An investigation of inflammatory markers in eating disorders

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Abstract

The aetiology of eating disorders (EDs) is unclear. It has been proposed that alterations in certain inflammatory processes could be a potential biological correlate of EDs, which may contribute to the development and/or maintenance of EDs. This thesis aimed to investigate the role of markers of these inflammatory mechanisms in EDs by (a) examining potential alterations in a broad range of cytokines and other inflammatory markers in people with anorexia nervosa (AN), bulimia nervosa (BN) and binge eating disorder (BED); (b) assessing whether longitudinal alterations in inflammatory markers co-occur with changes in ED psychopathology in patients with AN; and (c) exploring how stress, diet and genetic factors – all variables that have been reported to influence inflammatory marker concentrations in healthy and other clinical populations – are associated with inflammatory markers in people with EDs.

The first aim was addressed in: a systematic review and meta-analysis exploring cytokine alterations in people with EDs, in comparison to healthy individuals (Chapter 2); two studies examining cross-sectional differences in inflammatory marker concentrations in people with AN and healthy comparison groups (Chapter 3); and a cross-sectional analysis of concentrations of C-reactive protein (CRP) in people with lifetime diagnoses of AN, BN, and BED (Chapter 6). The second aim was addressed in an observational longitudinal study, measuring inflammatory marker concentrations at three time points over a period of six months, in patients with AN undergoing specialist ED treatment (Chapter 4). The final aim was addressed in: three studies assessing the role of several types of event-related stress (exposure to traumatic and stressful life events, including childhood maltreatment) on inflammatory marker concentrations in people with current and/or a history of EDs (Chapter 5); two studies assessing the association between dietary inflammation (i.e., the inflammatory potential of an individual's diet) and inflammatory markers in people with lifetime diagnoses of EDs and females with a current AN diagnosis (Chapter 6); and lastly, a nested case-control study using data from the UK Biobank, a population-based cohort, to explore the relationship between a polygenic risk score for CRP and ED status (Chapter 7).

The data provided some support for alterations of inflammatory markers, such as interleukin (IL)-6, IL-7 and C-reactive protein (CRP), as biological correlates of specific EDs; however, findings were inconsistent. It was further found that changes in certain cytokines (IL-6 and IL-7) co-occurred with improvements in ED symptoms in patients with AN, identifying possible state biomarkers of AN. Stress and the inflammatory potential of an individual's diet were not related to concentrations of inflammatory

markers in people with EDs. However, genetic factors may play a role in the relationship between inflammatory markers and EDs, as we reported that a polygenic risk score for circulating CRP was associated with having current and/or a lifetime BED diagnosis.

The findings extend current concepts related to biological correlates of EDs, and they provide direction for future research to explore the potential role of inflammatory markers as risk factors for EDs and as biomarkers of trait, state and treatment response in EDs.

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Abbreviations used throughout this thesis

Abbreviations are reintroduced in each chapter. In alphabetical order:

ACTH	Adrenocorticotropic hormone
AN	Anorexia nervosa
AN-BP	Anorexia nervosa binge-eating/purging type
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
AN-R	Anorexia nervosa restricting type
AVP	Arginine–vasopressin
BAME	Black, Asian and minority ethnic
BDI-II	Beck Depression Inventory-II
BDNF	Brain-derived neurotrophic factor
BED	Binge eating disorder
bFGF	Basic fibroblast growth factor
BH_4	Tetrahydrobiopterin
BIA	Bioelectrical impedance analysis
BMI	Body mass index
BN	Bulimia nervosa
CAFÉ	Compositional Analyses from Frequency Estimates
CBT-ED	Eating-disorder-focussed cognitive behavioural therapy
CHARGE	Cohorts for Heart and Aging Research in Genomic Epidemiology
CI	Confidence intervals
CRH	Corticotropin-releasing hormone
CRP	C-reactive protein
CTQ	Childhood Trauma Questionnaire
CytAN	Cytokines in Anorexia Nervosa
DASS-21	Depression Anxiety and Stress Scales – 21 Version
df	Degrees of freedom
DII	Dietary Inflammatory Index
DNA	Deoxyribonucleic acid
DSM	Diagnostic and Statistical Manual of Mental Disorders
ED	Eating disorder
EDDS	Eating Disorder Diagnostic Scale
EDE-Q	Eating Disorder Examination Questionnaire
EDTA	Ethylenediaminetetraacetic acid

ELISA	Enzyme-linked immunosorbent assay
EPIC	European Prospective Investigation of Cancer
ESTRA	Brain Activation in Eating Disorders
FDR	False discovery rate
FETA	Food Frequency Questionnaire European Prospective Investigation of Cancer Tool for Analysis
FFQ	Food Frequency Questionnaire
Flt-1	Fms-like tyrosine kinase-1
fMRI	Functional magnetic resonance imaging
g	Grams
GM-CSF	Granulocyte-macrophage colony-stimulating factor
GWAS	Genome-wide association study
HC	Healthy control
HPA	Hypothalamic–pituitary–adrenal
ICAM-1	Intercellular adhesion molecule-1
ICD	International Statistical Classification of Diseases
IDO	Indoleamine 2,3 dioxygenase
IFN	Interferon
IL	Interleukin
IoPPN	Institute of Psychiatry, Psychology & Neuroscience
IP-10	Interferon γ-induced protein-10
IQR	Interquartile range
kcal	Kilocalories
KCL	King's College London
kg	Kilograms
l	Litres
L-DOPA	L-3,4-dihydroxyphenylalanine
LEC	Revised Life Events Checklist
LEQ	Life events questionnaire
m^2	Metres (squared)
MCP	Monocyte chemoattractant protein
MDC	Macrophage-derived chemokine
MDD	Major depressive disorder
mg	Milligrams
MIC-1	Macrophage inhibitory cytokine-1
MIP	Macrophage inflammatory protein
ml	Millilitres

MRI	Magnetic resonance imaging
mRNA	Messenger ribonucleic acid
MUFA	Monounsaturated fatty acids
n	Number of observations
ng	Nanograms
NHS	National Health Service
NIHR	National Institute for Health Research
NOS	Newcastle-Ottawa Scale
OCD	Obsessive compulsive disorder
OR	Odds ratio
PANDAS	Paediatric Autoimmune Neuropsychiatric Disorders Associated with Streptococcal Infections
PCL	Post-Traumatic Stress Disorder Checklist
pg	Picograms
PlGF	Placental growth factor
POMC	Pro-opiomelanocortin
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PRS	Polygenic risk score
PTSD	Post-traumatic stress disorder
PUFA	Polyunsaturated fatty acids
PVN	Paraventricular nucleus
RANTES	Regulated on activation, normal T cell expressed and secreted
RDoC	Research Domain Criteria
r_g	Genetic correlation
RIA	Radioimmunoassay
ROSANA	Relationship between Overactivity, Stress and Anxiety in Anorexia Nervosa
SAA	Serum amyloid A
SD	Standard deviation
SE	Standard error
SGDP	Social, Genetic & Developmental Psychiatry
SMD	Standardised mean difference
SNP	Single-nucleotide polymorphism
SSRI	Selective serotonin reuptake inhibitor
STRATIFY	Brain Network Based Stratification of Reinforcement-Related Disorders
TARC	Thymus and activation-regulated chemokine
τb	Kendall's tau-b

TGF	Transforming growth factor
Th	T-helper
Tie-2	Tyrosine kinase-2
TLR	Toll-like receptor
TNF	Tumor necrosis factor
µg	Micrograms
UK	United Kingdom
µl	Microlitres
US	United States
VCAM-1	Vascular cell adhesion molecule-1
VEGF	Vascular endothelial growth factor

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Dissemination of research

Publications incorporated into thesis

Chapter 1: None.

Chapter 2: **Dalton, B.**, Bartholdy, S., Robinson, L., Solmi, M., Ibrahim, M.A.A., Breen, G., Schmidt, U. & Himmerich, H. (2018). A meta-analysis of cytokine concentrations in eating disorders. *Journal of Psychiatric Research, 103,* 252-264. DOI: 10.1016/j.jpsychires.2018.06.002.

Chapter 3: **Dalton, B.**, Campbell, I.C., Chung, R., Breen, G., Schmidt, U. & Himmerich, H. (2018). Inflammatory markers in anorexia nervosa: an exploratory study. *Nutrients, 10*, E1573. DOI: 10.3390/nu10111573.

Chapter 4: **Dalton, B.**, Leppanen, J., Campbell, I.C., Chung, R., Breen, G., Schmidt, U. & Himmerich, H. (2019). A longitudinal analysis of cytokines in anorexia nervosa. *Brain, Behavior, and Immunity, S0889-1591(19)30114-X.* DOI: 10.1016/j.bbi.2019.05.012.

Chapter 5: None

Chapter 6: None

Chapter 7: None

Chapter 8: None

PDF versions of the published articles are included in Appendix A Section 10.1.

Publications completed during PhD but not included in thesis

Patsalos, O., Mavrogiannidis, T., **Dalton, B.,** Field, C. J., & Himmerich, H. (submitted). An idea worth researching: Phosphatidylcholine containing long chain omega-3 fatty acids as a treatment adjunct for patients with anorexia nervosa. *Psychatria Danubina.*

Zavos, H., **Dalton, B.**, Jayaweera, K., Harber-Aschan, L., Pannala, G., Adikari, A., Hatch, S., Siribaddana, S., Sumathipala, A., Hotopf, M., & Rijsdijk, F. (in press). The relationship between independent and dependent life events and depression in Sri Lanka: A twin and singleton study. *Social Psychiatry and Psychiatric Epidemiology.*

Himmerich, H., Patsalos, O., Lichtblau, N., Ibrahim, M.A.A., **Dalton, B.** (2019). Cytokine research in depression: Principles, challenges and open questions. *Frontiers in Psychiatry, 10,* 30. DOI: 10.3389/fpsyt.2019.00030

Schmidt, F.M., Sander, C., Minkwitz, J., Mergl, R., **Dalton, B.**, Holdt, L.M., Teupser, D., Hegerl, U., & Himmerich, H. (2018). Serum markers of inflammation mediate the positive association between neuroticism and depression. *Frontiers in Psychiatry 9,* 609. DOI: 10.3389/fpsyt.2018.00609

Dalton, B.*, Bartholdy, S.*, McClelland, J.*, Kekic, M., Rennalls, S.J., Werthmann, J., Carter, B., O'Daly, O. G., Campbell, I. C., Davis, A.S., Glennon, D., Kern, N. & Schmidt, U. (2018). A randomised controlled feasibility trial of real versus sham repetitive transcranial magnetic stimulation treatment in adults with severe and enduring anorexia nervosa: The TIARA study. *BMJ Open, 8,* e021531. DOI: 10.1136/bmjopen-2018-021531 (**Joint first authors)*

Dalton, B., Bartholdy, S., Campbell, I. C. & Schmidt, U. (2018). Neurostimulation in clinical and sub-clinical eating disorders: A systematic update of the literature. *Current Neuropharmacology, 16,* 1174-1192. DOI: 10.2174/1570159X16666180108111532

Waheed, A.*, **Dalton, B.***, Wesemann, U., Ibrahim, M.A.A. & Himmerich, H. (2018). A systematic review of interleukin-1β in post-traumatic stress disorder: Evidence from human and animal studies. *Journal of Interferon and Cytokine Research, 38,* 1-11. DOI: 10.1089/jir.2017.0088 (**Joint first authors)*

Dalton, B., Campbell, I. C. & Schmidt, U. (2017). Neuromodulation and neurofeedback treatments in eating disorders and obesity. *Current Opinion in Psychiatry, 30,* 458-473. DOI: 10.1097/YCO.0000000000000361

Hussein, S.*, **Dalton, B.***, Willmund, G.D., Ibrahim, M.A.A. & Himmerich, H. (2017). A systematic review of tumor necrosis factor-α in post-traumatic stress disorder: Evidence

from human and animal studies. *Psychatria Danubina, 29,* 407-420. DOI: 10.24869/psyd.2017.407 (**Joint first authors)*

Wykes, T., Evans, J., Paton, C., Barnes, T. R. E., Taylor, D., Bentall, R., **Dalton, B.**, Ruffell, T., Rose, D. & Vitoratou, S. (2017). What side effects are problematic for patients prescribed antipsychotic medication? The Maudsley Side Effects (MSE) measure for antipsychotic medication. *Psychological Medicine,* 1-10. DOI: 10.1017/S0033291717000903

Kekic, M., McClelland, J., Bartholdy, S., Boysen, E., Musiat, P., **Dalton, B.**, Tiza, M., David, A. S., Campbell, I. C., & Schmidt, U. (2017). Transcranial direct current stimulation improves symptoms, mood, and self-regulatory control in bulimia nervosa: A proof-of-principle clinical trial. *PLOS ONE, 12,* e0167606. DOI: 10.1371/journal.pone.0167606

McClelland, J., **Dalton, B.**, Kekic, M., Bartholdy, S., Campbell, I. C., & Schmidt, U. (2016). A systematic review of temporal discounting in eating disorders and obesity: Behavioural and neuroimaging findings. *Neuroscience & Biobehavioral Reviews*, *71,* 506-528. DOI: 10.1016/j.neubiorev.2016.09.024

Himmerich, H., Wesemann, U., **Dalton, B.**, Holdt, L. M., Teupser, D. & Willmund, G. D. (2016). Exploring an association between hostility and serum concentrations of TNF-α and its soluble receptors. *Psychosomatic Research, 91,* 87-88. DOI: 10.1016/j.jpsychores.2016.11.001

Conference abstracts published

Dalton, B., Campbell, I.C., Chung, R., Breen, G., Schmidt, U. & Himmerich, H. (2019). Inflammatory Markers in Anorexia Nervosa: An Exploratory Study. *European Neuropsychopharmacology, 29,* S640-S641.

Conference and research presentations associated with thesis

Conference presentations:

Dalton, B., et al. (2019). Inflammatory Markers in Anorexia Nervosa: An Exploratory Study. Oral presentation at the International Conference of Eating Disorders (ICED), New York, USA.

Dalton, B. et al. (2019). Inflammatory Markers in Anorexia Nervosa: An Exploratory Study. Poster presentation at The European College of Neuropsychopharmacology Workshop for Early Career Scientists in Europe, Nice, France.

Dalton, B., et al. (2017). A systematic review of pro- and anti-inflammatory cytokines in eating disorders. Oral presentation at the World Psychiatric Association (WPA) XVII World Congress of Psychiatry, Berlin, Germany.

Dalton, B. (2017). Cytokines and eating disorders. Oral presentation as part of a symposium (titled "Immunological Aspects of Mood and Weight Regulation") at Arbeitsgemeinschaft für Neuropsychopharmakologie und Pharmakopsychiatrie, Münich, Germany.

PhD showcases:

Dalton, B., et al., (2018). Cytokines in anorexia nervosa. Oral presentation at the Biomedical Research Centre (BRC) Obesity, Lifestyle and Learning from Extreme Phenotypes (OBELIX) Theme Showcase.

Dalton, B., et al. (2018). A meta-analysis of cytokine concentrations in eating disorders. Poster presentation at the King's College London Institute of Psychiatry, Psychology & Neuroscience PhD Student Showcase.

Internal meetings:

The research included within this thesis has been presented at departmental meetings at the Institute of Psychiatry, Psychology & Neuroscience, King's College London (chaired by Professor Ulrike Schmidt and Professor Janet Treasure).

Declaration of the candidate's role

Chapter 1: General introduction

All work is the candidate's own. The candidate received constructive feedback from Professor Iain C. Campbell and her supervisors, Dr Hubertus Himmerich and Professor Ulrike Schmidt.

Chapter 2: A meta-analysis of cytokine concentrations in eating disorders

The candidate conceived the study with her supervisors, Dr Hubertus Himmerich and Professor Gerome Breen. The literature search was conducted by the candidate and Dr Savani Bartholdy. The candidate extracted the data, ran the meta-analyses and drafted the manuscript. Dr Lauren Robinson provided statistical guidance. The candidate received constructive feedback from Dr Savani Bartholdy, Dr Lauren Robinson, Dr Marco Solmi, Professor Mohammad A.A. Ibrahim, her supervisors (Dr Hubertus Himmerich and Professor Ulrike Schmidt), and from the peer reviewers at *Journal of Psychiatric Research*.

Chapter 3: Cross-sectional analyses of inflammatory markers in anorexia nervosa

Study 1: The study was conceived by the candidate and her supervisors, Dr Hubertus Himmerich, Professor Ulrike Schmidt and Professor Gerome Breen. This study made use of blood samples and data already collected for the Relationship between Overactivity, Stress and Anxiety in Anorexia Nervosa (ROSANA) study by the research team. The candidate did not contribute to the data collection for this study. Mr Raymond Chung performed cytokine measurement. The candidate conducted the statistical analyses and drafted the manuscript. The candidate received constructive feedback from Professor Iain C. Campbell, her supervisors (Dr Hubertus Himmerich and Professor Ulrike Schmidt), and from the peer reviewers at *Nutrients*.

Study 2: The candidate designed the study with her supervisor, Dr Hubertus Himmerich. The candidate obtained ethical approval. Recruitment and data collection and entry were performed by Ms Olivia Patsalos (main contributor) and the candidate. Blood samples were collected by the National Institute for Health Research (NIHR) Bioresource Centre Maudsley team. Mr Raymond Chung conducted cytokine measurement. The candidate performed the statistical analyses and drafted the chapter. The candidate received constructive feedback from her supervisors, Dr Hubertus Himmerich and Professor Ulrike Schmidt.

Chapter 4: A longitudinal analysis of cytokines in anorexia nervosa

The study was conceived by the candidate and her supervisors, Dr Hubertus Himmerich, Professor Ulrike Schmidt and Professor Gerome Breen. This study made use of blood samples and data already collected for the Relationship between Overactivity, Stress and Anxiety in Anorexia Nervosa (ROSANA) study by the research team. The candidate did not contribute to the data collection for this study. Mr Raymond Chung performed cytokine measurement. Dr John Hodsoll and Dr Jenni Leppanen provided guidance on statistical analyses. The candidate conducted analyses and drafted the manuscript. The candidate received constructive feedback from Dr Jenni Leppanen, Professor Iain C. Campbell, her supervisors (Dr Hubertus Himmerich, Professor Ulrike Schmidt, and Professor Gerome Breen), and from the peer reviewers at *Brain, Behavior and Immunity*.

Chapter 5: Examining the potential role of stress in the relationship between inflammatory markers and eating disorders

Study 1: The candidate designed the study with her supervisor, Dr Hubertus Himmerich. The candidate obtained ethical approval. Recruitment and data collection and entry were performed by Ms Olivia Patsalos (main contributor) and the candidate. Blood samples were collected by the National Institute for Health Research (NIHR) Bioresource Centre Maudsley team. Mr Raymond Chung conducted cytokine measurement. The candidate performed the statistical analyses and drafted the chapter. The candidate received constructive feedback from her supervisors, Dr Hubertus Himmerich and Professor Ulrike Schmidt.

Study 2: The candidate conceived the study, performed the statistical analyses and drafted the chapter. This study made use of data already collected for the UK Biobank. The candidate did not contribute to the data collection for this study. Data was extracted by Dr Christopher Hübel. The candidate received constructive feedback from her supervisors, Dr Hubertus Himmerich and Professor Ulrike Schmidt.

Study 3: The candidate conceived the study with Dr Lauren Robinson, Dr Sylvane Desrivières, Professor Ulrike Schmidt and Dr Hubertus Himmerich. The study made use of the data already collected for the Brain Activation in Eating Disorders (ESTRA) branch of the Brain Network Based Stratification of Reinforcement-Related Disorders (STRATIFY) study (Principal Investigator: Professor Gunter Schumann). The candidate did not contribute to the data collection for this study. Data was collected by Ms Madeleine Irish, Dr Lauren Robinson and the ESTRA research team. Data entry was performed by Ms Olivia Patsalos. The candidate performed the statistical analyses and drafted the chapter. The candidate received constructive feedback from her supervisors, Dr Hubertus Himmerich and Professor Ulrike Schmidt.

Chapter 6: Investigating the role of dietary inflammation in eating disorders

Study 1: The candidate conceived the study, performed the statistical analyses and drafted the chapter. This study made use of data already collected for the UK Biobank. The candidate did not contribute to the data collection for this study. Data was extracted by Dr Christopher Hübel. Dr Joseph Firth provided guidance on calculating the Dietary Inflammatory Index. Ms Daniela Mercado Beivide provided guidance on defining extreme dietary intake. The candidate received constructive feedback from her supervisors, Dr Hubertus Himmerich and Professor Ulrike Schmidt.

Study 2: The candidate designed the study with her supervisor, Dr Hubertus Himmerich. The candidate obtained ethical approval. Recruitment and data collection were performed by Ms Olivia Patsalos (main contributor) and the candidate. Data entry was performed by Ms Olivia Patsalos and Ms Christia Kyprianou (MSc Mental Health Studies student supervised by the candidate and Dr Hubertus Himmerich). Blood samples were collected by the National Institute for Health Research (NIHR) Bioresource Centre Maudsley team. Mr Raymond Chung performed cytokine measurement. Dr Joseph Firth provided guidance on calculating the Dietary Inflammatory Index. The candidate performed the statistical analyses and drafted the chapter. The candidate received constructive feedback from her supervisors, Dr Hubertus Himmerich and Professor Ulrike Schmidt. Ms Christia Kyprianou used the data from this study for her assessed dissertation contributing to her MSc in Mental Health Studies and received guidance from the candidate and Dr Hubertus Himmerich.

Chapter 7: Using polygenic risk scores to examine the relationship between C-reactive protein and eating disorders

The candidate conceived the study with Dr Christopher Hübel and her supervisor, Professor Gerome Breen. This study made use of data already collected for the UK Biobank. The candidate did not contribute to the data collection for this study. Data was extracted and polygenic risk scores were calculated by Dr Christopher Hübel. The candidate performed the statistical analyses and drafted the chapter. The candidate received constructive feedback from Dr Christopher Hübel and her supervisors, Dr Hubertus Himmerich, Professor Ulrike Schmidt and Professor Gerome Breen.

Chapter 8: General overview

All work is the candidate's own. The candidate received constructive feedback from Dr Lauren Robinson, Professor Iain C. Campbell and her supervisors, Dr Hubertus Himmerich and Professor Ulrike Schmidt.

Chapter 1. General introduction

This thesis explores the role of inflammatory markers in eating disorders (EDs). EDs are serious psychiatric conditions characterised by pathological eating and/or weight-control behaviours and disturbed body image, which significantly impairs quality of life and psychosocial functioning (American Psychiatric Association, 2013). They are associated with high psychiatric comorbidity (Ulfvebrand, Birgegard, Norring, Hogdahl, & von Hausswolff-Juhlin, 2015), various medical complications (Westmoreland, Krantz, & Mehler, 2016) and increased risk of mortality (Arcelus, Mitchell, Wales, & Nielsen, 2011). Current treatment options for EDs are predominantly psychological and/or behavioural therapies (including nutritional interventions) and are only moderately effective (Brockmeyer, Friederich, & Schmidt, 2018; Ghaderi et al., 2018; Slade et al., 2018). Furthermore, treatments, such as psychopharmacotherapy, targeting biological correlates of EDs are limited, particularly in anorexia nervosa where no single drug treatment is approved in any country (Crow, 2019; Himmerich & Treasure, 2018; Mitchell, Roerig, & Steffen, 2013). However, despite progress in genetics and epigenetics (Himmerich, Bentley, Kan, & Treasure, 2019a), brain imaging (Frank, 2019), and psychological (e.g., Cardi, Tchanturia, & Treasure, 2018; Westwood, Kerr-Gaffney, Stahl, & Tchanturia, 2017) research, the aetiology of EDs remains unclear. Thus, without greater knowledge of potential causal factors, opportunities for the development of alternative interventions, in particular biologically derived treatments, or for the improvement of existing treatments (e.g., personalised treatment approaches), are limited. Investigation into the biological correlates of EDs, that may contribute to the development and/or maintenance of the disorder, are therefore crucial.

This chapter summarises the ED diagnoses, epidemiology and pathogenesis, and current treatment approaches to EDs (Section 1.1). Following this, I[1] will provide an overview of inflammatory markers (Section 1.2). Finally, I will discuss the relevance of inflammatory markers to EDs (Section 1.3).

1.1 Eating disorders

The fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM)-5 (American Psychiatric Association, 2013) describes diagnostic criteria for the three main eating disorders: anorexia nervosa (AN), bulimia nervosa (BN) and binge eating disorder (BED)[2], all of which will be examined in this thesis.

[1] I will shift between the use of 'I' and 'we' over the course of this thesis. This is to reflect that much of the work in Chapters 2 to 7 was collaborative, as described on Page 29-31 (Declaration of the candidate's role).

[2] Four other less extensively studied Feeding and Eating Disorder diagnoses are described in the DSM-5: Avoidant/Restrictive Food Intake Disorder, Other Specified Feeding or Eating Disorder, Pica, and Rumination Disorder. However, they are beyond the scope of this thesis and will therefore not be discussed.

1.1.1 Anorexia nervosa

1.1.1.1 Diagnosis

According to the DSM-5 (American Psychiatric Association, 2013), AN is characterised by the restriction of energy intake relative to requirements, leading to a significantly low body weight in the context of age, sex, developmental trajectory, and physical health. Individuals present with an intense fear of gaining weight or of becoming fat, or persistent behaviour that interferes with weight gain, even though at a significantly low weight. Two subtypes of AN are described. Anorexia nervosa restricting type (AN-R) in which individuals do not engage in recurrent episodes of binge eating or purging and weight loss is accomplished primarily through dieting, fasting, and/or excessive exercise. Anorexia nervosa binge-eating/purging type describes presentations in which the individual engages in recurrent episodes of binge eating and/or purging behaviour (i.e., self-induced vomiting or the misuse of laxatives, diuretics, or enemas).

1.1.1.2 Comorbidity

Medical complications. AN-associated weight loss and malnutrition can adversely affect almost every system within the body and AN is therefore often accompanied by significant medical complications (Westmoreland et al., 2016). These include endocrine e.g., amenorrhoea and infertility; gastrointestinal e.g., impaired gastric motility, gastric dilation, defecatory complaints and disorders, and gastroparesis (Schalla & Stengel, 2019); cardiac e.g., bradycardia and sudden cardiac death; haematological e.g., anaemia, leucopoenia and electrolyte imbalances, some of which can become life-threatening (Himmerich, Schönknecht, Heitmann, & Sheldrick, 2010); musculoskeletal e.g., osteoporosis; neurological e.g., brain atrophy; and dermatological complications e.g., xerosis, lanugo hair growth on the spine and sides of the face, and thinning of the hair (Westmoreland et al., 2016).

Psychiatric comorbidities. AN is frequently associated with major depressive disorder (MDD), anxiety disorders, including obsessive compulsive disorder (OCD; Ulfvebrand et al., 2015), and autism spectrum disorders (Westwood & Tchanturia, 2017). For example, it has been reported that approximately 33% of people with AN-R and 49% with AN-BP also have diagnoses of a unipolar depression disorder and 45% of AN-R and 56% of AN-BP have an anxiety disorder diagnosis (Ulfvebrand et al., 2015).

1.1.1.3 Epidemiology

Lifetime prevalence of AN is estimated between 1 and 4% among European women, with considerable variation by age and ethnic/country origins (Keski-Rahkonen & Mustelin, 2016). Overall incidence rates of AN in females have been reported at approximately 12 per 100,000 person-years (Smink et al., 2016; Steinhausen & Jensen, 2015). During peak

age of onset, between the ages of 14 and 15, incidence rates of AN at primary care and service level have been reported to be approximately 206 per 100,000 person-years (Javaras et al., 2015; Micali, Hagberg, Petersen, & Treasure, 2013). It is important to note that as the incidence of EDs in the general population are relatively low, cases have often been identified using hospital records and case registers. Therefore, given that these estimates are based on individuals who seek treatment for their ED, and as patients may tend to deny or conceal their illness and avoid professional help, these may underestimate the prevalence and incidence of AN and other EDs (Smink, van Hoeken, & Hoek, 2012).

1.1.1.4 Course and outcome

Peak age of onset for AN is in adolescence or emerging adulthood between the ages 14 and 19 years (Javaras et al., 2015; Micali et al., 2013). Individuals with AN have an average illness duration of 8 years (Zipfel, Lowe, Reas, Deter, & Herzog, 2000). AN has the highest standardised mortality rate among all psychiatric disorders (Arcelus et al., 2011), with a standardised mortality ratio of approximately 5 (Himmerich et al., 2019b). This mortality rate is attributable to the elevated risk of suicide reported in AN patients (Yao et al., 2016; Zerwas et al., 2017) and the serious physical health issues associated with AN (as described above), in particular cardiovascular complications (Sachs, Harnke, Mehler, & Krantz, 2016), such as sudden cardiac death. A review of follow-up studies reported that of the surviving AN patients, on average, less than half made a full recovery, 33% improved, and 20% developed a chronic course of AN when assessed at a follow-up duration of four to ten years (Steinhausen, 2002). Recovery rates increased to approximately 70% when examined at a follow-up duration of longer than 10 years (Steinhausen, 2002). Similarly, a recent longitudinal study of ED patients showed that at a 9-year follow-up, 31.4% of AN patients had recovered, and this increased to 62.8% at a 22-year follow-up (Eddy et al., 2017). When considering symptom normalisation (follow-up duration of four to ten years), it was reported that 60% of surviving AN patients showed normalisation of weight, 57% normalisation of menstruation, and 47% normalisation of eating behaviours (Steinhausen, 2002). AN is a disabling disorder and can lead to persistent difficulties with psychosocial functioning, employment and financial dependency, and overall quality of life (Padierna, Quintana, Arostegui, Gonzalez, & Horcajo, 2000; Schmidt et al., 2016; Tchanturia et al., 2013; Treasure et al., 2015; Wentz, Gillberg, Anckarsater, Gillberg, & Rastam, 2009). Long-term sequelae of AN related to health and medical conditions, such as osteoporosis and gastrointestinal symptoms, can influence quality of life following improvement and recovery (Nilsson & Hägglöf, 2005; Schalla & Stengel, 2019; Treasure et al., 2015; Wentz et al., 2003).

1.1.1.5 Treatment

The available treatments for adults with AN largely focus on psychological and/or nutritional interventions. However, there is no leading treatment and current treatment options for adults with AN are only moderately effective, with low to moderate recovery rates, attrition, and high relapse rates (Brockmeyer et al., 2018; Byrne et al., 2017; Hay, Claudino, Touyz, & Abd Elbaky, 2015; Treasure et al., 2015; Zipfel, Giel, Bulik, Hay, & Schmidt, 2015).

There is little high quality evidence supporting the use of pharmacotherapy for the treatment of AN (Blanchet et al., 2019; Davis & Attia, 2017). Antidepressants seem to have little effect in treating comorbid depression symptoms in AN (Davis & Attia, 2017). However, some studies have found promising effects on weight gain and/or reductions in eating-related preoccupations with the use of olanzapine, aripiprazole, and dronabinol and also D-cycloserine as a psychotherapy adjunct (Attia et al., 2019; Davis & Attia, 2017; Himmerich & Treasure, 2018). Given the limited availability of treatment options, high mortality and poor long-term outcomes, the development of novel treatment approaches is needed (Brockmeyer et al., 2018; Le Grange, 2016; Schmidt et al., 2016).

1.1.2 Bulimia nervosa

1.1.2.1 Diagnosis

According to the DSM-5 criteria (American Psychiatric Association, 2013), BN is characterised by recurrent episodes of binge eating and inappropriate compensatory behaviours (e.g., self-induced vomiting; misuse of laxatives, diuretics, or other medications; fasting; or excessive exercise) to avoid weight gain, that occur, on average, at least once a week for three months. Binge eating episodes are discrete periods of time (e.g., within any 2-hour period) during which a person consumes an unusually large amount of food given the circumstances and experiences a loss of control over eating. Similar to in AN, in BN self-evaluation is unduly influenced by body shape and weight.

1.1.2.2 Comorbidity

Medical complications. Medical complications associated with BN behaviours (i.e., self-induced vomiting, laxative misuse), include dental and gastrointestinal problems, and electrolyte and acid-base imbalances, which can lead to serious cardiac abnormalities (Westmoreland et al., 2016).

Psychiatric comorbidities. Psychiatric comorbidities are common in BN, including anxiety disorders, MDDs, impulse-control related disorders and the highest rate of substance misuse disorders across all EDs (Kessler et al., 2013; Nazar et al., 2008; Ulfvebrand et al., 2015). Elevated rates of suicide have also been reported in individuals with BN compared to females reporting no ED (Pisetsky, Thornton, Lichtenstein, Pedersen, & Bulik, 2013).

1.1.2.3 Epidemiology

Lifetime prevalence of BN is around 2% for females, with an incidence rate of approximately 300 per 100,000 person-years (Keski-Rahkonen et al., 2009; Stice, Marti, & Rohde, 2013).

1.1.2.4 Course and outcome

BN typically develops during adolescence and early adulthood, with peak onset at around 16 to 20 years old (Stice et al., 2013) and a median duration of illness of approximately 6.5 years (Kessler et al., 2013). Although not as markedly as in AN, elevated mortality rates are reported in people with BN compared to the general population (Arcelus et al., 2011; Steinhausen, 2009), with a standardised mortality ratio of 2.52 (Himmerich et al., 2019c). The elevated mortality rates are mostly attributable to medical complications (Westmoreland et al., 2016) and suicide (Pisetsky et al., 2013). With regards to the course of BN in surviving patients, it has been reported that approximately 47.5% make a full recovery, 26% improve, and 26% develop a chronic form of the disorder (follow-up duration of four to ten years; Steinhausen, 2009). Higher depression, longer duration of illness, a history of substance use problems, and a high frequency of binge eating has been associated with poorer treatment outcomes (Accurso et al., 2016; Agras et al., 2000; Keel & Brown, 2010; Shapiro et al., 2007). BN is a disabling psychiatric disorder, with significant impacts on quality of life, impairments in role functioning and more generally at a societal level, a high economic and global burden (Ágh et al., 2016; Erskine, Whiteford, & Pike, 2016; Kessler et al., 2013).

1.1.2.5 Treatment

Psychological therapy (e.g., guided self-help, individual eating-disorder-focussed cognitive behavioural therapy [CBT-ED]) is the recommended treatment for adults with BN (National Institute for Health and Care Excellence, 2017) and the antidepressant Fluoxetine has shown some benefit as a treatment for BN (Aigner, Treasure, Kaye, Kasper, & World Federation of Societies of Biological Psychiatry Task Force on Eating Disorders, 2011; Shapiro et al., 2007). Recent reviews and meta-analyses have concluded that psychotherapy, including group behavioural therapy, group CBT-ED, individual CBT-ED and guided cognitive behavioural self-help, are more effective than other treatments (e.g., pharmacotherapy) in achieving full remission at the end of treatment for BN (Slade et al., 2018).

1.1.3 Binge eating disorder

1.1.3.1 Diagnosis

BED has only recently been recognised as an official diagnostic category (American Psychiatric Association, 2013). Similarly to BN, BED is characterised by recurrent,

psychologically distressing episodes of objective binge eating that occurs, on average, at least once a week for three months (American Psychiatric Association, 2013). Binge eating episodes involve eating much more rapidly than normal; eating until feeling uncomfortably full; eating large amounts of food in the absence of physical hunger; eating alone or in secret due to feelings of embarrassment and shame; and feeling disgusted, depressed or guilty following the episode. In contrast to BN, BED does not involve inappropriate compensatory behaviours.

1.1.3.2 Comorbidity

Medical complications. Due to the high-caloric foods consumed during binge episodes and the lack of compensatory behaviours, BED often co-occurs with overweight and obesity (Hay, Girosi, & Mond, 2015; Mustelin, Raevuori, Hoek, Kaprio, & Keski-Rahkonen, 2015; Villarejo et al., 2012) and is therefore associated with obesity-related medical complications e.g., type 2 diabetes mellitus, and diseases of the circulatory, respiratory and gastrointestinal systems (Raevuori et al., 2015; Thornton et al., 2017).

Psychiatric comorbidities. BED is associated with significant functional impairment, suicidality, emotional distress and high psychiatric comorbidity, in particular mood and anxiety related disorders (Kessler et al., 2013; Stice et al., 2013; Ulfvebrand et al., 2015).

1.1.3.3 Epidemiology

BED is the most common ED with a lifetime prevalence of 1-4% (Cossrow et al., 2016; Keski-Rahkonen & Mustelin, 2016; Stice et al., 2013). In a community sample of adolescent females, incidence rates of BED were 343 per 100,000 person-years (Stice et al., 2013).

1.1.3.4 Course and outcome

Peak age of onset is late adolescence to early adulthood, with a mean age of onset of approximately 23 to 24 years old, which is slightly later than reported for AN and BN, and a median illness duration of 4.3 years (Kessler et al., 2013). Given the relatively recent inclusion of BED in the DSM-5 (American Psychiatric Association, 2013), few long-term follow-up studies on BED exist; therefore, little is known about the course and outcomes of BED (Smink, van Hoeken, & Hoek, 2013).

