number: 01

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 each part 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. 13/08/2008.

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.

ATTIS Study

Subject/ Screening No. ☐☐☐ **Subject Initials** ☐☐☐ **Site** ☐

Visit 1 Screening

Visit date	☐☐☐☐☐☐☐☐ DAY MONTH YEAR

CONSENT

Date of Consent	☐☐☐☐☐☐☐☐ DAY MONTH YEAR

DEMOGRAPHICS

Gender	Male ☐ Female ☐
Date of Birth	☐☐☐☐☐☐☐☐ DAY MONTH YEAR
Race	☐ African American/African Heritage ☐ American Indian or Alaskan Native ☐ Asian – Central/South Asian Heritage ☐ Asian – East Asian Heritage ☐ Asian – Japanese Heritage ☐ Asian – South East Asian Heritage ☐ Native Hawaiian or Other Pacific Islander ☐ White – Arabic/North African Heritage ☐ White – White/Caucasian/European Heritage

ETHNICITY

	☐ Hispanic or Latin ☐ Non Hispanic or Latino

Visit 1 Screening

MEDICAL HISTORY

List relevant previous and current medical conditions (including allergies or drug sensitivity) and surgery (within the last year) that the subject has experienced

Are there any medical conditions to report? ☐ Yes* ***If Yes, please describe below.*** ☐ No

Medical Condition	Date Started	Continuing	Date Stopped (if applicable)
	DAY MONTH YEAR	☐	DAY MONTH YEAR
	DAY MONTH YEAR	☐	DAY MONTH YEAR
	DAY MONTH YEAR	☐	DAY MONTH YEAR
	DAY MONTH YEAR	☐	DAY MONTH YEAR
	DAY MONTH YEAR	☐	DAY MONTH YEAR
	DAY MONTH YEAR	☐	DAY MONTH YEAR
	DAY MONTH YEAR	☐	DAY MONTH YEAR

CURRENT MEDICATIONS

Is the subject currently taking current medications, or has the subject taken medications within 30 days prior to the start of the study? ☐ Yes ☐ No

Name	Reason	Date Started	Continuing	Date stopped
			☐	
			☐	
			☐	
			☐	

ATTIS Study

Subject/ Screening No. ☐☐☐ Subject Initials ☐☐☐ Site ☐

Visit 1 Screening

VITAL SIGNS

Blood Pressure 1st measurement	☐☐☐ / ☐☐☐ mmHg
Blood Pressure 2nd measurement	☐☐☐ / ☐☐☐ mmHg
Blood Pressure 3rd measurement	☐☐☐ / ☐☐☐ mmHg

HEIGHT & WEIGHT

Height	☐☐☐.☐ cm
Weight	☐☐☐.☐ kg
BMI	☐☐.☐ kg/m^2

BODY COMPOSITION

Waist circumference 1st measurement	☐☐☐.☐ cm
Waist circumference 2nd measurement	☐☐☐.☐ cm
Waist circumference 3rd measurement	☐☐☐.☐ cm
Body fat	☐☐.☐ %

BLOOD SAMPLE TAKEN

Blood collection performed?	☐ No (Comment Below) ☐ Yes, Complete below Comment*:_____		
Date & Time of Sample	__ __/__ __/_____ __ (DD/MM/YYYY)	_____:_____ HH:MM	
BLOOD COLLECTION (27.5 ml)	ANALYSIS		
4 ml Fluoride Oxalate Grey	Glucose	☐ No ☐ Yes	
4 ml EDTA Lavender	Full blood count	☐ No ☐ Yes	
8.5 ml No Anticoagulant Gold	Full lipid profile	☐ No ☐ Yes	
BLOOD SEPARATION		Number of Eppendorf tubes (at least two tubes)	Stored in box number (-80°C)
For Glucose: 1 ml in yellow lid tubes	☐ No ☐ Yes		
For full blood count: 4 ml EDTA Lavender	☐ No ☐ Yes		
For full lipid profile: 1 ml in pink lid tubes	☐ No ☐ Yes		

ATTIS Study

Subject/ Screening No. ☐☐☐ Subject Initials ☐☐☐ Site ☐

Visit 1 Screening

INCLUSION CRITERIA

Please mark the correct answers to the following questions:

	Yes	No
1. Aged between 30-70 years	☐	☐
2. Regular snack consumers (defined as those that consume ≥ 2 snack products per day	☐	☐
3. Above average risk of developing CVD (as defined by score of >2.5 on Framingham risk score) *NB- fill out this question after screening blood results are back.*	☐	☐
4. Able to understand the information sheet and willing to comply with study protocol	☐	☐
5. Able to give informed consent	☐	☐

ATTIS Study

Subject/ Screening No. ☐☐☐ Subject Initials ☐☐☐ Site ☐

Visit 1 Screening

EXCLUSION CRITERIA

	Yes	No
1. Non-snack consumers (assessed as subjects consuming <2 snack products per day by a specific FFQ at screening, adapted from the short Health Survey for England (2007) Eating Habits Questionnaire, appendix 4).	☐	☐
2. A reported history of myocardial infarction or cancer.	☐	☐
3. Being fitted with a heart pacemaker.	☐	☐
4. Presence of metal inside the body (implants, devices, shrapnel, metal particles in eyes from welding etc.).	☐	☐
5. History of black-outs/epilepsy.	☐	☐
6. Diabetes mellitus (fasting plasma glucose >7 mmol/L).	☐	☐
7. Chronic coronary, renal or bowel disease or history of cholestatic liver disease or pancreatitis.	☐	☐
8. Presence of gastrointestinal disorder or use of a drug, which is likely to alter gastrointestinal motility or nutrient absorption.	☐	☐
9. History of substance abuse or alcoholism (past history of alcohol intake >60 units/men or 50 units/women).	☐	☐
10. Currently pregnant, planning pregnancy, breastfeeding or having had a baby in the last 12 months.	☐	☐
11. Allergy or intolerance to nuts.	☐	☐
12. Unwilling to follow the protocol and/or give informed consent.	☐	☐
13. Weight change of > 3 kg in preceding 2 months. BMI <18 kg/m^2 (underweight) or >40 kg/m^2 (morbidly obese due to potential technical difficulties making FMD and ABP measurements).	☐	☐
14. Current smokers or individuals who quit smoking within the last 6 months.	☐	☐
15. Participation in other research trials involving dietary or drug intervention and/ or blood collection in the past 3 months.	☐	☐
16. Unable or unwilling to comply with study protocol.	☐	☐

ATTIS Study

Subject/ Screening No.		Subject Initials		Site	

Visit 1 Screening

FITNESS & ELIGIBILITY TO PARTICIPATE IN STUDY

In the investigators opinion, on the basis of the screening assessments and Inclusion and Exclusion criteria is the subject eligible and fit to participate in the next part of the study? ☐ Yes ☐ No*

If no, the subject should be discontinued from the study and the Study Conclusion page completed.

Qualified Signature: _____ Date: _____

ATTIS Study

Subject/ Screening No. ☐☐☐ Subject Initials ☐☐☐ Site ☐

Visit 2 MRU (Run-in)

Visit date ☐☐☐☐☐☐☐☐

SUBJECT ELIGIBILITY

Have there been any deviations from the protocol since the last visit?	☐ Yes*	☐ No
**If yes, please provide details on the Comments page*		
Has the subject adhered to all dietary and lifestyle restrictions since last visit?	☐ Yes	☐ No**
***If No, please provide details on the Comments page*		
Is the subject eligible to continue in the study?	☐ Yes	☐ No***
****If No, the subject should be discontinued from the study*		

VITAL SIGNS

Blood Pressure 1st measurement	☐☐☐ / ☐☐☐ mmHg
Blood Pressure 2nd measurement	☐☐☐ / ☐☐☐ mmHg
Blood Pressure 3rd measurement	☐☐☐ / ☐☐☐ mmHg

HEIGHT & WEIGHT

Height	☐☐☐.☐ cm
Weight	☐☐☐.☐ kg
BMI	☐☐.☐ kg/m^2

BODY COMPOSITION

Waist circumference	☐☐☐.☐ cm
Hip circumference	☐☐☐.☐ cm
Body fat	☐☐.☐ %

ATTIS Study

Subject/ Screening No. | | | | Subject Initials | | | | Site | |

Visit 2 MRU (Run-in)

ABP MEASUREMENT

Ambulatory blood pressure cuff fitted and participant willing to wear for 24 hrs Yes No*

Participant instructed how to use device and given diary card Yes No

ABP monitor code

If no, the subject should be discontinued from the study and the Study Conclusion page completed.

SNACKS

1) **Participant energy requirements**
   a) Daily energy requirements

   b) Snack requirements at 20% energy

2) **Snack requirements**
   a) Number of snacks per day to meet 20% requirements

   b) Number of snacks for two week intervention

   c) Snacks given (type and number):

Sweet cakes	*Savoury scones*
Lemon_____	Garlic and herb_____
Caramel _____	Cheese_____
Banana_____	Chili _____
Orange _____	Plain_____

Snacking information sheet given: Yes/ No

Qualified Signature: _____ Date: _____

ATTIS Study

Subject/ Screening No. ☐☐☐ Subject Initials ☐☐☐ Site ☐

Visit 3 CRF (Baseline)

Visit date ☐☐☐☐☐☐☐☐

SUBJECT ELIGIBILITY

Have there been any deviations from the protocol since the last visit?	☐ Yes* ☐ No
**If yes, please provide details on the Comments page*	
Has the subject adhered to all dietary and lifestyle restrictions since last visit?	☐ Yes ☐ No**
***If No, please provide details on the Comments page*	
Is the subject eligible to continue in the study?	☐ Yes ☐ No***
****If No, the subject should be discontinued from the study*	

VITAL SIGNS

Blood Pressure 1st measurement	☐☐☐ / ☐☐☐ mmHg
Blood Pressure 2nd measurement	☐☐☐ / ☐☐☐ mmHg
Blood Pressure 3rd measurement	☐☐☐ / ☐☐☐ mmHg

HEIGHT & WEIGHT

Height	☐☐☐.☐ cm
Weight	☐☐☐.☐ kg
BMI	☐☐.☐ kg/m^2

BODY COMPOSITION

Waist circumference	☐☐☐.☐ cm
Hip circumference	☐☐☐.☐ cm
Body fat	☐☐.☐ %

Visit 3 CRF (Baseline)

FMD MEASUREMENTS

FMD performed?	☐ No (Comment Below) ☐ Yes, Complete below Comment*:_____
ECG normal?	☐ No (Comment Below) ☐ Yes, Complete below Comment*:_____
Date & Time of measurement 1:	__ __/__ __ __/__ __ __ __ (DD / MMM / YYYY) _____:_____ HH:MM
Date & Time of measurement 2 (with GTN):	__ __/__ __ __/__ __ __ __ (DD / MMM / YYYY) _____:_____ HH:MM
Position: Supine Device/probe (if different than usual): Vivid i	
Diameter of artery	Average baseline: ☐.☐☐ mm After occlusion : ☐.☐☐ mm Percentage of difference: ☐☐.☐ %
Integral blood flow	Average baseline: ☐.☐☐ After occlusion : ☐.☐☐ Percentage of increment: ☐☐☐.☐ %
Comments	

ATTIS Study

Subject/ Screening No. ☐☐☐ Subject Initials ☐☐☐ Site ☐

Visit 3 CRF (Baseline)

BLOOD SAMPLING

Blood collection performed?	☐ No (Comment Below) ☐ Yes, Complete below Comment*:_____	
Date & Time of Sample	__ __/__ __/__ __ __ __ (DD/MM/YYYY)	_____:_____ HH:MM
		_____:_____ HH:MM
		_____:_____ HH:MM

BLOOD COLLECTION (27.5 mL)	ANALYSIS	0 min (fasting)	5 mins	10 mins
2 ml Fl Ox Grey	Glucose	☐ No ☐ Yes	☐ No ☐ Yes	☐ No ☐ Yes
2.5 ml EDTA Lavender	TAG/NEFA	☐ No ☐ Yes	☐ No ☐ Yes	☐ No ☐ Yes
2.5 ml Serum Gold	Insulin	☐ No ☐ Yes	☐ No ☐ Yes	☐ No ☐ Yes
6.5 ml Serum Gold	Fetuin-A, Resistin, ALT, GGT, Leptin, Adiponectin, Lipids	☐ No ☐ Yes		

BLOOD SEPARATION	0 min (fasting)	5 mins	10 mins	Number of Eppendorf tubes (at least two tubes for each) 0 min/5 mins/10 mins			Stored in box number (-80°C)
For Glucose: 0.5 ml in yellow lid tubes	☐ No ☐ Yes	☐ No ☐ Yes	☐ No ☐ Yes				
For TAG/NEFA: 0.5 ml in pink lid tubes	☐ No ☐ Yes	☐ No ☐ Yes	☐ No ☐ Yes				
For Insulin: 0.5 ml in blue lid tubes	☐ No ☐ Yes	☐ No ☐ Yes	☐ No ☐ Yes				
For Fetuin A, Resistin, ALT, GGT: 0.5 ml in green lid tubes	☐ No ☐ Yes	☐ No ☐ Yes	☐ No ☐ Yes				
For Leptin and Adiponectin: 0.5 ml in green lid tubes	☐ No ☐ Yes	☐ No ☐ Yes	☐ No ☐ Yes				
For FLIP: 0.5 ml in pink lid tubes	☐ No ☐ Yes	☐ No ☐ Yes	☐ No ☐ Yes				

ATTIS Study

Subject/ Screening No. ☐☐☐ Subject Initials ☐☐☐ Site ☐

Visit 3 CRF (Baseline)

MRI PARTICIPANTS ONLY

MRI/MRS performed?	☐ No (Comment Below) ☐ Yes, Complete below Comment*:_____
Date & Time of scanning:	__ __/ __ __ __/ __ __ __ __ (DD / MMM / YYYY) _____:_____ HH:MM
Total Body	Subcutaneous fat (via MRI) ☐ % Visceral fat (via MRI) ☐ % Total body fat (via MRI) ☐ %
Liver	Liver fat (via MRI) ☐ % Liver fat (via MRS) ☐ % Intrahepatocellular lipid (IHCL) (via MRS) ☐ mmol/kg wet weight Lipid species (name and quantity (mmol)) (via MRS) ☐ Comment ☐

Subject/ Screening No. | Subject Initials | Site

Muscle	Muscle fat (via MRI) % Muscle fat (via MRS) % Intramyocellular lipid (IHCL) (via MRS) mmol/kg wet weight Lipid species (name and quantity (mmol)) (via MRS) Comment
Pancreas	Pancreatic fat (via MRI) % Comment
General Comment	

ATTIS Study

Subject/ Screening No. ☐☐☐ Subject Initials ☐☐☐ Site ☐

Visit 3 CRF (Baseline)

STOOL SAMPLE PARTICIPANTS ONLY

Did participant provide stool sample Yes No*

If no, detail why not below

_____

Ambulatory blood pressure given and participant willing to wear for 24hrs Yes No

Participant instructed how to use device and given diary card Yes No

SHORT CHAIN FATTY ACIDS (SCFA)

Name of SCFA	Quantity (gram)

ATTIS Study

Subject/ Screening No. ☐☐☐ Subject Initials ☐☐☐ Site ☐

Visit 3 CRF (Baseline)

HRV MEASUREMENT PREP FOR HOME

HRV monitor fitted and on recording mode?	Yes	No
Participant willing to wear for 24hrs	Yes	No
Participant instructed how to use device and given diary card	Yes	No

HRV PARAMETERS

Physical stress

	Mean	95% CI
IBI and HR		
IBI (ms)		
HR (bpm)		
Time domain		
TI		
SDNN (ms)		
SDANN (ms)		
RMSSD (ms)		
PNN50 (%)		
Frequency domain		
LF (ms^2)		
HF (ms^2)		
VLF (ms^2)		
Non-linear method		
SD1:SD2 (Poincaré ratio)		

Mental stress

	Mean	95% CI
IBI and HR		
IBI (ms)		
HR (bpm)		
Time domain		
TI		
SDNN (ms)		
SDANN (ms)		
RMSSD (ms)		
PNN50 (%)		
Frequency domain		
LF (ms^2)		
HF (ms^2)		
VLF (ms^2)		
Non-linear method		
SD1:SD2 (Poincaré ratio)		

24 hours

	Mean	95% CI
IBI and HR		
IBI (ms)		
HR (bpm)		
Time domain		
TI		
SDNN (ms)		
SDANN (ms)		
RMSSD (ms)		
PNN50 (%)		
Frequency domain		
LF (ms^2)		
HF (ms^2)		
VLF (ms^2)		
Non-linear method		
SD1:SD2 (Poincaré ratio)		

ATTIS Study

Subject/ Screening No.		Subject Initials		Site	

Visit 3 CRF (Baseline)

SUBJECT RANDOMISATION

Was subject randomised?	☐ Yes ☐ No **If Yes, please complete the following:*
Date of randomisation	☐☐☐☐☐☐☐☐ DAY MONTH YEAR
Randomisation	*Cake snacks* ☐ *Almonds* ☐

ATTIS Study

Subject/ Screening No. | | | | Subject Initials | | | | Site | |

Visit 3 CRF (Baseline)

IF RANDOMISED TO CONSUME ALMONDS

1) **Participant energy requirements**
   a) Daily energy requirements

   b) Almond requirements at 20% energy

2) **Almond requirements**
   a) Amount of almonds (grams / number of packs) per day to meet 20% requirements
   /
   b) Almonds required of 6 week intervention (grams / number of packs)
   /
   c) Amount of almonds (grams / number of packs) given at this visit:
   /
   d) Amount of almond (grams / number of packs) left to give:
   /

Snacking information sheet given: Yes/ No

ATTIS Study

Subject/ Screening No. ☐☐☐ Subject Initials ☐☐☐ Site ☐

Visit 3 CRF (Baseline)

IF RANDOMISED TO CONTINUE WITH CAKE SNACKS

1) **Participant energy requirements**
   a) Daily energy requirements
   ☐
   b) Snack requirements at 20% energy
   ☐

2) **Snack requirements**
   a) Number of snacks per day to meet 20% requirements
   ☐
   b) Number of snacks for two week intervention
   ☐
   c) Snacks given (type and number):

Sweet cakes	*Savoury scones*
Lemon_____	Garlic and herb_____
Caramel _____	Cheese_____
Plain_____	
Banana _____	
Orange_____	

Snacking information sheet given: Yes/ No

ATTIS Study

Subject/ Screening No. ☐☐☐ Subject Initials ☐☐☐ Site ☐

Visit 4 MRU

Visit date ☐☐☐☐☐☐☐☐

VITAL SIGNS

Blood Pressure 1st measurement	☐☐☐ / ☐☐☐ mmHg
Blood Pressure 2nd measurement	☐☐☐ / ☐☐☐ mmHg
Blood Pressure 3rd measurement	☐☐☐ / ☐☐☐ mmHg

HEIGHT & WEIGHT

Weight	☐☐☐.☐ kg
BMI	☐☐.☐ kg/m^2

BODY COMPOSITION

Waist circumference	☐☐☐.☐ cm
Hip circumference	☐☐☐.☐ cm
Body fat	☐☐.☐ %

SNACK QUANTITY

Muffin	☐ (number of muffins)
Almond	☐ (grams) / ☐ (number of packs)

ATTIS Study

Subject/ Screening No. ☐☐☐ Subject Initials ☐☐☐ Site ☐

Visit 5 MRU

Visit date ☐☐☐☐☐☐☐☐

VITAL SIGNS

Blood Pressure 1st measurement	☐☐☐ / ☐☐☐ mmHg
Blood Pressure 2nd measurement	☐☐☐ / ☐☐☐ mmHg
Blood Pressure 3rd measurement	☐☐☐ / ☐☐☐ mmHg

HEIGHT & WEIGHT

Weight	☐☐☐.☐ kg
BMI	☐☐.☐ kg/m²

BODY COMPOSITION

Waist circumference	☐☐☐.☐ cm
Hip circumference	☐☐☐.☐ cm
Body fat	☐☐.☐ %

SNACK QUANTITY

Muffin	☐ (number of muffins)
Almond	☐ (grams) / ☐ (number of packs)

ATTIS Study

Subject/ Screening No. ☐☐☐ Subject Initials ☐☐☐ Site ☐

Visit 6 CRF (Endpoint)