1.1.3.5 Treatment

As with BN, psychological therapy is the recommended treatment for adults with BED (National Institute for Health and Care Excellence, 2017) and has been associated with positive treatment outcomes i.e., reduced binge eating frequency and increased binge eating abstinence (Brownley et al., 2016). Lisdexamfetamine has shown some benefit as a pharmacological treatment for BED (Brownley et al., 2016), however, it is only approved in certain countries (Himmerich & Treasure, 2018). Antidepressants have also shown

promise in reducing binge eating (Davis & Attia, 2017). A recent comprehensive meta-analysis of treatments for BED reported that psychotherapy showed large effects on binge eating episodes and abstinence from binge eating, structured self-help treatment followed with medium-to-large effects, and pharmacotherapy had small effects on outcomes, when compared to wait lists or placebo (Hilbert et al., 2019).

1.2 Inflammatory markers: An overview

Cytokines are the category of inflammatory markers that are the main focus of this thesis. However, as part of the immune response, cytokines interact with several other inflammatory molecules. Therefore, I will briefly describe other inflammatory molecule (acute-phase reactants and cellular adhesion molecules) categories, which will be examined in Chapter 3, Chapter 5, Chapter 6, and Chapter 7.

1.2.1 The immune system

The immune system is typically divided into two branches: the innate immune system (non-specific general defence) and the adaptive immune system (specialised acquired defence). These systems work closely together but differ in several features. The innate immune system is thought of as the first line of defence to non-self pathogens/antigens, responding immediately to prevent the circulation of foreign pathogens throughout the body. The innate immune system defences consist of several components, including external barriers like the skin and mucosal membranes and internally through defence cells from the white blood cell group (leukocytes) and various substances in the blood and in body fluids. If the innate immune system is unable to clear the infection, it acts to contain the infection while an adaptive immune response develops.

The adaptive immune system is the second line of defence and responds to specific pathogens after four to seven days. The effect of the adaptive immune response is sustained in the long-term as the system can remember the pathogen/antigen. If the foreign pathogen/antigen is already known to the body, the defence response is quicker. The adaptive immune system makes specific antibodies for pathogens/antigens (humoral immunity) and this leads to a specific cell-mediated immune response (cellular immunity), which includes T lymphocytes, B lymphocytes and cytokines. Autoimmune diseases can develop when the adaptive immune system makes an error and attacks itself rather than the non-self pathogens.

Many cells work together as part of the innate and adaptive immune systems. Indeed, cytokines and other inflammatory markers have functions within both of these systems.

1.2.2 Cytokines

Cytokines play a role as soluble intercellular signalling proteins with particular importance in the immune system. They are crucial in the regulation of physiological responses to foreign pathogens/antigens, infection and injury. Messenger molecules included within the category of cytokines are chemokines, interferons (IFN), interleukins (IL), and tumor necrosis factors (TNF). Cytokines are produced by a range of immune cells within the body, including macrophages, B lymphocytes, T lymphocytes, and mast cells. Cytokines are also produced by other cells, including endothelial cells, fibroblasts, epithelial cells, adipocytes, and stromal cells within the body's periphery, and microglia and astrocytes in the brain. Thus, cytokine production can occur throughout the whole human organism, rather than being restricted to a specific organ.

1.2.2.1 Characteristics of cytokine signalling

Cytokines are key mediators and regulators of the immune response via their cell signalling properties. A given cytokine can be produced and released by multiple cell types. For example, IL-6 is produced by T-cells, macrophages, endothelial cells, skeletal muscle and other cells (Jücker et al., 1991; Pedersen & Febbraio, 2008). Likewise, a single cell type can produce different cytokines. For instance, adipocytes can produce IL-1β, IL-6 and TNF-α (Coppack, 2001).

Most cytokines act on other cells by autocrine or paracrine signalling. Autocrine signalling involves cytokines acting on the cells that secrete them. For example, IL-1 is secreted by macrophages and then binds to IL-1 receptors on macrophages, which leads to further stimulation of these cells (King, 2007). In paracrine signalling, the cytokine is produced by a cell and then acts on another nearby cell. For example, T-helper 2 (Th2) cells secrete IL-4, which then stimulates nearby B lymphocytes (Janeway, Travers, Walport, & Shlomchik, 2005). Cytokines can also act on distant cells, which is known as endocrine signalling. For example, cytokines such as IL-1, IL-6 and TNF-α, are released into the blood and act on distant organs, such as the liver, to initiate the acute-phase response (Moshage, 1997).

Cytokines can interact and have effects on their target cells in multiple ways (Ozaki & Leonard, 2002). Cytokines can act pleiotropically, whereby a cytokine can have different effects on multiple cell types. For example, IL-4 activates B cells but promotes differentiation into Th2 cells (Janeway et al., 2005) and IL-10 can be both immunosuppressive and immunostimulatory. Cytokine pleiotropy can be explained by several mechanisms, including the presence of receptors for a cytokine on multiple cells, the ability of certain cytokines to activate more than one type of receptor, or the ability of cytokines to stimulate multiple signalling pathways that have differential effects on different functions (Ozaki & Leonard, 2002). Cytokines can also be redundant in the sense

that multiple cytokines have the same effect. For instance, IL-4 and IL-6 enhance B cell proliferation (Vazquez, Catalan-Dibene, & Zlotnik, 2015) and both IL-1α and IL-1β activate T cells (Janeway et al., 2005). Cytokine redundancy can in part be explained by multiple cytokines sharing an individual receptor and the sharing of signalling pathways (Ozaki & Leonard, 2002). Another mode of cytokine signalling is synergy, which describes combined effects of cytokines when they act together. For example, IL-21 synergistically acts with IL-15 to promote T cell proliferation (Zeng et al., 2005). Cytokines can also act antagonistically whereby one cytokine inhibits the effect of another e.g., IL-4 effects can be inhibited by IFN-γ (Schmitt et al., 1994). Finally, cytokine cascade induction, in which activation of one cytokine produced by one cell type induces cytokine production by other cell types, is another important mode of cell signalling. For example, IL-4 induces the expression of Th2 cytokines, including IL-3, IL-5 and IL-13 (Lorentz & Bischoff, 2001).

1.2.2.2 Cytokines within the immune system

There is no firm and generally accepted categorisation of cytokines (Cavaillon, 2001). While cytokines can be categorised based on their biological structure, relevant groupings based on their immunological function generally prove more useful in clinical and experimental settings. These functional categories are often referred to in the psychoimmunological literature. As described above, cytokines and their signalling are complex with cytokines having multiple and overlapping functions (Cavaillon, 2001). As such, the four categories described as follows, based on their immunological function, may be overly simplified, for example IFN-γ and IL-6 both have pro- and anti-inflammatory properties, but this will provide some suggestion of how cytokines can be classified. T-helper 1 (Th1) cytokines include IFN-γ, IL-2, IL-12, and TNF-β and are involved in cell cytotoxicity, the induction of cell-mediated immunity, and the activation of macrophages. Th2 cytokines include IL-4, IL-5, IL-6, IL-9, IL-10, and IL-13, and are involved in the induction of antibodies and the stimulation of the Th2 branch of the immune system. Pro-inflammatory cytokines, including IFN-α, IL-1, IL-6, IL-8, IL-17, IL-21, IL-22, and TNF-α, promote inflammation in which immune cells of the innate immune system are recruited to the site of infection and/or tissue injury. Anti-inflammatory cytokines, such as IL-10 and transforming growth factor (TGF)-β, are influenced by regulatory T cells and control the pro-inflammatory cytokine response and reduce inflammation (Opal & DePalo, 2000). The main functions of the cytokines studied in this thesis can be seen in **Table 1.1**.

In addition to the categories of cytokines described above, chemokines are a distinct category of small cytokines. Chemokines are produced by cells with the primary function of recruiting leukocytes to sites of infection or tissue injury (Turner, Nedjai, Hurst, & Pennington, 2014). They also induce chemotaxis (defined as cell movement towards a

source of a chemical gradient) in a variety of cells such as eosinophils, fibroblasts, lymphocytes, monocytes, and neutrophils, and promote wound healing (Borish & Steinke, 2003). Some chemokines are considered to be pro-inflammatory (e.g., interferon gamma-induced protein [IP]-10, monocyte chemoattractant protein [MCP]) and can be induced during an immune response; others are considered homeostatic (e.g., macrophage inflammatory protein [MIP]-3β) and are involved in controlling the migration of cells during normal processes of tissue maintenance or development; and some chemokines are considered to be both inflammatory and homeostatic (e.g., thymus and activation regulated chemokine [TARC]; Borish & Steinke, 2003). The chemokines measured in this thesis are presented in **Table 1.1**.

When the innate or adaptive immune system is activated, predominantly macrophages and dendritic cells, respectively, produce cytokines, such as IL-1β, IL-6, IL-12 and TNF-α, to modulate the immune response and initiate inflammatory processes. These cytokines then induce the acute-phase response in the liver, which involves the release of acute-phase proteins, such as C-reactive protein (CRP) and serum amyloid A (SAA), which respond to inflammation, as described below in Section 1.2.3. Cytokine release in an immune response induces sickness behaviour (e.g., fever, as described in Section 1.3.1.1) to promote recovery. While this is an appropriate immune response, excessive or chronic cytokine production can have negative health consequences. Indeed, this has been associated with a number of disease states, including atherosclerosis (a chronic inflammatory disorder of the arteries) and depression (Dantzer, 2009; Ramji & Davies, 2015).

1.2.3 Acute-phase proteins

Acute-phase reactants are a category of proteins that respond to inflammation in the body (e.g., CRP, SAA), a response known as the acute-phase reaction (see **Table 1.1** for the acute-phase proteins measured in this thesis). For example, increased circulating concentrations of CRP are produced in response to inflammation and injury and decrease rapidly when the condition is resolved (Du Clos, 2000).

CRP is synthesised primarily by liver cells (Pepys & Hirschfield, 2003). CRP is a sensitive systemic marker of inflammation, tissue injury, and infection (Pepys & Baltz, 1983). Following an acute-phase stimulus, concentrations of CRP can increase up to 1,000-fold at sites of infection or inflammation (Sproston & Ashworth, 2018). Furthermore, chronic elevation of CRP at a concentration higher than 3 mg/l has been associated with increased risk of coronary heart disease and CRP concentrations have been associated with prognosis in patients with cardiovascular disease (Osman, L'Allier, Elgharib, & Tardif, 2006). Concentrations of CRP have also been found to be chronically elevated in

inflammatory conditions and infections. Thus, it is an established routine laboratory parameter for assessing inflammation in clinical practice.

There is increasing evidence of a relationship between CRP and several pro-inflammatory cytokines. For example, both IL-6 and TNF-α have been found to induce the production of CRP and conversely, CRP can lead to the production of IL-6 and TNF-α at sites of inflammation (Sproston & Ashworth, 2018).

1.2.4 Cellular adhesion molecules

Cellular adhesion molecules mediate the binding of cells in the immune system and are crucial for maintaining tissue structure and function (Murphy, Travers, & Walport, 2009). For example, they play an essential role in binding circulating leukocytes to the vascular endothelium at sites of inflammation and regulating the migration of leukocytes from blood vessels into adjacent inflamed tissue (Butcher, 1991; Meager, 1999; Springer, 1990). Examples include intercellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1, which mediate the adhesion of lymphocytes, monocytes, eosinophils, and basophils to vascular endothelium. Cellular adhesion molecules are widely distributed in human tissues and organs, including the central nervous system (Sakisaka & Takai, 2005). **Table 1.1** details the cellular adhesion molecules studied in this thesis.

When cellular adhesion molecules are not stimulated, it is less easy for immune cells to bind to endothelial cells. Several pro-inflammatory cytokines, such as IL-1β and TNF-α, increase the expression of cellular adhesion molecules and thus, increase the likelihood of cell adhesion (Meager, 1999; Pober, 1987). Therefore, cytokines are essential in facilitating the role of cellular adhesion molecules in regulating inflammatory processes. Indeed, overactivation of cellular adhesion molecule expression has been associated with several inflammatory conditions, such as those arising from excessive endothelial cell and leukocyte activation (e.g., Entman et al., 1992).

Table 1.1 The main function of the inflammatory markers studied in this thesis. Information adapted from Akdis et al. (2016); Eklund, Niemi, and Kovanen (2012); Griffith, Sokol, and Luster (2014); Murphy, Travers, and Walport (2009); Turner et al. (2014); and Sproston and Ashworth (2018). This table is a summary of the primary sources, target cells and major functions of inflammatory markers; therefore, the information provided is not exhaustive and the inflammatory markers listed are produced by a number of other cells with a variety of other functions

Inflammatory marker name	Abbreviation	Primary sources	Target cells	Major function
Acute-phase proteins				
C-reactive protein	CRP	Hepatocytes Macrophages Endothelial cells Lymphocytes Adipocytes	Damaged cell membranes	Downstream mediator of the acute-phase response Induces synthesis of several cytokines
Serum amyloid A	SAA	Hepatocytes	Neutrophils Mast cells	Induces synthesis of several cytokines Chemoattractant for neutrophils and mast cells
Cellular adhesion molecules				
Intercellular adhesion molecule-1	ICAM-1	Activated endothelial cells Epithelium Smooth muscle cells Activated T and B cells	Vascular endothelium Leukocytes	Mediates the adhesion of leukocytes to the vascular endothelium
Vascular adhesion molecule-1	VCAM-1	Activated endothelial cells Dendritic cells	Vascular endothelium Lymphocytes	Mediates the adhesion of lymphocytes, monocytes, eosinophils, and basophils to the vascular endothelium
Chemokines				
Eotaxin	CCL11	Activated mast cells	Eosinophil Basophil Th2 cells	Promotes eosinophil residence in peripheral tissues and their release from the bone marrow Induces eosinophil and basophil migration
Eotaxin-3	CCL26	Endothelial cells	Eosinophil Basophil	Induces eosinophil and basophil migration

Inflammatory marker name	Abbreviation	Primary sources	Target cells	Major function
Interferon gamma-induced protein-10	IP-10, CXCL10	Activated mast cells Activated neutrophils	Activated T cells Natural killer cells B cells Endothelial cells	Th1 response Trafficking of Th1 and natural killer cells Instigates antigen-independent tethering between T cells and dendritic cells
Monocyte Chemoattractant Protein-1	MCP-1, CCL2	Monocytes Macrophages Dendritic Cells Activated mast cells	T cells Monocytes Basophils Natural killer cells	Migration of inflammatory monocyte migrations Recruitment of monocytes
Monocyte Chemoattractant Protein-4	MCP-4, CCL13	Epithelial cells	T cells Monocytes Eosinophils Basophils	Th2 responses Induces chemotaxis in monocytes, eosinophils, T lymphocytes, and basophils Induces expression of adhesion molecules and the secretion of pro-inflammatory cytokines in epithelial, endothelial and muscular cells
Macrophage-derived chemokine	MDC, CCL22	Dendritic cells Macrophages Activated B lymphocytes	Natural killer cells T cells Endothelial cells Monocytes Regulatory T cell	Th2 response Migration of Th2 cells and regulatory T cells
Macrophage inflammatory protein-1α	MIP-1α, CCL3	Activated neutrophils and mast cells Macrophages Dendritic cells	Monocytes Macrophages T cells Natural killer cells	Migration of macrophage and natural killer cells T cell–dendritic cell interactions
Macrophage inflammatory protein-1β	MIP-1β, CCL4	Activated neutrophils and mast cells Macrophages Dendritic cells	Monocytes Macrophages T cells Natural killer cells	Migration of macrophage and natural killer cells T cell–dendritic cell interactions
Macrophage inflammatory protein-3α	MIP-3α, CCL20	Activated neutrophils and mast cells	T cells Activated B cells Natural killer T cells	Th17 responses Homing of B cells and dendritic cells to gut-associated lymphoid tissue

Inflammatory marker name	Abbreviation	Primary sources	Target cells	Major function
Thymus- and activation-regulated chemokine	TARC, CCL17	Dendritic cells	T cells Regulatory T cells	Th2 responses Induces chemotaxis in T cells
Cytokines				
Basic fibroblast growth factor	bFGF	Damaged cells	Endothelial cells Epithelial cells Fibroblasts	Cell proliferation of preadipocytes, endothelial cells, epithelial cells, fibroblasts, and neural stem cell Cell migration of astrocytes Wound healing
Fms Related Tyrosine Kinase-1	Flt-1	Epithelial cells Stromal cells	Monocytes Macrophages Endothelial cells	Migration of monocytes and macrophages
Granulocyte-macrophage colony-stimulating factor	GM-CSF	Macrophages T cells Fibroblasts	Stem cells	Production of granulocytes, monocytes, and eosinophils Stimulates growth and differentiation of dendritic cells
Interferon-α	IFN-α	All nucleated cells can produce IFN-α in response to viral infection	All cells	Defence against viral infection by orchestrating adaptive immune responses Stimulation of macrophage antibody-dependent cytotoxicity Activation of naive T cells
Interferon-γ	IFN-γ	Th1 cells Natural killer and natural killer T cells B cells	Epithelial cells Macrophages Natural killer cells T cells B cells	Anti-viral properties Increases neutrophil and monocyte function Upregulation of major histocompatibility complex -I and -II expression Promotion of cytotoxic activity and Th1 differentiation Inhibition of cell growth
Interleukin-1α	IL-1α	Macrophages Monocytes Lymphocytes Microglia	T cells Fibroblasts Epithelial and endothelial cells	Induction of pro-inflammatory proteins T-cell activation Macrophage activation Differentiation of Th17 cells
Interleukin-1β	IL-1β	Macrophages Monocytes Lymphocytes Microglia Neutrophils	T cells Fibroblasts Epithelial and endothelial cells	Induction of pro-inflammatory proteins Fever T-cell activation Macrophage activation Differentiation of Th17 cells
Interleukin-2	IL-2	Activated T cells Natural killer cells	Activated T and B cells	Proliferation of effector T and B cells Development of regulatory T cells

Inflammatory marker name	Abbreviation	Primary sources	Target cells	Major function
		Mast cells	Natural killer cells	Differentiation and proliferation of natural killer cells Growth factor for B cells and stimulus for antibody synthesis
Interleukin-4	IL-4	Th2 cells Basophils Eosinophils Mast cells	B cells T cells Macrophages	Proliferation of B and cytotoxic T cells Enhances major histocompatibility complex class II expression Stimulates Immunoglobulin G and Immunoglobulin E production
Interleukin-5	IL-5	Th2 cells Activated eosinophils and mast cells	Eosinophils Basophils Mast cells Regulatory T cells	Proliferation and maturation Stimulates Immunoglobulin A and Immunoglobulin M production Involvement in remodelling and wound healing
Interleukin-6	IL-6	Th cells Macrophages Monocytes Endothelial cells Muscle cells	Hepatocytes Leukocytes T cells B cells	Induction of acute-phase proteins in hepatocytes Leukocyte trafficking and activation T-cell differentiation, activation, and survival B-cell differentiation and production of Immunoglobulin G, M and A
Interleukin-7	IL-7	B cells Epithelial cells Monocytes Macrophages	Mature T cells Natural killer cells	C and T cell growth factor Synthesis induction of inflammatory mediators in monocytes
Interleukin-8	IL-8	Monocytes Macrophages Neutrophils Lymphocytes Endothelial cells Epithelial cells	Neutrophils Natural killer cells Basophils Eosinophils Mast cells Monocytes	Chemoattractant for neutrophils, natural killer cells, T cells, basophils, and eosinophils Mobilisation of hematopoietic stem cells Angiogenesis
Interleukin-10	IL-10	T cells B cells Monocytes Macrophages	T cells B cells Monocytes Macrophages Natural killer cells	Inhibits cytokine production and mononuclear cell function Anti-inflammatory Suppression of Immunoglobulin E and induction of Immunoglobulin G by B cells
Interleukin-12/ Interleukin-23p40	IL-12/IL-23p40	Dendritic cells Macrophages Monocytes	Activated natural killer cells and T cells	Regulate Th1 and Th17 responses Production of IFN-γ

Inflammatory marker name	Abbreviation	Primary sources	Target cells	Major function
		Neutrophils		
Interleukin-12p70	IL-12p70	Dendritic cells Macrophages Monocytes	Activated natural killer cells and T cells	Production of IFN-γ in T cells and natural killer cells
Interleukin-13	IL-13	T cells Natural killer cells Basophils Eosinophils	B cells Mast cells Epithelial cells Macrophages Eosinophils	Activation of eosinophils and mast cells Recruitment and survival of eosinophils Defence against parasite infections
Interleukin-15	IL-15	Monocytes Macrophages Skeletal muscle cells Nerve cells	Natural killer cells Monocytes Macrophages Neutrophils T cells	T-cell activation Proliferation and activation of natural killer cells Enhancement of Th2 differentiation Prevention of neutrophils and eosinophils from apoptosis
Interleukin-16	IL-16	T cells Eosinophils Mast cells Monocytes	T cells Monocytes Macrophages Eosinophils	Modulation of T cell response Chemoattractant for CD4+ T cells, CD8+ T cells, monocytes, mast cells, and eosinophils
Interleukin-17A	IL-17A	Th17 cells Natural killer cells Neutrophils	Epithelial and endothelial cells Monocytes Macrophages B and T lymphocytes	Induction of pro-inflammatory cytokines and chemokines Recruitment and activation of neutrophils
Interleukin-21	IL-21	T cells (predominantly Th17) Natural killer cells T cells	CD4+ T cells CD8+ T cells B cells Macrophages	B cell proliferation, differentiation, and survival T cell growth factor Natural killer T cell proliferation when combined with either IL-2 or IL-15
Interleukin-22	IL-22	Activated T cells (predominantly Th17 and Th22 cells)	Epithelial cells of the kidney, small intestine, liver, colon, lung, and	Pathogen defence Wound healing Tissue reorganisation

Inflammatory marker name	Abbreviation	Primary sources	Target cells	Major function
		Natural killer T cells	particularly pancreas and skin	
Interleukin-23	IL-23	Phagocytic cells Macrophages	T cells Natural killer and natural killer T cells Monocytes Macrophages	Stimulation of production of pro-inflammatory IL-17 Enhancement of T cell proliferation and promotion of memory T cells Activation of natural killer cells Regulation of antibody production
Interleukin-27	IL-27	Macrophages Epithelial cells	T cells Natural killer cells	Promoting Th1 cell differentiation Inhibition of Th17 cells
Interleukin-31	IL-31	Activated CD4+ T cells and CD8+ T cells Monocytes Macrophages	Epithelial cells Eosinophils Mast cells Basophils Monocytes	Induction of IL-6, IL-8, and chemokine production in eosinophils Upregulation of chemokine mRNA expression Induction of growth factor and chemokine expression in epithelial cells Inhibition of proliferation and apoptosis in epithelial cells
Placental growth factor	PlGF	Endothelial cells	Endothelial cells	Enhances survival, growth and migration of endothelial cells
Transforming growth factor-β	TGF-β	T cells B cells Epithelial cells Fibroblasts Macrophages	Activated T and B cells Epithelial and endothelial cells Monocytes Macrophages	Inhibition of T and B cell proliferation Coordination of the proper development of the cardiac system and bone formation Balance of pro-inflammatory and anti-inflammatory effects by decreasing cellular growth of immune cell precursors Regulation of the differentiation of several Th cell subsets and induction of regulatory T cells
Tyrosine-protein kinase-2	Tie-2	Endothelial cells	Endothelial cells	Participates in signal transduction for various cytokine receptors
Tumor necrosis factor-α (cachetin)	TNF-α	Activated macrophages Monocytes T cells B cells Astrocytes	Macrophages Tumour cells	Host defence Pro-inflammatory mediator by initiating a strong inflammatory response Immunosuppressive mediator by limiting the extent and duration of inflammatory processes and by inhibiting the development of autoimmune diseases
Tumor necrosis factor -β (lymphotoxin)	TNF-β	T cells B cells	Phagocytes Tumour cells	Chemotaxis Induces other cytokines

Inflammatory marker name	Abbreviation	Primary sources	Target cells	Major function
Vascular endothelial growth factor	VEGF	Macrophages Stromal cells	Endothelial cells Stem cells	Regulates angiogenesis by inducing proliferation, migration and permeability of endothelial cells Promotes wound healing

Abbreviations: Th = T-helper; mRNA = messenger ribonucleic acid.

1.2.5 Quantifying concentrations of inflammatory markers

Circulating concentrations of inflammatory markers can be quantified using a range of assays that allow for highly sensitive measurement. Investigators initially started to measure inflammatory markers, such as cytokines, using radioimmunoassays (RIAs; e.g., Wilson et al., 1986). Later, enzyme-linked immunosorbent assays (ELISA) were developed because of the radioactive emissions of the RIAs (for more information on the history of immunological concepts in psychiatry, see Steinberg, Kirkby, & Himmerich, 2015). ELISAs were one of the most extensively used methods for detecting inflammatory markers in biological fluids (e.g., serum, plasma). However, recently, advancements in laboratory technologies have led to the development of assays for the simultaneous quantification of multiple cytokines and other inflammatory markers, making it possible to measure a comprehensive panel of markers in serum or plasma samples in a short period of time (Aziz, 2015). Examples of such assays are the fluorescent bead-based immunoassay Luminex® (Bio-Rad) and the electro-chemiluminescence immunoassay V-plex® (Meso Scale Discovery), which is used in the investigations in this thesis and for which the methodological principles will be described in Section 3.3.1.5. In this thesis, concentrations of inflammatory markers were measured in serum and plasma only. Other studies have determined inflammatory marker expression and production *in vitro*. However, this is beyond the scope of this thesis.

A number of bio-behavioural factors can affect the measurement of inflammatory marker concentrations and production (Dugué, Leppanen, & Grasbeck, 1996; O'Connor et al., 2009; ter Horst et al., 2016). **Box 1.1** lists some of the key factors that empirical research has shown to influence the measurement of circulating inflammatory markers (O'Connor et al., 2009). As such, in this rapidly growing field of research, careful consideration needs to be given to these variables, deciding *a priori* as to how they will be managed in study design and statistical analyses. Indeed, O'Connor et al. (2009) has provided a framework and empirically-based recommendations regarding the key factors that should be assessed and described, controlled for, and/or included as exclusion criteria in research protocols investigating circulating markers of inflammation. Aside from the variables listed in **Box 1.1**, there are many factors that influence cytokine concentrations. These include the presence of physical and mental health conditions, psychological risk factors (e.g., stress), and genetics, as well as environmental factors like seasonality (Himmerich, Patsalos, Lichtblau, Ibrahim, & Dalton, 2019d; ter Horst et al., 2016). However, in psychoimmunology research, some of these variables are often a key component of the research question. Therefore, *a priori* decisions based on the research question need to be made, as to the additional factors that are assessed, controlled for and/or excluded (e.g., the presence of infections, infectious diseases and autoimmune conditions).

Box 1.1 Bio-behavioural factors that can affect the measurement of circulating markers of inflammation (Dugué et al., 1996; O'Connor et al., 2009; ter Horst et al., 2016)

Age
Sex
Hormonal status, including phase of menstrual cycle, use of oral contraceptives, pregnancy, menopausal status, and use of hormone replacement therapy
Socioeconomic status
Ethnicity
Body mass index (BMI)
Smoking
Physical activity and fitness levels
Diet and nutrition, including caffeine and alcohol consumption
Sleep quality, including disturbed sleep
Medication, including selective serotonin uptake inhibitors (SSRIs), aspirin and statins

1.2.6 How cytokines and cytokine-mediated signals access the brain

Cytokines do not readily pass through the blood brain barrier as they are relatively large proteins (≈5–20 kilodalton). However, cytokines and cytokine-mediated signalling have been shown to enter the brain and subsequently influence several biological systems, as will be described in Section 1.2.7. As such, three main pathways via which cytokines and cytokine-mediated signals from the periphery enter the brain have been described: the humoral, neural, and cellular pathways (Capuron & Miller, 2011; Dantzer, 2009; Miller, Haroon, Raison, & Felger, 2013; Miller & Raison, 2016; Quan & Banks, 2007). The humoral pathway describes how circulating pro-inflammatory cytokines pass through leaky regions of the blood brain barrier (i.e., they lack tight gap junctions), such as the choroid plexus and circumventricular organs. Cytokines then bind to saturable transport molecules on endothelial cells and are actively transported into brain parenchyma. The neural route involves activation of peripheral afferent nerve fibres, such as the vagus nerve, by peripheral cytokines, that in turn allow the effects of cytokine signals to directly affect brain. In the cellular pathway, peripheral pro-inflammatory cytokines stimulate microglia, the primary inflammatory cell type in the brain, which produce chemokines that in turn recruit activated immune cells, such as monocytes, to the brain parenchyma and vasculature (D'Mello, Le, & Swain, 2009). Given the research describing these pathways is

mainly experimental and uses animals, it is unclear which pathway is most relevant for cytokine signal transmission in humans (Haroon, Raison, & Miller, 2012).

1.2.7 Pathological mechanisms by which cytokines affect the brain

Once the signals from inflammatory markers have accessed the brain, they influence several biological processes that play a fundamental role in mental state and behaviour. These include effects on neurotransmitter metabolism, neuroendocrine function, synaptic plasticity, and neurocircuits that regulate mood, motor activity, motivation, anxiety and alarm (Capuron & Miller, 2011; Miller et al., 2013). It is accepted that the effect of cytokines on the brain is part of normal functioning and development; however, pathological consequences occur when there is excessive or chronic cytokine production and signalling.

1.2.7.1 Effects on neurotransmitters

Inflammation and pro-inflammatory cytokines have been shown to have an impact on the function of the monoamines dopamine, noradrenaline and serotonin, including the synthesis, reuptake and release of these neurotransmitters (Dunn, Wang, & Ando, 1999; Hayley, Merali, & Anisman, 2003; Miller et al., 2013; Zalcman et al., 1994). They have also been shown to influence glutamate function, an excitatory amino acid essential for neuroplasticity (Miller et al., 2013). These are relevant neurotransmitters to many psychiatric disorders as they are involved in mental states and behaviour, including the regulation of mood, sleep and appetite (serotonin); motivation (dopamine); and arousal, memory and attention processes, and anxiety (noradrenaline). Indeed, dysregulation of these monoamines and also glutamate have been implicated in EDs (Broft, Berner, Martinez, & Walsh, 2011; Gauthier et al., 2014; Godlewska et al., 2017; Kaye, 2008; Kaye et al., 2005; O'Hara, Campbell, & Schmidt, 2015).

Cytokines can influence the synthesis of neurotransmitters. For example, inflammatory cytokines, such as IFN-γ and TNF-α, have been shown to stimulate the production of indoleamine 2,3 dioxygenase (IDO; Fujigaki et al., 2006; Kim, Miller, Stefanek, & Miller, 2015). When activated, IDO breaks down tryptophan, an amino acid that is the primary precursor of serotonin, into kynurenine. It has been proposed that the breakdown of tryptophan contributes to reduced serotonin availability in depressed patients (Dantzer, O'Connor, Freund, Johnson, & Kelley, 2008). In patients treated with IFN-α (e.g., for the treatment of cancer), increases in kynurenine and decreases in tryptophan have been associated with the development of depressive symptoms (Capuron et al., 2003a; Capuron et al., 2002). Furthermore, administration of IFN-α has been shown to decrease concentrations of tetrahydrobiopterin (BH_4), which is essential for the conversion of

tyrosine to l-3,4-dihydroxyphenylalanine (L-DOPA), the precursor of dopamine, and reduce dopamine concentrations in rodents (Kitagami et al., 2003).

Through the activation of signalling pathways, such as p38 mitogen-activated protein kinase, pro-inflammatory cytokines have also been reported to increase the expression and function of serotonin, noradrenaline and dopamine transporters (i.e., reuptake pumps; Miller et al., 2013; Zhu, Blakely, & Hewlett, 2006). For example, *in vitro* research showed that IL-1β and TNF-α can increase the activity of the serotonin transporter in a dose- and time-dependent manner (Zhu et al., 2006). Additionally, increases in the activity of the serotonin transporter, leading to decreased synaptic availability of serotonin, has also been reported in mice administered the cytokine-inducer lipopolysaccharide and this co-occurred with depressive-like behaviours in the tail suspension test and the forced swim test (Zhu et al., 2010). Interestingly, SSRIs, which increase synaptic serotonin, have been shown to lead to decreases in inflammatory markers such as IL-1β, IL-4, IL-6, and IL-10 in depressed patients, suggesting a bidirectional relationship (Wiedlocha et al., 2018). Taken together, it is clear that cytokines can disrupt normal functioning of monoamines, and subsequent mental state and behaviour.

1.2.7.2 Effects on neuroendocrine function

Cytokines can profoundly impact the functioning of the neuroendocrine system, in particular the hypothalamic-pituitary-adrenal (HPA) axis (Capuron & Miller, 2011; Dunn, 2000). Acute administration of pro-inflammatory cytokines or cytokine inducers have been shown to activate the HPA axis; for example, stimulating the expression and release of neuroendocrine hormones such as corticotropin-releasing hormone (CRH), adrenocorticotropic hormone (ACTH), and cortisol (Besedovsky & del Rey, 1996). In humans, treatment with IFN-α injections was shown to stimulate the release of ACTH and cortisol (Capuron et al., 2003b; Raison et al., 2010) and those who went on to develop depression exhibited higher neuroendocrine responses than patients who did not become depressed (Capuron et al., 2003b). However, chronic administration of cytokines or cytokine inducers in both humans and laboratory animals is not associated with continued activation of the HPA axis. For example, chronic IFN-α treatment did not elicit a significant ACTH or cortisol response after 8-weeks of treatment (Capuron et al., 2003b). Rather, chronic cytokine exposure has been associated with flattening of the diurnal cortisol slope and increased evening cortisol concentrations, which was in turn related to increased depression and fatigue (Raison et al., 2010). Cytokines may influence the HPA axis by inhibiting the cortisol receptors and decreasing responsiveness to glucocorticoids (i.e., glucocorticoid resistance; Pace & Miller, 2009; Pariante et al., 1999). As glucocorticoids inhibit the inflammatory response, as part of a negative feedback loop, cytokine-induced

alterations in the function of the HPA axis and reduced glucocorticoid receptors may further exacerbate inflammatory responses (Capuron & Miller, 2011).

1.2.7.3 Effects on neural plasticity and neurogenesis

Neural plasticity refers to the brain's ability to continually respond and adapt to the environment, which may include neuronal remodelling, formation of novel synapses and the production of new neurons. Neurogenesis refers to the production of new neurons. While cytokines are important molecules in supporting neural development (Deverman & Patterson, 2009), excessive cytokine production, such as in chronic stress, has been shown to disrupt the production of neurotrophic factors (molecules that support neurogenesis e.g., brain-derived neurotrophic factor [BDNF]) and inhibit neurogenesis. In laboratory animals, this has been associated with depressive-like behaviours and impaired memory (Calabrese et al., 2014; Duman & Monteggia, 2006). Indeed, reduced neurogenesis is characteristic of laboratory animals exposed to chronic stress (Duman & Monteggia, 2006). For example, administration of IL-1β or acute stress suppressed neurogenesis and expression of neurotrophic factors. In contrast, blocking IL-1β signalling (administering an IL-1β inhibitor or in knockout mice) prevented the decrease in neurogenesis and downregulation of neurotrophic factors observed after acute stress (Barrientos et al., 2003; Ben Menachem-Zidon et al., 2008; Koo & Duman, 2008). IL-6 and TNF-α have also been shown to decrease neurogenesis (Monje, Toda, & Palmer, 2003). Thus, activation of inflammatory pathways in response to stress leads to the release of cytokines, which in turn disrupts fundamental aspects of neural plasticity (e.g., neurogenesis, neurotrophic factors) and subsequently mental state and behaviour.

1.2.7.4 Effects on neural circuits

Given the impact of cytokines on neurotransmitter systems, it is not surprising that neuroimaging studies have reported cytokine-induced alterations in brain circuits implicated in the regulation of human behaviour. These include the basal ganglia, dorsal anterior cingulate cortex, amygdala, dorsolateral prefrontal cortex, and hippocampus (Miller et al., 2013). One of the most reliably identified brain circuits shown to be influenced by cytokines is the basal ganglia and as such, will be discussed in more detail.

Research involving patients treated with IFN-α has reported increased glucose metabolism in the basal ganglia, which was associated with self-reported fatigue (Capuron et al., 2007; Juengling et al., 2000). Functional magnetic resonance imaging (fMRI) studies in this patient population have also shown decreased activation of the ventral striatal regions of the basal ganglia during a reward task and this decreased activation was associated with increased fatigue and anhedonia (Capuron et al., 2012). When healthy individuals are given inducers of the inflammatory response (e.g., endotoxin), changes in

activation of the basal ganglia have also been observed. For example, significant reductions in activity in the ventral striatum to reward tasks have been shown (Eisenberger et al., 2010). This finding has been reported to be sex-specific, with only females showing a change in basal ganglia activity and associated increases in inflammatory markers (Moieni et al., 2019). In a further study, in which participants were administered the typhoid vaccine, increased concentrations of IL-6 and prolonged reaction times on a cognitive task were seen, and these were associated with neural activity in the substantia nigra, a structure within the basal ganglia (Brydon, Harrison, Walker, Steptoe, & Critchley, 2008). Finally, in depressed patients, increased CRP concentrations have been associated with decreased functional connectivity between the ventral striatum and the ventromedial prefrontal cortex and between the dorsal striatal and ventromedial prefrontal cortex and the presupplementary motor area (Felger et al., 2016). These functional connectivity patterns were then associated with increased anhedonia, decreased motor speed and increased psychomotor slowing.

Taken together, the basal ganglia are influenced by cytokine signals which lead to changes in motivation and motor activity. From an evolutionary perspective, these reductions in motivation and motor activity would be beneficial, promoting social withdrawal and energy conservation when a person is sick or injured (Miller & Raison, 2016). However, while these behaviours are essential in an appropriate immunological response to infection or tissue injury, in the context of chronic inflammation, this response can become maladaptive, leading to chronic withdrawal (Miller, 2009), a symptom of psychiatric disorders like depression and EDs. Indeed, social withdrawal and isolation is often reported across all EDs (Arkell & Robinson, 2008; Levine, 2012; Ranzenhofer et al., 2012; Striegel-Moore, Silberstein, & Rodin, 1993; Swanson, Crow, Le Grange, Swendsen, & Merikangas, 2011). However, in contrast to reduced motor activity associated with cytokine-induced sickness behaviour, many patients with AN report increased physical activity (Keyes et al., 2015), although measurements of objective physical activity do not always support this (Gümmer et al., 2015; Keyes et al., 2015).

1.3 Inflammatory markers and eating disorders

Chapter 2 will summarise the research that has been conducted on cytokines in EDs. Here, I will discuss the rationale for the work in this thesis and possible mechanisms of effect. This is summarised in **Figure 1.1**. I will also briefly consider some implications of altered inflammatory markers in EDs.

1.3.1 Rationale for investigating inflammatory markers in eating disorders

The rationale for examining inflammatory markers in EDs consists of multiple interrelated factors. In addition to the alterations in neurotransmitter and neuroendocrine functioning

discussed in Section 1.2.7, which are characteristic of EDs (Kaye, 2008; Misra & Klibanski, 2014; Milano & Capasso, 2018), factors such as sickness behaviour; the role of cytokines in weight, body fat, feeding and appetite regulation; evidence for altered cytokine concentrations in comorbid psychiatric disorders; and associations between EDs and autoimmune diseases and infections, further justify the investigation of a potential pathophysiological role of inflammatory markers in EDs. There is also evidence from genetics studies implicating cytokines in AN; this is discussed in Section 1.3.2.3 and Chapter 7.

1.3.1.1 Sickness behaviour

Sickness behaviour refers to a highly organised adaptive strategy to fight injury and infection within an organism (Dantzer, 2009; Dantzer et al., 2008). Symptoms of sickness behaviour include reduction of food intake and anorexia, withdrawal from the physical and social environment, fever, activation of the neuroendocrine system, fatigue and disturbed sleep, low mood and irritability, and mild cognitive impairments, such as compromised attention, concentration and short-term memory (Dantzer et al., 2008). In response to infection, specific pathogen-associated molecular patterns (molecules involved in identifying foreign pathogens) activate cells of the innate immune system which produce pro-inflammatory cytokines, mainly IL-1, IL-6 and TNF-α. The pro-inflammatory cytokines then act on the brain by several pathways described in Section 1.2.6 and 1.2.7, triggering sickness behaviour (Capuron & Miller, 2011; Konsman, Parnet, & Dantzer, 2002; Wichers & Maes, 2002).

In animals, peripheral administration of cytokines (e.g., IL-1β, TNF-α) or a cytokine inducer (e.g., lipopolysaccharide) has been shown to result in non-specific symptoms of sickness and administration of cytokine antagonists reverses the effects of the cytokine inducer (for a review, see Aubert, 1999). Through the administration of cytokine inducers (e.g., endotoxin), experimentally-induced inflammation has also been investigated in humans. This has consistently demonstrated changes in neuropsychological functioning and characteristics of sickness behaviour e.g., depressive symptoms, fatigue, and a reduction in energy intake and willingness for action (Schedlowski, Engler, & Grigoleit, 2014). In severe and chronic illness (e.g., cancer, chronic kidney disease), there is prolonged and chronic exposure to elevated cytokines concentrations and production. The cytokines interact with hormones and neuropeptides in the body to induce cachexia, a wasting condition that results in extreme weight loss and is characterised by decreases in appetite and increases in fat and lean body mass metabolism (Grossberg, Scarlett, & Marks, 2010; Matthys & Billiau, 1997). From an adaptive evolutionary perspective, cachexia and anorexia may help conserve energy by impairing locomotion and reducing

heat loss, and the increased metabolism, with the associated tissue atrophy, may be providing energy for the immune system (Grossberg et al., 2010). Furthermore, administration of cytokines (e.g., IFN-α, IL-2) used as treatment for various medical conditions, such as cancer and hepatitis C, is also associated with side effects such as depression, increased fatigue and irritability, and loss of appetite (Wichers & Maes, 2002). Thus, the inflammatory response and associated sickness behaviour has been proposed to be a central motivational state that reorganises behaviour to promote recovery (Aubert, 1999; Dantzer, 2009; Kelley et al., 2003; Lasselin et al., 2017).

The sickness behaviour symptoms can mimic the behavioural phenotype observed in depression (Dantzer, 2009; Dantzer et al., 2008) and similar symptoms can also be observed in ED patients (e.g., Allison, Spaeth, & Hopkins, 2016; Krug et al., 2013; Ulfvebrand et al., 2015). Furthermore, it has been posited that persistent activation of the peripheral immune system, such as during cancer, autoimmune disorders, or systemic chronic infections, and the subsequent cytokine signalling to the brain can lead to an exacerbation of cytokine-induced sickness behaviour and the development of depression in vulnerable individuals (the model of cytokine-induced depression; Dantzer, 2009; Dantzer et al., 2008). Given the apparent overlap in sickness behaviour and ED-related symptoms and behaviours, it would be of value to investigate the role of cytokines in EDs. Furthermore, as described below (Section 1.3.1.5), an association between autoimmune disorders and the development of EDs has been observed (Hedman et al., 2019), suggesting that for some vulnerable individuals, cytokines may be involved in the development of EDs.

1.3.1.2 Regulation of appetite and feeding behaviour

It has been well documented that cytokines are involved in the regulation of appetite and feeding. Multiple cytokines have been shown to be anorexigenic and to have inhibitory effects on food intake (Buchanan & Johnson, 2007; Gautron & Layé, 2010; Langhans & Hrupka, 1999; Plata-Salaman, 2001; Wong & Pinkney, 2004). For example, in animals, reductions in food intake have been observed following peripheral and/or central administration of IL-1β (Langhans, Savoldelli, & Weingarten, 1993; Van der Meer, Sweep, Pesman, Borm, & Hermus, 1995), IL-6 (Wallenius, Wallenius, Sunter, Dickson, & Jansson, 2002a), and TNF-α (Bodnar et al., 1989). More specifically, the administration of IL-1β reduces meal frequency, size and duration and increases inter-meal intervals (Langhans et al., 1993; Plata-Salaman, 1994). Moreover, anorexigenic effects are attenuated in cytokine and/or cytokine receptor gene knockout mice (Chida, Osaka, Hashimoto, & Iwakura, 2006; Netea et al., 2006; Zorrilla & Conti, 2014) or following administration of cytokine antagonists (Laye et al., 2000; Plata-Salaman, 1994; Uehara et al., 1989). However,

research in animals has suggested that the anorexic effects of cytokines are typically short-term. For example, within a few days, a tolerance to chronic cytokine administration has been found to develop and food intake has recovered (Mrosovsky, Molony, Conn, & Kluger, 1989; Porter, Arnold, & Langhans, 1998).

With respect to appetite, administration of IL-1β and IL-6 has been reported to reduce food seeking behaviour and inhibit food motivated behaviour (Kent, Bret-Dibat, Kelley, & Dantzer, 1996; Nunes et al., 2014; Plata-Salaman, 2001; Yohn et al., 2016). In humans, a natural elevation in IL-6 concentration following exercise (IL-6 is released by skeletal muscle; Nielsen & Pedersen, 2007) has been associated with reduced appetite and energy intake (Almada et al., 2013; Hunschede, Kubant, Akilen, Thomas, & Anderson, 2017; Islam et al., 2017).

The impact on appetite and feeding regulation is due to both direct and indirect effects of cytokines (Fonseka, Muller, & Kennedy, 2016), a few mechanisms of which I will now briefly describe. Pro-inflammatory cytokines promote gastric stasis (i.e., delayed gastric emptying), which may contribute to these disruptions in typical food behaviours and intake patterns (Lang Lehrskov et al., 2018; McCarthy, 2000; Suto, Kiraly, & Tache, 1994). Cytokines also interact with orexigenic and anorexigenic hormones (e.g., leptin), neuropeptides (e.g., pro-opiomelanocortin [POMC], ghrelin) and neurotransmitters (e.g., dopamine, serotonin, noradrenaline), which are involved in regulating food intake and appetite (Amaral et al., 2006; Meguid et al., 2000; MohanKumar, MohanKumar, & Quadri, 1998; Romanatto et al., 2007; Wang et al., 2006; Zalcman et al., 1994). For example, IL-1, IL-6 and TNF-α have been shown to stimulate the secretion and expression of POMC (Katahira, Iwasaki, Aoki, Oiso, & Saito, 1998; Senaris et al., 2011) and leptin (Berkowitz et al., 1998; Finck, Kelley, Dantzer, & Johnson, 1998; Grunfeld et al., 1996; Kirchgessner, Uysal, Wiesbrock, Marino, & Hotamisligil, 1997; Sarraf et al., 1997), both of which suppress appetite (Sohn, 2015). Furthermore, IL-1β, and to a lesser extent TNF-α, have been reported to significantly suppress plasma concentrations and messenger ribonucleic acid (mRNA) expression of ghrelin (Iwakura, Bando, Ueda, & Akamizu, 2017; Wang et al., 2006), an appetite-stimulating neuropeptide (Sohn, 2015).

Cytokines also exert their effects directly on the central nervous system and on neurons and cytokine receptors in the hypothalamus, the 'feeding centre' of the brain (Amaral et al., 2006; Holden & Pakula, 1996; Langhans & Hrupka, 1999; Plata-Salaman, Sonti, Borkoski, Wilson, & French-Mullen, 1996; Yabuuchi, Minami, Katsumata, & Satoh, 1994). Circulating cytokines have been found to act on the luminal surface of the blood brain barrier to induce brain endothelial cells to release anorexigenic substances into the brain (Banks, 2001). Furthermore, direct administration of IL-1β into the ventromedial nucleus

of the hypothalamus has been shown to lead to a reduction in food and water consumption (Kent, Rodriguez, Kelley, & Dantzer, 1994) and injection of cytokine antagonists into this brain area has been found to reverse the anorexigenic effects of IL-1β and increase food intake (Varma et al., 2000). Additionally, IL-18 has been shown to act on neurons on the bed nucleus of the stria terminalis, a component of extended amygdala recently shown to influence feeding via its projections to the lateral hypothalamus (Francesconi et al., 2016).

Most previous research has focussed on how cytokines play a role in restrictive food intake. However, a recent study has suggested cytokines may also be associated with other eating behaviours and patterns, such as binge eating. Using a validated animal model of binge-like eating behaviour, in which cycles of restriction are combined with frustration stress, down-regulation of the anorexigenic IL-18 system in the hypothalamus was observed in the animals that developed binge eating (Alboni et al., 2017). It is important to acknowledge that this research does not highlight a causal role for cytokines in this eating behaviour and is currently limited to animal models.

Overall, cytokines have extensive effects on the regulation of appetite and feeding behaviours. At present, it is unclear whether cytokines impact these processes in EDs. Alterations in the biological processes underlying food intake and appetite are evident in EDs. For example, in AN, serum concentrations of leptin are suppressed and concentrations of ghrelin are elevated (Cuesto et al., 2017; Schorr & Miller, 2017), suggesting an orexigenic profile. These alterations appear to be opposed to the anorexigenic cytokine-induced patterns described above. However, appetitive sensations are not reliable markers of food intake and appetite can be difficult to quantify (Mattes, 2015). As such, questions remain as to how people with EDs experience appetite (e.g., feelings of hunger, fullness, satiety, desire to eat).

1.3.1.3 Weight and body fat regulation

Cytokines have also been linked with the regulation of body weight and fat (Fonseka et al., 2016). As described above, pro-inflammatory cytokine and/or cytokine receptor gene knockout mice (e.g., deficient in IL-1, IL-6, IL-18, etc.) have increased food intake; this has been associated with the development of obesity (increased body weight and excess fat mass; Chida et al., 2006; García et al., 2006; Netea et al., 2006; Wallenius et al., 2002b). However, research has shown that even on low-fat diets, these knockout mice continue to be overweight and gain excess weight (McGillicuddy et al., 2013; Zorrilla & Conti, 2014). Furthermore, knockout mice with excess IL-1 signalling have a lean phenotype through impaired body fat accumulation and were resistant to obesity when fed a high-fat diet (Matsuki, Horai, Sudo, & Iwakura, 2003; Somm et al., 2005), despite feeding behaviour remaining normal (Matsuki et al., 2003). These animals had smaller adipocytes and a

reduced expression of genes involved in adipogenesis (Somm et al., 2005). However, the research on whether alterations in energy expenditure occurs in these mice is contradictory (Matsuki et al., 2003; Somm et al., 2005). Furthermore, administration of cytokines, such as IL-6, has been associated with loss of body weight and fat mass in mice (Wallenius et al., 2002a). Taken together, this research suggests that cytokines have a role in weight and fat regulation, independent of food intake and the environment.

In humans, during normal fat accumulation, adipose tissue becomes a site of active inflammation, one aspect of which includes the secretion of pro-inflammatory cytokines (Fontana, 2009; Smitka & Marešová, 2015). These cytokines subsequently supress food intake (as described in Section 1.3.1.2) and induce energy expenditure (e.g., by increasing body temperature) via a feedback loop to prevent excessive fat accumulation and maintain homeostatic balance (Weingarten, 1996; Ye & Keller, 2010). Caloric restriction is accompanied by an anti-inflammatory effect, part of which involves the reduced production of pro-inflammatory cytokines (Fontana, 2009). Thus, obesity induces chronic local inflammation in adipose tissue, in a large part due to macrophages, which infiltrate the adipose tissue and are responsible for producing pro-inflammatory cytokines during fat accumulation, leading to chronic systemic inflammation (Kintscher et al., 2008; Surmi & Hasty, 2008; Wellen & Hotamisligil, 2003). However, this low-grade inflammation is not accompanied by weight loss. This may be explained by a resistance to or deficit in inflammatory signalling (Fonseka et al., 2016; Monteiro & Azevedo, 2010). Indeed, obesity is associated with a chronic state of low-grade inflammation: overweight/obese individuals have significantly higher circulating concentrations of CRP and several cytokines, including IL-6, IL-8, IL-18, and TNF-α, compared to lean individuals (Fontana, 2009). In line with this, a positive correlation between BMI and/or body weight and pro-inflammatory cytokines has also been reported (e.g., Ambeba et al., 2013; Himmerich et al., 2006b; Schmidt et al., 2015). As expected therefore, measures of adiposity have also been shown to be positively associated with a number of inflammatory markers, including CRP (independent of BMI; Wedell-Neergaard et al., 2018a) and IL-6 (Matia-García et al., 2016). Consistent with the role of cytokines in normal fat accumulation, caloric restriction and subsequent weight and fat loss in obese individuals has been associated with a reduction in IL-6 (Ambeba et al., 2013; Bastard et al., 2000). Furthermore, subsequent weight regain was associated with an increase in IL-6 (Ambeba et al., 2013). Although this finding has not been consistently reported (e.g., Lien et al., 2009).

A number of genetic variants of cytokine genes have also been associated with differences in both BMI and body fat mass (Andersson et al., 2010; Andersson et al., 2009; Lee et al., 2008; Manica-Cattani et al., 2010; Qi, Zhang, van Dam, & Hu, 2007; Strandberg et al., 2006;

Strandberg et al., 2008; Um, Rim, Kim, Kim, & Hong, 2011; Wolford, Colligan, Gruber, & Bogardus, 2003). For example, the IL-6 −174C variant, which produces less IL-6, has been associated with higher BMI (Berthier et al., 2003; Stephens et al., 2004), while the −174G variant, which is related to greater IL-6 production, has been associated with a lean phenotype and low waist circumference (Goyenechea, Parra, & Martinez, 2007).

Naturally elevated cytokine concentrations due to chronic illness or administration of cytokine blockers as medical treatment have also highlighted the role of cytokines in weight and fat regulation. As previously mentioned, prolonged production of cytokines associated with cancer and chronic infections has been implicated in the development of cachexia (Matthys & Billiau, 1997). In contrast, treatment with cytokine blockers (e.g., anti-TNF-α) has been reported to lead to increases in body weight and fat mass (Peluso & Palmery, 2016). Taking this further, a recent experimental study has shown that administering an IL-6 receptor blocker (which leads to elevated circulating concentrations of IL-6) to abdominally obese adults prevented the exercise-related reductions in visceral fat mass that were observed in obese adults who had been administered a placebo (Wedell-Neergaard et al., 2018b). This research highlights a role for cytokine signalling in the regulation of body weight and fat.

In EDs, patients often exhibit changes in body weight and fat mass, particularly in AN and BED. Indeed, a feature of AN is reduced body weight and depleted fat mass (Tannir et al., 2019). For example, adolescents with AN tend to lose more central body fat, while adults with AN lose more peripheral fat (El Ghoch, Calugi, Lamburghini, & Dalle Grave, 2014a). Weight restoration treatment (e.g., especially in inpatient settings where there are high calorie diets and activity restriction) in AN then often promotes a disproportionate increase in central adiposity with weight gain (El Ghoch et al., 2014b; Mayer et al., 2009; Mayer et al., 2005), although this abnormal fat distribution does normalise with time if weight recovery is maintained (El Ghoch et al., 2014a). In contrast, BED is highly associated with obesity with a prevalence of lifetime obesity in BED of approximately 87% (Villarejo et al., 2012). Thus, given the alterations in weight and fat mass in EDs, the reports of inflammatory marker involvement in these processes provides support for further investigation.

1.3.1.4 Altered inflammatory markers in psychiatric disorders associated with eating disorders

EDs are both clinically- and genetically-related to a number of other psychiatric disorders. As previously mentioned, EDs are highly comorbid with depression: approximately one third of ED patients have comorbid MDD (Ulfvebrand et al., 2015). Post-traumatic stress disorder (PTSD) is also comorbid with EDs, with comorbidity estimated to range between

4% and 62% (Brewerton, 2007; Swinbourne et al., 2012; Swinbourne & Touyz, 2007; Tagay, Schlottbohm, Reyes-Rodriguez, Repic, & Senf, 2014). In addition, a genome-wide association study (GWAS) identified a positive genetic correlation between AN and schizophrenia, which is suggestive of shared genetic variants influencing both phenotypes (Bulik-Sullivan et al., 2015), and psychotic experiences have been associated with greater odds of reporting disordered eating behaviours in late adolescence (Solmi, Melamed, Lewis, & Kirkbride, 2018). Cytokines have been extensively investigated in depression, PTSD and schizophrenia/psychosis: research has reported alterations in peripheral inflammatory marker concentrations and cytokine-related gene expression and genotypes in these disorders.

Depression. A recent meta-analysis of 82 studies found that peripheral concentrations of IL-6, IL-10, IL-12, IL-13, IL-18, MCP-1 and TNF-α were elevated and IFN-γ concentrations were reduced in patients with MDD compared to healthy individuals (Köhler et al., 2017). Concentrations of IL-1β, IL-2, IL-4, IL-5, IL-8, IL-17 and MIP-1α were not significantly different in individuals with MDD and healthy controls (HCs; Köhler et al., 2017). Cytokine concentrations have also been associated with depressive symptoms including social withdrawal, suicidality, reduced appetite, cognitive deficits, and sleepiness (Lichtblau, Schmidt, Schumann, Kirkby, & Himmerich, 2013). Antidepressant treatment has been reported to significantly decrease peripheral concentrations of IL-6, IL-10, MCP-1 and TNF-α in MDD patients (Köhler et al., 2018). There is also preliminary evidence that symptom improvements following psychotherapy is associated with a reduction in IFN-γ, IL-5, IL-6, IL-8, IL-10, and TNF-α in unmedicated MDD patients, which is suggestive that certain cytokines may be state markers of the disorder (Dahl et al., 2016; Del Grande da Silva et al., 2016). Increased peripheral concentrations of cytokines, such as TNF-α, has been associated with a poorer response to antidepressants (e.g., Eller, Vasar, Shlik, & Maron, 2008). In fact, IL-1β mRNA molecules have been identified as a biomarker reported to accurately and reliably predict antidepressant treatment response (Cattaneo et al., 2016). Research reporting that anti-cytokine treatment, compared to placebo, has been shown to have an antidepressant effect (Kappelmann, Lewis, Dantzer, Jones, & Khandaker, 2018) further highlights a potentially causal role for cytokines in depression.

Post-traumatic stress disorder. A meta-analysis of 20 studies reported that concentrations of IFN-γ, IL-1β and IL- 6 were higher in patients with PTSD than in healthy individuals and this pattern remained when participants with comorbid depression were excluded from analyses (Passos et al., 2015). The following inflammatory markers were found not to significantly differ between PTSD and HC participants: CRP, IL-2, IL-4, IL-8, IL-10, and TNF-α (Passos et al., 2015). Longitudinal cytokine research in PTSD is in its early stages

and findings are mixed, with some studies finding increases in cytokine concentrations with treatment and others reporting reductions (Hussein, Dalton, Willmund, Ibrahim, & Himmerich, 2017; Waheed, Dalton, Wesemann, Ibrahim, & Himmerich, 2018). Epigenetic research has reported differential methylation between patients with PTSD or those who go on to develop PTSD and HCs on several genes associated with inflammation and the regulation of inflammatory markers (Bam et al., 2016; Rusiecki et al., 2013; Smith et al., 2011), suggesting that the altered expression of pro-inflammatory immune markers in PTSD patients may be regulated by epigenetic mechanisms.

Schizophrenia and psychosis. A meta-analysis found that IL-1β, IL-6 and TNF-α were significantly elevated in first-episode psychosis patients and acutely and chronically ill patients with schizophrenia, compared to HCs (Goldsmith, Rapaport, & Miller, 2016). IL-2 did not differ between groups and inconsistent findings between these groups in alterations of IFN-γ and IL-10 were reported, perhaps highlighting an important role of illness stage in cytokine alterations. Relatedly, studies have identified associations between cytokine concentrations and a number of clinical features including illness duration and symptom severity (e.g., Hatziagelaki et al., 2019). Reductions in IL-1β and IL-6 concentrations were found following treatment and thus they may be state markers of schizophrenia (Goldsmith et al., 2016; Miller, Buckley, Seabolt, Mellor, & Kirkpatrick, 2011). With respect to cytokine genes, certain genotypes of the IL-6 rs1800795 polymorphism and the IL-1β rs1143627 polymorphism have been associated with schizophrenia risk, suggesting certain genotypes and allele frequencies may be protective against the development of schizophrenia (Hudson & Miller, 2018).

While distinct alterations in cytokine concentrations have been observed between depression, PTSD and schizophrenia, certain similarities exist: elevations in IL-6 have been reported in all three disorders, and increases in TNF-α are seen in schizophrenia patients and MDD patients when compared with HCs (Baumeister, Russell, Pariante, & Mondelli, 2014; Goldsmith et al., 2016; Passos et al., 2015). Furthermore, a meta-analysis reported that following treatment IL-6 concentrations significantly decrease in both schizophrenia and MDD (Goldsmith et al., 2016). Given the clinical and genetic association of EDs with depression, PTSD and schizophrenia, this provides justification for the study of cytokines in EDs.

1.3.1.5 Association between eating disorders with autoimmune diseases and infections

A bidirectional relationship between autoimmune disorders (e.g., arthritis, celiac disease, Crohn's disease, lupus, psoriasis, and type 1 diabetes) and EDs has been reported, such that autoimmune disease diagnoses increased subsequent risk of AN, BN and other EDs and vice versa (Hedman et al., 2019). Generally, this association has been consistently

reported (e.g., Wotton, James, & Goldacre, 2016; Zerwas et al., 2017), although there have been some mixed findings (e.g., Raevuori et al., 2014). Indeed, a recent study found no significant genetic associations between AN and autoimmune diseases (Tylee et al., 2018). Hospital-treated infections and less severe infections treated with anti-infective agents have also been associated with increased risk of subsequent AN and BN (Breithaupt et al., 2019). In addition, increased use of antimicrobial medication, used as an indicator of infections, has been reported prior to receiving ED treatment for BN and BED (Raevuori et al., 2016). Relatedly, OCD is highly comorbid with AN (Godier & Park, 2014; Ulfvebrand et al., 2015) and the development of OCD has occurred in children following streptococcal infections (Paediatric Autoimmune Neuropsychiatric Disorders Associated with Streptococcal Infections [PANDAS]). Current evidence suggests that dysregulated immune function, including alterations in inflammatory markers, may be one underlying mechanism shared by EDs, autoimmune diseases and infections. Thus, further exploration of the association between immune functioning and EDs should be encouraged.

On the basis of these factors, which implicate cytokines and other inflammatory markers in processes and disorders associated with EDs, further exploration of the role of inflammatory markers in EDs is warranted.

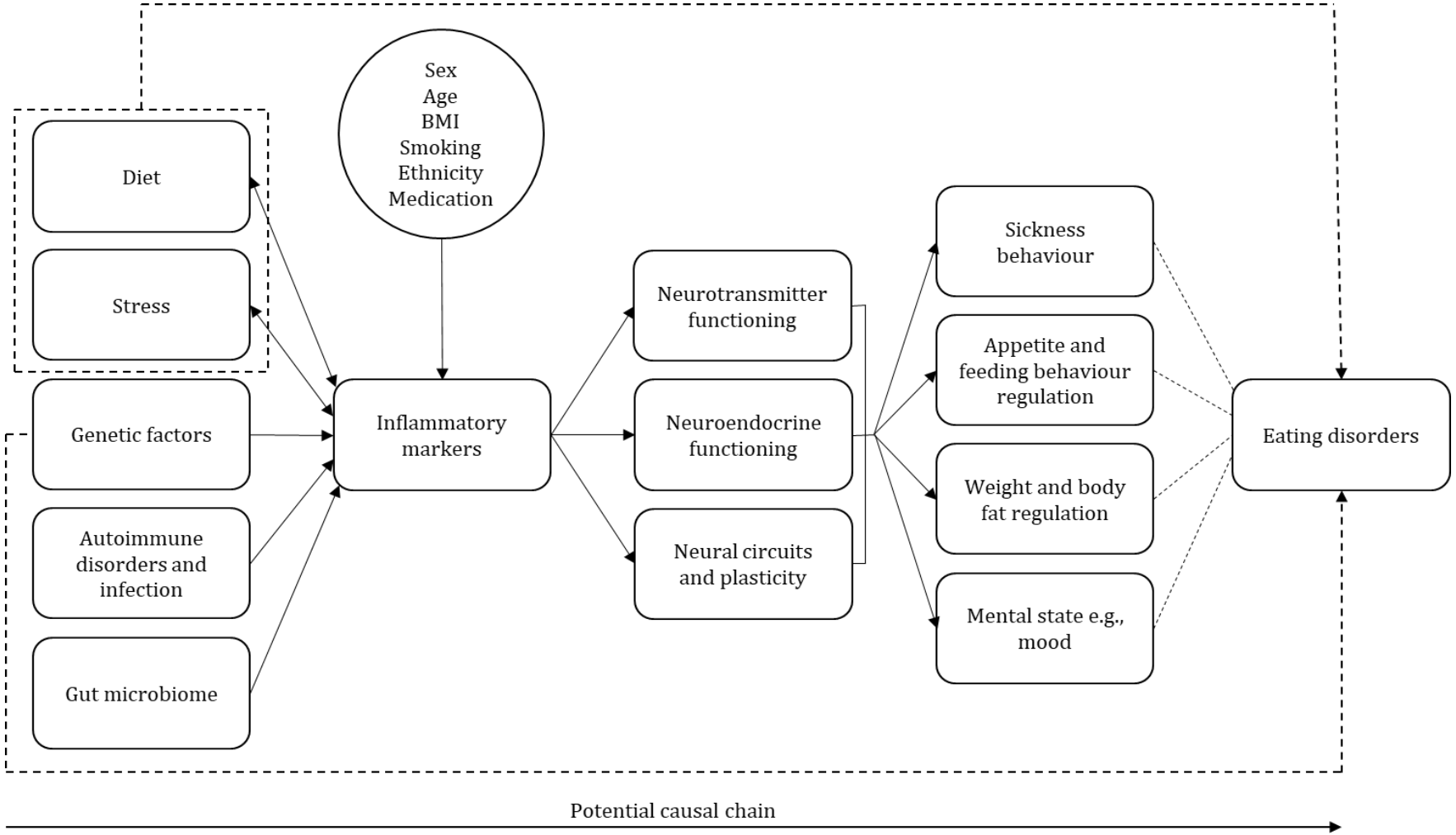


Figure 1.1 Schematic representation of the rationale for investigating the association between inflammatory markers and eating disorders, and the related mechanisms of effect. Abbreviations: BMI = body mass index.

1.3.2 Mechanisms of effect

There are several mechanisms by which inflammatory markers could be involved in the pathogenesis and maintenance of EDs. I will briefly describe four potential factors that may contribute to alterations in inflammatory markers in EDs and are mentioned throughout this thesis as possible explanatory considerations. The role of stress, dietary inflammation and genetics will be examined further in Chapter 5, Chapter 6, and Chapter 7, respectively.

1.3.2.1 Stress

Stress as a potential explanatory factor in the relationship between EDs and inflammatory markers will be discussed in detail in Chapter 5. Briefly, both acute and chronic stress has been reported to increase the production of pro-inflammatory cytokines in humans and animals (Glaser & Kiecolt-Glaser, 2005; Himmerich et al., 2013; Krügel, Fischer, Bauer, Sack, & Himmerich, 2014; Liu et al., 2012; Marsland, Walsh, Lockwood, & John-Henderson, 2017; Steptoe, Hamer, & Chida, 2007). Furthermore, prenatal stress exposure has been shown to correlate with offspring inflammatory parameters (Entringer et al., 2008; Hantsoo, Kornfield, Anguera, & Epperson, 2019). Indeed, it has been suggested that inflammation may be a potential mediator in the relationship between psychosocial stress during pregnancy and neuropsychiatric outcomes in offspring (Hantsoo et al., 2019).

1.3.2.2 Diet and the gut microbiome

In recent years, the gut microbiome has received increasing attention in psychiatric research, including in AN (Glenny, Bulik-Sullivan, Tang, Bulik, & Carroll, 2017; Herpertz-Dahlmann, Seitz, & Baines, 2017; Seitz, Trinh, & Herpertz-Dahlmann, 2019). The gut microbiome refers to the colony of bacterial gut microbes (and their genetic information) in the human digestive-tract (Bull & Plummer, 2014). The composition and function of the microbiome is highly sensitive to the host's nutritional status and diet (Belkaid & Hand, 2014; Lam, Maguire, Palacios, & Caterson, 2017) i.e., extreme and rapid changes in nutrient intake composition influences the diversity of an individual's gut microbiome (David et al., 2014; Li et al., 2016a; Wu et al., 2011a). Research has shown that stool samples from patients with AN exhibit reduced alpha diversity, a measure of the number of different bacterial species in the gut (i.e., within-sample diversity), as compared to HC participants (Kleiman et al., 2015; Mörkl et al., 2019; Mörkl et al., 2017). Studies that identified non-significant group differences showed a similar pattern of alterations in alpha diversity to the significant findings (Borgo et al., 2017; Mack et al., 2016). Beta diversity, a measure of the similarity in microbiome between different individuals (i.e., between-sample diversity), was shown to be increased in AN patients compared to HCs, thus indicating higher heterogeneity (Kleiman et al., 2015; Mack et al., 2016). Of

importance, the microbiome has been shown to influence body weight regulation hormones, and brain development and functioning (the gut-brain axis), and of particular relevance to the current work, inflammation and gut permeability (Seitz et al., 2019).

Reduced mucin, thinned intestinal walls, and altered tight junctions, which all contribute to increased intestinal permeability (also known as a 'leaky gut'), have been shown to facilitate the translocation of bacterial components and products (e.g., lipopolysaccharide) across the intestinal wall barrier into circulation (Jésus et al., 2014; Mack et al., 2016; Sherwin, Rea, Dinan, & Cryan, 2016). This bacteria triggers a microbiota driven pro-inflammatory state (Kelly et al., 2015), as the bacteria binds to pattern recognition receptors (e.g., toll-like receptors [TLR]-4) on circulating monocytes, macrophages and gut epithelial cells, which leads to pro-inflammatory cytokine production and release throughout the body (Kelly, Minuto, Cryan, Clarke, & Dinan, 2017; Lam et al., 2017; Rogier, Koenders, & Abdollahi-Roodsaz, 2015; Sherwin et al., 2016). Indeed, research using the activity-based anorexia animal model has suggested a role for TLR-4 in contributing to inflammation in AN: TLR-4 was reported to be upregulated on colonic epithelial cells and intestinal macrophages and this was found to lead to increased downstream mucosal cytokine production (Belmonte et al., 2016).

Nutrient intake alters the bacterial community within the gut (Cani & Everard, 2016; David et al., 2014; Li et al., 2016a). For example, a high fat diet is associated with an increase in pro-inflammatory bacteria (gram-negative bacteria) and reductions in beneficial bacteria, lower in inflammatory potential (Cani et al., 2007); whereas Mediterranean diets are associated with an increase in certain anti-inflammatory bacteria (Russell et al., 2011; Sandhu et al., 2017). Furthermore, different types of bacteria have differential influences (i.e., degrading or maintaining/improving) on the intestinal mucosa and walls, affecting the likelihood of a leaky gut and inflammation entering circulation. For example, high fat dietary patterns appear to increase the leakage of bacterial components across the intestinal barrier (Cani et al., 2007). In contrast, the microbe *akkermansia muciniphila* has been shown to improve the function of the intestinal barrier and thickness of the mucus layer (Cani & Everard, 2016). As well as dietary intake, acute and chronic stress have been reported to lead to increased gut permeability (Brzozowski et al., 2016; Herpertz-Dahlmann et al., 2017; Kelly et al., 2015; Linninge et al., 2018).

Studies using animal models of AN have shown increased colonic permeability, reduced gastric wall thickness, and weakening of the tight junctions (Jésus et al., 2014); all factors that contribute to a 'leaky gut'. However, a pilot study in humans did not identify any changes in blood zonulin concentrations, a biomarker of gut permeability (Sturgeon & Fasano, 2016), in patients with AN compared to healthy individuals (Mörkl et al., 2019).

Recent research has also suggested that people with AN have an elevated number of intestinal mucus-degrading bacteria in their gut, potentially reducing the thickness of the gut mucosa, which typically protects the gastric walls (Kleiman et al., 2015; Mack et al., 2016). This may further contribute to increased gut permeability.

These early stages of research investigating the microbiome in EDs suggest that the gut microbiota and specifically intestinal permeability may contribute to the chronic inflammation observed in AN. Although it must be mentioned that this is a complex relationship, as certain bacteria in the microbiome can have several neutralising effects on inflammation, for example, by counteracting some of the bacteria that induce inflammation or by improving the barrier effect of the gastrointestinal mucosa (Hakansson & Molin, 2011).

The potential role of dietary inflammation on the relationship between EDs and inflammatory markers will be further discussed in Chapter 6.

1.3.2.3 Genetic factors

Production of inflammatory markers is regulated at a genetic level (Li et al., 2016c). This means that individuals may have different capacities to produce cytokines based on their genes. Therefore, certain individuals may have a genetic predisposition for elevated cytokine production (Louis et al., 1998; Rask-Andersen, Olszewski, Levine, & Schiöth, 2010; Warle et al., 2003; Yilmaz, Yentur, & Saruhan-Direskeneli, 2005). For example, Kanbur et al. (2008) identified an association between a polymorphism of the TNF-α gene and AN, however, the evidence is inconsistent and based on small samples (Ando et al., 2001; Rask-Andersen et al., 2010; Slopien et al., 2014). Furthermore, an early GWAS found that two genes associated with AN are closely linked to cytokine signalling (Boraska et al., 2014; Lee et al., 2012; Wang, Yi, Guerini, Klee, & McBride, 1996), suggesting that the frequency of these genes are greater in AN than in control participants. Genetics and their role in the relationship between EDs and inflammatory markers will be discussed in more detail in Chapter 7.

1.3.2.4 Infection

Given the important role of the immune system in brain development, infections during the prenatal period and during early development may disrupt these processes and exert long-lasting alterations in inflammatory responses and thus, brain and behaviour (Danese & Lewis, 2017). Several animal studies have identified increased cytokine expression/production in offspring who experienced exposure to immune challenges prenatally (Arsenault, St-Amour, Cisbani, Rousseau, & Cicchetti, 2014; Garay, Hsiao, Patterson, & McAllister, 2013). In addition, following infection and systemic inflammation in the neonatal period, adult animals have shown an exaggerated immune response in the

face of an immunological challenge and long-term impairments in cognition e.g., memory (Bilbo & Schwarz, 2009). However, it is of note that not all animal studies reported an effect of prenatal inflammation on offspring inflammatory state (e.g., Labouesse, Langhans, & Meyer, 2015). In humans, as mentioned above, infections during childhood and early adolescence have been associated with increased risk for the development of subsequent EDs in later adolescence (Breithaupt et al., 2019).