Visit date ☐☐☐☐☐☐☐☐

VITAL SIGNS

Blood Pressure 1st measurement	☐☐☐ / ☐☐☐ mmHg
Blood Pressure 2nd measurement	☐☐☐ / ☐☐☐ mmHg
Blood Pressure 3rd measurement	☐☐☐ / ☐☐☐ mmHg

HEIGHT & WEIGHT

Height	☐☐☐.☐ cm
Weight	☐☐☐.☐ kg
BMI	☐☐.☐ kg/m^2

BODY COMPOSITION

Waist circumference	☐☐☐.☐ cm
Hip circumference	☐☐☐.☐ cm
Body fat	☐☐.☐ %

ATTIS Study

Subject/ Screening No. ☐☐☐ Subject Initials ☐☐☐ Site ☐

Visit 6 CRF (Endpoint)

FMD MEASUREMENTS

FMD performed?	☐ No (Comment Below) ☐ Yes, Complete below Comment*:_____
ECG normal?	☐ No (Comment Below) ☐ Yes, Complete below Comment*:_____
Date & Time of measurement 1:	__/__/____ ____:____ (DD / MMM / YYYY) HH:MM
Date & Time of measurement 2 (with GTN):	__/__/____ ____:____ (DD / MMM / YYYY) HH:MM
Position: Supine Device/probe (if different than usual): Vivid i	
Diameter of artery	Average baseline: ☐.☐☐ mm After occlusion : ☐.☐☐ mm Percentage of difference: ☐☐.☐ %
Integral blood flow	Average baseline: ☐.☐☐ After occlusion : ☐.☐☐ Percentage of increment: ☐☐☐.☐ %
Comments	

ATTIS Study

Subject/ Screening No. ☐☐☐ Subject Initials ☐☐☐ Site ☐

Visit 6 CRF (Endpoint)

BLOOD SAMPLING

Blood collection performed?	☐ No (Comment Below) ☐ Yes, Complete below Comment*:_____			
Date & Time of Sample	__/__/____ (DD/MM/YYYY)	_____:_____ HH:MM		
		_____:_____ HH:MM		
		_____:_____ HH:MM		

BLOOD COLLECTION (27.5 mL)	ANALYSIS	0 min (fasting)	5 mins	10 mins
2 ml Fl Ox Grey	Glucose	☐ No ☐ Yes	☐ No ☐ Yes	☐ No ☐ Yes
2.5 ml EDTA Lavender	TAG/NEFA	☐ No ☐ Yes	☐ No ☐ Yes	☐ No ☐ Yes
2.5 ml Serum Gold	Insulin	☐ No ☐ Yes	☐ No ☐ Yes	☐ No ☐ Yes
6.5 ml Serum Gold	Fetuin-A, Resistin, ALT, GGT, Leptin, Adiponectin, Lipids	☐ No ☐ Yes		

BLOOD SEPARATION	0 min (fasting)	5 mins	10 mins	Number of Eppendorf tubes (at least two tubes for each) 0 min/5 mins/10 mins			Stored in box number (-80°C)
For Glucose: 0.5 ml in yellow lid tubes	☐ No ☐ Yes	☐ No ☐ Yes	☐ No ☐ Yes				
For TAG/NEFA: 0.5 ml in pink lid tubes	☐ No ☐ Yes	☐ No ☐ Yes	☐ No ☐ Yes				
For Insulin: 0.5 ml in blue lid tubes	☐ No ☐ Yes	☐ No ☐ Yes	☐ No ☐ Yes				
For Fetuin A, Resistin, ALT, GGT: 0.5 ml in green lid tubes	☐ No ☐ Yes	☐ No ☐ Yes	☐ No ☐ Yes				
For Leptin and Adiponectin: 0.5 ml in green lid tubes	☐ No ☐ Yes	☐ No ☐ Yes	☐ No ☐ Yes				
For FLIP: 0.5 ml in pink lid tubes	☐ No ☐ Yes	☐ No ☐ Yes	☐ No ☐ Yes				

ATTIS Study

Subject/ Screening No. ☐☐☐ Subject Initials ☐☐☐ Site ☐

Visit 6 CRF (Endpoint)

MRI PARTICIPANTS ONLY

MRI/MRS performed?	☐ No (Comment Below) ☐ Yes, Complete below Comment*:_____
Date & Time of scanning:	__ __/__ __ __/__ __ __ __ (DD / MMM /YYYY) _____:_____ HH:MM
Total Body	Subcutaneous fat (via MRI) ☐ % Visceral fat (via MRI) ☐ % Total body fat (via MRI) ☐ %
Liver	Liver fat (via MRI) ☐ % Liver fat (via MRS) ☐ % Intrahepatocellular lipid (IHCL) (via MRS) ☐ mmol/kg wet weight Lipid species (name and quantity (mmol)) (via MRS) ☐ Comment ☐

Subject/ Screening No. | Subject Initials | Site

Muscle	Muscle fat (via MRI) % Muscle fat (via MRS) % Intramyocellular lipid (IHCL) (via MRS) mmol/kg wet weight Lipid species (name and quantity (mmol)) (via MRS) Comment
Pancreas	Pancreatic fat (via MRI) % Comment
General Comment	

ATTIS Study

Subject/ Screening No.		Subject Initials		Site	

Visit 6 CRF (Endpoint)

STOOL SAMPLE PARTICIPANTS ONLY

Did participant provide stool sample Yes No*

If no, detail why not below

_____

Ambulatory blood pressure given and participant willing to wear for 24hrs Yes No

Participant instructed how to use device and given diary card Yes No

SHORT CHAIN FATTY ACIDS (SCFA)

Name of SCFA	Quantity (gram)

ATTIS Study

Subject/ Screening No. ☐☐☐ Subject Initials ☐☐☐ Site ☐

Visit 6 CRF (Endpoint)

HRV MEASUREMENT PREP FOR HOME

HRV monitor fitted and on recording mode?	Yes	No
Participant willing to wear for 24hrs	Yes	No
Participant instructed how to use device and given diary card	Yes	No

HRV PARAMETERS

Physical stress

	Mean	95% CI
IBI and HR		
IBI (ms)		
HR (bpm)		
Time domain		
TI		
SDNN (ms)		
SDANN (ms)		
RMSSD (ms)		
PNN50 (%)		
Frequency domain		
LF (ms^2)		
HF (ms^2)		
VLF (ms^2)		
Non-linear method		
SD1:SD2 (Poincaré ratio)		

Mental stress

	Mean	95% CI
IBI and HR		
IBI (ms)		
HR (bpm)		
Time domain		
TI		
SDNN (ms)		
SDANN (ms)		
RMSSD (ms)		
PNN50 (%)		
Frequency domain		
LF (ms^2)		
HF (ms^2)		
VLF (ms^2)		
Non-linear method		
SD1:SD2 (Poincaré ratio)		

24 hours

	Mean	95% CI
IBI and HR		
IBI (ms)		
HR (bpm)		
Time domain		
TI		
SDNN (ms)		
SDANN (ms)		
RMSSD (ms)		
PNN50 (%)		
Frequency domain		
LF (ms^2)		
HF (ms^2)		
VLF (ms^2)		
Non-linear method		
SD1:SD2 (Poincaré ratio)		

ATTIS Study

Subject/ Screening No. ☐☐☐ Subject Initials ☐☐☐ Site ☐

All Visit

ADVERSE EVENTS

Did the subject experience any Adverse Events? ☐ Yes ☐ No *If Yes, please describe below*	
Adverse Event (AE)	☐
Onset Date & Time	☐☐☐☐☐☐☐☐ (DAY MONTH YEAR) ☐☐:☐☐ (24 hour clock)
Stop Date & Time*	☐☐☐☐☐☐☐☐ (DAY MONTH YEAR) ☐☐:☐☐ (24 hour clock)
Frequency	☐ Single Episode ☐ Intermittent
Intensity	☐ Mild ☐ Moderate ☐ Severe
Is there a reasonable possibility that the AE may have been cause by the Investigational Product?	☐ Yes ☐ No
Action Taken re: Investigational Product	☐ None ☐ Withdrawn ☐ Interrupted ☐ Amount Reduced
Outcome*	☐ Resolved* ☐ Ongoing ☐ Death*
Serious	☐ Yes ☐ No ***If Yes, all serious adverse events must be reported to the study manager within 24 hours and require additional reporting on an SAE form***
Did the subject withdraw from the study as a result of this AE?	☐ Yes ☐ No ***If Yes, all serious adverse events must be reported to the study manager within 24 hours and require additional reporting on an SAE form***
****If Outcome is Resolved, please complete Stop Date and Time and if Death, please enter Date of Death as Stop Date***	

Investigators Signature: _____ Date: _____

ATTIS Study

Subject/ Screening No. | | | | Subject Initials | | | | Site | |

All Visit

COMMENTS

Please include any additional information that has not been addressed on the previous Case Report Form pages.		
Page	**Section Heading**	**Comment**

ATTIS Study

Subject/ Screening No. ☐☐☐ Subject Initials ☐☐☐ Site ☐

All Visit

STUDY CONCLUSION

Did the subject complete the entire study? ☐ Yes ☐ No*

Date subject completed or withdrew from the study ☐☐☐☐☐☐☐☐ (DAY MONTH YEAR)

If No is marked, please indicate the primary reason below. Please mark only one.

Did not meet study criteria	☐	Please specify: ☐
Adverse Event	☐	Please complete Adverse Events page
Lost to Follow-up	☐	
Protocol Violation	☐	Please specify: ☐
Withdrawal of Consent	☐	Please specify: ☐
Other	☐	Please specify: ☐

INVESTIGATOR'S SIGNATURE

I confirm that I have reviewed all the data collected in this Case Report Form and take responsibility that the information is accurate and complete.

Investigator's Signature: _____ Date: _____

Appendix 8: Four-day food diary of ATTIS study

Guy's and St Thomas' NHS
NHS Foundation Trust
KING'S College LONDON

IRAS Project number: 212398 REC 16/LO/1910
Version 1. 07/07/2015

Appendix 5 – Four-day food diary

Participant code

Date form completed

4 Day food and drink diary

Study title: The impact of almond nut consumption on markers of cardiovascular and metabolic disease

Please return to:
Leanne Smith (Research Dietitian)
Room 4.46, Diabetes and Nutritional Sciences Division
King's College London, Franklin Wilkins Building, 150 Stamford Street, London SE1 9NH
Email: almondstudy@kcl.ac.uk Tel: 020 7848 4301

IRAS Project number: 212398 REC 16/LO/1910
Version 1. 07/07/2015

PLEASE READ THROUGH THESE PAGES BEFORE STARTING YOUR DIARY

We would like you to keep this diary of everything you eat and drink over 4 days. Please include all food consumed at home and outside the home e.g. work, college or restaurants. It is very important that you do not change what you normally eat and drink just because you are keeping this record. Please keep to your usual food habits.

Day and Date
Please write down the day and date at the top of the page each time you start a new day of recording.

Time Slots
Please note the time of each eating occasion into the space provided. For easy use each day is divided into sections, from the first thing in the morning to late evening and through the night.

Where and with whom?
Please tell us what **room or part of the house** you were in when you ate, e.g. kitchen, living room, and tell us **whether you ate at a table or not** and **whether you were watching television**. If you ate at your work canteen, a restaurant, fast food chain or your car, write that location down. We would also like to know **who you share your meals with**, e.g. whether you eat alone, with your partner, children, colleagues, or friends.

What do you eat?
Please describe the food you eat in as much detail as possible. Be as specific as you can. Pages 16 - 21 will help with the sort of detail we need, like **cooking methods** (fried, grilled, baked etc) and any **additions** (fats, sugar/sweeteners, sauces, pepper etc).

- **Homemade dishes**
  If you have eaten any **homemade dishes** e.g. chicken casserole, please record the name of the recipe, ingredients with amounts(including water or other fluids) for the whole recipe, the number of people the recipe serves, and the cooking method. Record how much of the whole recipe you have eaten in the portion size column.
- **Take-aways and eating out**
  If you have eaten **take-aways** or **made up dishes not prepared at home** such as at a restaurant or a friend's house, please record as much detail about the ingredients as you can e.g. vegetable curry containing chickpeas, aubergine, onion and tomato.

Brand name
Please note the **brand name** (if known). Most packed foods will list a brand name, e.g. Bird's eye, Hovis, or Supermarket own brands.

- ***Labels/Wrappers***
  Labels are an important source of information for us. It helps us a great deal if you enclose, in the plastic bag provided, labels from all **ready meals,** labels from **foods of lesser known brands** and also from any **supplements** you take.

Portion sizes
Examples for how to describe the **quantity** or **portion size** you had of a particular food or drink are shown on next pages.

For foods, quantity can be described using:

- **household measures**, e.g. one teaspoon (tsp) of sugar, two thick slices of bread, 4 tablespoons (tbsp) of peas, ½ cup of gravy. Be careful when describing amounts in spoons that you are referring to the correct spoon size. Compare the spoons you use with the life size pictures at the back of this diary.
- **weights from labels**, e.g. 4oz steak, 420g tin of baked beans, 125g pot of yoghurt.
- **number of items**, e.g. 4 fish fingers, 2 pieces of chicken nuggets, 1 regular size jam filled doughnut.
- **picture examples will be provided.**

For drinks, quantity can be described using:

- the **size of glass, cup etc** (e.g. large glass) or the **volume** (e.g. 300ml). Examples of typical drinks containers are on pages 26-27.
- **volumes from labels** (e.g. 330ml can of fizzy drink).

We would like to know the **amount that was actually eaten** which means taking **leftovers** into account. You can do this in two ways:
1. Record what was served and make notes of what was not eaten e.g. 3 tbsp of peas, only 2 tbsp eaten; 1 large sausage roll, ate only ½
2. Only record the amount actually eaten i.e. 2 tbsp of peas, ½ a large sausage roll

When to fill in the diary?
Please record your eating as you go, not from memory at the end of the day. Use written notes on a pad if you forget to take your diary with you. Each diary day covers a 24hr period, so please include any food or drinks that you may have had during the night. Remember to include foods and drinks between meals (snacks) including water.

IRAS Project number: 212398 REC 16/LO/1910
Version 1. 07/07/2015

Example

Day Tuesday	**Date** 1st of November 2016			
Time	**Where? With Whom? At table? TV on?**	**Food/Drink description & preparation**	**Brand Name**	**Portion size or quantity eaten**
		6am to 9am		
6.30am	*Kitchen Alone No TV Standing*	*Filter coffee, decaffeinated*	*Douwe Egberts*	*Mug*
		milk (fresh, semi-skimmed)		*A little*
		Sugar white	*Silverspoon*	*1 level tsp*
7.30am	*Kitchen Partner TV on At table*	*Filter coffee with milk and sugar*	*As above*	*As above*
		Cornflakes	*Tesco's own*	*Drowned*
		Milk (fresh, semi-skimmed)	*Tesco's own*	
		Toast, granarymedium sliced	*Hovis*	*1 slice*
		Light spread	*Flora*	*med spread*
		Marmalade	*Hartleys*	*1 heaped tsp*
		12 noon to 2pm		
12.30pm	*Tea room at work Colleagues*	*Ham salad sandwich from home*	*Tesco's own*	*2 slices*
		Bread, wholemeal, thick sliced	*Hovis*	*1 slice*
		Light spread	*Flora*	*thin spread*
		Low fat Mayonnaise	*Hellmans*	*2 teaspoons*
		Smoked ham thinly sliced	*Tesco's own*	*2 slices*
		Lettuce, iceberg		*1 leaf*
		Cucumber with skin	*Tropicana*	*4 thin slices*
		Unsweetened orange juice from canteen		*250ml carton*
		Apple with skin from home, Braeburn		*medium size, core left*
		5pm to 8pm		

6.30pm	*Pub* *Partner*	*Gin*	*Gordon's*	*Single measure*
		Tonic water diet	*Schweppes*	*1/2 small glass*
		Lager	*Draught*	*1 pint*
		Salted peanuts	*KP*	*1 handful*
8 pm	*Dining room* Family *No TV* *At table*	*Spaghetti, wholemeal*	*Tesco's own*	*6 tablespoons*
		Bolognese sauce (see recipe)		*4 tablespoons*
		Courgettes (fried in butter)		*3 halves*
		Tinned peaches in juice (juice drained)	*Prince's*	*1 tablespoon*
		Single cream UHT	*Sainsbury's own*	*200ml glass,*
		Orange squash No Added Sugar	*Sainsbury's own*	*1part squash, 3parts tap water*

IRAS Project number: 212398 REC 16/LO/1910
Version 1. 07/07/2015

DAY 1

Day	Date			
Time	Where? With Whom? At table? TV on?	Food/Drink description & preparation	Brand Name	Portion size or quantity eaten
6am to 9am				
9am to 12 noon				
12 noon to 2pm				

2pm to 5pm				
5pm to 8pm				
8 pm to 10pm				
10pm to 6am				

IRAS Project number: 212398 REC 16/LO/1910
Version 1. 07/07/2015

DAY 2

Day	Date			
Time	Where? With Whom? At table? TV on?	Food/Drink description & preparation	Brand Name	Portion size or quantity eaten
6am to 9am				
9am to 12 noon				
12 noon to 2pm				

2pm to 5pm				
5pm to 8pm				
8 pm to 10pm				
10pm to 6am				

IRAS Project number: 212398 REC 16/LO/1910
Version 1. 07/07/2015

DAY 3

Day	Date			
Time	Where? With Whom? At table? TV on?	Food/Drink description & preparation	Brand Name	Portion size or quantity eaten
6am to 9am				
9am to 12 noon				
12 noon to 2pm				

2pm to 5pm				
5pm to 8pm				
8 pm to 10pm				
10pm to 6am				

DAY 4

Day	Date			
Time	Where? With Whom? At table? TV on?	Food/Drink description & preparation	Brand Name	Portion size or quantity eaten
6am to 9am				
9am to 12 noon				
12 noon to 2pm				

2pm to 5pm				
5pm to 8pm				
8 pm to 10pm				
10pm to 6am				

Appendix 9: Four-day activity diary of ATTIS study

Guy's and St Thomas' NHS
NHS Foundation Trust

IRAS Project number: 212398 REC 16/LO/1910
Version 1 28/09/2016

KING'S College LONDON

Appendix 6 – Physical activity questionnaire

Participant code

Date form completed

Activity Diary

Study title: The impact of almond nut consumption on markers of cardiovascular and metabolic disease

Please complete this diary for 4 days.
How to fill in the diary:

1. Complete the date at the top of the page.
2. For each half hour please write down the main activity you were doing and where you were doing it.
3. Also include meals, naps and sleeping times.
4. Circle yes or no for each activity you have done nonstop for over 10 minutes.