1.3.3 Clinical implications of altered inflammatory markers in eating disorders

Possible clinically relevant implications of altered inflammatory marker concentrations and production in EDs include treatment response prediction and treatment targets. In related disorders, such as depression and psychosis, cytokine concentrations and mRNA expression levels have been reported to predict medication treatment response (Cattaneo et al., 2016; Cattaneo et al., 2013; Eller et al., 2008; Mondelli et al., 2015). To date, response to psychological therapies has not been explored, but in future following further research, cytokine concentrations may emerge as a biomarker of treatment response in EDs and provide a useful tool for personalising treatment plans. Furthermore, for individuals with EDs and elevated pro-inflammatory cytokine concentrations, this may be a potential treatment target as inflammatory markers are modifiable by environmental factors (e.g., diet, stress reduction) and medications. For example, if a particular cytokine can be identified as being consistently elevated in a certain ED and/or in a subgroup of patients with an ED, then cytokine blockers could become a potential future medication for the treatment of EDs. Indeed, nonsteroidal anti-inflammatory drugs, such as cytokine inhibitors, have shown some promise as an adjunct treatment in depression (Husain, Strawbridge, Stokes, & Young, 2017; Köhler et al., 2014). Other treatments using anti-inflammatory agents and interventions, such as use of omega-3 or meditation (to minimise stress), may also have potential in improving the symptoms associated with a pro-inflammatory profile (Allison, Sharma, & Timmons, 2019).

1.4 Thesis map

This thesis aims to explore the role of inflammatory markers in EDs by (a) investigating potential alterations in inflammatory markers in people with AN, BN and BED, (b) assessing whether longitudinal changes in cytokines co-occur with changes in AN psychopathology, and (c) examining how stress, diet and genetic factors – variables that have been reported to influence inflammatory marker concentrations in healthy individuals – are associated with cytokines and CRP in people with EDs. The thesis consists of eight chapters, inclusive of the present chapter, which provided a general overview of the potential relationship between inflammatory markers and EDs, and the

justification for the research performed in this thesis. For a simple visual representation of the content of this thesis, see **Figure 1.2**.

The first part of this thesis aims to explore cross-sectional differences in concentrations of inflammatory markers between patients with AN and other EDs and healthy individuals. This will be achieved in Chapters 2 to 3 and Chapter 6. Chapter 2 systematically reviews previous research on cytokine concentrations in EDs using meta-analysis. Based on the findings from Chapter 2, two studies in Chapter 3 measure a broad range of inflammatory markers, including cytokines and acute-phase proteins, in people with a current diagnosis of AN. Many of these inflammatory markers have not previously been measured in people with AN. The association between these markers and psychopathology (e.g., ED symptom severity) will also be considered. Study 2 of Chapter 3 additionally explores the role of demographic factors in the relationship between inflammatory markers and EDs, which have typically not been considered in previous research. In addition to its main aim discussed below, Chapter 6 assesses group differences in CRP concentrations in people with lifetime ED diagnoses (AN, BN, and BED).

The second part of this thesis aims to explore whether concentrations of inflammatory markers change over time in patients with AN, receiving specialist ED treatment. Unlike many previous studies, this research will explore co-occurring changes in both the physical (e.g., BMI) and cognitive/psychological (e.g., ED symptom severity and general psychopathology) aspects of AN presentation.

The third part of this thesis aims to explore factors that have been reported to influence cytokine concentrations and production in healthy individuals within several ED samples. This will be achieved in Chapters 5 to 7. Chapter 5 investigates the role of several types of event-related stress in inflammatory marker concentrations. Specifically, in relation to a range of inflammatory markers, Study 1 measures exposure to traumatic events and associated PTSD symptom severity in people with AN; Study 2 assesses trauma exposure as a child and as an adult in people with lifetime EDs; and Study 3 considers experience of childhood maltreatment trauma and exposure to stressful life events in emerging adults with ED diagnoses. Chapter 6 assesses the association between dietary inflammation (i.e., the inflammatory potential of an individual's diet) and inflammatory markers in patients with lifetime diagnoses of EDs (Study 1) and females with a current AN diagnosis (Study 2). Chapter 7 exploits the data from the UK Biobank, a population-based cohort, to explore the relationship between a polygenic risk score for CRP and ED status. These studies are the first to propose and explore the relationship between inflammatory markers and these influencing factors in EDs.

The final chapter (Chapter 8) provides a general overview of the findings from this thesis, conceptualises the findings within the broader literature, and discusses open questions that should be explored. The findings from the studies in this thesis extend current knowledge related to inflammatory markers in EDs and provides direction for future research.

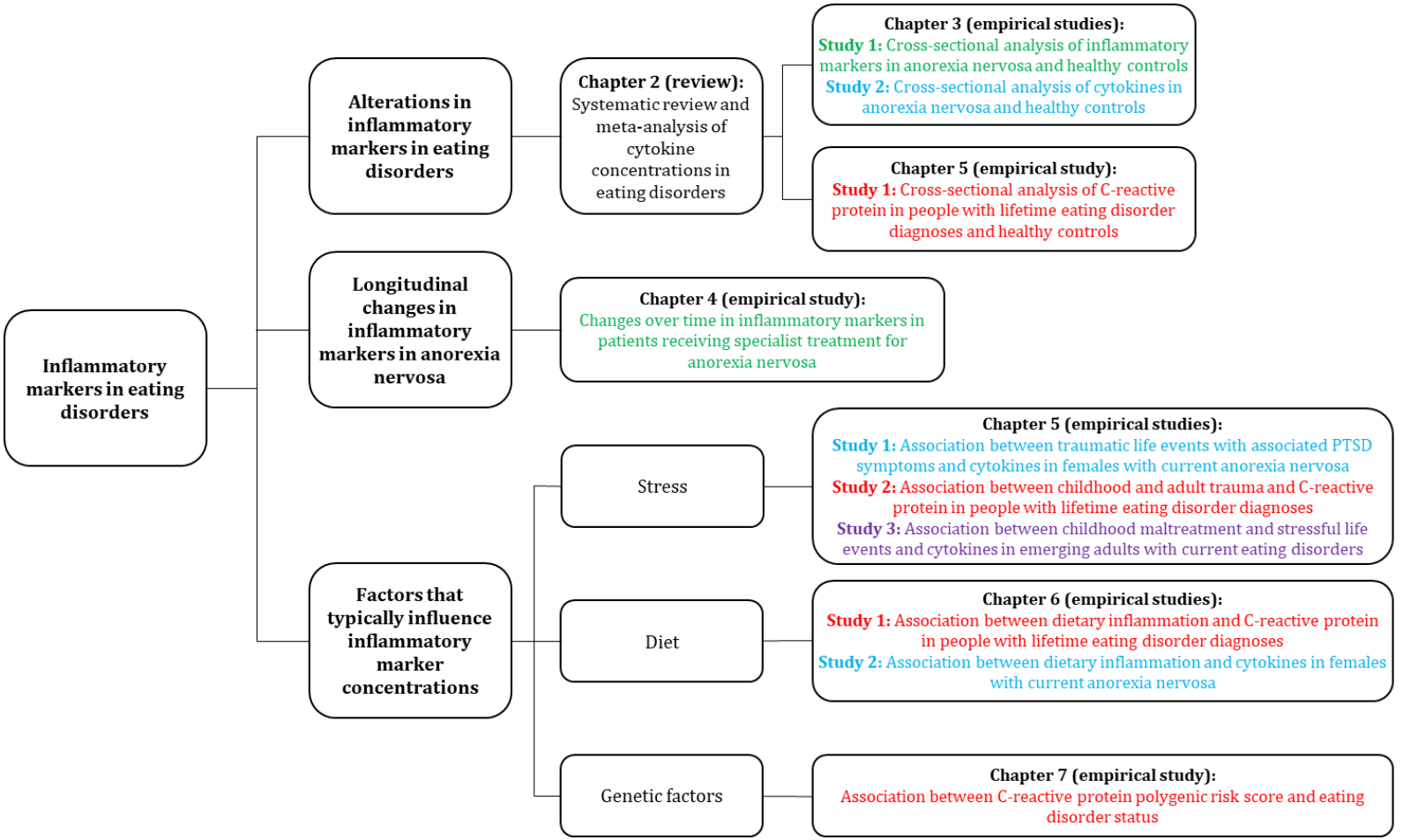


Figure 1.2 Schematic representation of thesis map. Coloured text indicates which research study data was obtained from, as follows: green = Relationship between Overactivity, Stress and Anxiety in Anorexia Nervosa (ROSANA) study; blue = Cytokines in Anorexia Nervosa (CytAN) study; red = the UK Biobank; and purple = Brain Activation in Eating Disorders (ESTRA) study. Abbreviations: PTSD = post-traumatic stress disorder.

Chapter 2. A meta-analysis of cytokine concentrations in eating disorders

As published in:

Dalton, B., Bartholdy, S., Robinson, L., Solmi, M., Ibrahim, M.A.A., Breen, G., Schmidt, U. & Himmerich, H. (2018). A meta-analysis of cytokine concentrations in eating disorders. *Journal of Psychiatric Research, 103*, 252-264. DOI: 10.1016/j.jpsychires.2018.06.002.

A copy of this article is shown in Appendix A Section 10.1.1.

The formatting of this publication has been amended here to ensure stylistic consistency throughout the thesis, however, the body text remains unchanged. Supplementary figures have been incorporated into the results section.

2.1 Abstract

Cytokines are signalling molecules, which play an important role in both immune system function, and brain development and function, and subsequently mental states and behaviour. Cytokines have been implicated in eating disorders (EDs) due to their role in psychological health, body weight and appetite regulation. This meta-analysis examined cross-sectional and longitudinal studies measuring concentrations of cytokines in individuals with EDs. Using PRISMA guidelines, we systematically reviewed relevant articles in PubMed, Web of Science, and MEDLINE. Random-effects meta-analyses were conducted for interleukin (IL)-1β, IL-6, transforming growth factor (TGF)-β, and tumor necrosis factor (TNF)-α, independently, firstly with all EDs combined and then stratified by ED diagnosis. Twenty-five studies were included: serum/plasma cytokine concentrations were measured in people with anorexia nervosa (AN) in 23 studies and bulimia nervosa (BN) in 4 studies. TNF-α and IL-6 were elevated in ED participants compared to healthy controls (HCs). Specifically, this pattern was seen only when comparing AN participants to HCs. Concentrations of these cytokines did not differ between people with BN and HCs. IL-1β and TGF-β did not differ between HCs and any ED group. Therefore, AN seems to be associated with elevated concentrations of TNF-α and IL-6. Considering the role of cytokines in appetite, mood regulation, and anxiety, these pro-inflammatory cytokines could be a potential future drug target to help people with AN, not only with weight gain, but also with various coexisting psychological problems. Future studies should consider confounding factors that affect cytokine concentrations and report ED-relevant clinical characteristics.

2.2 Introduction

Eating disorders (EDs) are serious mental illnesses characterised by pathological eating and weight control behaviours, and body image disturbances. More specifically, anorexia nervosa (AN) involves food restriction and weight-control behaviours resulting in severe weight loss. Both bulimia nervosa (BN) and binge eating disorder (BED) are characterised by frequent bingeing, with BN also involving inappropriate compensatory behaviours, which are not seen in those with BED (American Psychiatric Association, 2013). It is estimated that approximately 20 million people within the European Union have an ED (Schmidt et al., 2016). However, the current available treatments for EDs are limited. For example, with respect to psychopharmacological treatment options, medications are limited to fluoxetine for BN and lisdexamfetamine for BED, which are only approved in certain countries (Himmerich & Treasure, 2018). Ultimately, just over half of individuals with BN and AN treated in specialist ED services make a full recovery (Smink et al., 2013; Steinhausen, 2002; Steinhausen & Weber, 2009), underscoring the need for a greater understanding of ED pathophysiology and for novel alternative treatment strategies

Genetic, neurobiological, gastrointestinal, neuroendocrinological and immunological mechanisms have been implicated in the development and maintenance of EDs (Klein & Walsh, 2004; Slotwinska & Slotwinski, 2017). An immunological component, strongly interrelated to both the neuroendocrine and nervous systems, are cytokines. Cytokines have been shown to be altered in people with EDs compared to healthy individuals (Corcos et al., 2003), and are of particular current interest in EDs as genome wide association studies (GWAS) have identified significant genome-wide loci associated with AN that are closely linked with immune functioning and cytokine signalling (Duncan et al., 2017). Cytokines play a role as soluble intercellular signalling proteins with particular importance in the immune system. They are produced by a range of cells, including microglia and astrocytes, in both the brain and in the periphery (Lichtblau et al., 2013). There is no firm and generally accepted categorisation of cytokines (Cavaillon, 2001). However, functionally relevant groupings can be used: for example, pro-inflammatory cytokines e.g., tumor necrosis factor (TNF)-α, interleukin (IL)-1β and IL-6; anti-inflammatory cytokines e.g., IL-10; and chemokines e.g., CXC chemokine ligand (Zhang & An, 2007).

Cytokines produced in the body's periphery can access the brain via humoral, neural and cellular pathways (see Capuron & Miller, 2011, for a review), and thus, have an effect on mental state, including learning, memory, affect, and behaviour through several pathophysiological mechanisms (Kelley et al., 2003; Yirmiya & Goshen, 2011). These mechanisms include an influence on the metabolism and signal transduction of

neurotransmitters, modulation of neuroendocrine systems such as the hypothalamus-pituitary-adrenal (HPA) axis, induction of the release of hormones involved in feeding and appetite, and an impact on neural plasticity and neurogenesis (Capuron & Miller, 2011; Wong & Pinkney, 2004). Alterations in these biological systems may be particularly pertinent given that some of these have been linked to the pathophysiology of EDs (Klein & Walsh, 2004).

In recent years, evidence has shown that cytokines play an important role in mental health and the pathophysiology of mental disorders, including disorders which are highly comorbid with EDs such as depression (Dowlati et al., 2010; Lichtblau et al., 2013), anxiety disorders (Baldwin, Hou, Gordon, Huneke, & Garner, 2017; Quagliato & Nardi, 2018), post-traumatic stress disorder (PTSD; Hussein et al., 2017; Waheed et al., 2018), and sleep disorders (Weschenfelder, Sander, Kluge, Kirkby, & Himmerich, 2012). Cytokines have also been linked with body weight and its regulation (Fonseka et al., 2016); for example, plasma levels of pro-inflammatory cytokine IL-6 have been shown to correlate positively with body mass index (BMI; Himmerich et al., 2006b; Schmidt et al., 2015). Additionally, cytokines are involved in the regulation of food intake (Himmerich & Sheldrick, 2010) and appetite (Andréasson, Arborelius, Erlanson-Albertsson, & Lekander, 2007; Dent et al., 2012), which may be due to interactions with orexigenic and anorexigenic signals (Wong & Pinkney, 2004). Given the involvement of cytokines in psychological health, weight, and regulation of feeding behaviour and appetite, this provides a rationale for considering the role of cytokines in EDs.

Over the past two decades, several reviews have considered the role of cytokines in EDs (Brown, Bartrop, & Birmingham, 2008; Corcos et al., 2003; Holden & Pakula, 1996; Marcos, 1997; Slotwinska & Slotwinski, 2017). More recently, a meta-analysis has shown circulating concentrations of pro-inflammatory cytokines TNF-α, IL-1β & IL-6 to be elevated in people with AN, in comparison to healthy controls (HCs) and the concentration of these cytokines did not change with weight gain (Solmi et al., 2015). However, research in other EDs is mixed (Corcos et al., 2003) and has not been recently systematically collated. Therefore, the aim of this meta-analysis was to synthesise studies investigating cytokine concentrations in individuals with EDs, both in comparison to healthy individuals and longitudinally, and to update the previously described meta-analysis on cytokines in AN (Solmi et al., 2015). In line with this, the key research questions were: (i) do cytokine concentrations differ between people diagnosed with an ED and healthy individuals; and (ii) in ED participants, do cytokine concentrations change as a function of weight gain and/or symptom improvement?

2.3 Methods

This meta-analysis was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Moher, Liberati, Tetzlaff, Altman, & PRISMA Group, 2009). We used the Newcastle-Ottawa Scale (NOS; Wells et al., 2015) adapted for cross-sectional studies (**Box 2.1**) to determine the quality of included studies. The NOS is a widely used quality assessment tool for non-randomised studies of multiple designs.

2.3.1 Literature search

Three electronic databases (PubMed, ISI Web of Science Core Collection, and MEDLINE via Ovid SP) were searched from inception until 4th May 2018, using the following keywords, which were mapped to Medical Subject Headings with the Explode function where possible: eating disorder*, anorexia nervosa, bulimi*, binge eat* in combination with cytokine*, chemokine*, inflammat*, interleukin, interferon, IFN, tumor necrosis factor, TNF, transforming growth factor, TGF. These searches were supplemented by internet searches, hand-searches of reference lists of potentially relevant papers and reviews, and citation tracking in Google Scholar.

2.3.2 Inclusion/exclusion criteria

Studies in any language of any study design that assessed cytokine concentrations in the serum, plasma or cerebrospinal fluid (CSF) of individuals with a Diagnostic and Statistical Manual of Mental Disorders (DSM; American Psychiatric Association, 1980, 2000, 2013) or International Statistical Classification of Diseases (ICD; World Health Organization, 1992) diagnosis of an ED were eligible for inclusion. Publications were included if they reported cross-sectional comparisons of cytokine concentrations between ED groups and HCs or longitudinal assessments. Longitudinal studies were included if cytokine concentrations were measured at a minimum of two time-points, and BMI or ED symptoms were also assessed at both time points.

Studies were excluded if: i) they did not report group comparisons or longitudinal measurements of cytokine concentrations; ii) participants had an organic cause for their disordered eating e.g., cancer, immunological conditions, genetic disorder, etc.; iii) the sample was comprised of animals; or iv) they measured cytokine production or genetic expression but did not assess cytokine concentrations. Review articles, meta-analyses, conference proceedings/abstracts, book chapters, and unpublished theses were also not included.

Box 2.1 Adaption of the Newcastle-Ottawa Scale Wells et al. (2015) for this meta-analysis.

Newcastle-Ottawa Scale adapted for cross-sectional studies

Studies are allocated stars (up to a total of 11) based on meeting certain criteria, described below. The greater number of stars allocated indicates a higher quality.

Selection: (Maximum 5 stars)

1) Representativeness of the sample:
   a) Truly representative of the average in the target population (all subjects or random sampling) **
   b) Somewhat representative of the average in the target population (non-random sampling) *
   c) Selected group of users or no description of the sampling strategy.

2) Sample size:
   a) Justified and satisfactory *
   b) Not justified

3) Ascertainment of the exposure (disease i.e., ED):
   a) Validated measurement tool in both ED and HC group **
   b) Non-validated measurement tool, but the tool is available or described in both ED and HC group or validated measurement tool described in one group (ED or HC) *
   c) No description of the measurement tool or non-validated measurement tool, but the tool is available or described for only one group (ED or HC)

Comparability: (Maximum 3 stars)

4) The subjects in different outcome groups are comparable, based on the study design or analysis. Confounding factors are controlled.
   a) The study controls for the most important factor (age) *
   b) The study controls for an additional important factor (BMI) *
   c) The study controls for an additional important factor (smoking status) *

Outcome: (Maximum 3 stars)

5) Measurement of outcome:
   a) Validated measurement method (inter-assay coefficient of variation included) **
   b) Non-validated measurement method, but the method is available or described *
   c) No description of the measurement tool

6) Statistical test:
    a) The statistical test used to analyse the data is clearly described and appropriate, and the measurement of the association is presented, including confidence intervals and the probability level (*p* value). *
    b) The statistical test is not appropriate, not described or incomplete.

This scale was adapted from the NOS for cohort studies to perform a quality assessment of cross-sectional studies for use in the following systematic review: Herzog et al. (2013). We further adapted the scale from ascertainment of outcome (e.g., independent blind assessment/record linkage/self-report) to measurement of outcome to account for the methods used in this field of research.

2.3.3 Search selection

Titles and abstracts of retrieved publications were imported into EndNote. Duplicates were removed, and papers deemed highly unlikely to be relevant were disregarded. Full-text versions of the remaining articles were then obtained and screened according to the pre-specified eligibility criteria described above. All papers that did not meet the inclusion criteria were excluded, with the reasons documented (see **Figure 2.1**). The entire search process was conducted independently by two reviewers (B.D. and S.B.) and disagreements at the final stage were resolved by consensus.

2.3.4 Data extraction

The principal reviewer (B.D.) extracted data from all included studies into an electronic summary table, which was then checked by another reviewer (S.B.). Information collected related to: i) sample characteristics, including sample size, demographics, diagnostic criteria and clinical characteristics (e.g., illness duration, BMI), and medication status; and ii) parameters of interest, measurement methods, and concentrations of cytokines. Authors were contacted if the required data were not available in the publication.

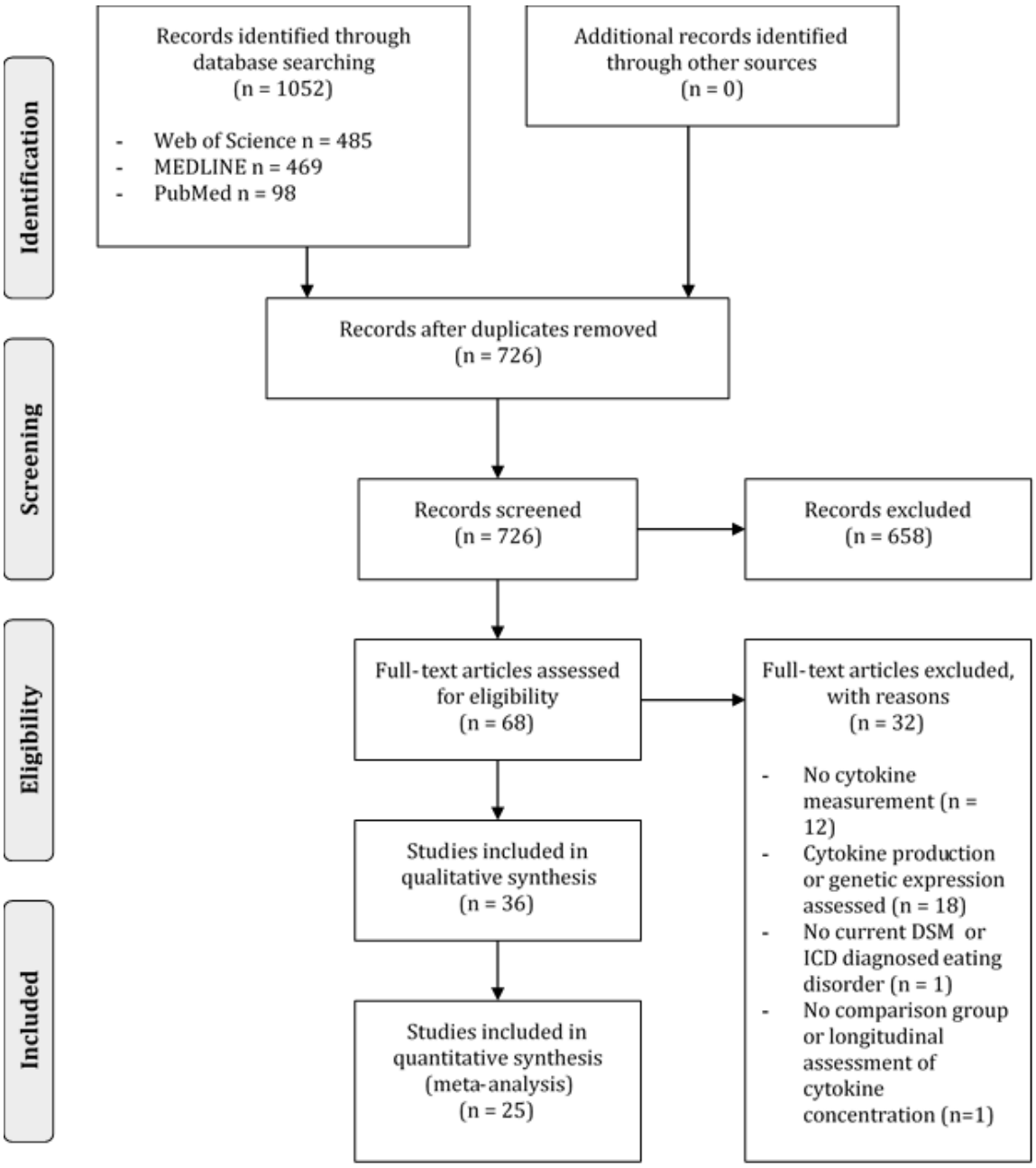


Figure 2.1 Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram. Abbreviations: DSM = Diagnostic and Statistical Manual of Mental Disorders; ICD = International Statistical Classification of Diseases.

2.3.5 Summary measure

The principle outcome measure was cytokine concentrations (pg/ml or ng/ml).

2.3.6 Synthesis of data

Individual meta-analyses were performed for each cytokine (with 2 or more available studies; The Cochrane Collaboration, 2011) for all EDs combined and then for each ED separately. For studies that included more than one diagnostic group (e.g., AN and BN participants) or AN subtype, the means and standard deviations for each group were pooled for the combined EDs and/or AN meta-analyses. Reported standard error means

were converted to SDs. The required data were not available to conduct meta-analyses on longitudinal measurements of cytokines, controlling for within-subject correlations.

2.3.7 Statistical analysis

All meta-analyses were conducted in Stata/SE 15.0 (StataCorp, 2017) using the 'metan' command. The standardised mean difference (SMD) was used as the summary statistic, which expresses the size of the effect in each study (EDs vs. HCs) relative to the variability observed in that study. SMD is used in meta-analyses when included studies assess the same outcome but measure it in a variety of ways and therefore it is necessary to standardise the results of the studies before they are combined (The Cochrane Collaboration, 2011). For all meta-analyses, a random effects model was specified using the DerSimonian & Laird method (DerSimonian & Laird, 1986) and SMDs were pooled using Hedges method (Hedges, 1981). The random effects model assumes both within-group variability and between-study heterogeneity. Positive SMDs were indicative of higher cytokine concentrations in the ED compared to the HC participants. A p-value <0.05 indicated a significant difference between the ED and HC groups.

Between study heterogeneity was assessed by calculating Higgins I^2 (Higgins, Thompson, Deeks, & Altman, 2003) based on Cochran's Q indexes. I^2 measures the percentage of total variation across studies due to heterogeneity. Moderate (50%) to high (75%–100%) heterogeneity was suspected between studies, and for this reason a random effects meta-analysis was used in all cases (Higgins et al., 2003). Significant between-study heterogeneity was further explored using sub-group analyses and/or meta-regressions using the Stata 'metareg' command. The meta-regressions investigated the effect of age, BMI, and illness duration on the SMD in cytokines between ED and HC groups. Subgroup analyses of the AN meta-analyses were stratified by AN subtype (restricting [AN-R] or binge-eating/purging [AN-BP]).

Publication bias was assessed using the Duval and Tweedie trim and fill method (Duval & Tweedie, 2000), which identifies and adjusts for funnel plot asymmetry, and Eggers test (Egger, Davey Smith, Schneider, & Minder, 1997) for small study effects.

2.4 Results

2.4.1 Characteristics of included studies and participants

We identified 25 studies (ED n=632, HC n=487), conducted in 9 countries, which met the inclusion criteria for a quantitative analysis and for which the required data were available (see **Figure 2.1** for PRISMA flow diagram). Study and sample characteristics are presented in **Table 2.1**. Two studies included multiple ED subgroups (Ahrén-Moonga et al., 2011; Brambilla, Bellodi, Brunetta, & Perna, 1998). Twenty-three studies reported

cross-sectional comparisons in cytokine concentrations between participants with AN (*n*=538) and HCs, identifying four new studies not included in the previous meta-analysis (Solmi et al., 2015). Within these, 12 reported AN subtype information: all assessed cytokine concentrations in participants with AN-R and 3 studies additionally reported on participants with AN-BP separately. Four studies compared cytokine concentrations in BN participants (*n*=75) to HCs. No studies assessed cytokine concentrations in patients with BED or other EDs.

The mean age of participants (reported in *n*=19 studies) with EDs and HCs was 21.39 ± 4.10 and 22.62 ± 5.18, respectively. All studies only included female participants. The mean BMI (reported in *n*=20 studies) of ED participants was 15.63 ± 1.83 kg/m^2 (AN participants, *n*=19 studies: 15.15 ± 1.17 kg/m^2, BN participants, *n*=2 studies: 21.25 ± 2.19 kg/m^2) and of HCs was 21.32 ± 1.98 kg/m^2. Mean illness duration for ED participants, reported in 8 studies, was 3.66 ± 2.59 years. ED diagnosis was based on the DSM-IV (*n*=24; American Psychiatric Association, 2000) or the DSM-III (*n*=1; American Psychiatric Association, 1980). Medication usage of ED participants was reported in 14 studies, of which participants in 11 studies were confirmed medication-free at assessment (Allende et al., 1998; Brambilla et al., 1998; Brambilla, Monti, & Franceschi, 2001; Dolezalova et al., 2007; Karczewska-Kupczewska et al., 2013; Karczewska-Kupczewska et al., 2012; Nagata, Yamada, Iketani, & Kiriike, 2006; Nakai, Hamagaki, Takagi, Taniguchi, & Kurimoto, 1999, 2000; Pomeroy et al., 1994; Shimizu et al., 2005). In two studies (Ahrén-Moonga et al., 2011; Nogueira et al., 2010), *n*=26 were reported to be taking antidepressants, neuroleptics, anxiolytics and/or sedatives, and in the remaining study, participants were not taking medication known to affect nutritional or bone status (Ostrowska et al., 2016).

Cytokines included in the meta-analyses were IL-1β, IL-6, TGF-β and TNF-α. Other cytokines (interferon [IFN]-γ, IL-1, IL-2, IL-4, IL-5, IL-7, IL-10, macrophage inhibitory cytokine [MIC]-1) were also measured in eligible studies, however, the data were not available for two or more studies and could not be included in a meta-analysis. Seventeen studies measured cytokine concentrations in serum, 8 in plasma and none in CSF. Measurement methods included immuno-assays (22 studies: enzyme immune-assay *n*=18, radio immune-assay *n*=2, undefined *n*=2) or bioassays (1 study). The remaining studies did not provide sufficient information to classify their measurement methods.

Table 2.1 Study and sample characteristics for studies included in the meta-analyses.

Study/country		Study design	Sample	*n*	Mean ± SD age (years)	Mean ± SD BMI (kg/m²)	Mean illness duration (years)	Diagnostic criteria	ED participants medication status	Parameters of interest	Measurement method
Agnello et al. Italy	2012	Cross-sectional	Female AN Female HC	39 25	26.0 ± 9.0 26.0 ± 3.0	13.9 ± 2 21.0 ± 2	NR -	DSM-IV	NR	TNF-α	Serum - double-antibody ELISA
Ahren-Moonga et al. Sweden	2011	Cross-sectional	Female ED - AN - BN Female HC	26 15 11 12	27.9 ± 8.0 NR NR 28.2 ± 7.2	NR NR NR NR	NR NR NR -	DSM-IV	n=16 antidepressants and/or sedatives	IL-6, TNF-α	Serum - high-sensitivity immunoassay
Allende et al. Spain	1998	Cross-sectional & longitudinal	Female AN BMI<17.5 Female AN BMI>17.5 Female HC	21 19 14	16.9 ± 2.9 16.7 ± 1.6 15.7 ± 1.2	15.8 ± 1.3 20.0 ± 1.9 20.6 ± 1.5	NR NR -	DSM-IV	Medication-free	IFN-γ, IL-1β, IL-2, IL-5, IL-10, TNF-α	Serum - ELISA
Brambilla et al. Italy	1998	Cross-sectional & longitudinal	Female AN-R Female AN-BP Female BN Female HC	9 17 24 26	25.0 ± 8.8 23.3 ± 4.7 23.6 ± 4.4 24.1 ± 3.2	14.8 ± 1.8 17.3 ± 1.8 22.8 ± 2.5 21.7 ± 1.2	6.7 ± 8.5 6.2 ± 3.8 4.2 ± 3.2 -	DSM-IV	Medication-free	IL-1β, IL-6, TNF-α	Plasma - immunoradiometric assays
Brambilla et al. Italy	2001	Cross-sectional	Female AN-R Female AN-BP Female HC	9 5 13	24.5 ± 6.4 19.2 ± 3.1 26.1 ± 1.9	13.4 ± 1.2 14.9 ± 2.3 20.9 ± 1.7	6.2 ± 4.7 2.6 ± 1.8 -	DSM-IV	Medication-free	IL-1β, IL-6, TNF-α,	Plasma - radioimmunoassay
Corcos et al. France	2001	Cross-sectional	Female AN Female HC	29 20	20.1 ± 10.2 23.7 ± 12.1	NR NR	NR -	DSM-IV	NR	IFN-γ, IL-1, IL-2, IL-4, IL-6, IL-10, TGF-β, TNF-α	Serum - ELISA sandwich-type
Dolezalova et al. Czech Republic	2007	Cross-sectional	Female AN Female HC	12 18	NR NR	16.37 ± 1.4 22.96 ± 2.8	NR -	DSM-IV	Medication-free	IL-6	Serum - human serum adipokine LINCOplex kit

Study/country		Study design	Sample	*n*	Mean ± SD age (years)	Mean ± SD BMI (kg/m²)	Mean illness duration (years)	Diagnostic criteria	ED participants medication status	Parameters of interest	Measurement method
Jiskra et al. Czech Republic	2000	Cross-sectional	Female AN-R Female HC	16 16	NR NR	15.0 ± 2.3 22.2 ± 2.5	NR -	DSM-IV	NR	TNF-α	Serum - ELISA
Karczewska-Kupczewska et al. Poland	2012	Cross-sectional	Female AN-R Female HC	20 28	22.3 ± 4.6 25.3 ± 4.9	15.7 ± 1.5 21.3 ± 1.9	1.4 ± 1.0 -	DSM-IV	Medication-free	TNF-α	Serum - immunoassay
Karczewska-Kupczewska et al. Poland	2013	Cross-sectional	Female AN-R Female HC	19 27	22.0 ± 4.8 23.5 ± 3.6	15.9 ± 1.2 22.0 ± 1.9	NR -	DSM-IV	Medication-free	IL-6	Serum - immunoenzymatic method
Krizova et al. Czech Republic	2002	Cross-sectional & longitudinal	Female AN-R Female HC	15 15	NR NR	14.1 ± 6.2 22.5 ± 9.7	NR -	DSM-IV	NR	TNF- α	Serum - ELISA
Krizova et al. Czech Republic	2008	Cross-sectional	Female AN Female HC	28 38	NR NR	15.7 ± 1.9 22.3 ± 2.5	NR -	DSM-IV	NR	TNF-α	Serum - ELISA
Misra et al. United States of America	2006	Cross-sectional & longitudinal	Female AN-R Female HC	23 20	16.2 ± 1.6 15.4 ± 1.8	16.7 ± 1.2 21.9 ± 3.6	0.7 ± 0.9 -	DSM-IV	NR	IL-6	Serum - high-sensitivity sandwich enzyme immunoassay
Nagata et al. Japan	2006	Cross-sectional & longitudinal	Female BN Female HC	20 14	23.1 ± 3.9 24.9 ± 3.5	19.7 ± 2.6 19.8 ± 0.8	NR -	DSM-IV	Medication-free	IL-6	Plasma - ELISA
Nakai et al. Japan	1999	Cross-sectional & longitudinal	Female AN Female HC	20 20	22.1 ± 4.5 20.2 ± 1.3	13.7 ± 1.8 19.9 ± 0.9	3.8 ± 3.6 -	DSM-IV	Medication-free	TNF-α	Plasma - enzyme immunoassay

Study/country		Study design	Sample	*n*	Mean ± SD age (years)	Mean ± SD BMI (kg/m²)	Mean illness duration (years)	Diagnostic criteria	ED participants medication status	Parameters of interest	Measurement method
Nakai et al. Japan	2000	Cross-sectional	Female BN Female HC	20 20	NR NR	NR NR	NR -	DSM-IV	Medication-free	TNF-α	Plasma - enzyme immunoassay
Nogueira et al. France	2010	Cross-sectional	Female AN-R Female AN-BP Female HC	15 9 14	21.1 ± 4.8 25.4 ± 6.9 24.0 ± 2.1	13.3 ± 1.3 13.8 ± 0.9 20.4 ± 1.8	NR NR -	DSM-IV	n=12 medication-free; n=10 antidepressants, neuroleptics and/or anxiolytics	IL-1β, TNF-α	Plasma - ELISA
Ostrowska et al. Poland	2015	Cross-sectional	Female AN Female HC	59 17	15.3 ± 1.6 15.7 ± 1.7	15.3 ± 1.8 20.4 ± 2.2	NR -	DSM-IV	NR	IL-1β, IL-6, TNF-α	Serum - high-sensitivity human ELISA
Ostrowska et al. Poland	2016	Cross-sectional	Female AN-R Female HC	60 20	15.3 ± 1.6 15.7 ± 1.7	15.3 ± 1.8 20.4 ± 2.2	1.0 ± NR	DSM-IV	No medication known to affect nutritional or bone status.	TGF-β	Serum - ELISA
Pomeroy et al. United States of America	1994	Cross-sectional & longitudinal	Female AN Female HC	16 11	23.3 ± 2.0 27.7 ± 6.6	NR NR	NR -	DSM-III	Medication-free	IL-6, TGF-β, TNF-α	Serum - ELISA (TNF-α) & bioassay (IL-6)
Shimizu et al. Japan	2005	Cross-sectional & longitudinal	Female AN-R Female HC	12 12	13.9 ± 1.1 13.7 ± 1.1	13.9 ± 2.1 17.7 ± 2.0	NR -	DSM-IV	Medication-free	TNF-α	Plasma - ELISA
Terra et al. Spain	2013	Cross-sectional	Female AN-R Female HC	28 33	27.4 ± 1.4 32.6 ± 1.3	16.8 ± 0.2 21.8 ± 0.3	8.3 ± 1.4	DSM-IV	NR	IL-6	Plasma - ELISA
Vaisman et al. Israel	2004	Cross-sectional & longitudinal	Female AN Female HC	7 7	NR NR	NR NR	NR -	DSM-IV	NR	IL-1β, IL-3, IL-6, TNF-α	Serum - solid phase ELISA

Study/country		Study design	Sample	*n*	Mean ± SD age (years)	Mean ± SD BMI (kg/m^2)	Mean illness duration (years)	Diagnostic criteria	ED participants medication status	Parameters of interest	Measurement method
Victor et al. Spain	2015	Cross-sectional	Female AN Female HC	24 36	22.4 ± 6.8 24.3 ± 3.4	16.3 ± 1.6 20.9 ± 1.4	NR -	DSM-IV	NR	IL-6, TNF-α	Serum - Luminex 200 flow analyser device
Yasuhara et al. Japan	2007	Cross-sectional & longitudinal	Female AN-R Female HC	7 11	23.2 ±7.8 23.0 ± 2.2	13.6 ± 1.8 19.8 ± 1.8	3.9 ± 4.2 -	DSM-IV	NR	IL-1β, IL-6, TNF-α	Serum - high sensitivity assay

Abbreviations: *n* = number of observations; SD = standard deviation; BMI = body mass index; ED = eating disorder; AN = anorexia nervosa; HC = healthy control; NR = not reported; BN = bulimia nervosa; AN-R = anorexia nervosa restricting subtype; AN-BP = anorexia nervosa binge-eating/purging subtype; DSM = Diagnostic and Statistical Manual of Mental Disorders; ELISA = enzyme-linked immunosorbent assay; TNF = tumor necrosis factor; IL = interleukin; TGF = transforming growth factor; IFN = interferon.