Example:

Time	Description of activity	Where?	At least 10 minutes of activity at a time without stopping?
8.00 – 8.30 AM	walking	street	(Yes) / No
8.30 – 9.00 AM	Sitting	bus	Yes / No
9.00 – 9.30 AM	Walking	street	(Yes) / No
9.30 – 10.00AM	Sitting (snack)	Work	Yes / No
10.00 – 10.30 AM	Sitting	Work	Yes / No
10.30 – 11.00 AM	Standing	work	Yes / No

IRAS Project number: 212398 REC 16/LO/19
Version 1 28/09/2016

Day 1
Date: __/__/____ Participant code:_____

Time	Description of activity	Where?	At least 10 minutes of activity at a time without stopping?
8.30 – 9.00 AM			Yes / No
9.00 – 9.30 AM			Yes / No
9.30 – 10.00AM			Yes / No
10.00 – 10.30 AM			Yes / No
10.30 – 11.00 AM			Yes / No
11.00 – 11.30 AM			Yes / No
11.30 – 12.00 PM			Yes / No
12.00 – 12.30 PM			Yes / No
12.30 – 1.00 PM			Yes / No
1.00 – 1.30 PM			Yes / No
1.30 – 2.00 PM			Yes / No
2.00 – 2.30 PM			Yes / No
2.30 – 3.00 PM			Yes / No
3.00 – 3.30 PM			Yes / No
3.30 – 4.00 PM			Yes / No
4.00 – 4.30 PM			Yes / No
4.30 – 5.00 PM			Yes / No
5.00 – 5.30 PM			Yes / No
5.30 – 6.00 PM			Yes / No
6.00 – 6.30 PM			Yes / No
6.30 – 7.00 PM			Yes / No
7.00 – 7.30 PM			Yes / No
7.30 – 8.00 PM			Yes / No
8.00 – 8.30 PM			Yes / No
8.30 – 9.00 PM			Yes / No
9.00 – 9.30 PM			Yes / No
9.30 – 10.00 PM			Yes / No
10.00 – 10.30 PM			Yes / No
10.30 – 11.00 PM			Yes / No
11.00 – 11.30 PM			Yes / No
11.30 – 12.00 AM			Yes / No
12.00 – 12.30 AM			Yes / No
12.30 – 1.00AM			Yes / No
1.00 – 1.30 AM			Yes / No
1.30 – 2.00 AM			Yes / No
2.00 – 2.30 AM			Yes / No
2.30 – 3.00 AM			Yes / No
3.00 – 3.30 AM			Yes / No
3.30 – 4.00 AM			Yes / No
4.00 – 4.30 AM			Yes / No
4.30 – 5.00 AM			Yes / No
5.00 – 5.30 AM			Yes / No
5.30 – 6.00 AM			Yes / No
6.00 – 6.30 AM			Yes / No
6.30 – 7.00 AM			Yes / No
7.00 – 7.30 AM			Yes / No
7.30 – 8.00 AM			Yes / No

8.00 – 8.30 AM			Yes / No

Day 2
Date: __/__/____ Participant code:_____

Time	Description of activity	Where?	At least 10 minutes of activity at a time without stopping?
8.30 – 9.00 AM			Yes / No
9.00 – 9.30 AM			Yes / No
9.30 – 10.00AM			Yes / No
10.00 – 10.30 AM			Yes / No
10.30 – 11.00 AM			Yes / No
11.00 – 11.30 AM			Yes / No
11.30 – 12.00 PM			Yes / No
12.00 – 12.30 PM			Yes / No
12.30 – 1.00 PM			Yes / No
1.00 – 1.30 PM			Yes / No
1.30 – 2.00 PM			Yes / No
2.00 – 2.30 PM			Yes / No
2.30 – 3.00 PM			Yes / No
3.00 – 3.30 PM			Yes / No
3.30 – 4.00 PM			Yes / No
4.00 – 4.30 PM			Yes / No
4.30 – 5.00 PM			Yes / No
5.00 – 5.30 PM			Yes / No
5.30 – 6.00 PM			Yes / No
6.00 – 6.30 PM			Yes / No
6.30 – 7.00 PM			Yes / No
7.00 – 7.30 PM			Yes / No
7.30 – 8.00 PM			Yes / No
8.00 – 8.30 PM			Yes / No
8.30 – 9.00 PM			Yes / No
9.00 – 9.30 PM			Yes / No
9.30 – 10.00 PM			Yes / No
10.00 – 10.30 PM			Yes / No
10.30 – 11.00 PM			Yes / No
11.00 – 11.30 PM			Yes / No
11.30 – 12.00 AM			Yes / No
12.00 – 12.30 AM			Yes / No
12.30 – 1.00AM			Yes / No
1.00 – 1.30 AM			Yes / No
1.30 – 2.00 AM			Yes / No
2.00 – 2.30 AM			Yes / No
2.30 – 3.00 AM			Yes / No
3.00 – 3.30 AM			Yes / No
3.30 – 4.00 AM			Yes / No
4.00 – 4.30 AM			Yes / No
4.30 – 5.00 AM			Yes / No
5.00 – 5.30 AM			Yes / No
5.30 – 6.00 AM			Yes / No
6.00 – 6.30 AM			Yes / No

6.30 – 7.00 AM			Yes / No
7.00 – 7.30 AM			Yes / No
7.30 – 8.00 AM			Yes / No
8.00 – 8.30 AM			Yes / No

Day 3
Date: __/__/____ Participant code:_____

Time	Description of activity	Where?	At least 10 minutes of activity at a time without stopping?
8.30 – 9.00 AM			Yes / No
9.00 – 9.30 AM			Yes / No
9.30 – 10.00AM			Yes / No
10.00 – 10.30 AM			Yes / No
10.30 – 11.00 AM			Yes / No
11.00 – 11.30 AM			Yes / No
11.30 – 12.00 PM			Yes / No
12.00 – 12.30 PM			Yes / No
12.30 – 1.00 PM			Yes / No
1.00 – 1.30 PM			Yes / No
1.30 – 2.00 PM			Yes / No
2.00 – 2.30 PM			Yes / No
2.30 – 3.00 PM			Yes / No
3.00 – 3.30 PM			Yes / No
3.30 – 4.00 PM			Yes / No
4.00 – 4.30 PM			Yes / No
4.30 – 5.00 PM			Yes / No
5.00 – 5.30 PM			Yes / No
5.30 – 6.00 PM			Yes / No
6.00 – 6.30 PM			Yes / No
6.30 – 7.00 PM			Yes / No
7.00 – 7.30 PM			Yes / No
7.30 – 8.00 PM			Yes / No
8.00 – 8.30 PM			Yes / No
8.30 – 9.00 PM			Yes / No
9.00 – 9.30 PM			Yes / No
9.30 – 10.00 PM			Yes / No
10.00 – 10.30 PM			Yes / No
10.30 – 11.00 PM			Yes / No
11.00 – 11.30 PM			Yes / No
11.30 – 12.00 AM			Yes / No
12.00 – 12.30 AM			Yes / No
12.30 – 1.00AM			Yes / No
1.00 – 1.30 AM			Yes / No
1.30 – 2.00 AM			Yes / No
2.00 – 2.30 AM			Yes / No
2.30 – 3.00 AM			Yes / No
3.00 – 3.30 AM			Yes / No
3.30 – 4.00 AM			Yes / No
4.00 – 4.30 AM			Yes / No
4.30 – 5.00 AM			Yes / No

5.00 – 5.30 AM			Yes / No
5.30 – 6.00 AM			Yes / No
6.00 – 6.30 AM			Yes / No
6.30 – 7.00 AM			Yes / No
7.00 – 7.30 AM			Yes / No
7.30 – 8.00 AM			Yes / No
8.00 – 8.30 AM			Yes / No

Day 4
Date: __/__/____ Participant code:_____

Time	Description of activity	Where?	At least 10 minutes of activity at a time without stopping?
8.30 – 9.00 AM			Yes / No
9.00 – 9.30 AM			Yes / No
9.30 – 10.00AM			Yes / No
10.00 – 10.30 AM			Yes / No
10.30 – 11.00 AM			Yes / No
11.00 – 11.30 AM			Yes / No
11.30 – 12.00 PM			Yes / No
12.00 – 12.30 PM			Yes / No
12.30 – 1.00 PM			Yes / No
1.00 – 1.30 PM			Yes / No
1.30 – 2.00 PM			Yes / No
2.00 – 2.30 PM			Yes / No
2.30 – 3.00 PM			Yes / No
3.00 – 3.30 PM			Yes / No
3.30 – 4.00 PM			Yes / No
4.00 – 4.30 PM			Yes / No
4.30 – 5.00 PM			Yes / No
5.00 – 5.30 PM			Yes / No
5.30 – 6.00 PM			Yes / No
6.00 – 6.30 PM			Yes / No
6.30 – 7.00 PM			Yes / No
7.00 – 7.30 PM			Yes / No
7.30 – 8.00 PM			Yes / No
8.00 – 8.30 PM			Yes / No
8.30 – 9.00 PM			Yes / No
9.00 – 9.30 PM			Yes / No
9.30 – 10.00 PM			Yes / No
10.00 – 10.30 PM			Yes / No
10.30 – 11.00 PM			Yes / No
11.00 – 11.30 PM			Yes / No
11.30 – 12.00 AM			Yes / No
12.00 – 12.30 AM			Yes / No
12.30 – 1.00AM			Yes / No
1.00 – 1.30 AM			Yes / No
1.30 – 2.00 AM			Yes / No
2.00 – 2.30 AM			Yes / No
2.30 – 3.00 AM			Yes / No
3.00 – 3.30 AM			Yes / No

3.30 – 4.00 AM			Yes / No
4.00 – 4.30 AM			Yes / No
4.30 – 5.00 AM			Yes / No
5.00 – 5.30 AM			Yes / No
5.30 – 6.00 AM			Yes / No
6.00 – 6.30 AM			Yes / No
6.30 – 7.00 AM			Yes / No
7.00 – 7.30 AM			Yes / No
7.30 – 8.00 AM			Yes / No
8.00 – 8.30 AM			Yes / No

Please return to:
Leanne Smith (Research Dietitian)
Room 4.46, Diabetes and Nutritional Sciences Division
King's College London, Franklin Wilkins Building, 150 Stamford Street, London SE1 9NH
Email: almondstudy@kcl.ac.uk Tel: 020 7848 4301

Appendix 10: Snack information sheet period of ATTIS study

SNACK INFORMATION SHEET FOR PARTICIPANTS

ATTIS STUDY

Thank you for attending to collect your snacks.

The number of snacks you have been given has been individually calculated to provide you with 20% of your energy requirements in place of your normal snacks. Please incorporate the study snacks into your day, remember that they are to replace your usual snacks (so please do not consume crisps, chocolate, pastries etc). ***NB: you are still permitted to eat fruit snacks: banana, apples, berries etc.*** You may wish to eat them at mid-morning, mid-afternoon and before bed for example. Try not to eat the snacks all in one go, or consume them as part of a meal, as we are trying to mimic snacking behaviour.

Do not consume any more of the snacks than the amount we have specified

Please do not alter your diet otherwise but continue with your usual breakfast/lunch/dinner meal routines. Please do not start any new diet regimes whilst on the trial.

CAKES/SCONES

We baked the cakes and kept them frozen so they are fresh. If you defrost them, they should keep for approximately 3-4 days. However, you may wish to defrost your required number of snacks each evening ready for consumption the next day.

TIP: The cakes/scones are tastier if taken directly from the freezer and placed in the microwave for 30 seconds- this give them a warm and 'just baked' texture.

Do not add extra toppings to the cakes/scones e.g. butter/jams/cream etc.

If you are struggling with the cake/scone flavours, please let the team know as we could exchange your snacks for other flavours. It is important that the cakes are eaten for the full duration.

ALMONDS

Do not crush the almonds or chop them finely. (This could give misleading results as it makes the fat in the almonds more accessible for your body to digest, so could lead to weight gain).

Do not add extra toppings to the almonds. Please do not introduce any other new nuts or nut milks into your diet.

Appendix 11: Snack consumption diary for run-in period of ATTIS study

IRAS project number: 212398 REC: 16/LO/1910

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Appendix 14 – Daily study day dairy for the run-in period

Participant code ☐ Date form completed ☐

Daily study day diary: run in period

Study title: The impact of almond nut consumption on markers of cardiovascular and metabolic disease

Please use this diary every day to capture eating your test muffins, in addition to other more general comments about your wellbeing and health.

Once completed, please return to:

Leanne Smith (Research Dietitian)
Room 4.46, Diabetes and Nutritional Sciences Division
King's College London, Franklin-Wilkins Building, 150 Stamford Street, London SE1 9NH
Email: almondstudy@kcl.ac.uk Tel: 020 7848 4301

IRAS project number: 212398 REC: 16/LO/1910

Guy's and St Thomas' NHS
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You are required to eat _____test muffins a day. Please eat as individual snacks throughout the day. It is important that you have the snacks every day and in the quantity suggested. We would like to know if you are not able to consume all the snacks as this may impact on the study results.

Please use the following table to indicate using ticks how many test muffin snacks you eat. If you missed any of the snacks, we would like to know.

Run in Week		Day						
		1	2	3	4	5	6	7
Week 1	Morning							
	Afternoon							
	Evening							
Week 2	Morning							
	Afternoon							
	Evening							

IRAS project number: 212398 REC: 16/LO/1910

Version 1 28/09/2016

Please use this table to capture any brief comments about your general health including any illness e.g. cold, fever, food poisoning, and any medications taken, or any events that prevented you consuming the snacks that day. Also any other details such as bloating, belching, wind etc. NB nil adverse effects are expected from the consumption of the test muffins/almonds, however if you experience symptoms that could be linked, please contact the investigators (and your GP for advice). If you are feeling well, please place a tick in the boxes.

Study week	Day						
	1	2	3	4	5	6	7
Week 1							
Week 2							

Appendix 12: Snack consumption diary for intervention period of ATTIS study

IRAS project number: 212398 REC: 16/LO/1910

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NHS Foundation Trust

Version 1 28/09/2016

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Appendix 15 – Daily study day dairy

Participant code

Date form completed

Daily study day diary

Study title: The impact of almond nut consumption on markers of cardiovascular and metabolic disease

Please use this diary every day to capture eating your test snacks, in addition to other more general comments about your wellbeing and health.

Once completed, please return to:

Leanne Smith (Research Dietitian)
Room 4.46, Diabetes and Nutritional Sciences Division
King's College London, Franklin-Wilkins Building, 150 Stamford Street, London SE1 9NH
Email: almondstudy@kcl.ac.uk Tel: 020 7848 4301

Version 1 28/09/2016

You are required to eat _____test muffins/almonds a day. Please eat as individual snacks throughout the day. It is important that you have the snacks every day and in the quantity suggested. We would like to know if you are not able to consume all the snacks as this may impact on the study results.

Please use the following table to indicate using ticks how many test muffin/almond snacks you eat. Please date the box. If you missed any of the snacks, we would like to know.

Study Week		Day						
		1	2	3	4	5	6	7
Week 1	Morning							
	Afternoon							
	Evening							
Week 2	Morning							
	Afternoon							
	Evening							
Week 3	Morning							
	Afternoon							
	Evening							
Week 4	Morning							
	Afternoon							
	Evening							
Week 5	Morning							
	Afternoon							
	Evening							
Week 6	Morning							
	Afternoon							
	Evening							

IRAS project number: 212398 REC: 16/LO/191

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Version 1 28/09/2016

Please use this table to capture any brief comments about your general health including any illness e.g. cold, fever, food poisoning, and any medications taken, or any events that prevented you consuming the snacks that day. Also any other details such as bloating, belching, wind etc. NB nil adverse effects are expected from the consumption of the test muffins/almonds, however if you experience symptoms that could be linked, please contact the investigators (and your GP for advice). If you are feeling well, please place a tick in the boxes.

Study week	Day						
	1	2	3	4	5	6	7
Week 1							
Week 2							
Week 3							
Week 4							
Week 5							
Week 6							

Appendix 13: Instructions for ambulatory blood pressure monitor use for ATTIS study participants

IRAS Project number: 212398

REC 16/LO/1910

Version 1 28/09/2016

Appendix 18: 24 hour Blood Pressure Monitoring - Participant Information Sheet

The aim of this test is to monitor what is happening to your blood pressure over a 24 hour period.

We will provide you with a diary card to fill in on the day of measurements, please fill in your activity level at the time of measurement, the time you go to bed, the time you get up and any unusual circumstances.

Procedure:

- **Night and day**; the blood pressure cuff has two settings; a day time setting and a night time setting. The monitor will automatically change to night time settings at 10pm and day time settings at 7am.
- **Frequency of cuff inflation**; During the day time the cuff will inflate every 30 minutes, during the night time the cuff will inflate every 1 hr.

During a measurement:

To avoid incorrect results **the arm must be kept still during measurements**.
If you are standing; let your arm hang loosely, whilst keeping it still.
If you are sitting; rest your arm loosely on a table or let your arm hang loosely, whilst keeping it still.

- Avoid opening and closing your hand during the measurement, and do not move your fingers.
- Ensure the air tube is not kinked while the measurement is being taken.
- If you are driving when a measurement starts, continue to drive normally and do not worry about keeping the arm still (please make a note on the diary card if this happens), or turn the monitor off whilst you are driving.
- While you are asleep, place the recorder on its side so that the air tube will not kink.
- In the event of failed measurements the monitor will repeat the measurement.

Between measurements:

- Engage in normal activities.
- Check that the yellow mark is still in position as the cuff may move during the day.

Using the blood pressure monitor

IRAS Project number: 212398

REC 16/LO/1910

Version 1 28/09/2016

How to put the cuff on upper arm:

- Put the cuff on the upper left arm so that the position of the yellow marking of the cuff is on the artery; the opposite side to your elbow (we will mark this with a pen).
- The cuff should be wrapped on the arm tightly, but still allow a finger to slide between the cuff and the arm.

How to turn the monitor on and off:

To start the 24-hr measurement press and hold the black 'AUTO ON/OFF' button until it bleeps and the letter 'A' appears on the display.

To turn the monitor off or pause it when you take the cuff off (during bathing or exercise) press and hold down the 'AUTO ON/OFF' button until the letter 'A' is no longer on the display. When you want to restart it press and hold the black 'AUTO ON/OFF' button until it bleeps and the letter 'A' appears on the display.

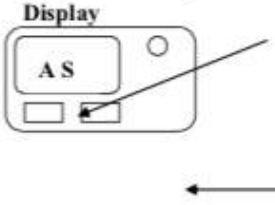
To deflate the cuff or terminate a recording press the red START/STOP button.

If the letter 'E' appears on the display at any time this means that there is an error and you will need to contact Leanne (Research Assistant). If the letter 'B' appears on the display at any time this means that the batteries need replacing.

If water gets into the unit, please remove the cuff and do not continue using the recorder. Turn off the unit and remove the batteries.

Some notes on operation:

Display



Red START/STOP button - can be used during a measurement to terminate a measurement and deflate the cuff

Black AUTO ON/OFF button – this will switch the monitor on to automatic mode (press for 3 seconds, until the letter 'A' appears on the display) or to cancel automatic mode (press again for 3 seconds, until the letter 'A' is no longer on display)

A - This is displayed when the unit is in automatic measurement mode

B – This is displayed when battery capacity is low

S – This is displayed when the unit is in sleep interval measurement mode

IF YOU EXPERIENCE ANY PAIN OR AN EXTREMELY UNPLEASANT SENSATION DURING MEASUREMENTS PLEASE TURN OFF THE UNIT IMMEDIATELY, USING THE ON/OFF KEY AND REMOVE THE CUFF.

In case of any problems please call Leanne on 020 7848 4301

Appendix 14: Activity diary for ambulatory blood pressure measurement of ATTIS study

IRAS Project number: 212398 REC: 16/LO/1910

Version 1. 28/09/2016

Appendix 17: Diary card of activity for 24 hour blood pressure

Participant ID: **Date:**

Please fill in your activity level at the time of measurement, the time you go to bed, the time you get up and any unusual circumstances.

Time you go to sleep…… Time you wake up……..

Did you take any exercise Y / N If Yes at what time……..

Time	Activity level (please circle)			Other
	Lying down	Sitting	Walking	
	Lying down	Sitting	Walking	
	Lying down	Sitting	Walking	

	Lying down	Sitting	Walking	
	Lying down	Sitting	Walking	
	Lying down	Sitting	Walking	
	Lying down	Sitting	Walking	
	Lying down	Sitting	Walking	
	Lying down	Sitting	Walking	

Notes:

Brief Instructions: Refer to the *24 hour Blood Pressure Monitoring - Subject Information Sheet* for full instructions. During the day time (7am – 10pm), the cuff will inflate every 30 minutes and during the night time (10pm-7am) every 1 hr. Between measurements engage in normal activities, also check that the yellow mark is still in position as the cuff may move during the day. The arm must be kept still during measurements. To start the 24-hr measurement press and hold the black 'AUTO ON/OFF' button until it bleeps and the letter 'A' appears on the display. To turn the monitor off or pause it whilst bathing etc press and hold down the 'AUTO ON/OFF' button until the letter 'A' is no longer on the display. To deflate the cuff or terminate a recording press the red START/STOP button.

In case of any problems please call Vita on 07874159803.

IF YOU EXPERIENCE ANY PAIN OR AN EXTREMELY UNPLEASANT SENSATION DURING MEASUREMENTS PLEASE TURN OFF THE UNIT IMMEDIATELY, USING THE ON/OFF KEY AND REMOVE THE CUFF.

Appendix 15: MRI safety questionnaire

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MRI Safety Questionnaire

Cardiac version

This must be completed by **ALL** persons entering the MRI scan room.
Please answer the following questions carefully, with a **black pen** and ask if anything is not clear. All information is held in the strictest confidence.