The quality ratings for each study are presented in **Table 2.2**. Few studies ensured representativeness of sample through their sampling method (*n*=6) or provided justification for their sample size (*n*=1). Most studies accounted for age as a confounding factor (*n*=21), however, the majority of studies did not control for additional important confounding factors (e.g., smoking and BMI). Thirteen studies used a validated method to measure outcome, with the remaining studies using a non-validated measurement method that was described in sufficient detail. In all studies, the statistical test used was clearly described and appropriate.

Table 2.2 Quality assessment of studies included in the meta-analysis using the Newcastle-Ottawa Scale adapted for cross-sectional studies.

Study		Selection				Comparability				Outcome		
		Representativeness of the sample	Sample size	Ascertainment of the exposure	TOTAL	Most important confound controlled for (age)	Additional important confound controlled for 1 (BMI)	Additional important confound controlled for 2 (smoking status)	TOTAL	Measurement of outcome	Statistical test	TOTAL
Agnello et al.	2012	*			*	*			*	*	*	**
Ahren-Moonga et al.	2011	*			*	*			*	*	*	**
Allende et al.	1998			**	**	*			*	*	*	**
Brambilla et al.	1998			*	*	*				**	*	***
Brambilla et al.	2001			**	**	*			*	**	*	***
Corcos et al.	2011			*	*						*	**
Dolezalova et al.	2007			*	*	*			*	**	*	***
Jiskra et al.	2000					*			*	*	*	**
Karczewska-Kupczewska et al.	2012		*		*	*		*	**	**	*	***
Karczewska-Kupczewska et al.	2013	*		*	**	*		*	**	**	*	***
Krizova et al.	2002					*			*	*	*	**
Krizova et al.	2008					*			*	*	*	**
Misra et al.	2006					*			*	**	*	***
Nagata et al.	2006			**	**		*		*	**	*	***
Nakai et al.	1999	*			*	*			*	**	*	***
Nakai et al.	2000	*			*	*			*	**	*	***

Study		Selection				Comparability				Outcome		
		Representativeness of the sample	Sample size	Ascertainment of the exposure	TOTAL	Most important confound controlled for (age)	Additional important confound controlled for 1 (BMI)	Additional important confound controlled for 2 (smoking status)	TOTAL	Measurement of outcome	Statistical test	TOTAL
Nogueira et al.	2010			*	*	*			*	*	*	**
Ostrowska et al.	2015			*	*	*			*	**	*	***
Ostrowska et al.	2016			*	*	*			*	**	*	***
Pomeroy et al.	1994									*	*	**
Shimizu et al.	2005									*	*	**
Terra et al.	2013			**	**	*			*	**	*	***
Vaisman et al.	2004					*			*	**	*	***
Victor et al.	2015			*	*	*	*		**	**	*	***
Yasuhara et al.	2007	*			*	*			*	*	*	**

Abbreviations: BMI = body mass index.

2.4.2 Meta-analysis results

Results are summarised in **Table 2.3** and in forest plots seen in **Figure 2.2** to **Figure 2.8**.

Table 2.3 Summary of comparative outcomes and heterogeneity.

Group	*n* (ED, HC)	SMD	95% CIs	*z*	*p*	Heterogeneity
All eating disorder diagnoses combined						
IL-1β	307 (205, 102)	0.77	-0.13 – 1.66	1.68	0.093	I^2 = 90.8%; X^2 = 65.22; df = 6; p < 0.0001
IL-6	589 (331, 258)	0.53	0.19 – 0.87	3.08	0.002	I^2 = 72.9%; X^2 = 44.34; df = 12; p < 0.0001
TGF-β	76 (45, 31)	0.24	-1.63 – 2.10	0.25	0.804	I^2 = 62.4%; X^2 = 13.12; df = 1; p < 0.0001
TNF-α	798 (454, 344)	0.56	0.17 – 0.94	2.80	0.005	I^2 = 84.2%; X^2 = 107.79; df = 17; p < 0.0001
Anorexia nervosa						
IL-1β	108 (44, 64)	0.49	0.08 – 0.89	2.37	0.018	I^2 = 0.0%; X^2 = 0.50; df = 3; p = 0.919
IL-6	520 (276, 244)	0.59	0.24 – 0.94	3.31	0.001	I^2 = 71.6%; X^2 = 38.78; df = 11; p < 0.0001
TGF-β	76 (45, 31)	0.24	-1.63 – 2.10	0.25	0.804	I^2 = 62.4%; X^2 = 13.12; df = 1; p < 0.0001
TNF-α	704 (380, 324)	0.48	0.09 – 0.87	2.43	0.015	I^2 = 82.8%; X^2 = 92.96; df = 16; p < 0.0001
Anorexia nervosa restricting subtype						
IL-1β	108 (44, 64)	0.49	0.08 – 0.89	2.37	0.018	I^2 = 0.0%; X^2 = 0.50; df = 3; p = 0.919
IL-6	229 (99, 130)	0.36	-0.07 – 0.78	1.62	0.105	I^2 = 57.8%; X^2 = 111.84; df = 5; p = 0.037
TNF-α	242 (107, 135)	0.12	-0.04 – 0.44	0.72	0.470	I^2 = 31.9%; X^2 = 10.27; df = 7; p = 0.174
Anorexia nervosa binge-eating/purging subtype						
IL-1β	84 (31, 84)	1.13	-0.30 – 2.57	1.55	0.122	I^2 = 85.4%; X^2 = 13.74; df = 2; p = 0.001
TNF-α	84 (31, 84)	-0.50	-1.31 – 0.30	1.22	0.221	I^2 = 63.4%; X^2 = 5.32; df = 2; p = 0.070
Bulimia nervosa						
IL-6	107 (55, 52)	0.13	-0.49 – 0.76	0.42	0.67	I^2 = 58.8%; X^2 = 4.86; df = 2; p = 0.088

Group	*n* (ED, HC)	SMD	95% CIs	*z*	*p*	Heterogeneity
TNF-α	107 (55, 52)	0.90	-0.26 – 2.06	1.52	0.130	I^2 = 87.2%; X^2 = 15.67; df = 2; $p < 0.0001$

Abbreviations: *n* = number of observations; TNF-α = tumor necrosis factor-alpha; IL-1β = interleukin-1 beta; IL-6 = interleukin-6; TGF-β = transforming growth factor-beta; N = number of observations; ED = eating disorder; HC = healthy control; SMD = standardised mean difference; CI = confidence intervals; df = degrees of freedom.

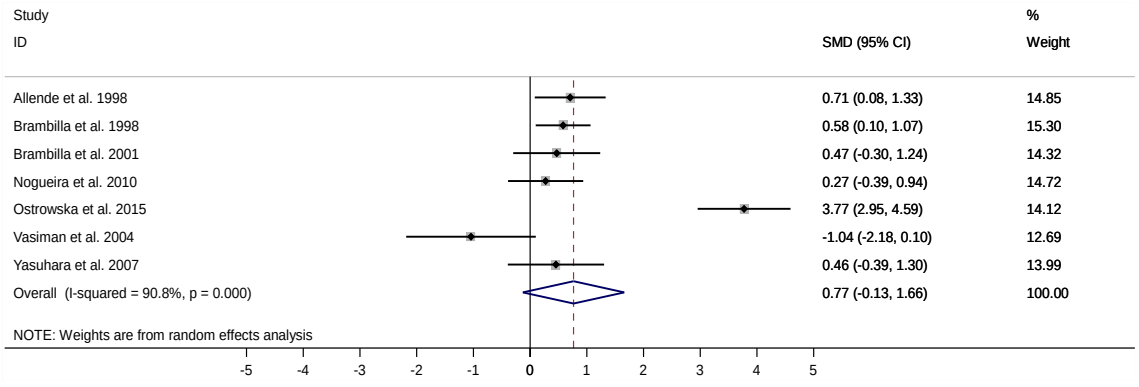
2.4.2.1 Across all eating disorders

Pro-inflammatory cytokines. IL-1β was measured in 7 studies including 205 ED participants and 102 HCs (Allende et al., 1998; Brambilla et al., 1998; Brambilla et al., 2001; Nogueira et al., 2010; Ostrowska et al., 2015; Vaisman et al., 2004; Yasuhara et al., 2007). The concentration of IL-1β did not differ between groups (SMD = 0.77, 95% confidence intervals [CIs] -0.13 to 1.66, $p = 0.093$, see **Figure 2.2a**).

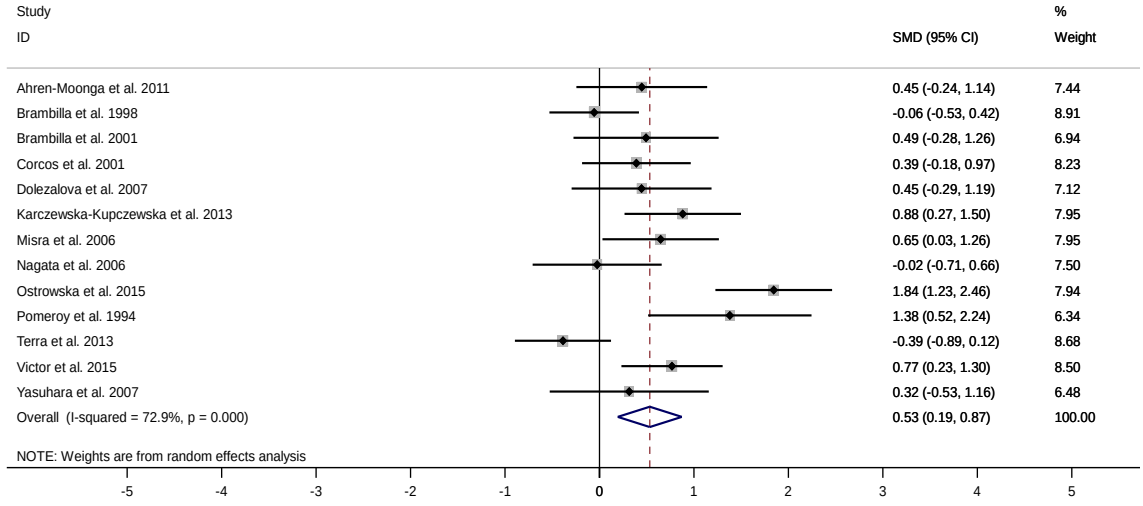
Thirteen studies measured IL-6 (Ahrén-Moonga et al., 2011; Brambilla et al., 1998; Brambilla et al., 2001; Corcos et al., 2001; Dolezalova et al., 2007; Karczewska-Kupczewska et al., 2013; Misra et al., 2006a; Nagata et al., 2006; Ostrowska et al., 2015; Pomeroy et al., 1994; Terra et al., 2013; Víctor et al., 2015; Yasuhara et al., 2007) and found concentrations of IL-6 to be significantly higher in participants with EDs (*n*=331) than HCs (*n*=258; SMD = 0.53, 95% CIs 0.19 to 0.87, $p = 0.002$; see **Figure 2.2b**).

Across 18 studies (Agnello et al., 2012; Ahrén-Moonga et al., 2011; Allende et al., 1998; Brambilla et al., 1998; Brambilla et al., 2001; Corcos et al., 2001; Jiskra et al., 2000; Karczewska-Kupczewska et al., 2012; Krízová et al., 2008; Krízová et al., 2002; Nakai et al., 1999, 2000; Nogueira et al., 2010; Ostrowska et al., 2015; Shimizu et al., 2005; Vaisman et al., 2004; Víctor et al., 2015; Yasuhara et al., 2007), there were significantly higher concentrations of TNF-α in ED participants (*n*=454) compared to HCs (*n*=344; SMD = 0.56, 95% CIs 0.17 to 0.94, $p = 0.005$; see **Figure 2.2c**).

(a) IL-1β



(b) IL-6



(c) TNF-α

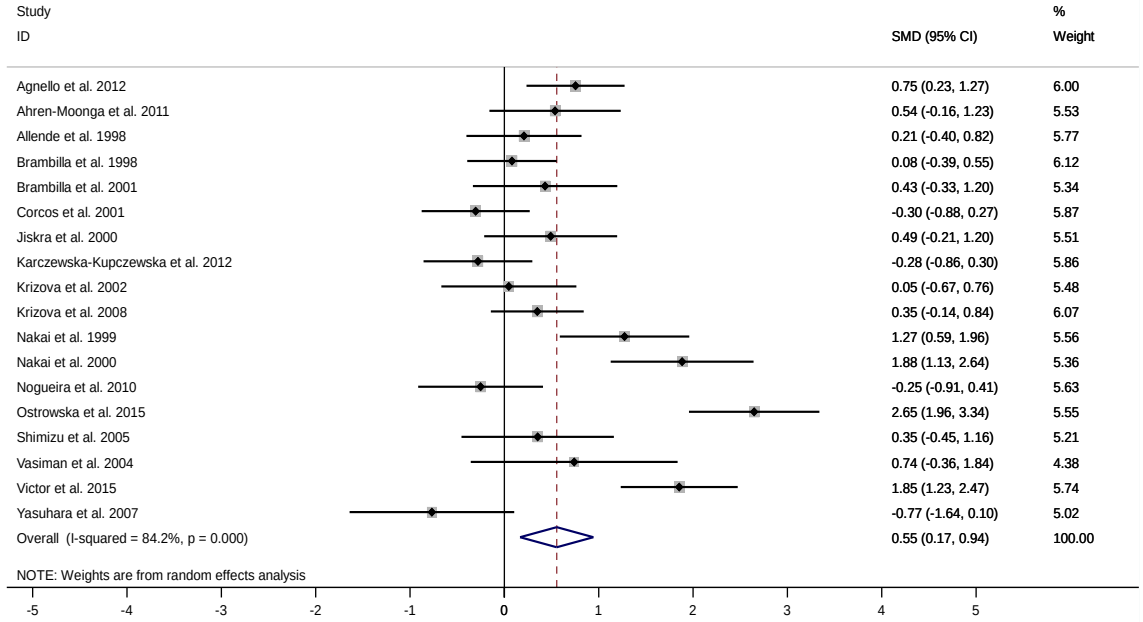


Figure 2.2 Forest plot of standardised mean difference in (a) interleukin-1β, (b) interleukin-6, and (c) tumor necrosis factor-α between eating disorder participants and healthy controls.Zero is the line of no effect, and points to the right of zero indicate an

elevation in (a) interleukin-1β, (b) interleukin-6, and (c) tumor necrosis factor-α in eating disorders compared to healthy controls. Abbreviations: IL = interleukin; SMD = standardised mean difference; CI = confidence intervals; TNF = tumor necrosis factor.

Other cytokines. Three studies that measured TGF-β (Corcos et al., 2001; Ostrowska et al., 2016; Pomeroy et al., 1994) suggested lower concentrations in ED participants (n=105; consists of exclusively AN participants) compared to HCs (n=51), though this difference did not reach statistical significance (SMD = −0.59, 95% CIs -2.37 to 1.20, p = 0.518; see **Figure 2.3**).

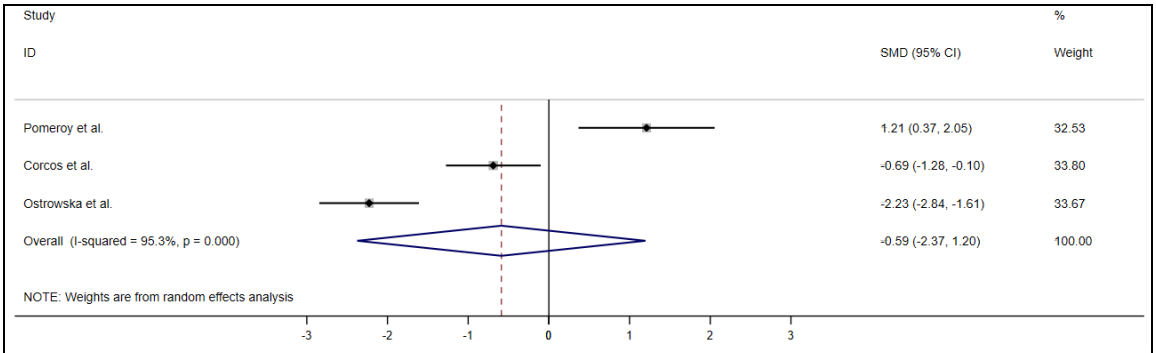


Figure 2.3 Forest plot of standardised mean difference in transforming growth factor-β between anorexia nervosa participants and healthy controls. Zero is the line of no effect, and points to the right of zero indicate an elevation in transforming growth factor-β in anorexia nervosa compared to healthy controls. Abbreviations: SMD = standardised mean difference; CI = confidence intervals.

2.4.2.2 Anorexia nervosa

Pro-inflammatory cytokines. IL-1β measurements were made in 162 AN participants and 102 HCs from 7 studies (Allende et al., 1998; Brambilla et al., 1998; Brambilla et al., 2001; Nogueira et al., 2010; Ostrowska et al., 2015; Vaisman et al., 2004; Yasuhara et al., 2007), extending the previous meta-analysis (Solmi et al., 2015) by 3 studies (additional 77 AN participants and 35 HCs). Between-group differences in IL-1β did not reach statistical significance in AN (SMD = 0.78, 95% CIs -0.17 to 1.72, p = 0.110; see **Figure 2.4a**), nor in subgroup analyses of AN-BP participants (SMD = 1.13, 95% CIs -0.30 to 2.57, p = 0.122), as shown in **Figure 2.4b** (Brambilla et al., 1998; Brambilla et al., 2001; Nogueira et al., 2010). Participants with AN-R showed significantly higher levels of IL-1β than HCs (SMD = 0.49, 95% CIs 0.08 to 0.89, p = 0.018, as can be seen in **Figure 2.4b** (Brambilla et al., 1998; Brambilla et al., 2001; Nogueira et al., 2010; Yasuhara et al., 2007).

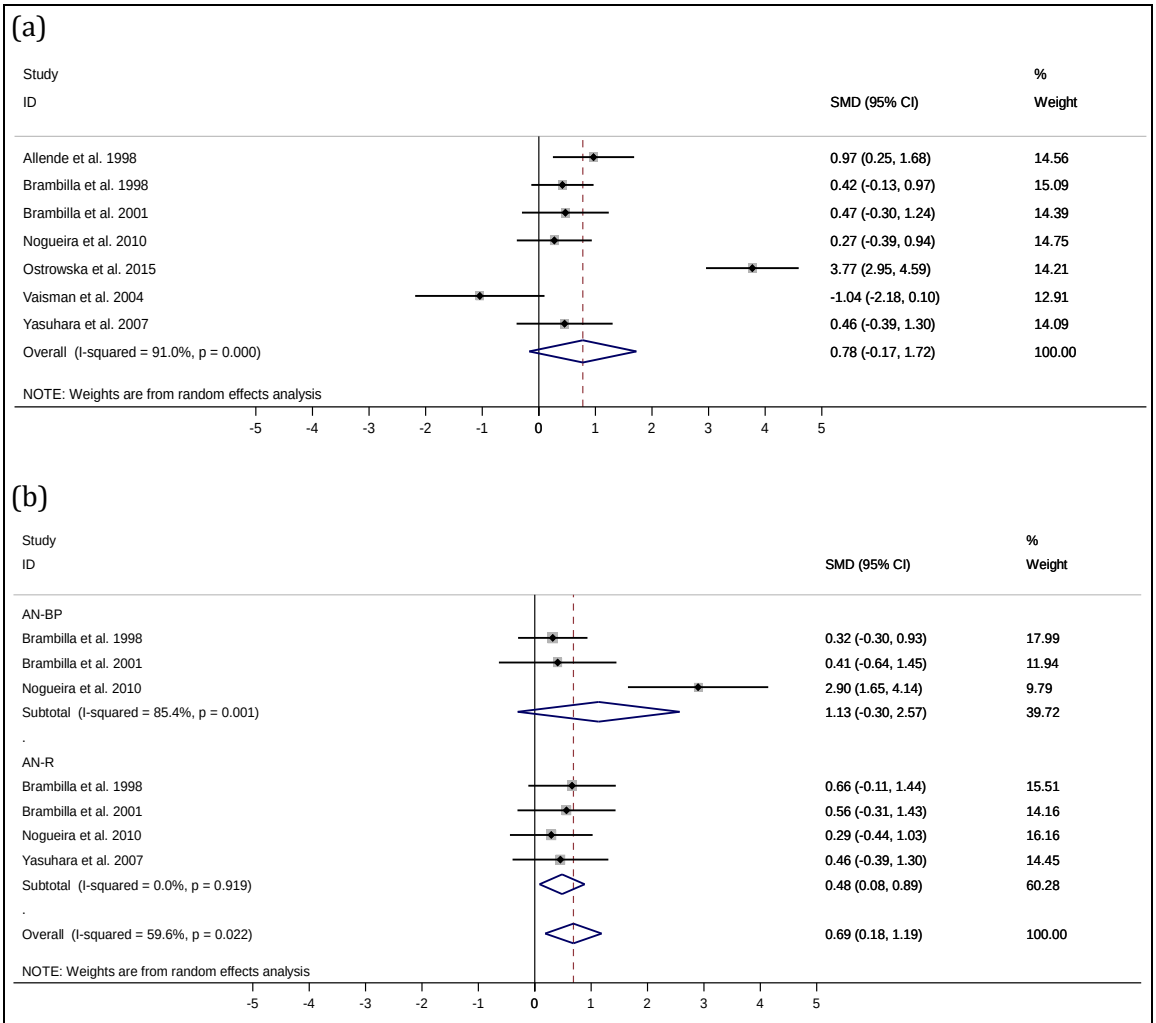


Figure 2.4 Forest plot of standardised mean difference in interleukin-1β between (a) anorexia nervosa participants and healthy controls, and (b) anorexia nervosa participants and healthy controls, stratified by anorexia nervosa subtype. Zero is the line of no effect, and points to the right of zero indicate an elevation in interleukin-1β in anorexia nervosa compared to healthy controls. Abbreviations: SMD = standardised mean difference; CI = confidence intervals; AN-BP = anorexia nervosa binge-eating/purging subtype; AN-R = anorexia nervosa restricting subtype.

Measurements of IL-6 were extracted from 12 studies, consisting of 276 AN participants and 244 HCs (Ahrén-Moonga et al., 2011; Brambilla et al., 1998; Brambilla et al., 2001; Corcos et al., 2001; Dolezalova et al., 2007; Karczewska-Kupczewska et al., 2013; Misra et al., 2006a; Ostrowska et al., 2015; Pomeroy et al., 1994; Terra et al., 2013; Víctor et al., 2015; Yasuhara et al., 2007); including an additional 94 AN and 64 HC participants (from 3 extra studies) compared to Solmi et al. (2015). There were significantly higher concentrations of IL-6 in AN participants compared to HCs (SMD = 0.59, 95% CIs 0.24 to 0.94, p = 0.001; see **Figure 2.5a**). Subgroup analyses found a trend towards higher levels of IL-6 in AN-R compared to HC participants, but this difference did not reach statistical

significance (SMD = 0.36, 95% CIs -0.07 to 0.78, p = 0.105; as seen in **Figure 2.5b** (Brambilla et al., 1998; Brambilla et al., 2001; Karczewska-Kupczewska et al., 2013; Misra et al., 2006a; Terra et al., 2013; Yasuhara et al., 2007).

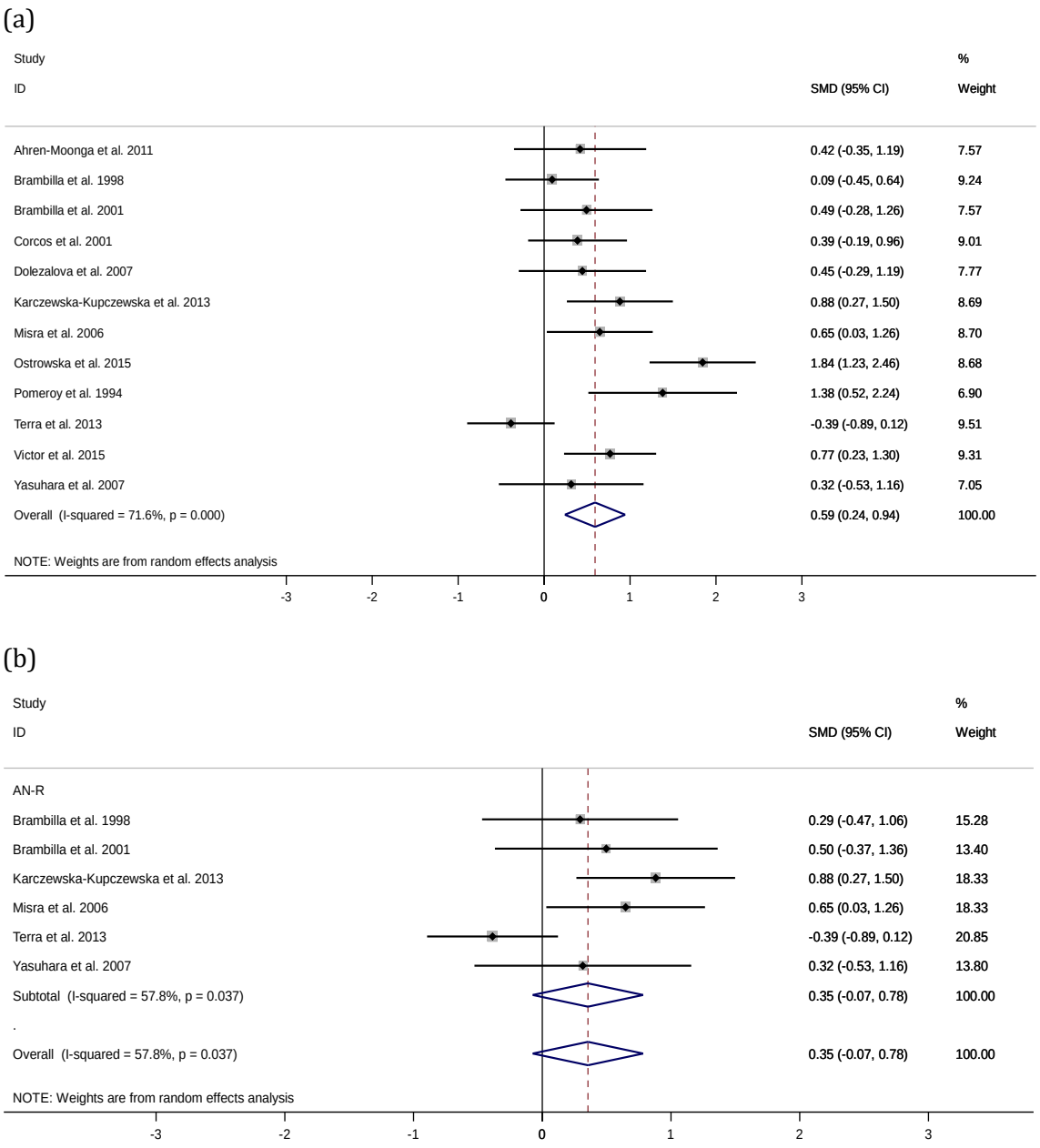


Figure 2.5 Forest plot of standardised mean difference in interleukin-6 between (a) anorexia nervosa participants and healthy controls, and (b) anorexia nervosa participants and healthy controls, stratified by anorexia nervosa subtype. Zero is the line of no effect, and points to the right of zero indicate an elevation in interleukin-6 in anorexia nervosa compared to healthy controls. Abbreviations: SMD = standardised mean difference; CI = confidence intervals; AN-R = anorexia nervosa-restricting type.

Across 17 studies (Agnello et al., 2012; Ahrén-Moonga et al., 2011; Allende et al., 1998; Brambilla et al., 1998; Brambilla et al., 2001; Corcos et al., 2001; Jiskra et al., 2000; Karczewska-Kupczewska et al., 2012; Krízová et al., 2008; Krízová et al., 2002; Nakai et al., 1999; Nogueira et al., 2010; Ostrowska et al., 2015; Shimizu et al., 2005; Vaisman et al., 2004; Víctor et al., 2015; Yasuhara et al., 2007), TNF-α concentrations were significantly higher in AN participants (*n*=380) compared to HCs (*n*=324; SMD = 0.48, 95% CIs 0.09 to 0.87, *p* = 0.015; see **Figure 2.6a**). This expands the sample of the previous meta-analysis (Solmi et al., 2015) by 145 AN participants and 106 HCs from 6 additional studies. Subgroup analyses showed that between-group differences for AN-R (Brambilla et al., 1998; Brambilla et al., 2001; Jiskra et al., 2000; Karczewska-Kupczewska et al., 2012; Krízová et al., 2002; Nogueira et al., 2010; Shimizu et al., 2005; Yasuhara et al., 2007) and AN-BP (Brambilla et al., 1998; Brambilla et al., 2001; Nogueira et al., 2010) compared to HCs did not reach statistical significance (AN-R: SMD = 0.12, 95% CIs -0.20 to 0.44, *p* = 0.470; AN-BP: SMD = −0.50, 95% CIs -1.31 to 0.30, *p* = 0.221; see **Figure 2.6b**).

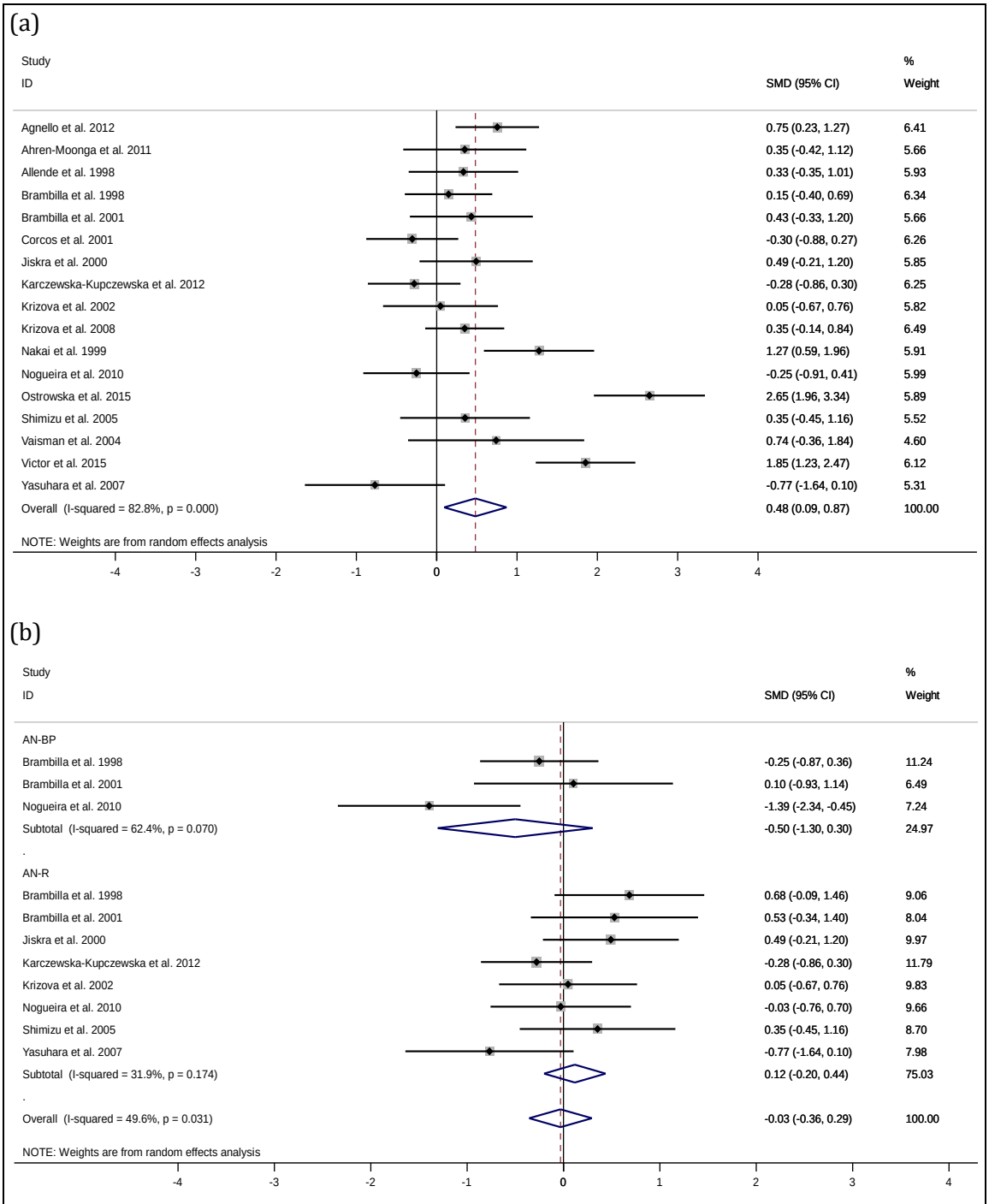


Figure 2.6 Forest plot of standardised mean difference in tumor necrosis factor-α between (a) anorexia nervosa participants and healthy controls, and (b) anorexia nervosa participants and healthy controls, stratified by anorexia nervosa subtype. Zero is the line of no effect, and points to the right of zero indicate an elevation in tumor necrosis factor-α in anorexia nervosa compared to healthy controls. Abbreviations: SMD = standardised mean difference; CI = confidence intervals; AN-BP = anorexia nervosa binge-eating/purging subtype; AN-R = anorexia nervosa restricting subtype.

Other cytokines. See analysis for TGF-β in Section 2.4.2.1 and **Figure 2.3**. This analysis included a recent study (Ostrowska et al., 2016) unavailable for the previous meta-analysis (Solmi et al., 2015), which increased the AN sample by 60 participants.

2.4.2.3 Bulimia nervosa

Pro-inflammatory cytokines. IL-6 concentrations were measured in 55 participants with BN and 52 HCs, taken from 3 studies (Ahrén-Moonga et al., 2011; Brambilla et al., 1998; Nagata et al., 2006). Between-group differences did not reach statistical significance (SMD = 0.13, 95% CIs -0.49 to 0.76, p = 0.67; see **Figure 2.7**).

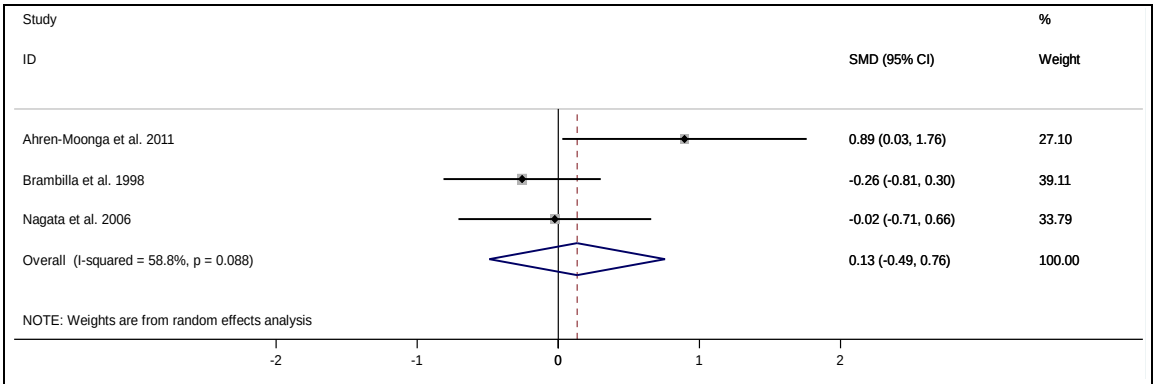


Figure 2.7 Forest plot of standardised mean difference in interleukin-6 between bulimia nervosa participants and healthy controls. Zero is the line of no effect, and points to the right of zero indicate an elevation in interleukin-6 in bulimia nervosa compared to healthy controls. Abbreviations: SMD = standardised mean difference; CI = confidence intervals.

Concentrations of TNF-α were extracted from 3 studies (Ahrén-Moonga et al., 2011; Brambilla et al., 1998; Nakai et al., 2000), suggesting higher concentrations of TNF-α concentrations in BN participants (n=55) compared to HCs (n=58); however, this did not reach statistical significance (SMD = 0.90, 95% CIs -0.26 to 2.06, p = 0.13; see **Figure 2.8**).

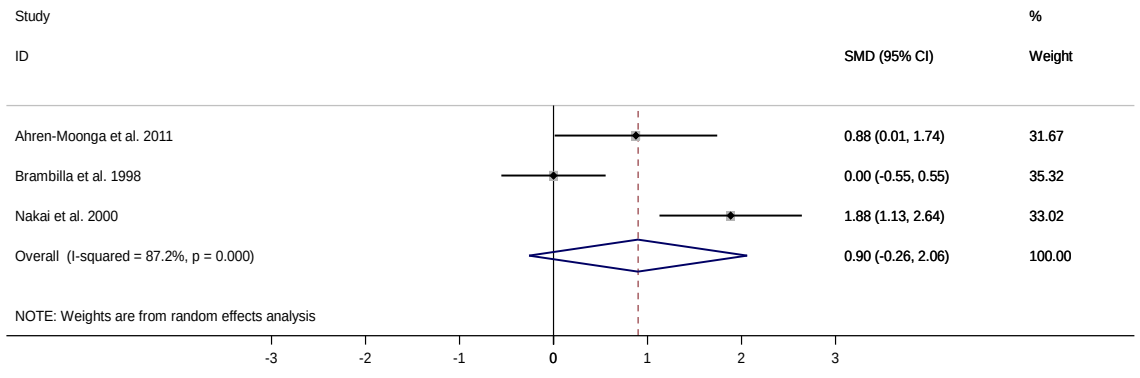


Figure 2.8 Forest plot of standardised mean difference in tumor necrosis factor-α between bulimia nervosa participants and healthy controls. Zero is the line of no effect, and points to the right of zero indicate an elevation in tumor necrosis factor-α in bulimia

nervosa compared to healthy controls. Abbreviations: SMD = standardised mean difference; CI = confidence intervals.

2.4.2.4 Meta-regressions

The results of the meta-regressions are presented in **Table 2.4**. Age significantly contributed to the SMDs for IL-6 for the combined ED group and AN group, which may account for some of the observed heterogeneity. The remaining results were non-significant, and BMI did not significantly contribute to the SMDs in any group.

Some meta-regressions were limited by the number of studies that could be included, particularly in relation to IL-1β, as only 6 studies were available. This violates the recommended guidelines of a requirement of 10 studies for a meta-regression (The Cochrane Collaboration, 2011). For this reason, illness duration could not be included in the meta-regressions as this limited the number of studies to *n*=5.

Table 2.4 Results of the conducted meta-regressions.

Cytokine	Number of included studies	Covariate	β coefficient	Lower 95% CIs	Upper 95% CIs	*p*
All eating disorder diagnoses combined						
TNF-α	11	Age	-0.083	-0.284	0.119	0.373
		BMI	0.003	-0.384	0.390	0.986
IL-1β	6	Age	-0.279	-0.712	0.154	0.132
		BMI	-0.053	-0.683	0.576	0.805
IL-6	9	Age	-0.132	-0.215	-0.049	**0.008**
		BMI	-0.106	-0.267	0.055	0.157
Anorexia nervosa						
TNF-α	11	Age	-0.080	-0.274	0.114	0.369
		BMI	0.204	-0.434	0.842	0.482
IL-1β	6	Age	-0.301	-0.701	0.098	0.096
		BMI	-0.017	-1.189	1.15	0.966
IL-6	8	Age	-0.135	-0.230	-0.039	**0.015**
		BMI	-0.109	-0.460	0.242	0.461

Significant findings at $p < 0.05$ highlighted in bold. Abbreviations: CI = confidence interval, TNF = tumor necrosis factor; IL = interleukin; BMI = body mass index.

2.4.2.5 Sensitivity analyses

Moderate to high heterogeneity (>50%) was observed in all meta-analyses conducted (except for AN-R IL-1β and TNF-α), as seen in **Table 2.3**. According to Egger's test for small study effects, there is presumed to be no publication bias, as all analyses were non-

significant. The trim and fill method showed that there were missing studies in the analysis of TNF-α concentrations in BN and TGF-β in AN. When the SMDs were re-estimated after adjusting for missing studies, they remained non-significant. All other analyses showed no evidence of missing data using the trim and fill method.

2.5 Discussion

2.5.1 Summary of findings

This is the first meta-analysis to investigate cytokine concentrations across all EDs. AN was most researched (*n*=23 studies), followed by fewer investigations of cytokine concentrations in BN (*n*=4 studies). No studies reported on cytokine concentrations in BED or other EDs. The only available cytokines to be included in meta-analyses were TNF-α, IL-6, IL-1β, and TGF-β. Generally, studies reported on a limited number of cytokines and several important cytokines are yet to be measured in ED samples; e.g., IL-17 is a key cytokine for immune response and seems to play a role in the development of other psychiatric disorders and their treatment (Borovcanin et al., 2012; Davami et al., 2016; Himmerich et al., 2011). This highlights the need for future studies to assess a broad range of cytokines. Moderate to high heterogeneity in most analyses may be accounted for by methodological issues, as discussed below (Section 2.5.4). The required data were not available to assess changes in cytokine concentrations longitudinally therefore, we were unable to investigate our second research question.

In whole-group analyses for EDs, both TNF-α and IL-6 concentrations were found to be elevated in comparison to HCs. In sub-group analyses, these differences were observed in the AN, but not in the BN group, suggesting that the findings in the combined ED group, may have been driven by the elevation in AN participants. Results in AN participants replicate those identified in the previous meta-analysis (Solmi et al., 2015). Both TNF-α and IL-6 are classed as pro-inflammatory cytokines involved in the acute-phase response and are secreted into the blood stream in response to an immunological challenge. TNF-α is produced by macrophages, natural killer cells and T cells and stimulates the release of other pro-inflammatory cytokines and neutrophils, and induces fever (Abbas, Lichtman, & Pillai, 2014). IL-6 is produced by macrophages, endothelial cells and T cells, and is involved in the proliferation of antibody-producing cells (Abbas et al., 2014).

Similar to the findings in Solmi et al. (2015), TGF-β, a multi-functional transforming growth factor, was not found to differ between ED and HC participants. However, this analysis was limited to three studies in AN participants and therefore it is premature to draw conclusions from these data. In addition, IL-1β, a pro-inflammatory cytokine, did not differ between ED, AN or BN participants and HCs. Including three additional studies in the current meta-analysis did not replicate the findings from the previous meta-analysis

(Solmi et al., 2015), which found elevated levels of IL-1β in AN compared to HCs. However, in subgroup analyses, IL-1β was found to be elevated in participants with AN-R, but not AN-BP, compared to HCs, as seen in Solmi et al. (2015). The significant findings limited to this restrictive subgroup may make sense given that IL-1β is anorexigenic and findings from animal studies have implicated IL-1β in reduced food intake: more specifically, administration of IL-1β results in reduced meal size and meal duration, but not meal frequency, and reduced food-seeking behaviour (Plata-Salaman, 2001; Wong & Pinkney, 2004).

Meta-regressions suggest that age may have contributed to the SMDs observed for IL-6 for both the combined ED and AN groups. Variations in age may therefore account for some of the observed heterogeneity, highlighting the importance of including a range of ages in investigations of cytokine concentrations in EDs. When included as a covariate, BMI did not appear to be a major factor influencing cytokine concentrations in people with EDs. In line with this, when BMI was used as a covariate in an included study, significantly elevated levels of TNF-α and IL-6 were still observed in AN participants compared to HCs (Víctor et al., 2015). However, it is important to consider that in the included studies, the participants' BMIs were generally within the underweight and normal-weight ranges. Therefore, our ability to make conclusions about the role of BMI in cytokine concentrations in people with EDs and overweight or obesity (e.g., BED and obesity/overweight often co-occur; Villarejo et al., 2012) are limited.

2.5.2 Mechanisms of effect

There are several mechanisms that may account for the elevated pro-inflammatory cytokines observed in those with EDs compared to HCs. Two such factors will be briefly discussed: stress, and the gut microbiota.

Firstly, stress can induce the release of and increase production of pro-inflammatory cytokines (Ménard, Pfau, Hodes, & Russo, 2017). This elevation has been found in a number of animal studies in which hyper-production of cytokines was induced by acute and chronic stress paradigms (Himmerich et al., 2013; Krügel et al., 2014; Liu et al., 2012). The mechanism as to how stress leads to an increase of pro-inflammatory cytokine production remains unclear. Importantly, psychological stress has been shown to augment the production of cytokines in humans (Glaser & Kiecolt-Glaser, 2005; Steptoe et al., 2007). Therefore, the presence of depression and/or anxiety, which are both highly comorbid with EDs, may play a role in the observed elevated concentrations of TNF-α and IL-6 (Dowlati et al., 2010; Felger & Lotrich, 2013; Furtado & Katzman, 2015; Kim & Won, 2017). Few studies assessed levels of stress, depression or anxiety and therefore, this cannot be determined.

Secondly, bacteria from the gut microbiota can stimulate the production of cytokines. This occurs when bacterial determinants, e.g., the lipopolysaccharide component of the bacteria's cell walls, bind to pattern recognition receptors, e.g., Toll-like receptor (TLR) 4, on circulating monocytes and macrophages (Sherwin et al., 2016) and gut epithelial cells. It is likely that dysregulation of intestinal microbiota is associated with EDs, given that the profile of gut microbiota is determined by the host's diet and EDs are characterised by dysregulated food intake (Lam et al., 2017). Furthermore, in AN, starvation is thought to provoke a 'leaky gut' in which the intestinal epithelial barrier is broken down (Herpertz-Dahlmann et al., 2017). This leads to 'leaking' of bacteria and/or their components from the gut into circulation which is then thought to elicit an inflammatory response i.e., stimulating cytokine production (Herpertz-Dahlmann et al., 2017; Sherwin et al., 2016). Little research has considered the role of gut microbiota in BN or BED. However, gut microbiota and regulation of the gut-brain axis have been proposed to play a significant role in stress and other psychiatric disorders that are highly comorbid with BN and BED, such as depression and anxiety, via their influence on inflammatory cytokines. This suggests such factors could contribute to alterations in cytokine production in these EDs (Alam, Abdolmaleky, & Zhou, 2017; Kelly et al., 2015).

Once cytokine alterations have occurred through these and/or other mechanisms, cytokines can affect brain function and development, and subsequently mental states and behaviour, through their influence on several systems (Capuron & Miller, 2011; Wong & Pinkney, 2004). Cytokines can influence the synthesis, release, and reuptake of relevant neurotransmitters, such as serotonin and dopamine (Felger & Lotrich, 2013). For example, cytokines have been shown to influence the synthesis of neurotransmitters by stimulating the production of Indoleamine 2,3 dioxygenase (IDO), which breaks down tryptophan, an essential amino acid. This depletion of tryptophan is thought to contribute to a reduced availability of serotonin (Capuron & Miller, 2011; Miller, Maletic, & Raison, 2009). Lower serotonin availability may contribute to low mood, anxiety, and sleep disturbances. In addition, pro-inflammatory cytokines have been shown to disrupt tetrahydrobiopterin (BH_4), which is an enzyme that is an essential cofactor for enzymes involved in the synthesis of monoamine neurotransmitters, including dopamine and serotonin (Haroon et al., 2012; Miller et al., 2013). This may be particularly pertinent, given that dysregulated serotonin and dopamine have been implicated in EDs (Broft et al., 2011; Gauthier et al., 2014; Kaye et al., 2005; O'Hara et al., 2015). Furthermore, the direct action of cytokines on the brain can influence neuroendocrine functioning. For example, cytokine exposure has been shown to activate the HPA axis, potentially by inhibiting glucocorticoid receptors (Capuron & Miller, 2011; Pace & Miller, 2009). This is of importance given that

hyperactivation of the HPA axis has been reliably observed in AN, and more mildly in BN patients, in the acute phase of illness (Lo Sauro, Ravaldi, Cabras, Faravelli, & Ricca, 2008).

2.5.3 Clinical implications

As we identified elevated concentrations of circulating TNF-α and IL-6 levels in people with AN compared to HCs, cytokines may represent a potential biomarker of AN. However, as cytokines have been found to be elevated in other psychiatric disorders (e.g., Baldwin et al., 2017; Dowlati et al., 2010; Dunjic-Kostic et al., 2013b; Passos et al., 2015) and inflammatory diseases (e.g., Aaltonen et al., 2012), cytokines may not be a specific biomarker for AN but rather a non-specific marker of overall illness severity or general treatment response.

Cytokines such as IL-6 and TNF-α have repeatedly been shown to reduce food intake in animals and humans after peripheral and central administration (Langhans & Hrupka, 1999; McCarthy, 2000). Therefore, it has been suggested that they contribute to the development of anorexia in various infectious, neoplastic and autoimmune diseases. This seems to be due to peripheral anorexigenic effects of cytokines, such as an increase in the production of leptin, a hormone produced by the adipose tissue (Finck et al., 1998; Grunfeld et al., 1996; Kirchgessner et al., 1997), but also due to the anorectic effects of pro-inflammatory cytokines on appetite-regulating hormones such as histamine, alpha-melanocyte stimulating hormone, ghrelin, and neuropeptide Y (Himmerich & Sheldrick, 2010; Huang, Hruby, & Tatro, 1999; Langhans & Hrupka, 1999; Sahu, Kalra, Crowley, & Kalra, 1988; Wong & Pinkney, 2004). However, in people with AN, orexigenic alterations in appetite-regulating hormones have been identified (e.g., Cuesto et al., 2017; Schorr & Miller, 2017). Therefore, it is unclear whether cytokines may contribute to changes in food intake in EDs.

Additionally, as mentioned, pro-inflammatory cytokines have been implicated in the pathophysiology of depression (Dowlati et al., 2010; Lichtblau et al., 2013), anxiety (Baldwin et al., 2017), and disturbed sleep (Weschenfelder et al., 2012). Patients with AN often do not want medication that induce weight gain, although this is necessary from a medical perspective, but rather want help with anxiety, low mood and sleep disturbances (Himmerich et al., 2018). Cytokines could theoretically be drug targets for the treatment of AN that addresses both the medical necessity for weight gain and patients' priorities of coexisting psychological problems. There is currently very little research investigating the therapeutic effect of cytokine blockers in AN (e.g., Barber, Sheeran, & Mulherin, 2003; Solmi, Santonastaso, Caccaro, & Favaro, 2013). However, these biologics have been shown to reduce depressive symptoms in patients with inflammatory diseases such as psoriasis (Tyring et al., 2006), and antidepressant-like effects of TNF-α blockers have been seen in

rats, using a chronic stress model for depression (Krügel, Fischer, Radicke, Sack, & Himmerich, 2013). In addition, anti-TNF-α medication has been shown to lead to increases in body weight in those with chronic inflammatory diseases (Ouchi, Parker, Lugus, & Walsh, 2011).

As seen in the included studies, some ED participants show similar cytokine concentrations to HCs (e.g., Agnello et al., 2012). Therefore, it is important to consider that only a subgroup of patients will present with an immunological basis to the disorder, displaying elevated cytokine concentrations compared to HCs and for these patients, cytokines may be a potential future treatment target (Himmerich & Treasure, 2018).

Taken together, a subgroup of individuals with AN may benefit from treatment targeted on reducing pro-inflammatory cytokines, such as TNF-α blockers (Berthold-Losleben, Heitmann, & Himmerich, 2009; Bou Khalil, de Muylder, & Hebborn, 2011), which are readily available for the treatment of inflammatory diseases (e.g., Aaltonen et al., 2012). However, carefully designed randomised controlled clinical trials will be required to investigate the role of cytokines as a treatment target in AN.

2.5.4 Methodological considerations

Several methodological issues, including technical factors and clinical confounders, may contribute to the moderate to high heterogeneity observed. Most studies did not account for confounding clinical and lifestyle factors that have been shown to affect cytokine production, e.g., age, menstruation, smoking status, medication, exercise, body fat, and concurrent diagnoses relating to physical and mental health (Dugué et al., 1996; Goebel, Mills, Irwin, & Ziegler, 2000; Haack et al., 1999; Munzer et al., 2013; Ouchi et al., 2011; Rom, Avezov, Aizenbud, & Reznick, 2013). Thus, future studies need to consider factors that may influence the measurement of cytokine concentrations within their study design and analyses.

Few studies measured or reported relevant clinical characteristics such as illness duration, age of illness onset, or ED symptom severity. Variability in such sample characteristics may contribute to the observed heterogeneity. In addition, participants' treatment status varied, and differences associated with these, such as the opportunity to engage in ED behaviours (e.g., restrictive eating, purging), may impact on cytokine concentrations (e.g., Canavan et al., 2005). Of interest, research in other psychiatric disorders has shown that certain clinical characteristics are associated with cytokine levels (e.g., Dunjic-Kostic et al., 2013a; Dunjic-Kostic et al., 2013b; Gill, Vythilingam, & Page, 2008). Therefore, future studies would benefit from including information on such clinical factors to further determine the role of cytokines in EDs.

With regards to the HC samples, not all studies used a validated measure to systematically screen and exclude for current and/or previous psychiatric disorders. This cannot rule out the presence of other psychiatric disorders, which as previously cited, have been shown to impact cytokine concentrations. Biases such as these in the HC sample may influence the accuracy of the results.

An additional consideration is that the specific methodologies in each of the laboratories used to measure cytokine concentrations is likely to vary considerably between studies, including the equipment used (e.g., immunoassays, bioassays). Different assay procedures may yield different results (Zhou, Fragala, McElhaney, & Kuchel, 2010) and certain platforms for cytokine assessment are more sensitive than others (Malekzadeh et al., 2017). However, use of random effects models in the current review accounts for such between-study heterogeneity.

2.5.5 Strengths and limitations

The primary strength of this meta-analysis was its objectivity and the systematic evaluation of cytokine concentrations across multiple EDs, utilising all available research to date in this area. In addition, a number of methodological considerations have been highlighted, which can be incorporated into future studies in order to advance our understanding of immunological factors involved in the pathophysiology of EDs. We have expanded on the previous meta-analysis of cytokines and AN (Solmi et al., 2015) by including all EDs, identifying 4 new studies assessing cytokines in AN participants, and also applying additional data from 2 previously included studies (Corcos et al., 2001; Vaisman et al., 2004) to provide a more comprehensive analysis. All analyses, except for the assessment of TNF-α in BN and TGF-β in AN, showed no evidence of publication bias.

With regards to limitations, few studies assessed concentrations of TGF-β and also more generally, cytokines in BN and AN-BP. It has been suggested that a minimum of 5 studies is required in order to achieve reasonable power for a random effects meta-analysis, which is greater than the power from the individual studies (Jackson & Turner, 2017). Therefore, the meta-analyses of TGF-β and these patient groups may lack power to detect an effect and the current results should be interpreted with caution. Furthermore, as associations between binge eating and purging symptoms and TNF-α concentrations have been identified (Lofrano-Prado et al., 2011), future studies should aim to expand the literature base of cytokines in BN. This also warrants the measurement of cytokine concentrations in individuals diagnosed with BED, for which no data are currently available. Additionally, in comparison to Solmi et al. (2015), we limited our research question to cytokines only and did not include data on the associated receptors. To provide a more full and complex

picture of the cytokine network, future reviews should consider the role of cytokine receptors across EDs.

The predictive capability of the meta-regressions was limited due to missing data on covariates of interest (e.g., BMI and illness duration) in several studies. While many longitudinal studies were eligible for inclusion, meta-analyses could not be conducted as the data needed to control for within-group correlations between time points were not available. As inflammatory processes, and in particular cytokines, have been suggested as a possible biomarker involved in illness staging and the neuroprogression of illness (McGorry et al., 2014), the opportunity to include longitudinal data may have provided greater insight into potential biological mechanisms underlying EDs and whether cytokine concentrations could be a marker of treatment response.

2.5.6 Conclusions and future directions

Current treatments for people with EDs are limited, and an improved understanding of the underlying biology may lead to novel treatment strategies. The current meta-analysis found that participants with EDs showed elevated concentrations of circulating TNF-α and IL-6, but not IL-1β or TGF-β, compared to HCs. This pattern of results was also observed in AN, but not BN participants when analyses were stratified by ED diagnosis. The majority of the meta-analyses showed moderate to high heterogeneity, which could be accounted for by various methodological issues, such as limited measurement and/or reporting of confounding factors in the measurement of cytokines (e.g., smoking status), variability in equipment and methods used to measure cytokines, and heterogeneity in sample characteristics.

Further studies should measure a broad range of cytokines and given that cytokines are part of a complex network, they should be analysed in functionally meaningful cytokine groupings. Furthermore, future research should consider measuring cytokines in all EDs, including BN and BED, and include separate analyses of AN subtypes. This research would also benefit from including psychological assessments of ED symptoms alongside cytokine measurement. This will permit further assessment of the role of cytokines in EDs and may provide the basis for investigations into immunomodulatory medication as a treatment for EDs.

Chapter 3. Cross-sectional analyses of inflammatory markers in anorexia nervosa

Study 1 has been published but has been amended here to allow for coherence in the chapter.

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A copy of this article is shown in Appendix A Section 10.1.2.

3.1 Abstract

Objective: Inflammatory markers, such as cytokines, have been suggested to play a pathophysiological role in anorexia nervosa (AN). However, to date, there has been a limited focus on a few pro-inflammatory cytokines that have been shown to be elevated in the serum or plasma of people with AN compared to healthy controls (HCs). Recent meta-analyses have highlighted the importance of measuring a broad range of inflammatory markers to improve our understanding of the relationship between inflammatory markers and AN. Study 1 is the first study to measure a comprehensive panel of inflammatory markers in AN and HC participants and explore the association between these inflammatory markers and clinical characteristics e.g., body mass index (BMI) and eating disorder (ED) symptoms. Study 2 aimed to examine the reproducibility of the findings from Study 1 and consider the effect of confounding sociodemographic factors in an adequately powered sample.

Method: In both studies, serum concentrations of a range of inflammatory markers (including cytokines, chemokines, and cellular adhesion molecules; *n*=41 in Study 1; *n*=36 in Study 2) were measured in people with AN (*n*=27 in Study 1; *n*=40 in Study 2) and HCs (*n*=13 in Study 1; *n*=34 in Study 2). Many of these inflammatory markers had not been previously quantified in people with AN. ED and general psychopathology symptoms were assessed. Demographic and anthropometric (e.g., BMI and body composition) data were obtained.

Results: Data from Study 1 showed that interleukin (IL)-6, IL-15, and vascular cell adhesion molecule (VCAM)-1 concentrations were nominally significantly elevated and concentrations of brain-derived neurotrophic factor (BDNF), tumor necrosis factor (TNF)-β, and vascular endothelial growth factor (VEGF) were nominally significantly lower in AN participants compared to HCs. In contrast, data from Study 2 did not replicate these results, instead finding that concentrations of IL-7, macrophage inflammatory protein (MIP)-1α and MIP-1β were significantly lower in AN patients compared to HCs, after controlling for sociodemographic covariates (age, ethnicity, smoking status) and adjustment for multiple comparisons. In both studies, most of the quantified markers were unchanged in people with AN, despite them being severely underweight with evident body fat loss, as well as having clinically significant ED symptoms and severe depression and anxiety symptoms.

Conclusion: These studies highlight the importance of measuring a broad range of inflammatory markers in AN and of accounting for confounding factors. IL-7, MIP-1α and

MIP-1β have been associated with ED-related phenotypes, including weight, fat mass, anxiety and depression. However, more research is needed to determine the replicability of these findings and further understand the role that these cytokines play in AN.

3.2 Introduction

Altered concentrations of inflammatory markers, in particular cytokines, have been reported in people with anorexia nervosa (AN; Chapter 2; Dalton et al., 2018a; Solmi et al., 2015). Cytokines are cell signalling molecules produced by a range of cells in the brain (e.g., microglia, astrocytes) and the periphery (e.g., by macrophages and T-lymphocytes) and are essential in coordinating responses to infection (Dinarello, 2000). In addition, changes in the circulating concentrations and production of cytokines have been associated with a range of disease states, including obesity (Schmidt et al., 2015) and diabetes (Xiao, Li, Cai, Chakrabarti, & Li, 2014), as well as depression (Lichtblau et al., 2013), schizophrenia (Müller, Weidinger, Leitner, & Schwarz, 2015), and eating disorders (EDs; Chapter 2; Dalton et al., 2018a; Solmi et al., 2015).

As discussed in the previous chapter, research in AN has primarily focussed on pro-inflammatory cytokines, which promote and up-regulate inflammatory reactions (Dinarello, 2000). Recent meta-analyses have concluded that the pro-inflammatory cytokines interleukin (IL)-6 and tumor necrosis factor (TNF)-α are elevated in people with AN, compared to healthy individuals (for reviews see: Chapter 2; Dalton et al., 2018a; Solmi et al., 2015). However, few studies have quantified the concentrations of cytokines in other categories, such as T-helper (Th) 1, Th2, and anti-inflammatory cytokines (e.g., IL-10), the latter of which play an immunomodulatory role by reducing inflammation (Opal & DePalo, 2000). An example of one such cytokine yet to be measured in people with AN is TNF-β, which is produced by Th1 cells. TNF-β performs a variety of important roles in immune regulation (Bauer et al., 2012; Ruddle, 2014), but has also been implicated in the regulation of the commensal gut microbiota (Kruglov et al., 2013; McCarthy et al., 2006; Upadhyay & Fu, 2013), which appears to be involved in the pathology of AN (Borgo et al., 2017; Herpertz-Dahlmann et al., 2017; Lam et al., 2017; Seitz et al., 2019). Additionally, a number of cytokines implicated in other disorders, such as depression and obesity, are yet to be measured in AN. One example is IL-17, a Th17 cytokine that has been reported to predict treatment response in people with depression (Jha et al., 2017b), and seems to be involved in the pathophysiology of schizophrenia (Borovcanin et al., 2012) and the molecular and cellular effects of antipsychotics (Himmerich et al., 2011).

Chemokines are a subcategory of smaller cytokines known to induce chemotaxis, with some also having a homeostatic function in relation to haematopoiesis, immune surveillance, and adaptive immune system responses (Borish & Steinke, 2003; Turner et al., 2014). The chemokines monocyte chemoattractant protein (MCP)-1, regulated on activation, normal T cell expressed and secreted (RANTES) and fractalkine have been

measured in two studies in people with AN (Pisetsky et al., 2014; Zhang, Tang, Gong, Liu, & Chen, 2017). Similarly, adhesion molecules, which mediate the binding of cells in the immune system (Murphy et al., 2009), have been measured in one study in a sample of people with AN (Víctor et al., 2015). Circulating concentrations of vascular cell adhesion molecule (VCAM)-1 were reported to be elevated in people with AN compared to healthy participants, but intercellular adhesion molecule (ICAM)-1 did not differ between the groups (Víctor et al., 2015). These additional subcategories of inflammatory markers have been insufficiently researched in AN.

As described in Chapter 1, cytokines and chemokines impact several biological domains implicated in the pathophysiology of AN, including the modulation of neurotransmitter systems, neuroendocrine functioning, and neural plasticity (Capuron & Miller, 2011; Himmerich & Treasure, 2018; Jeon & Kim, 2016; Kowalska, Karczewska-Kupczewska, & Straczkowski, 2011; Stuart, Singhal, & Baune, 2015). In the depression literature, it has been hypothesised that elevated pro-inflammatory cytokine concentrations may lead to symptoms of depression, partly via their disruption of growth factor production, e.g., brain-derived neurotropic factor (BDNF; Tong et al., 2012) and vascular endothelial growth factor (VEGF; Licht & Keshet, 2013), which has a subsequent effect on adult neurogenesis (Calabrese et al., 2014; Capuron & Miller, 2011). Disruption to these biological processes can then lead to alterations in mental state, including affect, learning and memory, and behaviour (e.g., depressive-like behaviours; Capuron & Miller, 2011; Donzis & Tronson, 2014).

EDs have both a behavioural (e.g., excessive exercise, self-induced vomiting, etc.) and cognitive/attitudinal (e.g., body image disturbances) component. However, only a few studies have assessed psychological aspects of EDs, as measured via self-report questionnaires, in relation to cytokines (Monteleone et al., 1999; Nakai et al., 2000). Rather, most studies have focussed on the association between physiological measures such as body mass index (BMI) and cytokine concentrations (e.g., Allende et al., 1998; Karczewska-Kupczewska et al., 2013). This is despite the established effect of cytokines on mental state and psychological health (Capuron & Miller, 2011), as mentioned above. There is also recent research showing a positive correlation between IL-6 serum concentrations and ED symptoms, both behavioural and cognitive, in college students (Lofrano-Prado et al., 2017). Relatedly, research in this area has not routinely considered the relationship between clinical characteristics (e.g., illness duration, age of onset, AN subtype) and cytokine concentrations. Of the few studies that have assessed this, no correlations between these parameters and circulating pro-inflammatory cytokines were

identified (e.g., Agnello et al., 2012; Brambilla et al., 2001; MacDowell et al., 2013). There is some literature in other psychiatric disorders, such as depression, schizophrenia, and post-traumatic stress disorder (PTSD), reporting an association between psychological symptom severity and/or other clinical characteristics with cytokine concentrations (Dunjic-Kostic et al., 2013a; Dunjic-Kostic et al., 2013b; Gill et al., 2008). However, more studies in AN are needed to examine the relationship between inflammatory markers and cognitive aspects and/or clinical characteristics of EDs.

In line with this, only one previous study has assessed the potential association between depression symptoms and cytokine concentrations in EDs (Ahrén-Moonga et al., 2011), finding that cytokine concentrations did not differ between depressed and non-depressed ED patients. A pro-inflammatory profile has been consistently identified in people with depression (Dowlati et al., 2010; Köhler et al., 2017) and the comorbidity between EDs and unipolar depression is of significant clinical relevance, as approximately 40% of people receiving treatment for AN also suffer from depression (Ulfvebrand et al., 2015). Therefore, given the lack of cytokine research in EDs considering depressive symptoms, it is unclear as to whether the alterations observed in cytokine concentrations in AN are due to the ED or symptoms of comorbid disorders, such as depression.

A variety of human biobehavioural factors, including BMI, age, psychoactive medication, and smoking status, can affect the measurement of immune system parameters, including concentrations of cytokines and other inflammatory markers (Dugué et al., 1996; Himmerich et al., 2019d; Kiecolt-Glaser & Glaser, 1988; O'Connor et al., 2009). These may be potential confounding factors in studies of the role of inflammatory markers in EDs, particularly given the low weight seen in AN, the tendency for cytokine research in EDs to focus on adolescents and young adults (Chapter 2; Dalton et al., 2018a), research indicating that people with EDs report higher rates of smoking than healthy individuals (Anzengruber et al., 2006), and the prescription of antidepressant and antipsychotic medication to patients with EDs (Davis & Attia, 2017). The review of studies in Chapter 2 demonstrates that these factors have often not been considered in previous research.

Taken together, few studies have considered a broad range of cytokines and other markers involved in inflammatory processes and their potential role in the biological profile of AN, nor have they considered the potential influence of additional psychosocial factors or illness severity and comorbidities on inflammatory marker measurement in AN. Therefore, the current chapter aims to address these shortcomings in two cross-sectional studies.

3.3 Study 1: An exploratory investigation of inflammatory markers in anorexia nervosa

The measurement of inflammatory markers in AN has been limited to a few pro-inflammatory cytokines (Chapter 2; Dalton et al., 2018a) and thus, research on other inflammatory markers in AN is lacking. Investigation of a panel of relevant cytokines and other inflammatory markers, rather than a focus on individual cytokines, may provide us with a more comprehensive indication of the state of the immune system in AN (Goldsmith et al., 2016). Indeed, measuring a broad range of inflammatory markers gives the opportunity for the detection of novel associations between cytokines and other immune markers with AN. It may also allow for the detection of patterns in alterations across inflammatory markers; for example, consistent under- or over-expression of pro- versus anti-inflammatory cytokines.

This exploratory cross-sectional study (Dalton et al., 2018b as shown in Appendix A Section 10.1.2) was the first to simultaneously assess a broad range of inflammatory markers in serum samples from AN and healthy control (HC) participants. The majority of these inflammatory markers have not been previously quantified in people with AN. Unlike much previous cytokine research in AN, this study also explored the association between current symptom severity and other clinical characteristics with markers of inflammation in people with AN.

3.3.1 Methods

3.3.1.1 Study design

The Relationship between Overactivity, Stress and Anxiety in Anorexia Nervosa (ROSANA) study (Keyes et al., 2015; U. Schmidt et al., 2017) was an observational longitudinal case-control study designed to identify psychological, physiological, endocrine and epigenetic factors that contribute to overactivity and the drive to increase activity in patients with AN. Four groups of women were recruited: AN outpatients, AN inpatients, individuals experiencing moderate anxiety, and HCs. Participants were enrolled in the study for a total of six months. They first completed a baseline assessment, in which BMI, ED and general psychopathology symptoms, body composition, and physical activity were assessed, and a blood sample was collected. Process measures were then obtained weekly for the first eight weeks, including activity and sleep diaries, and BMI. The baseline assessment described above was then repeated after three months and six months. For the purpose of the current study, data from the AN (both inpatients and outpatients) and HC participants at the baseline assessment were reported.

3.3.1.2 Participants

Between 2010 and 2013, 55 participants with AN (outpatients *n*=27; day-patients *n*=10; inpatients *n*=18) and 30 HCs were recruited (Keyes et al., 2015; Schmidt et al., 2017). Female adults with a primary diagnosis of AN (restricting or binge-eating/purging type) and a BMI <17.5 kg/m^2 were recruited within the first four weeks of treatment for AN via Specialist Eating Disorder Services in and around London. HCs were recruited via an email circular to students and staff at King's College London (KCL). Exclusion criteria for HCs were a history of or current mental health disorder, including EDs, and the presence of physical illness, which were assessed in the initial research session using a specially designed record form and the research version of the Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorders (DSM)-IV Axis I Disorders (First, Spitzer, Gibbon, & Williams, 2002), described below. All participants provided informed consent before study participation.

3.3.1.3 Ethical approval

The study was conducted in accordance with the Declaration of Helsinki and the study received ethical approval from the South East London Research Ethics Committee (REC reference: 09/H0807/4).

3.3.1.4 Measures

A number of additional measures related to physical activity were collected (for related findings see: Keyes et al., 2015; Schmidt et al., 2017); however, only the measures used in the current study are described below.

3.3.1.4.1 Demographic characteristics and screening

A specially designed record form was used to collect demographic data and additional information relating to the inclusion and exclusion criteria described above e.g., the presence of medical conditions. To determine smoking status, participants were asked if they smoked and if so, to report the number of cigarettes smoked per day. For AN participants, illness duration (in this case, time since diagnosis) was also recorded. All participants completed the research version of the Structured Clinical Interview for DSM-IV Axis I Disorders (First et al., 2002) to confirm diagnosis in the AN participants and to identify a history of and/or current mental health problems in the HCs. This assessment tool is widely used in both research and clinical settings and has moderate to excellent inter-rater reliability (Kübler, 2013; Lobbestael, Leurgans, & Arntz, 2011).

3.3.1.4.2 Anthropometry

Height and body weight were measured, and from these measurements, BMI (kg/m^2) was calculated. Body composition was measured using a portable and non-invasive Inbody S10 machine (Inbody Co., Ltd., Seoul, Korea), which uses the Bioelectrical Impedance Analysis (BIA) measurement method. This is a commonly used method for estimating body composition and following the input of height and weight details, this machine provides data on muscle and fat, bone mineral content, intracellular and extracellular water, protein, and minerals. In BIA, a weak electric current is passed through the body via electrodes placed on the ankles and hands of the participant. The voltage is then measured in order to calculate the impedance (resistance) of the body. Resistance is low in lean tissue (as it contains the majority of intracellular and extracellular fluid and is therefore a good conductor of electrical current), and fat mass is high in resistance as it is does not contain any water (and thus is not a conductor of electrical current). Based on the assumption that impedance (resistance) is proportional to total body water and that the body is a cylindrical-shaped conductor, calculations are used to determine fat free mass. The fat mass is indirectly measured by subtracting the fat free mass from the total weight. Given that adipose tissue has been implicated in the genesis of cytokines and produces certain pro-inflammatory cytokines (e.g., IL-6), we focussed on the association between inflammatory markers and body fat percentage and did not include other body composition parameters in our analyses.