Name:	Date of Birth:	Address:
Height:	Weight:	Post code:

SECTION A: MRI scanning uses strong magnetic fields. For your own safety and the safety of others it is very important that you do not go into the MRI scan room with any metal in or on your body or clothing.

If you had any surgery or device please comment stating the type of operation and date

Please answer ALL the questions, ticking your answer ☑	No	Yes	If yes, please comment
1. Do you have/ever had a heart pacemaker? These may stop working near the MRI scanner.	☐	☐	
2. Have you ever had any surgery on your head or brain?	☐	☐	
3. Have you ever had any surgery on your eyes?	☐	☐	
4. Have you ever had any surgery on your heart?	☐	☐	
5. Have you ever had any surgery on your limbs?	☐	☐	
6. Have you had any surgery in the past 2 months?	☐	☐	
7. Do you have any other foreign bodies inside you (e.g. implants, devices, shrapnel), not already listed above?	☐	☐	
8. Have you ever had any metal particles in your eyes? (e.g. from welding or metal work)	☐	☐	
9. Could you be pregnant?	☐	☐	
10. Do you wear dentures, a dental plate or a brace?	☐	☐	
11. Have you had blackouts, epilepsy or fits in the past 2 months?	☐	☐	
12. Do you have any tattoos?	☐	☐	
13. Do you have any transdermal patches (medicated adhesive patches like morphine patch or nicotine patch)?	☐	☐	
14. Are you wearing coloured contact lenses?	☐	☐	
15. Do you suffer from any allergies (e.g. drug allergies or latex)?	☐	☐	
16. Do you agree to remove all of the above before entering the MRI scan room (all metal objects, including coins, jewellery, body-piercings, hearing aids, clothing containing any metal, dental braces, artificial limbs or callipers)?	☐	☐	
17. Is there anything else you think we should know about in relation to your MRI scan?	☐	☐	
18. Do you have hypertension and/or diabetes?	☐	☐	
19. Are you a smoker or ex-smoker?	☐	☐	
20. Is there anyone in your family who suffers or has suffered from a cardiac condition?	☐	☐	

MRI AUTHORISED PERSON ONLY	If any yes, please state if the patient can safely enter the MRI Controlled area on:	• 1.5 T ☐ Yes ☐ No • 3T ☐ Yes ☐ No	☐ Not applicable

PATIENTS PLEASE CONTINUE OVERLEAF

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SECTION B: MRI Contrast – Patients who consent for an injection of MRI contrast

If you have any kidney problems, please bring with you your most recent blood test results

You may be given an injection of MRI dye during your scan, to help with the diagnosis of the MRI scan. The dye is a colourless liquid injected into your veins during your scan. The most common side effect is a metallic taste in the mouth during the injection. Rare side effects are nausea and allergic reactions. Please answer the questions below.

Please answer ALL the questions, ticking your answer	No	Yes	If yes, please comment
1. **Do you consent to an injection of contrast agent (dye) if required?**	☐	☐	
2. Do you have any kidney problems?	☐	☐	
3. Have you ever had an allergic reaction to contrast agent (dye) for MRI or X-Ray/CT in the past?	☐	☐	
4. Are you breast feeding?	☐	☐	

FOR CLINICAL STAFF ONLY: To be completed if any kidney problem is stated or inpatient otherwise mark as not applicable		
eGFR ml/min	Date	Checked by

SECTION C: Stress Agent Adenosine/Regadenoson/Dobutamine/Atropine

Adenosine/Dobutamine and Atropine are drugs that are used to help diagnose problems with blood flow to the heart muscle. For your safety, it is important that we know if you have any of the conditions mentioned below before we give you these drugs. If you would like any more information about dobutamine, atropine or adenosine, please ask the cardiologist/radiologist.

Please answer ALL the questions, ticking your answer	No	Yes	If yes, please comment
1. Do you have glaucoma, urinary retention/prostatic hypertrophy?	☐	☐	
3. Do you have known low blood pressure (<100mm of Hg)?	☐	☐	
4. Do you have asthma or chronic obstructive pulmonary disease (COPD) and require inhalers?	☐	☐	
5. Have you consumed any caffeinated products within the last 12 hours? (Coffee, tea, coke, chocolate, Red Bull)	☐	☐	

Cardiologist/Radiologist signature after assessing that the conditions above are fulfilled if stress agent is required.

.....

SIGNATURE		DATE
PATIENT (declaring to have read, understood, answered the questions above)		
MRI AUTHORISED PERSON (declaring the patient is safe to scan)		

CLINICAL RECORD- TO BE COMPLETED BY CLINICAL STAFF ONLY					
IV RECORD FOR OUTPATIENT					
Date of insertion		Time of insertion		Cannula size	
Site		Skin prep used		Volume of flush	
Sterile dressing		Number of attempts		Inserted by	
Date of removal		Time of removal		Removed by	
IV RECORD FOR INPATIENT					
Cannula checked by			Volume of flush		

AGENT ADMINISTERED RECORD			
	GADOVIST ☐YES ☐NO	ADENOSINE/RAPISCAN ☐YES ☐NO	DOBUTAMINE ☐YES ☐NO
Batch number			
Expiry date			
Volume			
Checked by			

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Appendix 16: 24 h ambulatory heart rate variability measurement sheet

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24 hour heart rate monitor data

Participant ID:

Please wear your heart rate monitor for 24 hours, the green light in the heart will be flashing to indicate it is on. When wearing the monitor, please go about your normal activities and exercise. Please do not wear this monitor swimming/ in the shower or for an activity where it will become very wet. If you wish to wash, we suggest carefully taking a bath/ having a flannel wash. Please could you detail the time at which you went to bed and the time you got up below.

The time to take off your monitor is detailed below. To switch the monitor off, please press and hold the middle button, until the green flashing light has stopped. Once you have taken the device off, please place it in the small bag you received with your ID code on it. We then ask that you either bring the monitor back to us (if convenient) or we can send out a courier to your home.

Time monitor started:

Bedtime with heart rate monitor:

Wake up time with heart rate monitor:

Time to take monitor off:

Appendix 17: Instructions for stool collection for ATTIS study participants

IRAS Project number: 212398
REC 16/LO/1910

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Appendix 16 Information sheet for participants on stool sample collection

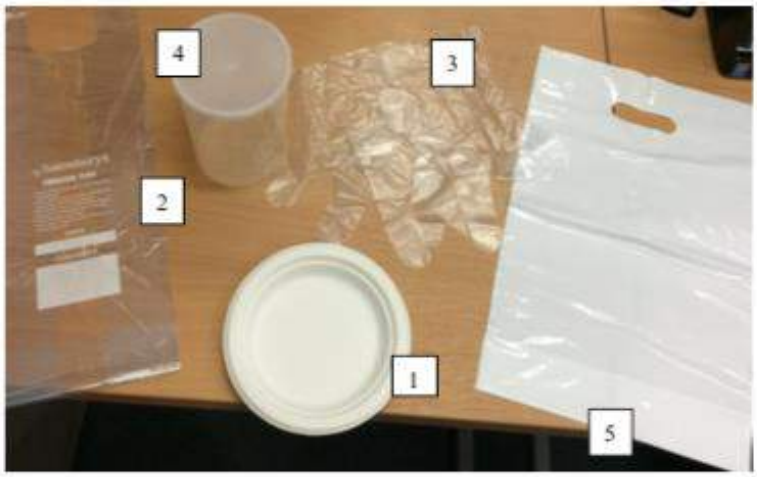
Collecting your stool

During the study we will ask you to collect your stool at the baseline and follow-up visits. It is best that we collect your stool at the study centre if possible as **we need to start processing it within 1-2 hours of being passed**.

We gather important information from these stool collections, including information about the bacteria in your gut.

Stool collection kit

1. Disposable paper plate
2. Clear plastic bag
3. Disposable gloves
4. Disposable plastic container
5. White bag



How to collect your stool

These instructions will also be given to you at your visit.

1. If you need to urinate, please do so before you collect your stool. YOU MUST ENSURE THAT YOU DO NOT URINATE AT THE SAME TIME AS COLLECTING YOUR STOOL SAMPLE - this would contaminate the sample.
2. Put on the disposable gloves.
3. Open the clear plastic bag

Version 1. 15/08/16

Appendix 16 Information sheet for participants on stool sample collection

4. Place the bag inside the plastic pot and allow the handles to hang over the outside of the pot. Push the bag down inside the pot so that only the handles hang over the side.

5. Place the paper plate in the toilet bowl, and try to wedge it in slightly to keep it in place – this will stop the pot going in the water.

6. Sit the pot containing the plastic bag on top of the paper plate.

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Appendix 16 Information sheet for participants on stool sample collection

7. Pass your stool into the bags inside the pot. REMEMBER - YOU MUST ENSURE THAT YOU DO NOT URINATE AT THE SAME TIME as this would contaminate the sample. You may find it helpful to steady the pot with your gloved hand when initially passing the stool.

8. Remove the pot from the toilet bowl. Fold the bag loosely inside the pot on top of the sample. Put the lid on the pot and ensure that it is fully sealed.

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Appendix 16 Information sheet for participants on stool sample collection

9. Place the sample pot inside the white bag provided (and inside further bags if you wish) to transport to the study centre (if you are not collecting the sample at your visit).

Thank you! ☺

Appendix 18: Acceptability questionnaire

IRAS Project number: 212398 REC 16/LO/1910
Version 2. 28/09/2016

Appendix 7 – Acceptability and use questionnaire

Participant code ☐ Date form completed ☐

Snack product acceptability and use questionnaire

Study title: The impact of almond nut consumption on markers of cardiovascular and metabolic disease

Purpose: This interviewer-administered form is used to collect participant's acceptability and the product (mini muffins and almonds) use feedback.

General information/Instructions: Please select one option for each question.

We would like to ask you some questions about your attitudes and experiences consuming the mini muffins and or almonds as a daily snack. Your honest answers will help us to investigate 'The impact of muffin snacks versus almond nut consumption on emerging markers of cardiovascular and metabolic disease' for future studies.

All the information will be kept confidential and will not be shared with anyone else besides the research study staff.

1. **Rate how well you liked the overall flavour of the mini muffins?**

☐ 1 I like it
☐ 2 Neither like nor dislike
☐ 3 Dislike

2. **How much did you like the following features of the mini muffins?**

	Like 1	Neither like nor dislike 2	Dislike 3
a. Colour			
b. Shape			
c. Texture			
d. Portion size			
e. Mouth feel			

3. **Did you like the packaging of the mini muffins?**

- ☐ 1 Yes
- ☐ 2 No
- ☐ 3 Somewhat

4. **Rate the portion size of the muffins as an everyday snack?**

- ☐ 1 Too little
- ☐ 2 Enough
- ☐ 3 More than enough

5. **How would you rate your satiety eating the muffins?**

- ☐ 1 Still Hungry
- ☐ 2 Comfortably full
- ☐ 3 Too full

6. **How often did you have problems storing the bag of mini muffins at home?**

- ☐ 1 Never
- ☐ 2 Sometimes
- ☐ 3 Often

7. **How difficult or easy was it to incorporate these mini muffins in your diet?**

- ☐ 1 Very difficult
- ☐ 2 Difficult
- ☐ 3 Easy
- ☐ 4 Very easy

8. **Did you have any of the following problems after eating the mini muffins?**

	Yes	A little	None
a. Reflux/ nausea			
b. Burps/belching/bloating			
c. Severe indigestion			

9. **Rate your willingness to continue eating these mini muffins as a snack?**

- ☐ 1 Not at all willing
- ☐ 2 Unsure
- ☐ 3 Extremely Willing

10. How often would you purchase these muffins as an alternative to your regular snacks if available in the market?

- ☐ 1 Never
- ☐ 2 Sometimes
- ☐ 3 Often

5. Any specific feature you like (specify)

_____
_____
_____

6. Any specific feature you dislike (specify)

_____
_____
_____

7. Do you have any suggestions for enhancement or improvement of any feature of the mini muffins?

_____
_____
_____
_____

Relating to almonds:

1. Rate the portion size of the almonds as an everyday snack?

- ☐ 1 Too little
- ☐ 2 Enough
- ☐ 3 More than enough

2. How would you rate your satiety eating the almonds?

- ☐ 1 Still Hungry
- ☐ 2 Comfortably full
- ☐ 3 Too full

3. How often did you have problems storing the bag of almonds at home?

- ☐ 1 Never
- ☐ 2 Sometimes
- ☐ 3 Often

4. How difficult or easy was it to incorporate the almonds in your diet?

- ☐ 1 Very difficult
- ☐ 2 Difficult
- ☐ 3 Easy
- ☐ 4 Very easy

Overall, were there any snacks that you particularly missed during the study? If so, which ones?

Version 3,
Dated 28/09/2016

Please return to:
Leanne Smith (Research Dietitian)
Room 4.46, Diabetes and Nutritional Sciences Division
King's College London, Franklin-Wilkins Building, 150 Stamford Street, London SE1 9NH
Email: almondstudy@kcl.ac.uk Tel: 020 7848 4301

Appendix 19: MRI/^{1}H-MRS scan protocols of the ATTIS study

The documents below are the protocols of MRI/^{1}H-MRS scan of the ATTIS study.

\\Non Cardiac Research\Whole body\ATTIS\ATTIS\FastViewLocalizer

TA: 0:42 PM: ISO Voxel size: 5.0×5.0×5.0 mmRel. SNR: 1.00 : flct

Properties

Prio recon	Off
Load images to viewer	On
Inline movie	Off
Auto store images	On
Load images to stamp segments	Off
Load images to graphic segments	On
Auto open inline display	On
Auto close inline display	On
Start measurement without further preparation	On
Wait for user to start	Off
Start measurements	Single measurement

Routine

Slice group	1
Slices	1
Dist. factor	100 %
Position	L0.0 A25.0 H47.0 mm
Orientation	Transversal
Phase enc. dir.	A >> P
AutoAlign	---
FoV read	480 mm
FoV phase	87.5 %
Slice thickness	5 mm
TR	3.31 ms
TE	2.19 ms
Filter	Distortion Corr.(2D)
Coil elements	BC

Contrast - Common

TR	3.31 ms
TE	2.19 ms

Resolution - Common

FoV read	480 mm
FoV phase	87.5 %
Slice thickness	5 mm
Base resolution	96
Phase resolution	100 %
Phase partial Fourier	6/8

Geometry - Common

Slice group	1
Slices	1
Dist. factor	100 %
Position	L0.0 A25.0 H47.0 mm
Orientation	Transversal
Phase enc. dir.	A >> P
FoV read	480 mm
FoV phase	87.5 %
Slice thickness	5 mm
TR	3.31 ms

Geometry - AutoAlign

Slice group	1
AutoAlign	---
Position	L0.0 A25.0 H47.0 mm
Orientation	Transversal
Phase enc. dir.	A >> P
Initial Position	L0.0 A25.0 H47.0

Geometry - AutoAlign

Phase	-25.0 mm
Read	0.0 mm
Shift	47.0 mm
Initial Rotation	0.00 deg
Initial Orientation	Transversal

Geometry - Tim CT

Tim CT mode	On
Range start	H
Range start	50 mm
Total FoV	H >> F
Total FoV	1500 mm
Slices	1
Slice thickness	5 mm
Dist. factor	100 %
FoV read	480 mm
FoV phase	87.5 %
Perform CTM adjustments	On
Table Speed	36 mm/s

System - Miscellaneous

Positioning mode	ISO
Table position	H
Table position	47 mm
MSMA	S - C - T
Sagittal	R >> L
Coronal	A >> P
Transversal	H >> F
Matrix Optimization	Off
AutoAlign	---
Coil Select Mode	Default

System - Adjustments

B0 Shim mode	Tune up
Adj. water suppr.	Off
Assume Dominant Fat	Off
Assume Silicone	Off
Perform CTM adjustments	On
Adjustment Tolerance	Maximum

System - Tx/Rx

Frequency 1H	63.685340 MHz
Correction factor	1
Img. Scale Cor.	1.000

Sequence - Part 1

Dimension	2D
Bandwidth	801.282051 Hz/Px

Sequence - Assistant

Mode	Off

\\Non Cardiac Research\Whole body\ATTIS\ATTIS\ax_t1_qdixon_1chest_F76

TA: 0:16 PM: ISO Voxel size: 2.2×2.2×3.5 mmPAT: 4 Rel. SNR: 1.00 : fl

Properties

Prio recon	Off
Load images to viewer	On
Inline movie	Off
Auto store images	On
Load images to stamp segments	On
Load images to graphic segments	Off
Auto open inline display	Off
Auto close inline display	Off
Start measurement without further preparation	On
Wait for user to start	Off
Start measurements	Single measurement

Routine

Slab group	1
Slabs	1
Dist. factor	20 %
Position	L0.0 A30.0 F76.0 mm
Orientation	Transversal
Phase enc. dir.	A >> P
AutoAlign	---
Phase oversampling	0 %
Slice oversampling	28.6 %
Slices per slab	56
FoV read	500 mm
FoV phase	72.3 %
Slice thickness	3.5 mm
TR	15.60 ms
TE 1	2.38 ms
TE 2	4.76 ms
TE 3	7.14 ms
TE 4	9.52 ms
TE 5	11.90 ms
TE 6	14.28 ms
Averages	1
Concatenations	1
Filter	Distortion Corr.(2D), Prescan Normalize
Coil elements	BO1,2;NE2;SP1,2

Contrast - Common

TR	15.60 ms
TE 1	2.38 ms
TE 2	4.76 ms
TE 3	7.14 ms
TE 4	9.52 ms
TE 5	11.90 ms
TE 6	14.28 ms
Flip angle	4.0 deg
Fat suppr.	None
Water suppr.	None
Dixon	On
Dixon evaluation	On

Contrast - Dynamic

Averages	1
Averaging mode	Long term
Reconstruction	Magnitude
Measurements	1
Multiple series	Off

Resolution - Common

FoV read	500 mm
FoV phase	72.3 %
Slice thickness	3.5 mm
Base resolution	224
Phase resolution	79 %
Slice resolution	50 %
Phase partial Fourier	7/8
Slice partial Fourier	7/8
Trajectory	Cartesian
View sharing	Off
Interpolation	Off

Resolution - iPAT

PAT mode	CAIPIRINHA
Accel. factor PE	2
Ref. lines PE	24
Accel. factor 3D	2
Ref. lines 3D	24
Reordering Shift 3D	1
Reference scan mode	GRE/separate
CAIPIRINHA mode	Body Tra
Total PAT factor	4

Resolution - Filter Image

Image Filter	Off
Distortion Corr.	On
Mode	2D
Unfiltered images	Off
Prescan Normalize	On
Unfiltered images	Off
Normalize	Off
B1 filter	Off

Resolution - Filter Rawdata

Raw filter	Off
Elliptical filter	Off
POCS	Off

Geometry - Common

Slab group	1
Slabs	1
Dist. factor	20 %
Position	L0.0 A30.0 F76.0 mm
Orientation	Transversal
Phase enc. dir.	A >> P
Slice oversampling	28.6 %
Slices per slab	56
FoV read	500 mm
FoV phase	72.3 %
Slice thickness	3.5 mm
TR	15.60 ms
Multi-slice mode	Sequential
Series	Ascending
Concatenations	1

Geometry - AutoAlign

Slab group	1
AutoAlign	---
Position	L0.0 A30.0 F76.0 mm
Orientation	Transversal

Geometry - AutoAlign

Phase enc. dir.	A >> P
Initial Position	L0.0 A30.0 F76.0
Phase	-30.0 mm
Read	0.0 mm
Shift	-76.0 mm
Initial Rotation	0.00 deg
Initial Orientation	Transversal

Geometry - Saturation

Fat suppr.	None
Water suppr.	None
Dixon	On
Dixon evaluation	On
Special sat.	None

System - Miscellaneous

Positioning mode	ISO
Table position	F
Table position	76 mm
MSMA	S - C - T
Sagittal	R >> L
Coronal	A >> P
Transversal	H >> F
Coil Combine Mode	Adaptive Combine
Save uncombined	Off
Matrix Optimization	Off
Coil Focus	Flat
AutoAlign	---
Coil Select Mode	On - AutoCoilSelect