3.3.1.4.3 Eating disorder behaviours

ED symptoms were assessed using the Eating Disorder Examination-Questionnaire (EDE-Q; Fairburn, 2008). This questionnaire has 36 items assessing ED symptoms and behaviours over the previous 28 days. A global score can be calculated to give an overall indication of level of symptomology. Items can also be categorised and scored into the following four subscales: restraint, eating concern, weight concern, and shape concern. Higher scores indicate greater ED symptom severity and the commonly used clinical cut-off score for the EDE-Q is four (e.g., Luce, Crowther, & Pole, 2008; Mond, Hay, Rodgers, & Owen, 2006).

The EDE-Q has been reported to have adequate to good criterion, concurrent, convergent and divergent validity (Aardoom, Dingemans, Slof Op't Landt, & Van Furth, 2012; Berg, Peterson, Frazier, & Crow, 2012; Mond, Hay, Rodgers, Owen, & Beumont, 2004) and adequate to excellent internal consistency (Luce & Crowther, 1999; Rose, Vaewsorn, Rosselli-Navarra, Wilson, & Weissman, 2013). However, test-retest reliability varies on the basis of ED symptoms, with strong test-retest reliability for attitudinal features of EDs, but

lower test-retest reliability for behavioural symptoms (Luce & Crowther, 1999; Rose et al., 2013). The original, theoretically-derived factor structure of the EDE-Q, described above, has received limited empirical support and research has proposed several alternatives (e.g., Carey et al., 2019; Rand-Giovannetti, Cicero, Mond, & Latner, 2017). Despite these shortcomings, the EDE-Q is widely used and is the only outcome tool recommended for the assessment of EDs by the National Institute for Mental Health in England (2008).

3.3.1.4.4 General psychopathology

Related psychopathology was assessed using the Depression Anxiety and Stress Scales – 21 Version (DASS-21; Lovibond & Lovibond, 1995). This is a 21-item questionnaire measuring symptoms of general psychopathology over the previous seven days. Respondents rate the extent to which each item has applied to them over the past week on a scale of 0 ("Did not apply to me at all") to 3 ("Applied to me most of the time"). As well as a total score, a score for three subscales—depression, anxiety, and stress—can be calculated. Cut-off scores have been proposed to indicate the level of severity of depression, anxiety, and stress (Lovibond & Lovibond, 1995). Higher scores are indicative of more severe symptoms. The DASS-21 is widely used to screen for symptoms of depression, anxiety, and stress at varying levels of severity and has been reported to have acceptable to excellent internal consistency and concurrent and divergent validity in clinical and non-clinical samples (Antony, Bieling, Cox, Enns, & Swinson, 1998).

3.3.1.5 Quantification of inflammatory markers

Blood samples were collected, and serum was separated by centrifugation. Serum was stored at -80°C prior to use and was thawed at room temperature, with 0.15ml of thawed serum being used for the current analyses. The concentrations of 42 inflammatory markers were quantified simultaneously using multiplex enzyme-linked immunosorbent assay (ELISA)-based technology provided by the Meso Scale Discovery V-PLEX Human Biomarker 40-Plex Kit and a customised human duplex kit assaying BDNF and interferon (IFN)-α, following the manufacturer's instructions (Meso Scale Diagnostics, LLC., Rockville, MD, USA). The methodological principles of this electro-chemiluminescence V-PLEX immunoassay provided by Meso Scale Discovery which was used in several investigations in this thesis (V-PLEX Human Cytokine 40-Plex Kit: Study 1 in Chapter 3 and Chapter 4, and V-plex Human Biomarker 36-Plex Kit: Study 2 in Chapter 3, Study 1 and Study 3 in Chapter 5, and Study 2 in Chapter 6) will be briefly described. These assays are validated sandwich immunoassays. Plates are pre-coated with capture antibodies on independent well-defined spots. The sample and a detection antibody solution conjugated with electrochemiluminescent labels are added to the plate over the course of one or more

incubation periods. Analytes in the sample bind to capture antibodies immobilised on the working electrode surface. Recruitment of the detection antibodies by the bound analytes completes the sandwich. A buffer is then added, which creates the appropriate chemical environment for electrochemiluminescence. The plate is loaded into a Meso Scale Discovery instrument, which applies a voltage to the plate electrodes causing the captured labels to emit light. The instrument then measures the intensity of emitted light, which is proportional to the amount of analyte present in the sample. This provides a quantitative measure (pg/ml) of each analyte in the sample. Research has indicated that these assays have high sensitivity and has suggested that they have an overall better performance than other technologies (e.g., Dabitao, Margolick, Lopez, & Bream, 2011).

Seven-point standard curves were run in duplicate on each plate in order to calculate absolute pg/ml values for inflammatory markers in the samples assayed. Cases and controls were randomised across batches, and plates were scanned on the Meso Scale Discovery MESO Quickplex SQ 120 reader at the Social Genetic and Developmental Psychiatry (SGDP) Centre, Institute of Psychiatry, Psychology & Neuroscience (IoPPN), KCL. The inflammatory markers measured in the 40-plex array were: basic fibroblast growth factor (bFGF), C-reactive protein (CRP), Eotaxin, Eotaxin-3, Fms-like tyrosine kinase (Flt)-1, granulocyte-macrophage colony-stimulating factor (GM-CSF), ICAM-1, IFN-γ, IL-1α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12/IL-23p40, IL-12p70, IL-13, IL-15, IL-16, IL-17A, interferon γ-induced protein (IP)-10, MCP-1, MCP-4, macrophage inflammatory protein (MIP)-1α, MIP-1β, placental growth factor (PlGF), serum amyloid A (SAA), thymus and activation-regulated chemokine (TARC), tyrosine kinase (Tie)-2, TNF-α, TNF-β, VCAM-1, VEGF, VEGF-C, and VEGF-D. The assay for macrophage-derived chemokine (MDC) was not included in the current panel due to quality control issues. Measurement of IL-8 was duplicated across two plates; therefore, statistical analyses were performed on the duplicate with the lowest proportion of samples with undetectable cytokine concentrations.

3.3.1.6 Statistical analysis

Thirty-two AN and 14 HC participants had available blood samples. One HC participant was excluded from the analyses due to having a BMI below 18.5 kg/m^2, i.e., in the underweight range. Five AN participants were excluded from analyses due to reported autoimmune and/or inflammatory diseases. Therefore, the current cross-sectional analyses were based on a sample consisting of 27 participants with a diagnosis of AN and 13 HCs.

All statistical analyses were performed in Stata 15 (StataCorp, 2017). Standard curves were used to determine absolute quantities (pg/ml) of each inflammatory marker. Cytokines where greater than 30% of the data was missing were excluded from analyses as this would reduce the sample size significantly and it indicates that these parameters may not be useful as biomarkers.

3.3.1.6.1 Cross-sectional comparisons

For demographics and clinical characteristics, group comparisons were assessed using t-tests or Mann-Whitney U-tests depending on the distribution of the data. The level of significance was set at $p < 0.05$.

Due to the presence of outliers and non-normal distributions, Mann-Whitney U tests were employed to compare concentrations of inflammatory markers between the AN and HC groups. The false discovery rate (FDR) of 0.1, using the Benjamini-Hochberg procedure, was used to adjust for multiple testing.

3.3.1.6.2 Exploratory correlational analyses

Associations between clinical characteristics (BMI, body fat percentage, EDE-Q total score, DASS-21 total score, illness duration) and inflammatory marker concentrations were assessed using Kendall's tau-b (τb) correlation coefficient. The association between illness duration and concentrations of inflammatory markers was assessed only in the AN participants. The Benjamini-Hochberg procedure with an FDR of 0.1 was applied separately to analyses for each clinical characteristic as a correction for multiple testing of inflammatory markers.

3.3.2 Results

3.3.2.1 Participant characteristics

Demographic, anthropometric, and clinical characteristics of the AN participants and HCs are presented in **Table 3.1**. All participants were female. Mean age did not significantly differ between the AN and HC groups ($U = 144$, $z = -1.36$, $p = 0.1735$). Seven participants with AN reported being a current smoker. As expected, AN participants had lower BMI ($t(38) = 7.88$, $p < 0.0001$) and percentage body fat ($U = -22$, $z = 3.63$, $p = 0.0003$), and higher EDE-Q scores (global score: $U = 85.5$, $z = -4.87$, $p < 0.0001$) than HCs. The EDE-Q global score for the AN participants was greater than the commonly used clinical cut-off score of four (e.g., Luce et al., 2008; Mond et al., 2006). AN participants also reported greater depression, anxiety, and stress than HCs on the DASS-21 (total score: $U = 92.5$, $z = -4.67$, $p < 0.0001$). Proposed cut-off scores (Lovibond & Lovibond, 1995) suggest that the level of severity that AN participants reported was severe for depression, anxiety, and stress.

Table 3.1 Demographic, anthropometric, and clinical characteristics for anorexia nervosa participants and healthy controls.

Demographic, anthropometric, and clinical characteristics	Healthy controls (*n*=13)	Anorexia nervosa (*n*=27)	*p*
Demographics			
Age [years] (mean ± SD)	25.54 ± 4.52	31.48 ± 11.40	0.1735
Current smoker (*n*)	0[a]	7	
Anthropometrics			
BMI [kg/m^2] (mean ± SD)	20.88 ± 1.68	15.33 ± 2.25	**<0.0001**
Body fat [%] (mean ± SD)	17.08 ± 6.05[b]	7.76 ± 6.07	**0.0003**
Clinical characteristics			
Duration of diagnosis [years] (mean ± SD)		11.64 ± 11.54[c]	
AN-R/AN-BP (*n*)		12/15	
Current outpatient/inpatient (*n*)		16/11	
EDE-Q Global (mean ± SD)	0.66 ± 0.70	4.20 ± 1.27	**<0.0001**
EDE-Q Restraint (mean ± SD)	0.62 ± 0.88	4.04 ± 1.77	**<0.0001**
EDE-Q Eating Concern (mean ± SD)	0.26 ± 0.48	3.82 ± 1.28	**<0.0001**
EDE-Q Weight Concern (mean ± SD)	0.74 ± 0.83	4.13 ± 1.59	**<0.0001**
EDE-Q Shape Concern (mean ± SD)	1.02 ± 0.85	4.82 ± 1.24	**<0.0001**
DASS-21 Total (mean ± SD)	13.85 ± 13.89	72.30 ± 33.32	**<0.0001**
DASS-21 Depression (mean ± SD)	3.54 ± 5.43	24.59 ± 13.73	**<0.0001**
DASS-21 Anxiety (mean ± SD)	3.08 ± 4.94	19.48 ± 11.84	**0.0001**
DASS-21 Stress (mean ± SD)	7.23 ± 5.20	28.22 ± 10.69	**<0.0001**

[a] *n*=6 missing; [b] *n*=1 missing; [c] *n*=3 missing. Results in bold text indicate statistically significant group differences at $p < 0.05$. Abbreviations: *n* = number of observations; SD = standard deviation; BMI = body mass index; kg = kilogram; m = metre; AN-R = anorexia nervosa restricting type; AN-BP = anorexia nervosa binge-eating/purging type; EDE-Q = Eating Disorder Examination-Questionnaire; DASS-21 = Depression Anxiety and Stress Scales – 21 Version.

3.3.2.2 Inflammatory markers

Three cytokines (IFN-α, IL-2, IL-13) were found to have greater than 30% missing data (i.e., greater than 30% of the sample had undetectable concentrations of the cytokine; see **Table 3.2**) and were excluded from statistical analyses. Known quantities within the standard curves correlated highly with quantities predicted by fluorescence intensity ($r^2 > 0.99$; except for IP-10 on both plates [$r^2 > 0.97$] and Tie-2 on the second plate [$r^2 = 0.842$]).

Table 3.2 Cytokine measurement metrics in the whole sample.

Inflammatory marker	Undetectable[a] [*n* (%)]	Lowest detectable concentration (pg/ml)	Highest detectable concentration (pg/ml)
Lowly expressed inflammatory markers (<1 pg/ml)			
GM-CSF	5 (12.50)	0.112	1130
IFN-α	37 (92.50)	1.04	2500
IL-1β	10 (25.00)	0.0335	575
IL-2	21 (52.50)	0.0578	1530
IL-4	2 (5.00)	0.0263	283
IL-6	0	0.0470	765
IL-10	0	0.0390	374
IL-12p70	4 (10.00)	0.0824	501
TNF-β	0	0.0855	732
Low to moderately expressed inflammatory markers (1-20 pg/ml)			
bFGF	0	0.106	1960
Eotaxin-3	0	0.974	7520
IFN-γ	0	0.392	1400
IL-1α	0	0.0851	487
IL-5	4 (10.00)	0.284	833
IL-7	0	0.124	972
IL-13	14 (35.00)	1.09	496
IL-15	0	0.162	796
IL-17A	0	0.337	6560
PlGF	0	0.191	1220
TNF-α	0	0.0813	368
Moderate to highly expressed inflammatory markers (21-400 pg/ml)			
Eotaxin	0	4.26	1820
Flt-1	0	1.49	8250
IL-8	0	0.0495	599
IL-12/IL-23p40	0	0.310	3710
IL-16	0	0.376	3400
IP-10	0	0.0844	2640
MCP-1	0	0.0402	530
MCP-4	0	2.04	729
MIP-1α	0	3.87	1110
MIP-1β	0	0.368	1120
TARC	0	0.129	1900

Inflammatory marker	Undetectable[a] [*n* (%)]	Lowest detectable concentration (pg/ml)	Highest detectable concentration (pg/ml)
VEGF	0	0.638	1750
Highly expressed inflammatory markers (401-25,000 pg/ml)			
BDNF	0	19.4	100000
Tie-2	0	43.1	82400
VEGF-C	0	12.4	21800
VEGF-D	0	4.56	24200
Very highly expressed inflammatory markers (25,001- 100,000,000 pg/ml)			
CRP	0	2.89	216000
ICAM-1	0	1.55	53900
SAA	0	12.4	222000
VCAM-1	0	7.09	56800

[a]Below fit curve range. Abbreviations: *n* = number of observations; pg = picogram; ml = millilitre; GM-CSF = granulocyte-macrophage colony-stimulating factor; IFN = interferon; IL = interleukin; TNF = tumor necrosis factor; bFGF = basic fibroblast growth factor; PlGF = placental growth factor; Flt-1 = Fms-like tyrosine kinase-1; IP = interferon γ-induced protein; MCP = monocyte chemoattractant protein; MIP = macrophage inflammatory protein; TARC = thymus and activation-regulated chemokine; VEGF = vascular endothelial growth factor; BDNF = brain-derived neurotrophic factor; Tie-2 = tyrosine kinase-2; CRP = C-reactive protein; ICAM-1 = intercellular adhesion molecule-1; SAA = serum amyloid A; VCAM-1 = vascular cell adhesion molecule-1.

3.3.2.2.1 Cross-sectional comparisons of inflammatory markers

The median concentrations (pg/ml) of the quantified inflammatory markers, along with the interquartile range, for HCs and AN participants are shown in **Table 3.3**. The identified cross-sectional group differences in cytokine concentrations did not meet formal significance thresholds (i.e., with correction for multiple comparisons), therefore, nominally significant findings (i.e., without correction for multiple testing) are presented. Median concentrations of IL-6 ($U = 92$, $z = -2.41$, $p = 0.016$), IL-15 ($U = 85$, $z = -2.61$, $p = 0.009$), and VCAM-1 ($U = 106$, $z = -2.01$, $p = 0.032$) were found to be higher in AN participants compared to HCs (see **Figure 3.1**). Median serum concentrations of BDNF ($U = 101$, $z = 2.15$, $p = 0.0320$), TNF-β ($U = 76$, $z = 2.87$, $p = 0.004$) and VEGF ($U = 102$, $z = 2.12$, $p = 0.030$) were lower in AN participants than in HCs (see **Figure 3.2**). No other inflammatory parameters were found to differ between groups.

Table 3.3 Median serum concentrations (pg/ml, with interquartile range) of inflammatory markers for healthy control and anorexia nervosa participants, with significance value of group comparison.

Inflammatory marker	**Healthy controls**		**Anorexia nervosa**		***p***
	n	Median (IQR[a])	*n*	Median (IQR[a])	
a. Concentrations nominally significantly lower in AN compared to HCs					
BDNF	13	17375.24 (12018.46, 19881.18)	27	12799.75 (10123.93, 16071.80)	0.0315
TNF-β	13	0.86 (0.71, 1.03)	27	0.60 (0.49, 0.69)	0.0041
VEGF	13	471.96 (381.18, 601.94)	27	288.82 (245.54, 370.95)	0.0338
b. Concentrations nominally significantly higher in AN compared to HCs					
IL-6	13	0.38 (0.15, 0.44)	27	0.49 (0.35, 1.25)	0.0159
IL-15	13	2.54 (2.28, 2.67)	27	2.90 (2.70, 3.51)	0.0090
VCAM-1	13	612378.30 (554193.80, 646531.70)	27	709059.60 (580691.20, 801523.30)	0.0448
c. Concentrations not significantly different between groups					
bFGF	13	11.01 (6.56, 17.29)	27	12.13 (8.08, 21.47)	0.3334
CRP	13	332422.10 (160182.60, 1657423.00)	27	342975.80 (94006.89, 5502771.00)	0.9424
Eotaxin	13	208.55 (160.77, 222.58)	27	175.47 (155.93, 279.31)	0.8511
Eotaxin-3	13	20.55 (17.35, 23.83)	27	15.15 (11.36, 25.58)	0.1058
Flt-1	13	61.83 (55.00, 66.64)	27	68.69 (51.53, 79.91)	0.1224
GM-CSF	13	0.15 (0.11, 0.32)	22	0.19 (0.12, 0.29)	0.8779
ICAM-1	13	658988.00 (567918.10, 751345.20)	27	701224.80 (620767.10, 864811.80)	0.1224
IFN-γ	13	3.94 (2.96, 4.60)	27	4.64 (2.89, 8.61)	0.1448

Inflammatory marker	Healthy controls		Anorexia nervosa		*p*
	n	Median (IQR[a])	*n*	Median (IQR[a])	
IL-1α	13	1.13 (0.65, 1.97)	27	1.01 (0.65, 1.29)	0.4105
IL-1β	9	0.20 (0.05, 0.38)	21	0.19 (0.07, 0.25)	0.7343
IL-4	13	0.08 (0.04, 0.11)	25	0.05 (0.3, 0.08)	0.1481
IL-5	12	1.09 (0.71, 1.58)	24	1.29 (0.72, 1.72)	0.7626
IL-7	13	13.66 (10.20, 16.31)	27	12.58 (10.46, 17.27)	0.8966
IL-8	13	33.51 (14.00, 76.10)	27	23.09 (10.27, 103.21)	0.4271
IL-10	13	0.18 (0.15, 0.25)	27	0.28 (0.11, 0.37)	0.3120
IL-12/IL-23p40	13	117.69 (88.80, 138.62)	27	92.00 (68.06, 118.65)	0.0806
IL-12p70	11	0.18 (0.12, 0.21)	25	0.19 (0.11, 0.36)	0.7572
IL-16	13	160.52 (140.62, 199.51)	27	183.59 (145.14, 339.36)	0.1702
IL-17A	13	1.78 (1.26, 3.20)	27	1.91 (1.08, 2.59)	0.9539
IP-10	13	110.93 (91.53, 149.22)	27	115.99 (98.45, 207.33)	0.3191
MCP-1	13	208.55 (165.87, 292.65)	27	191.53 (164.41, 241.73)	0.3480
MCP-4	13	142.79 (93.31, 173.56)	27	120.65 (86.10, 169.37)	0.6133
MIP-1α	13	25.01 (21.50, 29.23)	27	23.02 (20.10, 35.20)	0.8624
MIP-1β	13	102.15 (69.16, 131.99)	27	81.06 (65.78, 110.10)	0.3334
PlGF	13	3.38 (2.96, 4.53)	27	3.85 (3.07, 4.89)	0.6754
SAA	13	2683488.00 (2071528.00, 3290097.00)	27	2681116.00 (907155.40, 10400000.00)	0.8061
TARC	13	365.16 (227.07, 546.58)	27	370.33 (263.01, 641.46)	0.7617

Inflammatory marker	Healthy controls		Anorexia nervosa		*p*
	n	Median (IQR[a])	*n*	Median (IQR[a])	
Tie-2	13	5847.41 (4869.40, 7654.15)	27	6212.88 (4839.31, 7868.05)	0.7617
TNF-α	13	1.59 (1.43, 1.93)	27	1.64 (1.34, 2.42)	0.8966
VEGF-C	13	574.92 (480.12, 599.83)	27	449.26 (387.74, 630.84)	0.2919
VEGF-D	13	790.06 (589.67, 947.75)	27	757.22 (587.05, 1,000.25)	0.8061

[a]25th and 75th percentile reported. Abbreviations: IQR = interquartile range; AN = anorexia nervosa; HC = healthy control; BDNF = brain-derived neurotrophic factor; TNF = tumor necrosis factor; VEGF = vascular endothelial growth factor; IL = interleukin; VCAM-1 = vascular cell adhesion molecule-1; bFGF = basic fibroblast growth factor; CRP = C-reactive protein; Flt-1 = Fms-like tyrosine kinase-1; GM-CSF = granulocyte-macrophage colony-stimulating factor; ICAM-1 = intercellular adhesion molecule-1, IFN-γ = interferon- γ; IP-10 = interferon γ-induced protein-10; MCP = monocyte chemoattractant protein; MIP = macrophage inflammatory protein; PlGF = placental growth factor; SAA = serum amyloid A; TARC = thymus and activation-regulated chemokine; Tie-2 = tyrosine kinase-2.

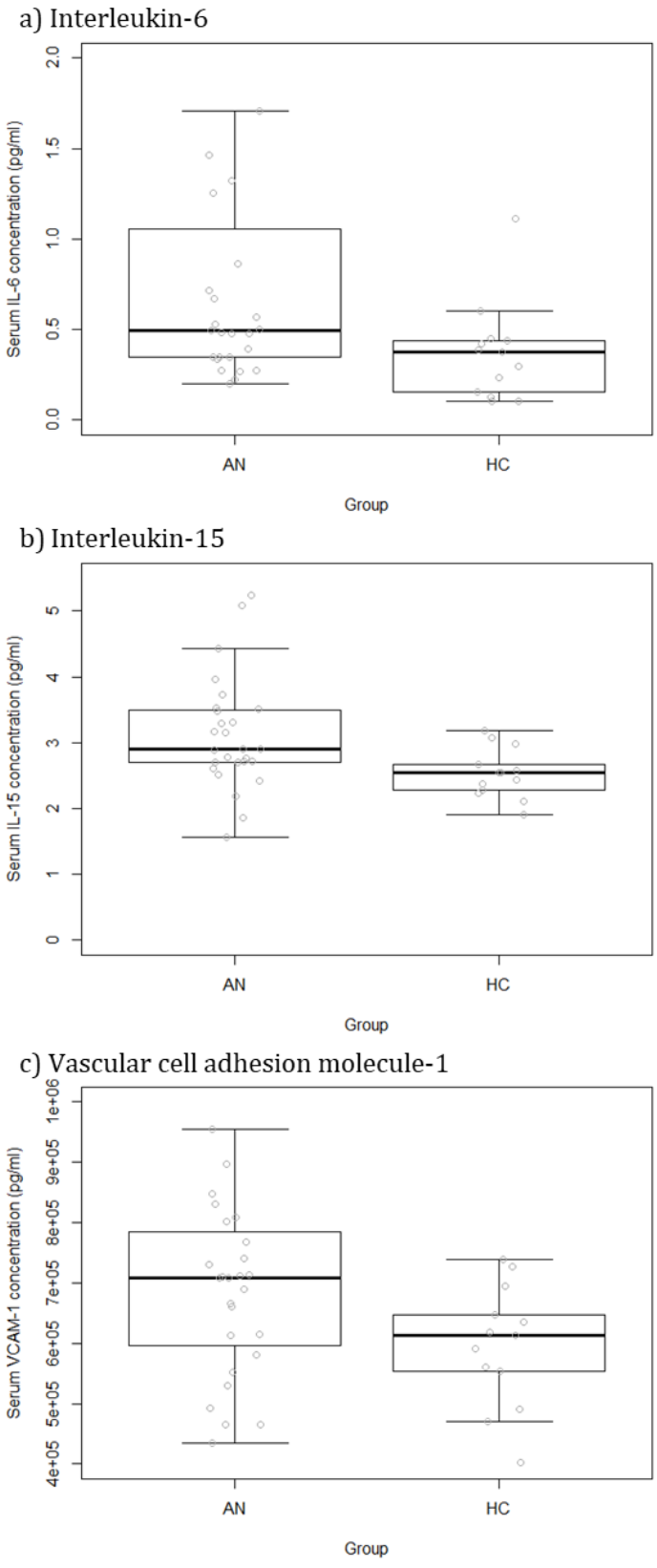

Figure 3.1 Box plots of inflammatory markers with significantly increased concentrations in anorexia nervosa compared to healthy controls. Individual data points are shown in grey. Abbreviations: AN = anorexia nervosa; HC = healthy controls; pg = picogram; ml = millilitre; IL = interleukin; VCAM-1 = vascular cell adhesion molecule-1.

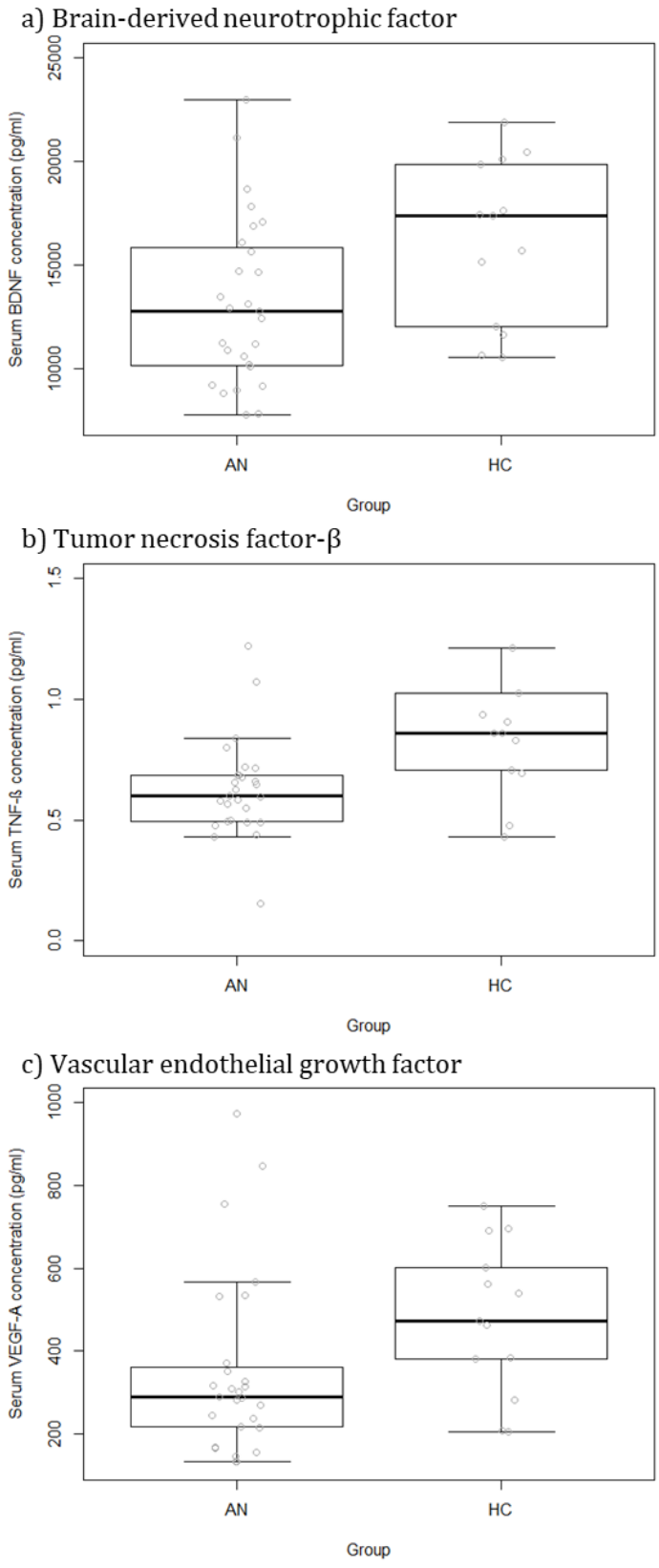

Figure 3.2 Box plots of inflammatory markers with significantly decreased concentrations in anorexia nervosa compared to healthy controls. Individual data points are shown in grey. Abbreviations: AN = anorexia nervosa; HC = healthy controls; pg = picogram; ml = millilitre; BDNF = brain-derived neurotrophic factor; TNF-β = tumor necrosis factor-β; VEGF = vascular endothelial growth factor.

3.3.2.2.2 Associations between clinical characteristics and inflammatory markers

Results of Kendall's tau-b correlations for the associations between clinical characteristics and concentrations of inflammatory markers are shown in **Table 3.4**. Following correction for multiple testing, BDNF, IL-6, IL-10, IL-12/IL-23p40, IL-15, and TNF-β were significantly correlated with BMI. BDNF and IL-12/IL-23p40 were positively correlated and IL-6, IL-10, IL-15, and TNF-β were negatively associated with BMI. BDNF, IL-12/IL-23p40 and TNF-β were also positively correlated with body fat percentage. After adjusting for multiple comparisons, no inflammatory markers were significantly associated with ED symptom severity (as measured by the EDE-Q global score), general psychopathology symptoms (as measured by the DASS-21 total score), or illness duration. Although, there were some nominally significant associations: ICAM-1, IL-16, and TNF-β with EDE-Q global score; Flt-1, ICAM-1, IL-16, and TNF-β with DASS-21 total score; and IL-2, MIP-1α, and VEGF with illness duration.

Table 3.4 Associations between clinical characteristics and concentrations of inflammatory markers.

Inflammatory marker	BMI			Body fat percentage			EDE-Q Global score			DASS-21 Total score			Illness duration		
	n	τb	*p*	*n*	τb	*p*	*n*	τb	*p*	*n*	τb	*p*	*n*	τb	*p*
BDNF	**40**	**0.34**	**0.0021**	**39**	**0.32**	**0.0053**	40	-0.12	0.2943	40	-0.15	0.1915	24	-0.09	0.5675
bFGF	40	-0.02	0.8888	39	0.09	0.4388	40	0.08	0.4558	40	0.17	0.1295	24	0.17	0.2634
CRP	40	0.02	0.8521	39	0.01	0.9119	40	-0.03	0.8157	40	-0.08	0.4988	24	-0.04	0.7845
Eotaxin	40	-0.04	0.7266	39	-0.02	0.8925	40	-0.01	0.9443	40	0.07	0.5598	24	-0.07	0.6367
Eotaxin-3	40	0.22	0.0475	39	0.20	0.0853	40	0.05	0.6919	40	0.03	0.7795	24	-0.05	0.7654
Flt-1	40	-0.07	0.5445	39	-0.10	0.3964	40	0.20	0.0691	40	0.26	0.0223	24	-0.11	0.4710
GM-CSF	35	0.06	0.6188	34	-0.19	0.1405	35	-0.04	0.7763	35	0.03	0.8200	19	-0.16	0.3613
ICAM-1	40	-0.08	0.4844	39	-0.14	0.2237	40	0.26	0.0198	40	0.22	0.0449	24	0.04	0.8230
IFN-γ	40	-0.22	0.0530	39	-0.11	0.3440	40	0.10	0.3513	40	0.05	0.6577	24	0.08	0.6017
IL-1α	40	0.12	0.2836	39	0.07	0.5635	40	-0.01	0.9443	40	-0.00	1.0000	24	-0.11	0.4710
IL-1β	30	-0.07	0.5925	30	-0.07	0.6230	30	-0.12	0.3819	30	-0.03	0.8442	20	0.19	0.2685
IL-4	38	0.20	0.0741	37	0.23	0.0595	38	-0.06	0.6149	38	-0.15	0.2036	23	-0.21	0.1768
IL-5	36	-0.14	0.2414	35	-0.09	0.4950	36	-0.05	0.7029	36	0.09	0.4615	22	0.11	0.4978
IL-6	**40**	**-0.30**	**0.0064**	39	-0.29	0.0110	40	0.15	0.1620	40	0.19	0.0844	24	0.22	0.1425
IL-7	40	0.08	0.4917	39	0.05	0.6760	40	-0.08	0.4772	40	-0.19	0.0908	24	-0.11	0.4863
IL-8	40	0.01	0.9443	39	0.05	0.6850	40	0.01	0.9257	40	0.15	0.1837	24	0.21	0.1566
IL-10	**40**	**-0.31**	**0.0050**	39	-0.19	0.1072	40	-0.04	0.7178	40	0.06	0.6159	24	0.21	0.1638
IL-12/IL-23p40	**40**	**0.31**	**0.0048**	**39**	**0.38**	**0.0010**	40	0.01	0.9257	40	-0.11	0.3389	24	-0.13	0.4121
IL-12p70	36	-0.02	0.8701	36	0.01	0.9448	36	0.07	0.5488	36	0.05	0.7127	22	0.13	0.4125
IL-15	**40**	**-0.30**	**0.0064**	39	-0.18	0.1129	40	0.13	0.2346	40	0.16	0.1548	24	-0.02	0.9406
IL-16	40	-0.11	0.3393	39	-0.02	0.8731	40	0.26	0.0211	40	0.27	0.0174	24	0.20	0.1877
IL-17A	40	0.05	0.6663	39	0.03	0.8058	40	0.07	0.5216	40	-0.02	0.8428	24	-0.19	0.2049

Inflammatory marker	BMI			Body fat percentage			EDE-Q Global score			DASS-21 Total score			Illness duration		
	n	τb	*p*	*n*	τb	*p*	*n*	τb	*p*	*n*	τb	*p*	*n*	τb	*p*
IP-10	40	-0.07	0.5445	39	-0.02	0.8731	40	0.00	1.0000	40	-0.06	0.6243	24	-0.15	0.3082
MCP-1	40	0.13	0.2632	39	0.04	0.2332	40	-0.02	0.8339	40	0.04	0.7440	24	-0.07	0.6726
MCP-4	40	0.15	0.1764	39	-0.01	0.9706	40	0.02	0.8704	40	0.02	0.8887	24	-0.09	0.5675
MIP-1α	40	0.07	0.5138	39	-0.01	0.9314	40	-0.05	0.6578	40	-0.08	0.4986	24	-0.30	0.0467
MIP-1β	40	0.19	0.0889	39	0.14	0.2430	40	0.04	0.7619	40	-0.01	0.9628	24	-0.10	0.5343
PlGF	40	-0.04	0.7619	39	-0.07	0.5308	40	-0.11	0.3277	40	-0.01	0.9072	24	0.00	1.0000
SAA	40	0.06	0.6245	39	0.05	0.7032	40	-0.05	0.6579	40	-0.07	0.5137	24	-0.24	0.1062
TARC	40	0.04	0.7092	39	0.13	0.2849	40	0.12	0.2837	40	0.11	0.3273	24	0.13	0.3843
Tie-2	40	-0.01	0.9628	39	-0.10	0.3964	40	0.08	0.4845	40	0.15	0.1761	24	0.02	0.9406
TNF-α	40	0.04	0.7179	39	0.06	0.6057	40	0.09	0.4350	40	0.09	0.4210	24	-0.04	0.8036
TNF-β	**40**	**0.39**	**0.0004**	**39**	**0.34**	**0.0032**	40	-0.26	0.0169	40	-0.31	0.0053	24	-0.04	0.8036
VCAM-1	40	-0.13	0.2632	39	-0.13	0.2634	40	0.19	0.0889	40	0.14	0.2079	24	-0.11	0.4710
VEGF	40	0.18	0.0980	39	0.07	0.5470	40	-0.19	0.0889	40	-0.18	0.1075	24	0.29	0.0496
VEGF-C	40	0.22	0.0502	39	0.26	0.0278	40	0.01	0.9443	40	-0.01	0.9628	24	0.11	0.4710
VEGF-D	40	-0.12	0.2942	39	-0.07	0.5635	40	0.02	0.8704	40	0.06	0.5917	24	0.23	0.1295

Results in bold text indicate formally statistically significant correlations (i.e., after correcting for multiple testing). Abbreviations: BMI = body mass index; EDE-Q = Eating Disorders Examination Questionnaire; DASS-21 = Depression Anxiety and Stress Scales – 21 Version; *n* = number of observations; τb = Kendall's tau-b; BDNF = brain-derived neurotrophic factor; bFGF = basic fibroblast growth factor; CRP = C-reactive protein; Flt-1 = Fms-like tyrosine kinase-1; GM-CSF = granulocyte-macrophage colony-stimulating factor; ICAM-1 = intercellular adhesion molecule-1, IFN-γ = interferon- γ; IL = interleukin; IP-10 = interferon γ-induced protein-10; MCP = monocyte chemoattractant protein; MIP = macrophage inflammatory protein; PlGF = placental growth factor; SAA = serum amyloid A; TARC = thymus and activation-regulated chemokine; Tie-2 = tyrosine kinase-2; TNF = tumor necrosis factor; VCAM-1 = vascular cell adhesion molecule-1; VEGF = vascular endothelial growth factor.