System - Adjustments

B0 Shim mode	Standard
Adjust with body coil	Off
Confirm freq. adjustment	Off
Assume Dominant Fat	Off
Assume Silicone	Off
Adjustment Tolerance	Auto

System - Adjust Volume

Position	L0.0 A30.0 F76.0 mm
Orientation	Transversal
Rotation	0.00 deg
A >> P	362 mm
R >> L	500 mm
F >> H	196 mm
Reset	Off

System - Tx/Rx

Frequency 1H	63.685340 MHz
Correction factor	1
Gain	Low
Img. Scale Cor.	1.000
Reset	Off
? Ref. amplitude 1H	0.000 V

Physio - PACE

Resp. control	Off
Concatenations	1

Inline - Common

View sharing	Off
Flip angle	4.0 deg
Measurements	1

Inline - Common

Burn time-to-center	Off
Temporal interpolation	1
3D centric reordering	Off
Time to center	7.9 s

Inline - Inline

Subtract	Off
Measurements	1
StdDev	Off
Liver registration	Off
Save original images	On

Inline - MIP

MIP-Sag	Off
MIP-Cor	Off
MIP-Tra	Off
MIP-Time	Off
Save original images	On

Inline - Soft Tissue

Wash - In	Off
Wash - Out	Off
TTP	Off
PEI	Off
MIP - time	Off
Measurements	1

Inline - Composing

Inline Composing	On
Composing Function	Adaptive
Composing Group	1
Last Step	Off
Normalize	None
Series Description	Dixon
Distortion Corr.	On
Mode	2D
Unfiltered images	Off

Sequence - Part 1

Introduction	Off
Dimension	3D
Elliptical scanning	Off
Asymmetric echo	Off
Contrasts	6
Readout mode	Bipolar
Optimization	Equidistant
Multi-slice mode	Sequential
Bandwidth 1	1060 Hz/Px
Bandwidth 2	1060 Hz/Px
Bandwidth 3	1060 Hz/Px
Bandwidth 4	1060 Hz/Px
Bandwidth 5	1060 Hz/Px
Bandwidth 6	1060 Hz/Px

Sequence - Part 2

RF pulse type	Normal
Gradient mode	Fast
Excitation	Slab-sel.
RF spoiling	On
Incr. Gradient spoiling	Off

Sequence - Assistant

Mode	Off

\\Non Cardiac Research\Whole body\ATTIS\ATTIS\ax_t1_qdixon_2abdo_F272

TA: 0:16 PM: ISO Voxel size: 2.2×2.2×3.5 mmPAT: 4 Rel. SNR: 1.00 : fl

Properties

Prio recon	Off
Load images to viewer	On
Inline movie	Off
Auto store images	On
Load images to stamp segments	On
Load images to graphic segments	Off
Auto open inline display	Off
Auto close inline display	Off
Start measurement without further preparation	On
Wait for user to start	Off
Start measurements	Single measurement

Routine

Slab group	1
Slabs	1
Dist. factor	20 %
Position	L0.0 A30.0 F272.0 mm
Orientation	Transversal
Phase enc. dir.	A >> P
AutoAlign	---
Phase oversampling	0 %
Slice oversampling	28.6 %
Slices per slab	56
FoV read	500 mm
FoV phase	72.3 %
Slice thickness	3.5 mm
TR	15.60 ms
TE 1	2.38 ms
TE 2	4.76 ms
TE 3	7.14 ms
TE 4	9.52 ms
TE 5	11.90 ms
TE 6	14.28 ms
Averages	1
Concatenations	1
Filter	Distortion Corr.(2D), Prescan Normalize
Coil elements	BO1-3;SP2-4

Contrast - Common

TR	15.60 ms
TE 1	2.38 ms
TE 2	4.76 ms
TE 3	7.14 ms
TE 4	9.52 ms
TE 5	11.90 ms
TE 6	14.28 ms
Flip angle	4.0 deg
Fat suppr.	None
Water suppr.	None
Dixon	On
Dixon evaluation	On

Contrast - Dynamic

Averages	1
Averaging mode	Long term
Reconstruction	Magnitude
Measurements	1
Multiple series	Off

Resolution - Common

FoV read	500 mm
FoV phase	72.3 %
Slice thickness	3.5 mm
Base resolution	224
Phase resolution	79 %
Slice resolution	50 %
Phase partial Fourier	7/8
Slice partial Fourier	7/8
Trajectory	Cartesian
View sharing	Off
Interpolation	Off

Resolution - iPAT

PAT mode	CAIPIRINHA
Accel. factor PE	2
Ref. lines PE	24
Accel. factor 3D	2
Ref. lines 3D	24
Reordering Shift 3D	1
Reference scan mode	GRE/separate
CAIPIRINHA mode	Body Tra
Total PAT factor	4

Resolution - Filter Image

Image Filter	Off
Distortion Corr.	On
Mode	2D
Unfiltered images	Off
Prescan Normalize	On
Unfiltered images	Off
Normalize	Off
B1 filter	Off

Resolution - Filter Rawdata

Raw filter	Off
Elliptical filter	Off
POCS	Off

Geometry - Common

Slab group	1
Slabs	1
Dist. factor	20 %
Position	L0.0 A30.0 F272.0 mm
Orientation	Transversal
Phase enc. dir.	A >> P
Slice oversampling	28.6 %
Slices per slab	56
FoV read	500 mm
FoV phase	72.3 %
Slice thickness	3.5 mm
TR	15.60 ms
Multi-slice mode	Sequential
Series	Ascending
Concatenations	1

Geometry - AutoAlign

Slab group	1
AutoAlign	---
Position	L0.0 A30.0 F272.0 mm
Orientation	Transversal

Geometry - AutoAlign

Phase enc. dir.	A >> P
Initial Position	L0.0 A30.0 F272.0
Phase	-30.0 mm
Read	0.0 mm
Shift	-272.0 mm
Initial Rotation	0.00 deg
Initial Orientation	Transversal

Geometry - Saturation

Fat suppr.	None
Water suppr.	None
Dixon	On
Dixon evaluation	On
Special sat.	None

System - Miscellaneous

Positioning mode	ISO
Table position	F
Table position	272 mm
MSMA	S - C - T
Sagittal	R >> L
Coronal	A >> P
Transversal	H >> F
Coil Combine Mode	Adaptive Combine
Save uncombined	Off
Matrix Optimization	Off
Coil Focus	Flat
AutoAlign	---
Coil Select Mode	On - AutoCoilSelect

System - Adjustments

B0 Shim mode	Standard
Adjust with body coil	Off
Confirm freq. adjustment	Off
Assume Dominant Fat	Off
Assume Silicone	Off
Adjustment Tolerance	Auto

System - Adjust Volume

Position	L0.0 A30.0 F272.0 mm
Orientation	Transversal
Rotation	0.00 deg
A >> P	362 mm
R >> L	500 mm
F >> H	196 mm
Reset	Off

System - Tx/Rx

Frequency 1H	63.685340 MHz
Correction factor	1
Gain	Low
Img. Scale Cor.	1.000
Reset	Off
? Ref. amplitude 1H	0.000 V

Physio - PACE

Resp. control	Off
Concatenations	1

Inline - Common

View sharing	Off
Flip angle	4.0 deg
Measurements	1

Inline - Common

Burn time-to-center	Off
Temporal interpolation	1
3D centric reordering	Off
Time to center	7.9 s

Inline - Inline

Subtract	Off
Measurements	1
StdDev	Off
Liver registration	Off
Save original images	On

Inline - MIP

MIP-Sag	Off
MIP-Cor	Off
MIP-Tra	Off
MIP-Time	Off
Save original images	On

Inline - Soft Tissue

Wash - In	Off
Wash - Out	Off
TTP	Off
PEI	Off
MIP - time	Off
Measurements	1

Inline - Composing

Inline Composing	On
Composing Function	Adaptive
Composing Group	1
Last Step	Off
Normalize	None
Series Description	Dixon
Distortion Corr.	On
Mode	2D
Unfiltered images	Off

Sequence - Part 1

Introduction	Off
Dimension	3D
Elliptical scanning	Off
Asymmetric echo	Off
Contrasts	6
Readout mode	Bipolar
Optimization	Equidistant
Multi-slice mode	Sequential
Bandwidth 1	1060 Hz/Px
Bandwidth 2	1060 Hz/Px
Bandwidth 3	1060 Hz/Px
Bandwidth 4	1060 Hz/Px
Bandwidth 5	1060 Hz/Px
Bandwidth 6	1060 Hz/Px

Sequence - Part 2

RF pulse type	Normal
Gradient mode	Fast
Excitation	Slab-sel.
RF spoiling	On
Incr. Gradient spoiling	Off

Sequence - Assistant

Mode	Off

\\Non Cardiac Research\Whole body\ATTIS\ATTIS\ax_t1_qdixon_2abdo_F272_hires_pancreas

TA: 0:13 PM: ISO Voxel size: 1.1×1.1×3.5 mmPAT: 4 Rel. SNR: 1.00 : fl

Properties

Prio recon	Off
Load images to viewer	On
Inline movie	Off
Auto store images	On
Load images to stamp segments	On
Load images to graphic segments	On
Auto open inline display	Off
Auto close inline display	Off
Start measurement without further preparation	On
Wait for user to start	Off
Start measurements	Single measurement

Routine

Slab group	1
Slabs	1
Dist. factor	20 %
Position	L0.0 A30.0 F272.0 mm
Orientation	Transversal
Phase enc. dir.	A >> P
AutoAlign	---
Phase oversampling	0 %
Slice oversampling	28.6 %
Slices per slab	56
FoV read	500 mm
FoV phase	72.3 %
Slice thickness	3.5 mm
TR	6.36 ms
TE 1	2.38 ms
TE 2	4.76 ms
Averages	1
Concatenations	1
Filter	Distortion Corr.(2D), Prescan Normalize
Coil elements	BO1-3;SP2-4

Contrast - Common

TR	6.36 ms
TE 1	2.38 ms
TE 2	4.76 ms
Flip angle	12.0 deg
Fat suppr.	None
Water suppr.	None
Dixon	On
Dixon evaluation	Off

Contrast - Dynamic

Averages	1
Averaging mode	Long term
Reconstruction	Magnitude
Measurements	1
Multiple series	Off

Resolution - Common

FoV read	500 mm
FoV phase	72.3 %
Slice thickness	3.5 mm
Base resolution	448
Phase resolution	79 %
Slice resolution	50 %

Resolution - Common

Phase partial Fourier	7/8
Slice partial Fourier	7/8
Trajectory	Cartesian
View sharing	Off
Interpolation	Off

Resolution - iPAT

PAT mode	CAIPIRINHA
Accel. factor PE	2
Ref. lines PE	24
Accel. factor 3D	2
Ref. lines 3D	24
Reordering Shift 3D	1
Reference scan mode	GRE/separate
CAIPIRINHA mode	Body Tra
Total PAT factor	4

Resolution - Filter Image

Image Filter	Off
Distortion Corr.	On
Mode	2D
Unfiltered images	Off
Prescan Normalize	On
Unfiltered images	Off
Normalize	Off
B1 filter	Off

Resolution - Filter Rawdata

Raw filter	Off
Elliptical filter	Off
POCS	Off

Geometry - Common

Slab group	1
Slabs	1
Dist. factor	20 %
Position	L0.0 A30.0 F272.0 mm
Orientation	Transversal
Phase enc. dir.	A >> P
Slice oversampling	28.6 %
Slices per slab	56
FoV read	500 mm
FoV phase	72.3 %
Slice thickness	3.5 mm
TR	6.36 ms
Multi-slice mode	Sequential
Series	Ascending
Concatenations	1

Geometry - AutoAlign

Slab group	1
AutoAlign	---
Position	L0.0 A30.0 F272.0 mm
Orientation	Transversal
Phase enc. dir.	A >> P
Initial Position	L0.0 A30.0 F272.0
Phase	-30.0 mm
Read	0.0 mm
Shift	-272.0 mm
Initial Rotation	0.00 deg

Geometry - AutoAlign

Initial Orientation	Transversal

Geometry - Saturation

Fat suppr.	None
Water suppr.	None
Dixon	On
Dixon evaluation	Off
Special sat.	None

System - Miscellaneous

Positioning mode	ISO
Table position	F
Table position	272 mm
MSMA	S - C - T
Sagittal	R >> L
Coronal	A >> P
Transversal	H >> F
Coil Combine Mode	Adaptive Combine
Save uncombined	Off
Matrix Optimization	Off
Coil Focus	Flat
AutoAlign	---
Coil Select Mode	On - AutoCoilSelect

System - Adjustments

B0 Shim mode	Standard
Adjust with body coil	Off
Confirm freq. adjustment	Off
Assume Dominant Fat	Off
Assume Silicone	Off
Adjustment Tolerance	Auto

System - Adjust Volume

Position	L0.0 A30.0 F272.0 mm
Orientation	Transversal
Rotation	0.00 deg
A >> P	362 mm
R >> L	500 mm
F >> H	196 mm
Reset	Off

System - Tx/Rx

Frequency 1H	63.685340 MHz
Correction factor	1
Gain	Low
Img. Scale Cor.	1.000
Reset	Off
? Ref. amplitude 1H	0.000 V

Physio - PACE

Resp. control	Off
Concatenations	1

Inline - Common

View sharing	Off
Flip angle	12.0 deg
Measurements	1
Burn time-to-center	Off
Temporal interpolation	1
3D centric reordering	Off
Time to center	6.8 s

Inline - Inline

Subtract	Off
Measurements	1
StdDev	Off
Liver registration	Off
Save original images	On

Inline - MIP

MIP-Sag	Off
MIP-Cor	Off
MIP-Tra	Off
MIP-Time	Off
Save original images	On

Inline - Soft Tissue

Wash - In	Off
Wash - Out	Off
TTP	Off
PEI	Off
MIP - time	Off
Measurements	1

Inline - Composing

Inline Composing	Off
Distortion Corr.	On
Mode	2D
Unfiltered images	Off

Sequence - Part 1

Introduction	Off
Dimension	3D
Elliptical scanning	Off
Asymmetric echo	Off
Contrasts	2
Readout mode	Bipolar
Optimization	Equidistant
Multi-slice mode	Sequential
Bandwidth 1	1010 Hz/Px
Bandwidth 2	1010 Hz/Px

Sequence - Part 2

RF pulse type	Normal
Gradient mode	Fast
Excitation	Slab-sel.
RF spoiling	On
Incr. Gradient spoiling	On

Sequence - Assistant

Mode	Off

\\Non Cardiac Research\Whole body\ATTIS\ATTIS\ax_t1_qdixon_3abdo-pelvis_F468

TA: 0:16 PM: ISO Voxel size: 2.2×2.2×3.5 mmPAT: 4 Rel. SNR: 1.00 : fl

Properties

Prio recon	Off
Load images to viewer	On
Inline movie	Off
Auto store images	On
Load images to stamp segments	On
Load images to graphic segments	Off
Auto open inline display	Off
Auto close inline display	Off
Start measurement without further preparation	On
Wait for user to start	Off
Start measurements	Single measurement

Routine

Slab group	1
Slabs	1
Dist. factor	20 %
Position	L0.0 A30.0 F468.0 mm
Orientation	Transversal
Phase enc. dir.	A >> P
AutoAlign	---
Phase oversampling	0 %
Slice oversampling	28.6 %
Slices per slab	56
FoV read	500 mm
FoV phase	72.3 %
Slice thickness	3.5 mm
TR	15.60 ms
TE 1	2.38 ms
TE 2	4.76 ms
TE 3	7.14 ms
TE 4	9.52 ms
TE 5	11.90 ms
TE 6	14.28 ms
Averages	1
Concatenations	1
Filter	Distortion Corr.(2D), Prescan Normalize
Coil elements	BO1-3;SP2-4

Contrast - Common

TR	15.60 ms
TE 1	2.38 ms
TE 2	4.76 ms
TE 3	7.14 ms
TE 4	9.52 ms
TE 5	11.90 ms
TE 6	14.28 ms
Flip angle	4.0 deg
Fat suppr.	None
Water suppr.	None
Dixon	On
Dixon evaluation	On

Contrast - Dynamic

Averages	1
Averaging mode	Long term
Reconstruction	Magnitude
Measurements	1
Multiple series	Off

Resolution - Common

FoV read	500 mm
FoV phase	72.3 %
Slice thickness	3.5 mm
Base resolution	224
Phase resolution	79 %
Slice resolution	50 %
Phase partial Fourier	7/8
Slice partial Fourier	7/8
Trajectory	Cartesian
View sharing	Off
Interpolation	Off

Resolution - iPAT

PAT mode	CAIPIRINHA
Accel. factor PE	2
Ref. lines PE	24
Accel. factor 3D	2
Ref. lines 3D	24
Reordering Shift 3D	1
Reference scan mode	GRE/separate
CAIPIRINHA mode	Body Tra
Total PAT factor	4

Resolution - Filter Image

Image Filter	Off
Distortion Corr.	On
Mode	2D
Unfiltered images	Off
Prescan Normalize	On
Unfiltered images	Off
Normalize	Off
B1 filter	Off

Resolution - Filter Rawdata

Raw filter	Off
Elliptical filter	Off
POCS	Off

Geometry - Common

Slab group	1
Slabs	1
Dist. factor	20 %
Position	L0.0 A30.0 F468.0 mm
Orientation	Transversal
Phase enc. dir.	A >> P
Slice oversampling	28.6 %
Slices per slab	56
FoV read	500 mm
FoV phase	72.3 %
Slice thickness	3.5 mm
TR	15.60 ms
Multi-slice mode	Sequential
Series	Ascending
Concatenations	1

Geometry - AutoAlign

Slab group	1
AutoAlign	---
Position	L0.0 A30.0 F468.0 mm
Orientation	Transversal

Geometry - AutoAlign

Phase enc. dir.	A >> P
Initial Position	L0.0 A30.0 F468.0
Phase	-30.0 mm
Read	0.0 mm
Shift	-468.0 mm
Initial Rotation	0.00 deg
Initial Orientation	Transversal

Geometry - Saturation

Fat suppr.	None
Water suppr.	None
Dixon	On
Dixon evaluation	On
Special sat.	None

System - Miscellaneous

Positioning mode	ISO
Table position	F
Table position	468 mm
MSMA	S - C - T
Sagittal	R >> L
Coronal	A >> P
Transversal	H >> F
Coil Combine Mode	Adaptive Combine
Save uncombined	Off
Matrix Optimization	Off
Coil Focus	Flat
AutoAlign	---
Coil Select Mode	On - AutoCoilSelect

System - Adjustments

B0 Shim mode	Standard
Adjust with body coil	Off
Confirm freq. adjustment	Off
Assume Dominant Fat	Off
Assume Silicone	Off
Adjustment Tolerance	Auto

System - Adjust Volume

Position	L0.0 A30.0 F468.0 mm
Orientation	Transversal
Rotation	0.00 deg
A >> P	362 mm
R >> L	500 mm
F >> H	196 mm
Reset	Off

System - Tx/Rx

Frequency 1H	63.685340 MHz
Correction factor	1
Gain	Low
Img. Scale Cor.	1.000
Reset	Off
? Ref. amplitude 1H	0.000 V

Physio - PACE

Resp. control	Off
Concatenations	1

Inline - Common

View sharing	Off
Flip angle	4.0 deg
Measurements	1

Inline - Common

Burn time-to-center	Off
Temporal interpolation	1
3D centric reordering	Off
Time to center	7.9 s

Inline - Inline

Subtract	Off
Measurements	1
StdDev	Off
Liver registration	Off
Save original images	On

Inline - MIP

MIP-Sag	Off
MIP-Cor	Off
MIP-Tra	Off
MIP-Time	Off
Save original images	On

Inline - Soft Tissue

Wash - In	Off
Wash - Out	Off
TTP	Off
PEI	Off
MIP - time	Off
Measurements	1

Inline - Composing

Inline Composing	On
Composing Function	Adaptive
Composing Group	1
Last Step	Off
Normalize	None
Series Description	Dixon
Distortion Corr.	On
Mode	2D
Unfiltered images	Off

Sequence - Part 1

Introduction	Off
Dimension	3D
Elliptical scanning	Off
Asymmetric echo	Off
Contrasts	6
Readout mode	Bipolar
Optimization	Equidistant
Multi-slice mode	Sequential
Bandwidth 1	1060 Hz/Px
Bandwidth 2	1060 Hz/Px
Bandwidth 3	1060 Hz/Px
Bandwidth 4	1060 Hz/Px
Bandwidth 5	1060 Hz/Px
Bandwidth 6	1060 Hz/Px

Sequence - Part 2

RF pulse type	Normal
Gradient mode	Fast
Excitation	Slab-sel.
RF spoiling	On
Incr. Gradient spoiling	Off

Sequence - Assistant

Mode	Off

\\Non Cardiac Research\Whole body\ATTIS\ATTIS\ax_t1_qdixon_4pelvis-thigh_F664

TA: 0:16 PM: ISO Voxel size: 2.2×2.2×3.5 mmPAT: 4 Rel. SNR: 1.00 : fl

Properties

Prio recon	Off
Load images to viewer	On
Inline movie	Off
Auto store images	On
Load images to stamp segments	On
Load images to graphic segments	Off
Auto open inline display	Off
Auto close inline display	Off
Start measurement without further preparation	On
Wait for user to start	Off
Start measurements	Single measurement

Routine

Slab group	1
Slabs	1
Dist. factor	20 %
Position	L0.0 A30.0 F664.0 mm
Orientation	Transversal
Phase enc. dir.	A >> P
AutoAlign	---
Phase oversampling	0 %
Slice oversampling	28.6 %
Slices per slab	56
FoV read	500 mm
FoV phase	72.3 %
Slice thickness	3.5 mm
TR	15.60 ms
TE 1	2.38 ms
TE 2	4.76 ms
TE 3	7.14 ms
TE 4	9.52 ms
TE 5	11.90 ms
TE 6	14.28 ms
Averages	1
Concatenations	1
Filter	Distortion Corr.(2D), Prescan Normalize
Coil elements	BO1-3;SP2-4

Contrast - Common

TR	15.60 ms
TE 1	2.38 ms
TE 2	4.76 ms
TE 3	7.14 ms
TE 4	9.52 ms
TE 5	11.90 ms
TE 6	14.28 ms
Flip angle	4.0 deg
Fat suppr.	None
Water suppr.	None
Dixon	On
Dixon evaluation	On

Contrast - Dynamic

Averages	1
Averaging mode	Long term
Reconstruction	Magnitude
Measurements	1
Multiple series	Off

Resolution - Common

FoV read	500 mm
FoV phase	72.3 %
Slice thickness	3.5 mm
Base resolution	224
Phase resolution	79 %
Slice resolution	50 %
Phase partial Fourier	7/8
Slice partial Fourier	7/8
Trajectory	Cartesian
View sharing	Off
Interpolation	Off

Resolution - iPAT

PAT mode	CAIPIRINHA
Accel. factor PE	2
Ref. lines PE	24
Accel. factor 3D	2
Ref. lines 3D	24
Reordering Shift 3D	1
Reference scan mode	GRE/separate
CAIPIRINHA mode	Body Tra
Total PAT factor	4

Resolution - Filter Image

Image Filter	Off
Distortion Corr.	On
Mode	2D
Unfiltered images	Off
Prescan Normalize	On
Unfiltered images	Off
Normalize	Off
B1 filter	Off

Resolution - Filter Rawdata

Raw filter	Off
Elliptical filter	Off
POCS	Off

Geometry - Common

Slab group	1
Slabs	1
Dist. factor	20 %
Position	L0.0 A30.0 F664.0 mm
Orientation	Transversal
Phase enc. dir.	A >> P
Slice oversampling	28.6 %
Slices per slab	56
FoV read	500 mm
FoV phase	72.3 %
Slice thickness	3.5 mm
TR	15.60 ms
Multi-slice mode	Sequential
Series	Ascending
Concatenations	1

Geometry - AutoAlign

Slab group	1
AutoAlign	---
Position	L0.0 A30.0 F664.0 mm
Orientation	Transversal

Geometry - AutoAlign

Phase enc. dir.	A >> P
Initial Position	L0.0 A30.0 F664.0
Phase	-30.0 mm
Read	0.0 mm
Shift	-664.0 mm
Initial Rotation	0.00 deg
Initial Orientation	Transversal

Geometry - Saturation

Fat suppr.	None
Water suppr.	None
Dixon	On
Dixon evaluation	On
Special sat.	None

System - Miscellaneous

Positioning mode	ISO
Table position	F
Table position	664 mm
MSMA	S - C - T
Sagittal	R >> L
Coronal	A >> P
Transversal	H >> F
Coil Combine Mode	Adaptive Combine
Save uncombined	Off
Matrix Optimization	Off
Coil Focus	Flat
AutoAlign	---
Coil Select Mode	On - AutoCoilSelect

System - Adjustments

B0 Shim mode	Standard
Adjust with body coil	Off
Confirm freq. adjustment	Off
Assume Dominant Fat	Off
Assume Silicone	Off
Adjustment Tolerance	Auto

System - Adjust Volume

Position	L0.0 A30.0 F664.0 mm
Orientation	Transversal
Rotation	0.00 deg
A >> P	362 mm
R >> L	500 mm
F >> H	196 mm
Reset	Off

System - Tx/Rx

Frequency 1H	63.685340 MHz
Correction factor	1
Gain	Low
Img. Scale Cor.	1.000
Reset	Off
? Ref. amplitude 1H	0.000 V

Physio - PACE

Resp. control	Off
Concatenations	1

Inline - Common

View sharing	Off
Flip angle	4.0 deg
Measurements	1

Inline - Common

Burn time-to-center	Off
Temporal interpolation	1
3D centric reordering	Off
Time to center	7.9 s

Inline - Inline

Subtract	Off
Measurements	1
StdDev	Off
Liver registration	Off
Save original images	On

Inline - MIP

MIP-Sag	Off
MIP-Cor	Off
MIP-Tra	Off
MIP-Time	Off
Save original images	On

Inline - Soft Tissue

Wash - In	Off
Wash - Out	Off
TTP	Off
PEI	Off
MIP - time	Off
Measurements	1

Inline - Composing

Inline Composing	On
Composing Function	Adaptive
Composing Group	1
Last Step	Off
Normalize	None
Series Description	Dixon
Distortion Corr.	On
Mode	2D
Unfiltered images	Off

Sequence - Part 1

Introduction	Off
Dimension	3D
Elliptical scanning	Off
Asymmetric echo	Off
Contrasts	6
Readout mode	Bipolar
Optimization	Equidistant
Multi-slice mode	Sequential
Bandwidth 1	1060 Hz/Px
Bandwidth 2	1060 Hz/Px
Bandwidth 3	1060 Hz/Px
Bandwidth 4	1060 Hz/Px
Bandwidth 5	1060 Hz/Px
Bandwidth 6	1060 Hz/Px

Sequence - Part 2

RF pulse type	Normal
Gradient mode	Fast
Excitation	Slab-sel.
RF spoiling	On
Incr. Gradient spoiling	Off

Sequence - Assistant

Mode	Off

\\Non Cardiac Research\Whole body\ATTIS\ATTIS\ax_t1_qdixon_5thigh-knee_F860

TA: 0:16 PM: ISO Voxel size: 2.2×2.2×3.5 mmPAT: 4 Rel. SNR: 1.00 : fl

Properties

Prio recon	Off
Load images to viewer	On
Inline movie	Off
Auto store images	On
Load images to stamp segments	On
Load images to graphic segments	Off
Auto open inline display	Off
Auto close inline display	Off
Start measurement without further preparation	On
Wait for user to start	Off
Start measurements	Single measurement

Routine

Slab group	1
Slabs	1
Dist. factor	20 %
Position	L0.0 A30.0 F860.0 mm
Orientation	Transversal
Phase enc. dir.	A >> P
AutoAlign	---
Phase oversampling	0 %
Slice oversampling	28.6 %
Slices per slab	56
FoV read	500 mm
FoV phase	72.3 %
Slice thickness	3.5 mm
TR	15.60 ms
TE 1	2.38 ms
TE 2	4.76 ms
TE 3	7.14 ms
TE 4	9.52 ms
TE 5	11.90 ms
TE 6	14.28 ms
Averages	1
Concatenations	1
Filter	Distortion Corr.(2D), Prescan Normalize
Coil elements	BO1-3;SP2-4

Contrast - Common

TR	15.60 ms
TE 1	2.38 ms
TE 2	4.76 ms
TE 3	7.14 ms
TE 4	9.52 ms
TE 5	11.90 ms
TE 6	14.28 ms
Flip angle	4.0 deg
Fat suppr.	None
Water suppr.	None
Dixon	On
Dixon evaluation	On

Contrast - Dynamic

Averages	1
Averaging mode	Long term
Reconstruction	Magnitude
Measurements	1
Multiple series	Off

Resolution - Common

FoV read	500 mm
FoV phase	72.3 %
Slice thickness	3.5 mm
Base resolution	224
Phase resolution	79 %
Slice resolution	50 %
Phase partial Fourier	7/8
Slice partial Fourier	7/8
Trajectory	Cartesian
View sharing	Off
Interpolation	Off

Resolution - iPAT

PAT mode	CAIPIRINHA
Accel. factor PE	2
Ref. lines PE	24
Accel. factor 3D	2
Ref. lines 3D	24
Reordering Shift 3D	1
Reference scan mode	GRE/separate
CAIPIRINHA mode	Body Tra
Total PAT factor	4

Resolution - Filter Image

Image Filter	Off
Distortion Corr.	On
Mode	2D
Unfiltered images	Off
Prescan Normalize	On
Unfiltered images	Off
Normalize	Off
B1 filter	Off

Resolution - Filter Rawdata

Raw filter	Off
Elliptical filter	Off
POCS	Off

Geometry - Common

Slab group	1
Slabs	1
Dist. factor	20 %
Position	L0.0 A30.0 F860.0 mm
Orientation	Transversal
Phase enc. dir.	A >> P
Slice oversampling	28.6 %
Slices per slab	56
FoV read	500 mm
FoV phase	72.3 %
Slice thickness	3.5 mm
TR	15.60 ms
Multi-slice mode	Sequential
Series	Ascending
Concatenations	1

Geometry - AutoAlign

Slab group	1
AutoAlign	---
Position	L0.0 A30.0 F860.0 mm
Orientation	Transversal

Geometry - AutoAlign

Phase enc. dir.	A >> P
Initial Position	L0.0 A30.0 F860.0
Phase	-30.0 mm
Read	0.0 mm
Shift	-860.0 mm
Initial Rotation	0.00 deg
Initial Orientation	Transversal

Geometry - Saturation

Fat suppr.	None
Water suppr.	None
Dixon	On
Dixon evaluation	On
Special sat.	None

System - Miscellaneous

Positioning mode	ISO
Table position	F
Table position	860 mm
MSMA	S - C - T
Sagittal	R >> L
Coronal	A >> P
Transversal	H >> F
Coil Combine Mode	Adaptive Combine
Save uncombined	Off
Matrix Optimization	Off
Coil Focus	Flat
AutoAlign	---
Coil Select Mode	On - AutoCoilSelect

System - Adjustments

B0 Shim mode	Standard
Adjust with body coil	Off
Confirm freq. adjustment	Off
Assume Dominant Fat	Off
Assume Silicone	Off
Adjustment Tolerance	Auto

System - Adjust Volume

Position	L0.0 A30.0 F860.0 mm
Orientation	Transversal
Rotation	0.00 deg
A >> P	362 mm
R >> L	500 mm
F >> H	196 mm
Reset	Off

System - Tx/Rx

Frequency 1H	63.685340 MHz
Correction factor	1
Gain	Low
Img. Scale Cor.	1.000
Reset	Off
? Ref. amplitude 1H	0.000 V

Physio - PACE

Resp. control	Off
Concatenations	1

Inline - Common

View sharing	Off
Flip angle	4.0 deg
Measurements	1

Inline - Common

Burn time-to-center	Off
Temporal interpolation	1
3D centric reordering	Off
Time to center	7.9 s

Inline - Inline

Subtract	Off
Measurements	1
StdDev	Off
Liver registration	Off
Save original images	On

Inline - MIP

MIP-Sag	Off
MIP-Cor	Off
MIP-Tra	Off
MIP-Time	Off
Save original images	On

Inline - Soft Tissue

Wash - In	Off
Wash - Out	Off
TTP	Off
PEI	Off
MIP - time	Off
Measurements	1

Inline - Composing

Inline Composing	On
Composing Function	Adaptive
Composing Group	1
Last Step	Off
Normalize	None
Series Description	Dixon
Distortion Corr.	On
Mode	2D
Unfiltered images	Off

Sequence - Part 1

Introduction	Off
Dimension	3D
Elliptical scanning	Off
Asymmetric echo	Off
Contrasts	6
Readout mode	Bipolar
Optimization	Equidistant
Multi-slice mode	Sequential
Bandwidth 1	1060 Hz/Px
Bandwidth 2	1060 Hz/Px
Bandwidth 3	1060 Hz/Px
Bandwidth 4	1060 Hz/Px
Bandwidth 5	1060 Hz/Px
Bandwidth 6	1060 Hz/Px

Sequence - Part 2

RF pulse type	Normal
Gradient mode	Fast
Excitation	Slab-sel.
RF spoiling	On
Incr. Gradient spoiling	Off

Sequence - Assistant

Mode	Off

\\Non Cardiac Research\Whole body\ATTIS\ATTIS\ax_t1_qdixon_6knee-calf_F1056

TA: 0:16 PM: ISO Voxel size: 2.2×2.2×3.5 mmPAT: 4 Rel. SNR: 1.00 : fl

Properties

Prio recon	Off
Load images to viewer	On
Inline movie	Off
Auto store images	On
Load images to stamp segments	On
Load images to graphic segments	On
Auto open inline display	Off
Auto close inline display	Off
Start measurement without further preparation	On
Wait for user to start	Off
Start measurements	Single measurement

Routine

Slab group	1
Slabs	1
Dist. factor	20 %
Position	L0.0 A30.0 F1056.0 mm
Orientation	Transversal
Phase enc. dir.	A >> P
AutoAlign	---
Phase oversampling	0 %
Slice oversampling	28.6 %
Slices per slab	56
FoV read	500 mm
FoV phase	72.3 %
Slice thickness	3.5 mm
TR	15.60 ms
TE 1	2.38 ms
TE 2	4.76 ms
TE 3	7.14 ms
TE 4	9.52 ms
TE 5	11.90 ms
TE 6	14.28 ms
Averages	1
Concatenations	1
Filter	Distortion Corr.(2D), Prescan Normalize
Coil elements	BO1-3;SP2-4

Contrast - Common

TR	15.60 ms
TE 1	2.38 ms
TE 2	4.76 ms
TE 3	7.14 ms
TE 4	9.52 ms
TE 5	11.90 ms
TE 6	14.28 ms
Flip angle	4.0 deg
Fat suppr.	None
Water suppr.	None
Dixon	On
Dixon evaluation	On

Contrast - Dynamic

Averages	1
Averaging mode	Long term
Reconstruction	Magnitude
Measurements	1
Multiple series	Off

Resolution - Common

FoV read	500 mm
FoV phase	72.3 %
Slice thickness	3.5 mm
Base resolution	224
Phase resolution	79 %
Slice resolution	50 %
Phase partial Fourier	7/8
Slice partial Fourier	7/8
Trajectory	Cartesian
View sharing	Off
Interpolation	Off

Resolution - iPAT

PAT mode	CAIPIRINHA
Accel. factor PE	2
Ref. lines PE	24
Accel. factor 3D	2
Ref. lines 3D	24
Reordering Shift 3D	1
Reference scan mode	GRE/separate
CAIPIRINHA mode	Body Tra
Total PAT factor	4

Resolution - Filter Image

Image Filter	Off
Distortion Corr.	On
Mode	2D
Unfiltered images	Off
Prescan Normalize	On
Unfiltered images	Off
Normalize	Off
B1 filter	Off

Resolution - Filter Rawdata

Raw filter	Off
Elliptical filter	Off
POCS	Off

Geometry - Common

Slab group	1
Slabs	1
Dist. factor	20 %
Position	L0.0 A30.0 F1056.0 mm
Orientation	Transversal
Phase enc. dir.	A >> P
Slice oversampling	28.6 %
Slices per slab	56
FoV read	500 mm
FoV phase	72.3 %
Slice thickness	3.5 mm
TR	15.60 ms
Multi-slice mode	Sequential
Series	Ascending
Concatenations	1

Geometry - AutoAlign

Slab group	1
AutoAlign	---
Position	L0.0 A30.0 F1056.0 mm
Orientation	Transversal

Geometry - AutoAlign

Phase enc. dir.	A >> P
Initial Position	L0.0 A30.0 F1056.0
Phase	-30.0 mm
Read	0.0 mm
Shift	-1056.0 mm
Initial Rotation	0.00 deg
Initial Orientation	Transversal

Geometry - Saturation

Fat suppr.	None
Water suppr.	None
Dixon	On
Dixon evaluation	On
Special sat.	None

System - Miscellaneous

Positioning mode	ISO
Table position	F
Table position	1056 mm
MSMA	S - C - T
Sagittal	R >> L
Coronal	A >> P
Transversal	H >> F
Coil Combine Mode	Adaptive Combine
Save uncombined	Off
Matrix Optimization	Off
Coil Focus	Flat
AutoAlign	---
Coil Select Mode	On - AutoCoilSelect

System - Adjustments

B0 Shim mode	Standard
Adjust with body coil	Off
Confirm freq. adjustment	Off
Assume Dominant Fat	Off
Assume Silicone	Off
Adjustment Tolerance	Auto

System - Adjust Volume

Position	L0.0 A30.0 F1056.0 mm
Orientation	Transversal
Rotation	0.00 deg
A >> P	362 mm
R >> L	500 mm
F >> H	196 mm
Reset	Off

System - Tx/Rx

Frequency 1H	63.685340 MHz
Correction factor	1
Gain	Low
Img. Scale Cor.	1.000
Reset	Off
? Ref. amplitude 1H	0.000 V

Physio - PACE

Resp. control	Off
Concatenations	1

Inline - Common

View sharing	Off
Flip angle	4.0 deg
Measurements	1

Inline - Common

Burn time-to-center	Off
Temporal interpolation	1
3D centric reordering	Off
Time to center	7.9 s

Inline - Inline

Subtract	Off
Measurements	1
StdDev	Off
Liver registration	Off
Save original images	On

Inline - MIP

MIP-Sag	Off
MIP-Cor	Off
MIP-Tra	Off
MIP-Time	Off
Save original images	On

Inline - Soft Tissue

Wash - In	Off
Wash - Out	Off
TTP	Off
PEI	Off
MIP - time	Off
Measurements	1

Inline - Composing

Inline Composing	On
Composing Function	Adaptive
Composing Group	1
Last Step	Off
Normalize	None
Series Description	Dixon
Distortion Corr.	On
Mode	2D
Unfiltered images	Off

Sequence - Part 1

Introduction	Off
Dimension	3D
Elliptical scanning	Off
Asymmetric echo	Off
Contrasts	6
Readout mode	Bipolar
Optimization	Equidistant
Multi-slice mode	Sequential
Bandwidth 1	1060 Hz/Px
Bandwidth 2	1060 Hz/Px
Bandwidth 3	1060 Hz/Px
Bandwidth 4	1060 Hz/Px
Bandwidth 5	1060 Hz/Px
Bandwidth 6	1060 Hz/Px

Sequence - Part 2

RF pulse type	Normal
Gradient mode	Fast
Excitation	Slab-sel.
RF spoiling	On
Incr. Gradient spoiling	Off

Sequence - Assistant

Mode	Off

\\Non Cardiac Research\Whole body\ATTIS\ATTIS\ax_t1_qdixon_7calf-foot_F1252

TA: 0:16 PM: ISO Voxel size: 2.2×2.2×3.5 mmPAT: 4 Rel. SNR: 1.00 : fl

Properties

Prio recon	Off
Load images to viewer	On
Inline movie	Off
Auto store images	On
Load images to stamp segments	On
Load images to graphic segments	On
Auto open inline display	Off
Auto close inline display	Off
Start measurement without further preparation	On
Wait for user to start	Off
Start measurements	Single measurement

Routine

Slab group	1
Slabs	1
Dist. factor	20 %
Position	L0.0 A30.0 F1252.0 mm
Orientation	Transversal
Phase enc. dir.	A >> P
AutoAlign	---
Phase oversampling	0 %
Slice oversampling	28.6 %
Slices per slab	56
FoV read	500 mm
FoV phase	72.3 %
Slice thickness	3.5 mm
TR	15.60 ms
TE 1	2.38 ms
TE 2	4.76 ms
TE 3	7.14 ms
TE 4	9.52 ms
TE 5	11.90 ms
TE 6	14.28 ms
Averages	1
Concatenations	1
Filter	Distortion Corr.(2D), Prescan Normalize
Coil elements	BO1-3;SP2-4

Contrast - Common

TR	15.60 ms
TE 1	2.38 ms
TE 2	4.76 ms
TE 3	7.14 ms
TE 4	9.52 ms
TE 5	11.90 ms
TE 6	14.28 ms
Flip angle	4.0 deg
Fat suppr.	None
Water suppr.	None
Dixon	On
Dixon evaluation	On

Contrast - Dynamic

Averages	1
Averaging mode	Long term
Reconstruction	Magnitude
Measurements	1
Multiple series	Off

Resolution - Common

FoV read	500 mm
FoV phase	72.3 %
Slice thickness	3.5 mm
Base resolution	224
Phase resolution	79 %
Slice resolution	50 %
Phase partial Fourier	7/8
Slice partial Fourier	7/8
Trajectory	Cartesian
View sharing	Off
Interpolation	Off

Resolution - iPAT

PAT mode	CAIPIRINHA
Accel. factor PE	2
Ref. lines PE	24
Accel. factor 3D	2
Ref. lines 3D	24
Reordering Shift 3D	1
Reference scan mode	GRE/separate
CAIPIRINHA mode	Body Tra
Total PAT factor	4

Resolution - Filter Image

Image Filter	Off
Distortion Corr.	On
Mode	2D
Unfiltered images	Off
Prescan Normalize	On
Unfiltered images	Off
Normalize	Off
B1 filter	Off

Resolution - Filter Rawdata

Raw filter	Off
Elliptical filter	Off
POCS	Off

Geometry - Common

Slab group	1
Slabs	1
Dist. factor	20 %
Position	L0.0 A30.0 F1252.0 mm
Orientation	Transversal
Phase enc. dir.	A >> P
Slice oversampling	28.6 %
Slices per slab	56
FoV read	500 mm
FoV phase	72.3 %
Slice thickness	3.5 mm
TR	15.60 ms
Multi-slice mode	Sequential
Series	Ascending
Concatenations	1

Geometry - AutoAlign

Slab group	1
AutoAlign	---
Position	L0.0 A30.0 F1252.0 mm
Orientation	Transversal

Geometry - AutoAlign

Phase enc. dir.	A >> P
Initial Position	L0.0 A30.0 F1252.0
Phase	-30.0 mm
Read	0.0 mm
Shift	-1252.0 mm
Initial Rotation	0.00 deg
Initial Orientation	Transversal

Geometry - Saturation

Fat suppr.	None
Water suppr.	None
Dixon	On
Dixon evaluation	On
Special sat.	None

System - Miscellaneous

Positioning mode	ISO
Table position	F
Table position	1252 mm
MSMA	S - C - T
Sagittal	R >> L
Coronal	A >> P
Transversal	H >> F
Coil Combine Mode	Adaptive Combine
Save uncombined	Off
Matrix Optimization	Off
Coil Focus	Flat
AutoAlign	---
Coil Select Mode	On - AutoCoilSelect

System - Adjustments

B0 Shim mode	Standard
Adjust with body coil	Off
Confirm freq. adjustment	Off
Assume Dominant Fat	Off
Assume Silicone	Off
Adjustment Tolerance	Auto

System - Adjust Volume

Position	L0.0 A30.0 F1252.0 mm
Orientation	Transversal
Rotation	0.00 deg
A >> P	362 mm
R >> L	500 mm
F >> H	196 mm
Reset	Off

System - Tx/Rx

Frequency 1H	63.685340 MHz
Correction factor	1
Gain	Low
Img. Scale Cor.	1.000
Reset	Off
? Ref. amplitude 1H	0.000 V

Physio - PACE

Resp. control	Off
Concatenations	1

Inline - Common

View sharing	Off
Flip angle	4.0 deg
Measurements	1

Inline - Common

Burn time-to-center	Off
Temporal interpolation	1
3D centric reordering	Off
Time to center	7.9 s

Inline - Inline

Subtract	Off
Measurements	1
StdDev	Off
Liver registration	Off
Save original images	On

Inline - MIP

MIP-Sag	Off
MIP-Cor	Off
MIP-Tra	Off
MIP-Time	Off
Save original images	On

Inline - Soft Tissue

Wash - In	Off
Wash - Out	Off
TTP	Off
PEI	Off
MIP - time	Off
Measurements	1

Inline - Composing

Inline Composing	On
Composing Function	Adaptive
Composing Group	1
Last Step	Off
Normalize	None
Series Description	Dixon
Distortion Corr.	On
Mode	2D
Unfiltered images	Off

Sequence - Part 1

Introduction	Off
Dimension	3D
Elliptical scanning	Off
Asymmetric echo	Off
Contrasts	6
Readout mode	Bipolar
Optimization	Equidistant
Multi-slice mode	Sequential
Bandwidth 1	1060 Hz/Px
Bandwidth 2	1060 Hz/Px
Bandwidth 3	1060 Hz/Px
Bandwidth 4	1060 Hz/Px
Bandwidth 5	1060 Hz/Px
Bandwidth 6	1060 Hz/Px

Sequence - Part 2

RF pulse type	Normal
Gradient mode	Fast
Excitation	Slab-sel.
RF spoiling	On
Incr. Gradient spoiling	Off

Sequence - Assistant

Mode	Off

\\Non Cardiac Research\Whole body\ATTIS\ATTIS\ax_t1_qdixon_8foot_F1448

TA: 0:16 PM: ISO Voxel size: 2.2×2.2×3.5 mmPAT: 4 Rel. SNR: 1.00 : fl

Properties

Prio recon	Off
Load images to viewer	On
Inline movie	Off
Auto store images	On
Load images to stamp segments	On
Load images to graphic segments	Off
Auto open inline display	Off
Auto close inline display	Off
Start measurement without further preparation	On
Wait for user to start	Off
Start measurements	Single measurement

Routine

Slab group	1
Slabs	1
Dist. factor	20 %
Position	L0.0 A30.0 F1448.0 mm
Orientation	Transversal
Phase enc. dir.	A >> P
AutoAlign	---
Phase oversampling	0 %
Slice oversampling	28.6 %
Slices per slab	56
FoV read	500 mm
FoV phase	72.3 %
Slice thickness	3.5 mm
TR	15.60 ms
TE 1	2.38 ms
TE 2	4.76 ms
TE 3	7.14 ms
TE 4	9.52 ms
TE 5	11.90 ms
TE 6	14.28 ms
Averages	1
Concatenations	1
Filter	Distortion Corr.(2D), Prescan Normalize
Coil elements	BO1-3;SP2-4

Contrast - Common

TR	15.60 ms
TE 1	2.38 ms
TE 2	4.76 ms
TE 3	7.14 ms
TE 4	9.52 ms
TE 5	11.90 ms
TE 6	14.28 ms
Flip angle	4.0 deg
Fat suppr.	None
Water suppr.	None
Dixon	On
Dixon evaluation	On

Contrast - Dynamic

Averages	1
Averaging mode	Long term
Reconstruction	Magnitude
Measurements	1
Multiple series	Off

Resolution - Common

FoV read	500 mm
FoV phase	72.3 %
Slice thickness	3.5 mm
Base resolution	224
Phase resolution	79 %
Slice resolution	50 %
Phase partial Fourier	7/8
Slice partial Fourier	7/8
Trajectory	Cartesian
View sharing	Off
Interpolation	Off

Resolution - iPAT

PAT mode	CAIPIRINHA
Accel. factor PE	2
Ref. lines PE	24
Accel. factor 3D	2
Ref. lines 3D	24
Reordering Shift 3D	1
Reference scan mode	GRE/separate
CAIPIRINHA mode	Body Tra
Total PAT factor	4

Resolution - Filter Image

Image Filter	Off
Distortion Corr.	On
Mode	2D
Unfiltered images	Off
Prescan Normalize	On
Unfiltered images	Off
Normalize	Off
B1 filter	Off

Resolution - Filter Rawdata

Raw filter	Off
Elliptical filter	Off
POCS	Off

Geometry - Common

Slab group	1
Slabs	1
Dist. factor	20 %
Position	L0.0 A30.0 F1448.0 mm
Orientation	Transversal
Phase enc. dir.	A >> P
Slice oversampling	28.6 %
Slices per slab	56
FoV read	500 mm
FoV phase	72.3 %
Slice thickness	3.5 mm
TR	15.60 ms
Multi-slice mode	Sequential
Series	Ascending
Concatenations	1

Geometry - AutoAlign

Slab group	1
AutoAlign	---
Position	L0.0 A30.0 F1448.0 mm
Orientation	Transversal

Geometry - AutoAlign

Phase enc. dir.	A >> P
Initial Position	L0.0 A30.0 F1448.0
Phase	-30.0 mm
Read	0.0 mm
Shift	-1448.0 mm
Initial Rotation	0.00 deg
Initial Orientation	Transversal

Geometry - Saturation

Fat suppr.	None
Water suppr.	None
Dixon	On
Dixon evaluation	On
Special sat.	None

System - Miscellaneous

Positioning mode	ISO
Table position	F
Table position	1448 mm
MSMA	S - C - T
Sagittal	R >> L
Coronal	A >> P
Transversal	H >> F
Coil Combine Mode	Adaptive Combine
Save uncombined	Off
Matrix Optimization	Off
Coil Focus	Flat
AutoAlign	---
Coil Select Mode	On - AutoCoilSelect

System - Adjustments

B0 Shim mode	Standard
Adjust with body coil	Off
Confirm freq. adjustment	Off
Assume Dominant Fat	Off
Assume Silicone	Off
Adjustment Tolerance	Auto

System - Adjust Volume

Position	L0.0 A30.0 F1448.0 mm
Orientation	Transversal
Rotation	0.00 deg
A >> P	362 mm
R >> L	500 mm
F >> H	196 mm
Reset	Off

System - Tx/Rx

Frequency 1H	63.685340 MHz
Correction factor	1
Gain	Low
Img. Scale Cor.	1.000
Reset	Off
? Ref. amplitude 1H	0.000 V

Physio - PACE

Resp. control	Off
Concatenations	1

Inline - Common

View sharing	Off
Flip angle	4.0 deg
Measurements	1

Inline - Common

Burn time-to-center	Off
Temporal interpolation	1
3D centric reordering	Off
Time to center	7.9 s

Inline - Inline

Subtract	Off
Measurements	1
StdDev	Off
Liver registration	Off
Save original images	On

Inline - MIP

MIP-Sag	Off
MIP-Cor	Off
MIP-Tra	Off
MIP-Time	Off
Save original images	On

Inline - Soft Tissue

Wash - In	Off
Wash - Out	Off
TTP	Off
PEI	Off
MIP - time	Off
Measurements	1

Inline - Composing

Inline Composing	On
Composing Function	Adaptive
Composing Group	1
Last Step	On
Normalize	None
Series Description	Dixon
Distortion Corr.	On
Mode	2D
Unfiltered images	Off

Sequence - Part 1

Introduction	Off
Dimension	3D
Elliptical scanning	Off
Asymmetric echo	Off
Contrasts	6
Readout mode	Bipolar
Optimization	Equidistant
Multi-slice mode	Sequential
Bandwidth 1	1060 Hz/Px
Bandwidth 2	1060 Hz/Px
Bandwidth 3	1060 Hz/Px
Bandwidth 4	1060 Hz/Px
Bandwidth 5	1060 Hz/Px
Bandwidth 6	1060 Hz/Px

Sequence - Part 2

RF pulse type	Normal
Gradient mode	Fast
Excitation	Slab-sel.
RF spoiling	On
Incr. Gradient spoiling	Off

Sequence - Assistant

Mode	Off

\\Non Cardiac Research\Whole body\ATTIS\ATTIS\liver_localizer_for_MRS

TA: 5.0 s PM: ISO Voxel size: 1.6×1.6×5.0 mmPAT: Off Rel. SNR: 1.00 : fl

Properties

Prio recon	On
Load images to viewer	On
Inline movie	Off
Auto store images	On
Load images to stamp segments	On
Load images to graphic segments	On
Auto open inline display	Off
Auto close inline display	Off
Start measurement without further preparation	Off
Wait for user to start	Off
Start measurements	Single measurement

Routine

Slice group	1
Slices	1
Dist. factor	20 %
Position	Isocenter
Orientation	Sagittal
Phase enc. dir.	A >> P
Slice group	2
Slices	1
Dist. factor	20 %
Position	Isocenter
Orientation	Transversal
Phase enc. dir.	A >> P
Slice group	3
Slices	1
Dist. factor	20 %
Position	Isocenter
Orientation	Coronal
Phase enc. dir.	R >> L
AutoAlign	---
Phase oversampling	0 %
FoV read	400 mm
FoV phase	100.0 %
Slice thickness	5.0 mm
TR	9.0 ms
TE	4.50 ms
Averages	1
Concatenations	3
Filter	Prescan Normalize, Elliptical filter
Coil elements	HE1-4

Contrast - Common

TR	9.0 ms
TE	4.50 ms
TD	0 ms
MTC	Off
Magn. preparation	None
Flip angle	20 deg
Fat suppr.	None
Water suppr.	None
SWI	Off

Contrast - Dynamic

Averages	1
Averaging mode	Short term
Reconstruction	Magnitude
Measurements	1

Contrast - Dynamic

Multiple series	Off

Resolution - Common

FoV read	400 mm
FoV phase	100.0 %
Slice thickness	5.0 mm
Base resolution	256
Phase resolution	50 %
Phase partial Fourier	Off
Interpolation	Off

Resolution - iPAT

PAT mode	None

Resolution - Filter Image

Image Filter	Off
Distortion Corr.	Off
Prescan Normalize	On
Unfiltered images	Off
Normalize	Off
B1 filter	Off

Resolution - Filter Rawdata

Raw filter	Off
Elliptical filter	On

Geometry - Common

Slice group	1
Slices	1
Dist. factor	20 %
Position	Isocenter
Orientation	Sagittal
Phase enc. dir.	A >> P
Slice group	2
Slices	1
Dist. factor	20 %
Position	Isocenter
Orientation	Transversal
Phase enc. dir.	A >> P
Slice group	3
Slices	1
Dist. factor	20 %
Position	Isocenter
Orientation	Coronal
Phase enc. dir.	R >> L
FoV read	400 mm
FoV phase	100.0 %
Slice thickness	5.0 mm
TR	9.0 ms
Multi-slice mode	Sequential
Series	Ascending
Concatenations	3

Geometry - AutoAlign

Slice group	1
Slice group	2
Slice group	3
AutoAlign	---
Position	Isocenter
Orientation	Coronal

Geometry - AutoAlign

Phase enc. dir.	R >> L
Initial Position	L0.0 P0.0 F272.0
Phase	0.0 mm
Read	-272.0 mm
Shift	0.0 mm
Initial Rotation	0.00 deg
Initial Orientation	Sagittal

Geometry - Saturation

Saturation mode	Standard
Fat suppr.	None
Water suppr.	None
Special sat.	None

System - Miscellaneous

Positioning mode	ISO
Table position	F
Table position	272 mm
MSMA	S - C - T
Sagittal	R >> L
Coronal	A >> P
Transversal	F >> H
Coil Combine Mode	Adaptive Combine
Save uncombined	Off
Matrix Optimization	Off
AutoAlign	---
Coil Select Mode	On - AutoCoilSelect

System - Adjustments

B0 Shim mode	Tune up
Adjust with body coil	Off
Confirm freq. adjustment	Off
Assume Dominant Fat	Off
Assume Silicone	Off
Adjustment Tolerance	Auto

System - Adjust Volume

Position	Isocenter
Orientation	Transversal
Rotation	0.00 deg
A >> P	263 mm
R >> L	350 mm
F >> H	350 mm
Reset	Off

System - Tx/Rx

Frequency 1H	63.685340 MHz
Correction factor	1
Gain	High
Img. Scale Cor.	1.000
Reset	Off
? Ref. amplitude 1H	0.000 V

Physio - Signal1

1st Signal/Mode	None
TR	9.0 ms
Concatenations	3
Segments	1

Physio - Cardiac

Tagging	None
Magn. preparation	None
Fat suppr.	None

Physio - Cardiac

Dark blood	Off
FoV read	400 mm
FoV phase	100.0 %
Phase resolution	50 %

Physio - PACE

Resp. control	Off
Concatenations	3

Inline - Common

Subtract	Off
Measurements	1
StdDev	Off
Liver registration	Off
Save original images	On

Inline - MIP

MIP-Sag	Off
MIP-Cor	Off
MIP-Tra	Off
MIP-Time	Off
Save original images	On

Inline - Soft Tissue

Wash - In	Off
Wash - Out	Off
TTP	Off
PEI	Off
MIP - time	Off
Measurements	1

Inline - Composing

Inline Composing	Off
Distortion Corr.	Off

Sequence - Part 1

Introduction	On
Dimension	2D
Phase stabilisation	Off
Asymmetric echo	Off
Contrasts	1
Flow comp.	No
Multi-slice mode	Sequential
Bandwidth	260 Hz/Px

Sequence - Part 2

Segments	1
Acoustic noise reduction	None
RF pulse type	Normal
Gradient mode	Normal
Excitation	Slice-sel.
RF spoiling	On

Sequence - Assistant

Mode	Off

\\Non Cardiac Research\Whole body\ATTIS\ATTIS\MRS_se_30_liver

TA: 0:14 PM: REF Vol: 15 ×15 ×15 mmRel. SNR: 1.00 : svs_se

Properties

Prio recon	Off
Load images to viewer	On
Inline movie	Off
Auto store images	On
Load images to stamp segments	Off
Load images to graphic segments	Off
Auto open inline display	Off
Auto close inline display	Off
Start measurement without further preparation	Off
Wait for user to start	Off
Start measurements	Single measurement

Routine

Position	L0.0 P0.0 H30.0 mm
Orientation	Transversal
Rotation	0 deg
Vol R >> L	15 mm
Vol R >> L	15 mm
Vol F >> H	15 mm
TR	2000 ms
TE	30 ms
Averages	4
Filter	Prescan Normalize
Coil elements	HE1-4

Contrast

TR	2000 ms
TE	30 ms
Averages	4
Flip angle	90 deg
Water suppr.	Water sat.
Water suppr. BW	35 Hz
Spectral suppr.	None
Measurements	1

Resolution - Common

Prescan Normalize	On
Vector size	1024

Geometry - Common

Position	L0.0 P0.0 H30.0 mm
Orientation	Transversal
Rotation	0 deg
Vol R >> L	15 mm
Vol A >> P	15 mm
Vol F >> H	15 mm

Geometry - AutoAlign

AutoAlign	---
Initial Position	L0.0 P0.0 H30.0
Phase	0 mm
Read	0 mm
Shift	30 mm
Initial Rotation	0.00 deg
Initial Orientation	Transversal

Geometry - Navigator

System - Miscellaneous

Positioning mode	REF
Table position	H
Table position	0 mm
MSMA	S - C - T
Sagittal	R >> L
Coronal	A >> P
Transversal	F >> H
Save uncombined	Off
Save single averages	Off
AutoAlign	---
Coil Select Mode	On - AutoCoilSelect

System - Adjustments

B0 Shim mode	Brain
Adj. water suppr.	On
Adjust with body coil	Off
Confirm freq. adjustment	On
Only after freq. change	On
Assume Dominant Fat	Off
Assume Silicone	Off
Adjustment Tolerance	Auto

System - Adjust Volume

Position	L0.0 P0.0 H30.0 mm
Orientation	Transversal
Rotation	0.00 deg
A >> P	15 mm
R >> L	15 mm
F >> H	15 mm
Reset	Off

System - Tx/Rx

Frequency 1H	63.685340 MHz
Gain	High
Img. Scale Cor.	1.000
Reset	Off
? Ref. amplitude 1H	0.000 V

Physio - Signal1

1st Signal/Mode	None
TR	2000 ms

Physio - PACE

Resp. control	Off

Sequence - Common

Preparation scans	3
Delta frequency	-2.7 ppm
Ref. scan mode	Off
Phase cycling	Auto
Bandwidth	1000 Hz
Acquisition duration	1024 ms
Remove oversampling	On

\\Non Cardiac Research\Whole body\ATTIS\ATTIS\MRS_se_30_liver

TA: 0:14 PM: FIX Vol: 15 ×15 ×15 mmRel. SNR: 1.00 : svs_se

Properties

Prio recon	Off
Load images to viewer	On
Inline movie	Off
Auto store images	On
Load images to stamp segments	Off
Load images to graphic segments	Off
Auto open inline display	Off
Auto close inline display	Off
Start measurement without further preparation	On
Wait for user to start	Off
Start measurements	Single measurement

Routine

Position	L0.0 P0.0 H30.0 mm
Orientation	Transversal
Rotation	0 deg
Vol R >> L	15 mm
Vol R >> L	15 mm
Vol F >> H	15 mm
TR	2000 ms
TE	30 ms
Averages	4
Filter	Prescan Normalize
Coil elements	HE1-4

Contrast

TR	2000 ms
TE	30 ms
Averages	4
Flip angle	90 deg
Water suppr.	Water sat.
Water suppr. BW	35 Hz
Spectral suppr.	None
Measurements	1

Resolution - Common

Prescan Normalize	On
Vector size	1024

Geometry - Common

Position	L0.0 P0.0 H30.0 mm
Orientation	Transversal
Rotation	0 deg
Vol R >> L	15 mm
Vol A >> P	15 mm
Vol F >> H	15 mm

Geometry - AutoAlign

AutoAlign	---
Initial Position	L0.0 P0.0 H30.0
Phase	0 mm
Read	0 mm
Shift	30 mm
Initial Rotation	0.00 deg
Initial Orientation	Transversal

Geometry - Navigator

System - Miscellaneous

Positioning mode	FIX
Table position	H
Table position	0 mm
MSMA	S - C - T
Sagittal	R >> L
Coronal	A >> P
Transversal	F >> H
Save uncombined	Off
Save single averages	Off
AutoAlign	---
Coil Select Mode	Off - All

System - Adjustments

B0 Shim mode	Brain
Adj. water suppr.	On
Adjust with body coil	Off
Confirm freq. adjustment	On
Only after freq. change	On
Assume Dominant Fat	Off
Assume Silicone	Off
Adjustment Tolerance	Auto

System - Adjust Volume

Position	L0.0 P0.0 H30.0 mm
Orientation	Transversal
Rotation	0.00 deg
A >> P	15 mm
R >> L	15 mm
F >> H	15 mm
Reset	Off

System - Tx/Rx

Frequency 1H	63.685340 MHz
Gain	High
Img. Scale Cor.	1.000
Reset	Off
? Ref. amplitude 1H	0.000 V

Physio - Signal1

1st Signal/Mode	None
TR	2000 ms

Physio - PACE

Resp. control	Off

Sequence - Common

Preparation scans	3
Delta frequency	-2.7 ppm
Ref. scan mode	Off
Phase cycling	Auto
Bandwidth	1000 Hz
Acquisition duration	1024 ms
Remove oversampling	On

\\Non Cardiac Research\Whole body\ATTIS\ATTIS\MRS_se_30_liver

TA: 0:14 PM: FIX Vol: 15 ×15 ×15 mmRel. SNR: 1.00 : svs_se

Properties

Prio recon	Off
Load images to viewer	On
Inline movie	Off
Auto store images	On
Load images to stamp segments	Off
Load images to graphic segments	Off
Auto open inline display	Off
Auto close inline display	Off
Start measurement without further preparation	On
Wait for user to start	Off
Start measurements	Single measurement

Routine

Position	L0.0 P0.0 H30.0 mm
Orientation	Transversal
Rotation	0 deg
Vol R >> L	15 mm
Vol R >> L	15 mm
Vol F >> H	15 mm
TR	2000 ms
TE	30 ms
Averages	4
Filter	Prescan Normalize
Coil elements	HE1-4

Contrast

TR	2000 ms
TE	30 ms
Averages	4
Flip angle	90 deg
Water suppr.	Water sat.
Water suppr. BW	35 Hz
Spectral suppr.	None
Measurements	1

Resolution - Common

Prescan Normalize	On
Vector size	1024

Geometry - Common

Position	L0.0 P0.0 H30.0 mm
Orientation	Transversal
Rotation	0 deg
Vol R >> L	15 mm
Vol A >> P	15 mm
Vol F >> H	15 mm

Geometry - AutoAlign

AutoAlign	---
Initial Position	L0.0 P0.0 H30.0
Phase	0 mm
Read	0 mm
Shift	30 mm
Initial Rotation	0.00 deg
Initial Orientation	Transversal

Geometry - Navigator

System - Miscellaneous

Positioning mode	FIX
Table position	H
Table position	0 mm
MSMA	S - C - T
Sagittal	R >> L
Coronal	A >> P
Transversal	F >> H
Save uncombined	Off
Save single averages	Off
AutoAlign	---
Coil Select Mode	Off - All

System - Adjustments

B0 Shim mode	Brain
Adj. water suppr.	On
Adjust with body coil	Off
Confirm freq. adjustment	On
Only after freq. change	On
Assume Dominant Fat	Off
Assume Silicone	Off
Adjustment Tolerance	Auto

System - Adjust Volume

Position	L0.0 P0.0 H30.0 mm
Orientation	Transversal
Rotation	0.00 deg
A >> P	15 mm
R >> L	15 mm
F >> H	15 mm
Reset	Off

System - Tx/Rx

Frequency 1H	63.685340 MHz
Gain	High
Img. Scale Cor.	1.000
Reset	Off
? Ref. amplitude 1H	0.000 V

Physio - Signal1

1st Signal/Mode	None
TR	2000 ms

Physio - PACE

Resp. control	Off

Sequence - Common

Preparation scans	3
Delta frequency	-2.7 ppm
Ref. scan mode	Off
Phase cycling	Auto
Bandwidth	1000 Hz
Acquisition duration	1024 ms
Remove oversampling	On

\\Non Cardiac Research\Whole body\ATTIS\ATTIS\MRS_se_30_liver

TA: 0:14 PM: FIX Vol: 15 ×15 ×15 mmRel. SNR: 1.00 : svs_se

Properties

Prio recon	Off
Load images to viewer	On
Inline movie	Off
Auto store images	On
Load images to stamp segments	Off
Load images to graphic segments	Off
Auto open inline display	Off
Auto close inline display	Off
Start measurement without further preparation	On
Wait for user to start	Off
Start measurements	Single measurement

Routine

Position	L0.0 P0.0 H30.0 mm
Orientation	Transversal
Rotation	0 deg
Vol R >> L	15 mm
Vol R >> L	15 mm
Vol F >> H	15 mm
TR	2000 ms
TE	30 ms
Averages	4
Filter	Prescan Normalize
Coil elements	HE1-4

Contrast

TR	2000 ms
TE	30 ms
Averages	4
Flip angle	90 deg
Water suppr.	Water sat.
Water suppr. BW	35 Hz
Spectral suppr.	None
Measurements	1

Resolution - Common

Prescan Normalize	On
Vector size	1024

Geometry - Common

Position	L0.0 P0.0 H30.0 mm
Orientation	Transversal
Rotation	0 deg
Vol R >> L	15 mm
Vol A >> P	15 mm
Vol F >> H	15 mm

Geometry - AutoAlign

AutoAlign	---
Initial Position	L0.0 P0.0 H30.0
Phase	0 mm
Read	0 mm
Shift	30 mm
Initial Rotation	0.00 deg
Initial Orientation	Transversal

Geometry - Navigator

System - Miscellaneous

Positioning mode	FIX
Table position	H
Table position	0 mm
MSMA	S - C - T
Sagittal	R >> L
Coronal	A >> P
Transversal	F >> H
Save uncombined	Off
Save single averages	Off
AutoAlign	---
Coil Select Mode	Off - All

System - Adjustments

B0 Shim mode	Brain
Adj. water suppr.	On
Adjust with body coil	Off
Confirm freq. adjustment	On
Only after freq. change	On
Assume Dominant Fat	Off
Assume Silicone	Off
Adjustment Tolerance	Auto

System - Adjust Volume

Position	L0.0 P0.0 H30.0 mm
Orientation	Transversal
Rotation	0.00 deg
A >> P	15 mm
R >> L	15 mm
F >> H	15 mm
Reset	Off

System - Tx/Rx

Frequency 1H	63.685340 MHz
Gain	High
Img. Scale Cor.	1.000
Reset	Off
? Ref. amplitude 1H	0.000 V

Physio - Signal1

1st Signal/Mode	None
TR	2000 ms

Physio - PACE

Resp. control	Off

Sequence - Common

Preparation scans	3
Delta frequency	-2.7 ppm
Ref. scan mode	Off
Phase cycling	Auto
Bandwidth	1000 Hz
Acquisition duration	1024 ms
Remove oversampling	On

\\Non Cardiac Research\Whole body\ATTIS\ATTIS\MRS_se_30_liver_h2o

TA: 0:10 PM: FIX Vol: 15 ×15 ×15 mmRel. SNR: 1.00 : svs_se

Properties

Prio recon	Off
Load images to viewer	On
Inline movie	Off
Auto store images	On
Load images to stamp segments	Off
Load images to graphic segments	Off
Auto open inline display	Off
Auto close inline display	Off
Start measurement without further preparation	On
Wait for user to start	Off
Start measurements	Single measurement

Routine

Position	L0.0 P0.0 H30.0 mm
Orientation	Transversal
Rotation	0 deg
Vol R >> L	15 mm
Vol R >> L	15 mm
Vol F >> H	15 mm
TR	2000 ms
TE	30 ms
Averages	2
Filter	Prescan Normalize
Coil elements	HE1-4

Contrast

TR	2000 ms
TE	30 ms
Averages	2
Flip angle	90 deg
Water suppr.	Only RF off
Water suppr. BW	35 Hz
Spectral suppr.	None
Measurements	1

Resolution - Common

Prescan Normalize	On
Vector size	1024

Geometry - Common

Position	L0.0 P0.0 H30.0 mm
Orientation	Transversal
Rotation	0 deg
Vol R >> L	15 mm
Vol A >> P	15 mm
Vol F >> H	15 mm

Geometry - AutoAlign

AutoAlign	---
Initial Position	L0.0 P0.0 H30.0
Phase	0 mm
Read	0 mm
Shift	30 mm
Initial Rotation	0.00 deg
Initial Orientation	Transversal

Geometry - Navigator

System - Miscellaneous

Positioning mode	FIX
Table position	H
Table position	0 mm
MSMA	S - C - T
Sagittal	R >> L
Coronal	A >> P
Transversal	F >> H
Save uncombined	Off
Save single averages	Off
AutoAlign	---
Coil Select Mode	Default

System - Adjustments

B0 Shim mode	Brain
Adj. water suppr.	On
Adjust with body coil	Off
Confirm freq. adjustment	On
Only after freq. change	On
Assume Dominant Fat	Off
Assume Silicone	Off
Adjustment Tolerance	Auto

System - Adjust Volume

Position	L0.0 P0.0 H30.0 mm
Orientation	Transversal
Rotation	0.00 deg
A >> P	15 mm
R >> L	15 mm
F >> H	15 mm
Reset	Off

System - Tx/Rx

Frequency 1H	63.685340 MHz
Gain	High
Img. Scale Cor.	1.000
Reset	Off
? Ref. amplitude 1H	0.000 V

Physio - Signal1

1st Signal/Mode	None
TR	2000 ms

Physio - PACE

Resp. control	Off

Sequence - Common

Preparation scans	3
Delta frequency	0.0 ppm
Ref. scan mode	Off
Phase cycling	Auto
Bandwidth	1000 Hz
Acquisition duration	1024 ms
Remove oversampling	On

\\Non Cardiac Research\Whole body\ATTIS\ATTIS\calf_localizer_for_MRS

TA: 3.8 s PM: ISO Voxel size: 0.8×0.8×5.0 mmPAT: Off Rel. SNR: 1.00 : fl

Properties

Prio recon	Off
Load images to viewer	On
Inline movie	Off
Auto store images	On
Load images to stamp segments	On
Load images to graphic segments	On
Auto open inline display	Off
Auto close inline display	Off
Start measurement without further preparation	Off
Wait for user to start	Off
Start measurements	Single measurement

Routine

Slice group	1
Slices	1
Dist. factor	20 %
Position	R90.9 P42.3 F1253.8 mm
Orientation	Sagittal
Phase enc. dir.	A >> P
Slice group	2
Slices	1
Dist. factor	20 %
Position	R90.9 P42.3 F1253.8 mm
Orientation	Transversal
Phase enc. dir.	A >> P
AutoAlign	---
Phase oversampling	0 %
FoV read	200 mm
FoV phase	100.0 %
Slice thickness	5.0 mm
TR	9.0 ms
TE	4.50 ms
Averages	1
Concatenations	2
Filter	Prescan Normalize, Elliptical filter
Coil elements	PA2-4

Contrast - Common

TR	9.0 ms
TE	4.50 ms
TD	0 ms
MTC	Off
Magn. preparation	None
Flip angle	20 deg
Fat suppr.	None
Water suppr.	None
SWI	Off

Contrast - Dynamic

Averages	1
Averaging mode	Short term
Reconstruction	Magnitude
Measurements	1
Multiple series	Off

Resolution - Common

FoV read	200 mm

Resolution - Common

FoV phase	100.0 %
Slice thickness	5.0 mm
Base resolution	256
Phase resolution	50 %
Phase partial Fourier	Off
Interpolation	Off

Resolution - iPAT

PAT mode	None

Resolution - Filter Image

Image Filter	Off
Distortion Corr.	Off
Prescan Normalize	On
Unfiltered images	Off
Normalize	Off
B1 filter	Off

Resolution - Filter Rawdata

Raw filter	Off
Elliptical filter	On

Geometry - Common

Slice group	1
Slices	1
Dist. factor	20 %
Position	R90.9 P42.3 F1253.8 mm
Orientation	Sagittal
Phase enc. dir.	A >> P
Slice group	2
Slices	1
Dist. factor	20 %
Position	R90.9 P42.3 F1253.8 mm
Orientation	Transversal
Phase enc. dir.	A >> P
FoV read	200 mm
FoV phase	100.0 %
Slice thickness	5.0 mm
TR	9.0 ms
Multi-slice mode	Sequential
Series	Ascending
Concatenations	2

Geometry - AutoAlign

Slice group	1
Slice group	2
AutoAlign	---
Position	R90.9 P42.3 F1253.8 mm
Orientation	Transversal
Phase enc. dir.	A >> P
Initial Position	R90.9 P42.3 F1253.8
Phase	42.3 mm
Read	-1253.8 mm
Shift	-90.9 mm
Initial Rotation	0.00 deg
Initial Orientation	Sagittal

Geometry - Saturation

Saturation mode	Standard
Fat suppr.	None
Water suppr.	None
Special sat.	None

System - Miscellaneous

Positioning mode	ISO
Table position	F
Table position	1253 mm
MSMA	S - C - T
Sagittal	R >> L
Coronal	A >> P
Transversal	F >> H
Coil Combine Mode	Adaptive Combine
Save uncombined	Off
Matrix Optimization	Off
AutoAlign	---
Coil Select Mode	On - AutoCoilSelect

System - Adjustments

B0 Shim mode	Tune up
Adjust with body coil	Off
Confirm freq. adjustment	Off
Assume Dominant Fat	Off
Assume Silicone	Off
Adjustment Tolerance	Auto

System - Adjust Volume

Position	Isocenter
Orientation	Transversal
Rotation	0.00 deg
A >> P	263 mm
R >> L	350 mm
F >> H	350 mm
Reset	Off

System - Tx/Rx

Frequency 1H	63.685340 MHz
Correction factor	1
Gain	High
Img. Scale Cor.	1.000
Reset	Off
? Ref. amplitude 1H	0.000 V

Physio - Signal1

1st Signal/Mode	None
TR	9.0 ms
Concatenations	2
Segments	1

Physio - Cardiac

Tagging	None
Magn. preparation	None
Fat suppr.	None
Dark blood	Off
FoV read	200 mm
FoV phase	100.0 %
Phase resolution	50 %

Physio - PACE

Resp. control	Off
Concatenations	2

Inline - Common

Subtract	Off
Measurements	1
StdDev	Off
Liver registration	Off
Save original images	On

Inline - MIP

MIP-Sag	Off
MIP-Cor	Off
MIP-Tra	Off
MIP-Time	Off
Save original images	On

Inline - Soft Tissue

Wash - In	Off
Wash - Out	Off
TTP	Off
PEI	Off
MIP - time	Off
Measurements	1

Inline - Composing

Inline Composing	Off
Distortion Corr.	Off

Sequence - Part 1

Introduction	On
Dimension	2D
Phase stabilisation	Off
Asymmetric echo	Off
Contrasts	1
Flow comp.	No
Multi-slice mode	Sequential
Bandwidth	260 Hz/Px

Sequence - Part 2

Segments	1
Acoustic noise reduction	None
RF pulse type	Normal
Gradient mode	Normal
Excitation	Slice-sel.
RF spoiling	On

Sequence - Assistant

Mode	Off

\\Non Cardiac Research\Whole body\ATTIS\ATTIS\MRS_se_30_right_calf

TA: 2:22 PM: REF Vol: 10 ×10 ×10 mmRel. SNR: 1.00 : svs_se

Properties

Prio recon	Off
Load images to viewer	On
Inline movie	Off
Auto store images	On
Load images to stamp segments	Off
Load images to graphic segments	Off
Auto open inline display	Off
Auto close inline display	Off
Start measurement without further preparation	Off
Wait for user to start	Off
Start measurements	Single measurement

Routine

Position	R118.0 P93.0 H0.0 mm
Orientation	Transversal
Rotation	0 deg
Vol R >> L	10 mm
Vol R >> L	10 mm
Vol F >> H	10 mm
TR	2000 ms
TE	30 ms
Averages	64
Filter	Prescan Normalize
Coil elements	HE1-4

Contrast

TR	2000 ms
TE	30 ms
Averages	64
Flip angle	90 deg
Water suppr.	Water sat.
Water suppr. BW	35 Hz
Spectral suppr.	None
Measurements	1

Resolution - Common

Prescan Normalize	On
Vector size	1024

Geometry - Common

Position	R118.0 P93.0 H0.0 mm
Orientation	Transversal
Rotation	0 deg
Vol R >> L	10 mm
Vol A >> P	10 mm
Vol F >> H	10 mm

Geometry - AutoAlign

AutoAlign	---
Initial Position	R118.0 P93.0 H0.0
Phase	93 mm
Read	118 mm
Shift	0 mm
Initial Rotation	0.00 deg
Initial Orientation	Transversal

Geometry - Navigator

System - Miscellaneous

Positioning mode	REF
Table position	H
Table position	0 mm
MSMA	S - C - T
Sagittal	R >> L
Coronal	A >> P
Transversal	F >> H
Save uncombined	Off
Save single averages	Off
AutoAlign	---
Coil Select Mode	On - AutoCoilSelect

System - Adjustments

B0 Shim mode	Brain
Adj. water suppr.	On
Adjust with body coil	Off
Confirm freq. adjustment	On
Only after freq. change	On
Assume Dominant Fat	Off
Assume Silicone	Off
Adjustment Tolerance	Auto

System - Adjust Volume

Position	R118.0 P93.0 H0.0 mm
Orientation	Transversal
Rotation	0.00 deg
A >> P	10 mm
R >> L	10 mm
F >> H	10 mm
Reset	Off

System - Tx/Rx

Frequency 1H	63.685340 MHz
Gain	High
Img. Scale Cor.	1.000
Reset	Off
? Ref. amplitude 1H	0.000 V

Physio - Signal1

1st Signal/Mode	None
TR	2000 ms

Physio - PACE

Resp. control	Off

Sequence - Common

Preparation scans	3
Delta frequency	-2.7 ppm
Ref. scan mode	Save all
No. of ref. scans	4
Phase cycling	Auto
Bandwidth	1000 Hz
Acquisition duration	1024 ms
Remove oversampling	On