3.3.3 Discussion

We measured a range of markers involved in inflammatory processes, including cytokines, chemokines, acute-phase reactants, and cellular adhesion molecules, in people with AN and HCs. Cross-sectional group differences did not meet formal significance thresholds; however, several nominally significant group differences were observed. Median concentrations of BDNF, IL-6, IL-15, TNF-β, VCAM-1, and VEGF were found to differ between AN and HCs, with IL-6, IL-15, and VCAM-1 being elevated in AN, and BDNF, TNF-β, and VEGF being reduced, compared to HCs. No other inflammatory markers differed between AN participants and HCs.

To the best of our knowledge, this was the first time serum concentrations of IL-15, TNF-β, and VEGF have been measured and were found to be altered in AN patients. Therefore, the potential significance of these findings is discussed in more detail.

The finding of elevated IL-15 in AN, compared to HCs, has recently been reproduced in an adolescent sample (Roczniak et al., 2019). IL-15, a T cell growth factor, has been suggested to be involved in the modulation of serotonergic transmission (Pan et al., 2013; Wu et al., 2011b), which may underlie the depressive symptoms and sleep disturbances that are often present in people with AN, and fits with existing evidence of serotonergic alterations in AN (Gauthier et al., 2014; Kaye et al., 2005). Elevations of IL-15 concentrations have also been reported in patients with schizophrenia (de Witte et al., 2014). This is interesting in light of the possible genetic overlaps between AN and schizophrenia that have been identified in genome-wide association study (GWAS) data (Bulik-Sullivan et al., 2015). IL-15 has also been implicated in cross-talk between fat and muscle and reported to have an anabolic role, i.e., decreasing fat and increasing muscle mass (Pedersen, 2011). Indeed, systematic elevation of IL-15 has been reported to promote loss of adipose tissue, without affecting lean mass (Quinn, Anderson, Strait-Bodey, Stroud, & Argiles, 2009). It is unclear, therefore, why it is increased in the anorectic group given that they already show a severe loss of body fat. It may, for example, be raised as a way of trying to maintain muscle mass in the ill state. In support of this hypothesis, even with the loss of body fat, it has been shown that AN patients with a BMI greater than 16.5 kg/m^2 have comparable total body skeletal muscle and lean body mass to healthy women (El Ghoch et al., 2017).

TNF-β (also known as lymphotoxin-α) has several roles in immune regulation (Ruddle, 2014) and is involved in the regulation of cell survival, proliferation, differentiation, and apoptosis (Bauer et al., 2012). A TNF-β polymorphism (+252G/A) has been proposed to increase the risk of developing schizophrenia (Kadasah, Arfin, Rizvi, Al-Asmari, & Al-Asmari, 2017). TNF-β also has a role in maintaining lipid homeostasis, which is potentially important in AN. However, what is perhaps of most interest is that it is involved in

regulating intestinal microbiota (Kruglov et al., 2013; McCarthy et al., 2006; Upadhyay & Fu, 2013), especially as the gut microbiome has been implicated in the pathophysiology of AN (e.g., Borgo et al., 2017; as described in Section 1.3.2.2). It is unclear as to why this cytokine is reduced in AN as TNF-β is functionally pro-inflammatory. However, animal research found that TNF-β deficient mice were smaller and leaner than wild type counterparts following a high-fat/high-sucrose diet and that they were protected from diet-induced obesity (Pamir, McMillen, Edgel, Kim, & LeBoeuf, 2012). Similarly, in this sample, TNF-β was positively associated with BMI and body fat percentage, such that lower TNF-β was related to lower BMI and body fat. However, administration of TNF-β to rats has been shown to limit food intake (Kapas & Krueger, 1992). These results suggest that TNF-β does play some role in weight and food intake regulation; however, the limited and conflicting research in this area precludes further conclusions. Furthermore, research has suggested concentrations of TNF-β may be related to hormonal status: it has been shown that post-menopausal women, who have decreased levels of dehydroepiandrosterone and oestrogen (as seen in AN; Gordon et al., 2002; Misra & Klibanski, 2014), also have reduced concentrations of circulating TNF-β, compared to fertile women (Cioffi et al., 2002). Hormonal status in AN in relation to cytokines has only been researched in a single study, in which cytokine concentrations were undetectable (Gordon et al., 2002); therefore, further research is warranted.

VEGF induces angiogenesis, vasculogenesis, and endothelial cell growth, and also influences vascular permeability, similar to VCAM-1 (Ferrara, 2004). VEGF has been suggested to enhance adult neurogenesis and hippocampus-dependent learning and memory (Licht & Keshet, 2013), which may be important in both responsivity to illness and in relation to therapy. Altered concentrations of VEGF have also been found in patients with depression (Sharma, da Costa e Silva, Soares, Carvalho, & Quevedo, 2016) and schizophrenia (Frydecka et al., 2018).

Taken together, changes in IL-15, TNF-β, and VEGF could potentially contribute to the development of AN symptoms, such as low mood and disturbed sleep, as well as its clinical consequences, such as impaired learning and memory. Furthermore, they provide a link between the biological pathophysiology of AN with depression and schizophrenia, which are clinically- and genetically-related psychiatric disorders. This association may be suggestive of inflammatory markers being indicators of transdiagnostic symptoms, such as low mood, rather than specific psychiatric diagnoses. Also, such a biological association between these disorders could have clinical and therapeutic relevance. For example, we could consider the question as to whether antipsychotics, such as olanzapine, which is approved for the treatment of schizophrenia and alters cytokine production (Kluge et al.,

2009), might help patients with AN (Himmerich et al., 2017; Himmerich & Treasure, 2018). This may also be of particular interest in AN as olanzapine has been shown to alter the gut microbiome, which could additionally contribute to weight gain (Davey et al., 2013; Davey et al., 2012; Flowers, Evans, Ward, McInnis, & Ellingrod, 2017; Morgan et al., 2014).

The findings of reduced BDNF and increased IL-6 and VCAM-1 serum concentrations in our sample of patients with AN, compared to HCs, were of less novelty. However, they indicate the reliability of our findings, as these results have also been reported previously.

As described, there are already a number of studies that have consistently identified an association between AN and IL-6 (Chapter 2; Dalton et al., 2018a). IL-6 is a pleiotropic cytokine exerting anti- and pro-inflammatory effects and is an inducer of the acute-phase response (Opal & DePalo, 2000), which has been shown to have suppressive effects on food intake (Wong & Pinkney, 2004) and inhibit adipogenesis (Ohsumi et al., 1994). Our results replicated the findings of increased concentrations of IL-6 in people with AN, as compared to HCs. Although, in comparison to previous research in which elevated IL-6 concentrations were identified in obesity and normal-weight subjects (Park, Park, & Yu, 2005), a negative correlation between IL-6 concentrations and BMI was found. This suggests that the relationship between IL-6 and BMI is highly complex.

The results also replicate the findings of Víctor et al. (2015) who previously reported increased VCAM-1 serum concentrations in patients with AN. VCAM-1 is a cellular adhesion molecule with a key role in leukocyte recruitment from blood into tissue and is thus important for cellular immune response (Wittchen, 2009). Because of its wide distribution in human tissues and organs, VCAM-1 has been implicated in the development of a variety of pathophysiological states in the brain and in the body periphery, including autoimmune diseases, cardiovascular disease, and infections (Allavena, Noy, Andrews, & Pullen, 2010).

The reduced serum concentrations of BDNF in AN participants compared to HCs are consistent with previous findings (Brandys, Kas, van Elburg, Campbell, & Adan, 2011). BDNF is a neurotrophin implicated in both central and peripheral nervous system development. It is well-established that BDNF and cytokines cross-regulate each other. Certain pro-inflammatory cytokines can suppress the expression of BDNF (Calabrese et al., 2014) and BDNF-dependent synaptic plasticity (Tong et al., 2012). It is thought that the detrimental effect of pro-inflammatory cytokines on neuroplasticity may be mediated by BDNF (Calabrese et al., 2014). Taking together the evidence in AN of elevated pro-inflammatory cytokines (Chapter 2; Dalton et al., 2018a; Solmi et al., 2015), reduced concentrations of BDNF and VEGF (Brandys et al., 2011), and reduced hippocampal

volumes (Burkert, Koschutnig, Ebner, & Freidl, 2015; Connan et al., 2006), a key area for adult neurogenesis, it could be hypothesised that this mechanism may be at play in AN, as proposed in the depression literature (Audet & Anisman, 2013; Calabrese et al., 2014). This hypothesis would be consistent with the high prevalence (approximately 40%) of depression in patients with AN (Ulfvebrand et al., 2015).

BDNF and several cytokines (IL-6, IL-10, IL-12/IL-23p40, IL-15, and TNF-β) were significantly correlated with BMI. Specifically, BDNF and IL-12/IL-23p40 were positively associated with BMI, such that BMI increased as concentration of these inflammatory markers also increased; whereas IL-6, IL-10, IL-15, and TNF-β were negatively correlated with BMI, such that as BMI decreased, concentrations of these cytokines increased. BDNF, IL-12/IL-23p40 and TNF-β were also positively correlated with body fat percentage, in that increasing body fat percentage was associated with increases in concentrations of these inflammatory markers. There were no significant correlations between inflammatory markers and the remaining clinical characteristics (ED and general psychopathology symptoms, and illness duration) following correction for multiple comparisons. This suggests that cytokines may be more greatly associated with physiological, rather than psychological or clinical factors, and that the inflammatory marker alterations observed in AN may not be related to comorbid depression.

3.4 Study 2: A confirmatory investigation of cytokines in anorexia nervosa

In Study 1, we showed the value of measuring a broad range of cytokines, by identifying alterations in inflammatory markers that have not been previously measured in AN, specifically IL-15, TNF-β and VEGF (Dalton et al., 2018b). Study 2 (Cytokines in Anorexia Nervosa: CytAN) was designed as a confirmatory study to assess the reproducibility of the novel significant cytokine findings from Study 1 in a larger sample.

As well as assessing replicability, the current study aimed to continue to expand on previous data by assessing additional cytokines that have yet to be measured in AN. Thus, in this study a different assay kit was used that allowed us to explore a broader range of cytokines and chemokines, such as Th17 cytokines that have not been previously measured in AN, with less of a focus on cellular adhesion molecules and acute-phase proteins.

Chapter 2 emphasised the importance of potential confounding factors in the assessment of cytokines (O'Connor et al., 2009). Therefore, this study aimed to expand on previous research by including the potential confounding variables of age, ethnicity and smoking status in the statistical analyses, to determine the relationship between AN status and cytokines while controlling for these factors. This study also aimed to extend on the correlational analyses performed in Study 1 by conducting exploratory regression

analyses of the relationship between cytokines and clinical characteristics, while controlling for sociodemographic factors.

3.4.1 Methods

3.4.1.1 Participants

Participants were females over the age of 18 years with AN or no current/previous diagnosis of any psychiatric disorder (healthy comparison group). AN participants were recruited via Specialist Eating Disorder Services in South London and Maudsley NHS Foundation Trust, online and poster advertisements at KCL, the Beat research recruitment webpages, and through participation in other research projects. AN participants were required to have a current primary diagnosis of AN, according to the DSM-5 (American Psychiatric Association, 2013), and a BMI <18.5 kg/m^2. HCs were of healthy weight (BMI 18.5-24.5 kg/m^2) without a history of or current mental health condition, including EDs, and were recruited via an e-mail circular to students and staff at KCL and through online and poster advertisements. Exclusion criteria for all participants were current pregnancy and the presence of acute or chronic inflammatory conditions e.g., asthma, psoriasis, Crohn's Disease, inflammatory bowel disease, arthritis.

Group classification was established using self-report and checked via a telephone screening. The screening procedure was also used to determine eligibility against the other criteria described above. Screening questionnaires included the Eating Disorder Diagnostic Scale (EDDS; Stice, Telch, & Rizvi, 2000) to assess the presence of ED symptoms, and a brief inclusion/exclusion screen specific to this study, which included an assessment of physical health conditions (see Appendix F Section 10.6). HC participants additionally completed the research version of the Structured Clinical Interview for DSM-IV Axis I Disorders (First et al., 2002) to assess the presence of current or past psychiatric disorders. One hundred and five participants completed the screening questionnaire and 93 were eligible for inclusion at time of screening. Of these, 78 participants (40 AN and 38 HC) completed the study. All participants provided informed consent before study participation.

For the analyses planned in the current study, an *a priori* power analysis gave the total number of required participants as 33 (17 in each group). The power analysis was calculated using the following parameters: an ANCOVA of the *F*-test family, with an effect size of 0.78 (from IL-6 group difference in Study 1), a power of 0.80, an α level of 0.000138 to account for multiple comparisons of 36 cytokines, two groups and three covariates.

3.4.1.2 Ethical approval

The study was conducted in accordance with the Declaration of Helsinki and the study received ethical approval from the London - City & East Research Ethics Committee (REC reference: 17/LO/2107).

3.4.1.3 Procedure

Participants attended a single research session at the IoPPN, KCL lasting no longer than one and a half hours. In this session, participants first had their blood collected by trained phlebotomists from the National Institute of Health Research (NIHR) Bioresource Centre at the Maudsley. This blood collection included one 5ml serum tube. Participants then completed a purposely designed record form to collect demographic data, including ethnicity, smoking status, and current medication. Data on illness-related variables such as illness duration, lowest ever weight, and symptom-free periods, were also collected from AN participants. Participants then completed a questionnaire pack which included the Eating Disorder Examination-Questionnaire (EDEQ; Fairburn, 2008) to assess ED symptoms and behaviours, and the Depression Anxiety and Stress Scales – 21 Version (DASS-21; Lovibond & Lovibond, 1995), the Beck Depression Inventory (BDI)-II (Beck, Steer, & Brown, 1996), the Alcohol Use Disorders Identification Test (AUDIT; Babor, de la Fuente, Saunders, & Grant, 2001) and the Post-Traumatic Stress Disorder (PTSD) Checklist for DSM-5 (PCL-5; Weathers et al., 2013b) to assess other aspects of mental health. Questionnaires assessing diet (European Prospective Investigation of Cancer in Norfolk Food Frequency Questionnaire; Bingham et al., 1994; Bingham et al., 2001) and life events (revised Life Events Checklist for DSM-5; Weathers et al., 2013a) were also completed. Height and body weight were then measured, and from these measurements, BMI (kg/m^2) was calculated. Finally, body composition was assessed using a portable and non-invasive Inbody S10 machine, Biospace Co., Ltd (methods described previously in Section 3.3.1.4.2).

3.4.1.4 Measures

As described, participants completed a number of questionnaires to assess ED and general psychopathology symptoms. For the purpose of the current study, only data on the EDE-Q, DASS-21 and BDI-II were used. The EDE-Q and DASS-21 have been described in full in Section 3.3.1.4. Briefly, the EDE-Q was used to assess ED symptoms over the previous 28 days and the DASS-21 was used to assess symptoms of depression, anxiety and stress over the past 7 days.

The BDI-II (Beck et al., 1996) is a 21-item self-report questionnaire used to assess depressive symptoms. The items include affective, cognitive, somatic and vegetative symptoms that reflect the DSM-IV criteria (American Psychiatric Association, 2000) for major depression. Participants select one statement from each item that best describes the

way they have been feeling during the past two weeks. Items are rated on a 4-point scale ranging from 0 (symptom absent) to 3 (severe symptom) based on the severity of each item. Scores are calculated by adding together the highest ratings for all items, with a minimum score of 0 and a maximum score of 63. Higher scores indicate greater symptom severity. Cut-off scores have been reported to indicate the severity of depression symptoms, as follows: scores of 0–13 indicate minimal depression; 14–19, mild depression; 20–28, moderate depression; and 29–63, severe depression (Beck et al., 1996). The BDI-II is widely used as an indicator of the severity of depression and has been reported to be highly reliable and valid (Wang & Gorenstein, 2013). The BDI-II was used in the current study in addition to the DASS-21 to provide a more comprehensive assessment of depression symptom severity (the DASS-21 only includes seven items that assess depression symptoms).

3.4.1.5 Quantification of cytokine concentrations

Blood samples were collected, and processing of the blood samples was conducted by trained and well-experienced technical staff in the laboratory at the SGDP Centre. Serum was separated by centrifugation and stored at -80°C prior to use. All samples were anonymised and stored under secure conditions. Serum was thawed at room temperature for use. The concentrations of 36 cytokines were quantified simultaneously using multiplex ELISA-based technology provided by the Meso Scale Discovery V-PLEX Plus Human Biomarker 36-Plex Kit, following the manufacturer's instructions (Meso Scale Discovery, Maryland, USA). Seven-point standard curves were run in duplicate on each plate in order to calculate absolute pg/ml values of cytokines for the samples assayed. Cases and controls were randomised across the plate. The plate was scanned on the Meso Scale Discovery MESO Quickplex SQ 120 reader at the SGDP Centre, IoPPN, KCL. The cytokines measured in the assay were: Eotaxin, Eotaxin-3, GM-CSF, IFN-γ, IL-1α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-8 (HA), IL-10, IL-12/IL-23p40, IL-12p70, IL-13, IL-15, IL-16, IL-17A, IL-21, IL-22, IL-23, IL-27, IL-31, IP-10, MCP-1, MCP-4, MDC, MIP-1α, MIP-1β, MIP-3α, TARC, TNF-α, TNF-β, and VEGF. Measurement of IL-8 and IL-17A were duplicated across two plates. As such, analyses were performed on the duplicate with the lowest proportion of samples with undetectable cytokine concentrations.

3.4.1.6 Statistical analysis

Data from three HC participants were excluded from analyses due to n=2 having a BMI greater than 24.5 kg/m^2 and n=1 reporting use of psychoactive medication at the research session. Additionally, a blood sample could not be collected from one HC participant. Thus, the analyses used a final sample of 40 AN and 34 HC participants. Based on the *a priori*

power analysis described above, this study had a sufficient sample size for the planned analyses described below.

All statistical analyses were performed in Stata 15 (StataCorp, 2017). For non-normally distributed demographic and clinical characteristic data, median and interquartile ranges (25^{th} and 75^{th} percentile) are presented and cross-sectional comparisons were performed using Mann-Whitney U tests. For normally distributed demographic and clinical characteristic data, means and standard deviations are shown and between subjects t-tests were used to assess group differences. The chi-square test of homogeneity was used to assess group differences in probability distributions of nominal variables e.g., smoking status and ethnicity. Smoking status was dichotomised into current smoker or not a current smoker. Ethnicity was categorised into Caucasian and black, Asian and minority ethnic (BAME), as the majority of participants (67.57%) were Caucasian. The level of significance was set at $p < 0.05$.

Standard curves were used to determine absolute quantities (pg/ml) of each inflammatory marker. As in Study 1, cytokines where greater than 30% of the data was missing were excluded from analyses. A factorial ANCOVA was performed on each cytokine to assess group differences in cytokine values while controlling for the following covariates determined *a priori*: age, ethnicity and smoking status. Thus, adjusted means (taking into account the covariates) and their associated standard errors are shown for each cytokine. The Benjamini-Hochberg procedure with an FDR of 0.1 was applied as a correction for multiple comparisons. While cytokine values were biologically plausible, the factorial ANCOVAs were repeated after removing outliers (based on visual inspection of box plots), as they can negatively influence this statistical test.

Exploratory multiple regressions were performed with cytokine concentrations as the independent variable and clinical characteristics as the dependent variable (BMI, body fat percentage, EDE-Q global score, BDI-II total score, illness duration), controlling for sociodemographic covariates (age, ethnicity, smoking status). Separate regression models were performed for each cytokine. For these regressions, studentised residuals greater than ±3 standard deviations were deemed to be outliers and were removed, and assumptions were tested and met. The Benjamini-Hochberg procedure with an FDR of 0.1 was applied to the regression models per clinical characteristic as a correction for multiple comparisons.

3.4.2 Results

3.4.2.1 Participant demographics

Demographic, anthropometric, and clinical characteristics of the AN participants and HCs, along with statistical group comparisons, are presented in **Table 3.5**. The groups did not

differ in age ($U = 550$, $z = -1.41$, $p = 0.157$), nor their proportion of current smokers ($\chi^2(1) = 0.08$, $p = 0.782$). There was a significantly greater proportion of BAME participants in the HC than in the AN group ($\chi^2(1) = 12.07$, $p = 0.001$). As expected, AN participants had significantly lower weight ($t(72) = 11.59$, $p < 0.0001$), BMI ($U = 0$, $z = 7.38$, $p < 0.0001$) and body fat percentage ($U = 57.50$, $z = 6.75$, $p < 0.0001$) than HCs. Furthermore, measures of ED (using the EDE-Q; $U = 24$, $z = -7.12$, $p < 0.0001$), general psychopathology (using the DASS-21; $U = 25$, $z = -7.11$, $p < 0.0001$), and depression (using the BDI-II; $U = 20$, $z = -7.11$, $p < 0.0001$) symptom severity were significantly higher in AN than in HC participants.

Table 3.5 Demographic and clinical characteristics of the anorexia nervosa participants and healthy controls with group statistical comparisons.

Demographic and clinical characteristics	**Healthy Controls (*n*=34)**	**Anorexia Nervosa (*n*=40)**	
Age [years] [median (IQR[a])]	23.50 (21.00, 25.00)	25.00 (21.00, 29.00)	
Current smoker [*n*]	5	5	
Ethnicity [Caucasian/BAME] [*n*]	16 /18	34 / 6	*
Weight [kg] [mean ± SD]	58.57 ± 6.18	43.50 ± 5.01	*
BMI [kg/m^2] [median (IQR[a])]	20.95 (19.60, 22.60)	16.05 (14.95, 16.95)	*
Body fat [%] [median (IQR[a])]	24.15 (21.60, 29.10)	10.95 (8.15, 14.35)	*
Illness duration[b] [years] [median (IQR[a])]		4.50 (0.88, 10.00)	
AN subtype [AN-R/AN-BP] [*n*]		33/7	
Current treatment [inpatient/outpatient/none] [*n*]		1/31/8	
Antidepressant medication [*n*]		17	
Antipsychotic medication [*n*]		4	
EDE-Q Global [median (IQR[a])]	0.34 (0.18, 0.83)	4.05 (2.95, 4.65)	*
DASS-21 Total [median (IQR[a])]	6.00 (2.00, 14.00)	59.00 (33.00, 80.00)	*
BDI-II Total [median (IQR[a])]	2.00 (1.00, 4.00)	30.50 (22.00, 38.00)	*

[a]25th and 75th percentile reported. [b]Illness duration refers to years since AN diagnosis. * denotes a statistically significant group difference at $p < 0.05$. Abbreviations: *n* = number of observations; IQR = interquartile range; BAME = black, Asian, and minority ethnic; BMI = body mass index; kg = kilogram; m = metre; AN = anorexia nervosa; AN-R = anorexia nervosa restricting type; AN-BP = anorexia nervosa binge-eating/purging type; EDE-Q = Eating Disorder Examination-Questionnaire; DASS-21 = Depression Anxiety and Stress Scales – 21 Version; BDI-II = Beck Depression Inventory-II.

3.4.2.2 Cytokine concentrations

Eight cytokines (GM-CSF, IL-1α, IL-1β, IL-4, IL-5, IL-21, IL-23, IL-31) had undetectable concentrations in greater than 30% of the sample (see **Table 3.6**) and were not included in the statistical analyses. Chi-squared tests of homogeneity showed that groups did not differ in the proportion of undetectable cytokine concentrations. Known quantities within the standard curves correlated highly with quantities predicted by fluorescence intensity ($r^2 > 0.99$).

Table 3.6 Cytokine measurement metrics in the whole sample.

Cytokine	Undetectable[a] [*n* (%)]	Lowest detectable concentration (pg/ml)	Highest detectable concentration (pg/ml)
Lowly expressed cytokines (<1 pg/ml)			
GM-CSF	30 (40.54)	0.116	1070
IL-1α	53 (71.62)	0.920	454
IL-1β	62 (83.78)	0.0929	554
IL-2	4 (5.41)	0.0523	1440
IL-4	31 (41.89)	0.0423	218
IL-5	62 (83.78)	0.190	830
IL-6	0	0.110	742
IL-10	0	0.0451	378
IL-12p70	17 (22.97)	0.0795	549
IL-31	29 (39.19)	0.0700	962
TNF-β	5 (6.76)	0.144	711
Low to moderately expressed cytokines (1-20 pg/ml)			
Eotaxin-3	0	1.03	6240
IFN-γ	0	0.638	1280
IL-7	0	0.116	830
IL-8	0	0.0459	545
IL-13	15 (20.27)	0.778	529
IL-15	0	0.142	829
IL-17A	0	0.273	6240
IL-21	53 (71.62)	0.681	842
IL-22	6 (8.11)	0.494	551
IL-23	73 (98.65)	0.455	4600
MIP-3α	1 (1.35)	0.496	566
TNF-α	0	0.119	349
Moderate to highly expressed cytokines (21-400 pg/ml)			
Eotaxin	0	2.29	1720
IL-12/IL-23p40	0	0.367	3650
IL-16	0	0.564	2640
IP-10	0	0.171	2360
MCP-1	0	0.0438	562
MCP-4	0	4.24	724
MIP-1α	6 (8.11)	83.9	11800
MIP-1β	0	1.01	1180
TARC	0	0.674	1780
VEGF	0	1.15	1120

Cytokine	Undetectable[a] [*n* (%)]	Lowest detectable concentration (pg/ml)	Highest detectable concentration (pg/ml)
Highly expressed cytokines (401-25,000 pg/ml)			
IL-27	0	12.9	16100
MDC	0	5.75	11800

[a]Below fit curve range. Abbreviations: *n* = number of observations; pg = picogram; m = millilitre; GM-CSF = granulocyte-macrophage colony-stimulating factor; IL = interleukin; TNF = tumor necrosis factor; IFN = interferon; MIP = macrophage inflammatory protein; IP = interferon γ-induced protein; MCP = monocyte chemoattractant protein; TARC = thymus and activation-regulated chemokine; VEGF = vascular endothelial growth factor; MDC = macrophage-derived chemokine.

Table 3.7 shows the statistical significance of the group comparison from the factorial ANCOVAs with the adjusted means. After correction for multiple comparisons and adjustment for age, ethnicity and smoking status, IL-7 ($F(1, 69) = 12.15$, $p = 0.0009$), MIP-1α ($F(1, 63) = 7.84$, $p = 0.0068$), and MIP-1β ($F(1, 69) = 7.98$, $p = 0.0062$) were found to be significantly lower in the AN group, compared to the HC group. These findings remained significant after the removal of outliers (IL-7, $F(1, 66) = 7.52$, $p = 0.0078$; MIP-1α, $F(1, 62) = 7.53$, $p = 0.0079$; MIP-1β, $F(1, 66) = 8.86$, $p = 0.0041$). Following the removal of outliers and correction for multiple comparisons, the following cytokines were also found to be significantly lower in AN participants than in HCs: IL-12/IL-23p40 ($F(1, 65) = 33.46$, $p < 0.0001$), IL-12p70 ($F(1, 40) = 8.07$, $p = 0.0070$), IL-16 ($F(1, 64) = 7.94$, $p = 0.0064$), IL-17A ($F(1, 63) = 16.05$, $p = 0.0002$), IP-10 ($F(1, 62) = 5.02$, $p = 0.0286$), MCP-1 ($F(1, 68) = 7.92$, $p = 0.0064$), and MDC ($F(1, 67) = 9.22$, $p = 0.0034$). No other cytokines were found to significantly differ between groups.

Table 3.7 Adjusted mean (± standard error) concentrations of each cytokine with the statistical significance of the group comparison from the factorial ANCOVAs, with and without outliers included.

Cytokine	Analyses with outliers included				Analyses following removal of outliers			
	n	**Healthy control** (adjusted mean ± SE)	**Anorexia nervosa** (adjusted mean ± SE)	***p***	***n***	**Healthy control** (adjusted mean ± SE)	**Anorexia nervosa** (adjusted mean ± SE)	***p***
Eotaxin	74	182.83 ± 21.41	239.65 ± 15.66	0.0191	71	180.03 ± 17.27	218.91 ± 13.03	0.0565
Eotaxin-3	74	10.21 ± 8.59	22.72 ± 6.28	0.1193	62	15.50 ± 1.34	15.19 ± 0.88	0.8951
IFN-γ	74	10.51 ± 4.72	6.03 ± 3.45	0.4333	62	3.04 ± 0.42	2.78 ± 0.29	0.4372
IL-2	70	0.18 ± 0.11	0.28 ± 0.08	0.5713	64	0.19 ± 0.23	0.15 ± 0.02	0.2663
IL-6	74	1.88 ± 0.78	0.56 ± 0.57	0.1407	63	0.32 ± 0.04	0.27 ± 0.03	0.1059
IL-7	74	**18.43 ± 1.23**	**13.96 ± 0.90**	**0.0009**	71	**17.89 ± 1.17**	**14.59 ± 0.85**	**0.0078**
IL-8	74	10.31 ± 0.85	11.12 ± 0.63	0.2607	74	10.31 ± 0.85	11.12 ± 0.63	0.2607
IL-10	74	0.61 ± 0.13	0.45 ± 0.10	0.3522	63	0.27 ± 0.03	0.27 ± 0.02	0.9693
IL-12/IL-23p40	74	125.20 ± 13.50	98.87 ± 9.87	0.1346	70	**122.22 ± 6.64**	**80.69 ± 4.86**	**<0.0001**
IL-12p70	57	0.29 ± 0.09	0.19 ± 0.7	0.3147	45	**0.13 ± 0.02**	**0.07 ± 0.02**	**0.0070**
IL-13	59	2.13 ± 0.82	2.08 ± 0.62	0.9789	50	1.20 ± 0.17	0.91 ± 0.13	0.2073
IL-15	74	2.55 ± 0.16	2.59 ± 0.11	0.9573	72	2.59 ± 0.14	2.50 ± 0.11	0.4447
IL-16	74	167.72 ± 12.40	153.49 ± 9.06	0.2561	69	**173.91 ± 9.90**	**140.03 ± 7.45**	**0.0064**
IL-17A	74	2.52 ± 0.26	1.87 ± 0.19	0.0309	68	**2.53 ± 0.21**	**1.58 ± 0.17**	**0.0002**
IL-22	68	3.99 ± 1.79	2.41 ± 1.35	0.4738	59	2.01 ± 0.25	1.84 ± 0.20	0.8494
IL-27	74	1042.76 ± 96.89	1018.22 ± 70.85	0.8679	73	956.11 ± 90.70	1004.41 ± 64.45	0.4237
IP-10	74	278.78 ± 33.86	240.81 ± 24.76	0.4504	67	**245.38 ± 19.20**	**198.53 ± 13.16**	**0.0286**

Cytokine	Analyses with outliers included				Analyses following removal of outliers			
	n	**Healthy control** (adjusted mean ± SE)	**Anorexia nervosa** (adjusted mean ± SE)	***p***	***n***	**Healthy control** (adjusted mean ± SE)	**Anorexia nervosa** (adjusted mean ± SE)	***p***
MCP-1	74	302.42 ± 16.05	258.07 ± 11.74	0.0266	73	**304.12 ± 15.04**	**254.78 ± 11.04**	**0.0064**
MCP-4	74	117.69 ± 11.54	117.98 ± 8.44	0.9858	73	116.19 ± 10.20	112.23 ± 7.56	0.7589
MDC	74	1758.71 ± 102.09	1481.97 ± 74.66	0.0282	72	**1787.44 ± 97.30**	**1442.17 ± 70.23**	**0.0034**
MIP-1α	68	**274.84 ± 19.09**	**216.07 ± 15.01**	**0.0068**	67	**246.43 ± 18.20**	**221.31 ± 14.34**	**0.0079**
MIP-1β	74	**145.72 ± 12.85**	**102.18 ± 9.39**	**0.0062**	71	**132.14 ± 11.23**	**93.57 ± 8.22**	**0.0041**
MIP-3α	73	12.93 ± 3.82	5.85 ± 2.83	0.1792	54	4.98 ± 0.43	4.88 ± 0.24	0.9334
TARC	74	344.14 ± 38.06	331.76 ± 27.83	0.6645	74	344.14 ± 38.06	331.76 ± 27.83	0.6645
TNF-α	74	2.22 ± 0.14	1.89 ± 0.10	0.0391	72	2.13 ± 0.12	1.86 ± 0.09	0.0508
TNF-β	69	0.21 ± 0.02	0.17 ± 0.02	0.1775	69	0.21 ± 0.02	0.17 ± 0.02	0.1775
VEGF	74	110.97 ± 13.43	89.98 ± 9.82	0.2694	70	105.75 ± 11.56	91.68 ± 8.42	0.3504

Results highlighted in bold indicate statistically significant group differences after correction for multiple comparisons. Abbreviations: *n* = number of observations; SE = standard error; IFN = interferon; IL = interleukin; IP = interferon γ-induced protein; MCP = monocyte chemoattractant protein; MDC = macrophage-derived chemokine; MIP = macrophage inflammatory protein; TARC = thymus and activation-regulated chemokine; TNF = tumor necrosis factor; VEGF = vascular endothelial growth factor.

The findings from the exploratory linear regression analyses to examine whether cytokine concentrations were associated with clinical characteristics, after controlling for sociodemographic covariates are shown in **Table 3.8**. BMI, body fat percentage, ED symptom severity (as measured using the EDE-Q) and depression symptom severity (as measured by the BDI-II) were all significantly associated with IL-7 after controlling for age, ethnicity and smoking status and adjusting for multiple comparisons. Higher BMI and body fat percentage were associated with higher concentrations of IL-7 and lower ED and depression symptom severity were also associated with higher IL-7 serum concentrations. Body fat percentage was additionally associated with TNF-α, such that higher body fat percentage was related to higher concentrations of TNF-α. ED symptom severity was also associated significantly with MCP-1, in that lower MCP-1 was associated with higher ED symptom severity. The regression analyses showed no significant association between the concentration of any cytokine and illness duration.

Table 3.8 Analysis of associations between clinical characteristics and cytokine concentrations in the whole sample combined, controlling for age, ethnicity and smoking status.

	Body mass index			Body fat percentage			EDE-Q global score			BDI-II total score		
Cytokine	*n*	β coefficient *(95% CIs)*	*p*	*n*	β coefficient *(95% CIs)*	*p*	*n*	β coefficient *(95% CIs)*	*p*	*n*	β coefficient *(95% CIs)*	*p*
Eotaxin	74	-0.01 (-0.02, -0.00)	0.008	74	-0.02 (-0.04, -0.00)	0.030	74	0.01 (0.00, 0.01)	0.023	73	0.04 (0.00, 0.07)	0.042
Eotaxin-3	74	-0.01 (-0.03, 0.01)	0.197	74	-0.02 (-0.06, 0.03)	0.426	74	0.01 (-0.00, 0.02)	0.204	73	0.09 (-0.00, 0.18)	0.061
IFN-γ	74	0.01 (-0.02, 0.05)	0.453	74	0.06 (-0.03, 0.14)	0.174	74	-0.01 (-0.03, 0.01)	0.409	73	-0.06 (-0.23, 0.12)	0.517
IL-2	70	-0.06 (-1.63, 1.50)	0.935	70	1.12 (-2.72, 4.96)	0.561	70	0.16 (-0.79, 1.10)	0.739	69	2.08 (-5.95, 10.11)	0.606
IL-6	74	0.02 (-0.19, 0.23)	0.844	74	0.33 (-0.17, 0.83)	0.190	74	-0.09 (-0.21, 0.04)	0.190	73	-0.67 (-1.71, 0.37)	0.204
IL-7	**74**	**0.20 (0.08, 0.31)**	**0.001**	**73**	**0.53 (0.27, 0.78)**	**<0.001**	**74**	**-0.12 (-0.19, -0.05)**	**0.002**	**73**	**-1.08 (-1.65, -0.52)**	**<0.001**
IL-8	74	-0.17 (-0.35, 0.02)	0.084	74	-0.35 (-0.80, 0.10)	0.128	73	0.12 (0.00, 0.23)	0.043	73	0.40 (-0.56, 1.36)	0.405
IL-10	74	0.48 (-0.76, 1.71)	0.446	74	1.17 (-1.79, 4.14)	0.433	74	-0.29 (-1.06, 0.47)	0.444	73	-2.47 (-8.67, 3.73)	0.430
IL-12/IL-23p40	74	0.01 (-0.00, 0.02)	0.056	74	0.03 (0.01, 0.06)	0.019	74	-0.01 (-0.01, 0.00)	0.146	73	-0.01 (-0.07, 0.05)	0.812
IL-12p70	57	1.49 (-0.98, 3.96)	0.232	57	6.40 (0.74, 12.07)	0.028	57	-0.42 (-1.91, 1.08)	0.577	56	-3.64 (-15.14, 7.86)	0.528
IL-13	59	-0.01 (-0.28, 0.26)	0.939	59	0.05 (-0.57, 0.67)	0.865	59	0.03 (-0.13, 0.19)	0.682	58	-0.65 (-1.95, 0.65)	0.323

Cytokine	Body mass index			Body fat percentage			EDE-Q global score			BDI-II total score		
	n	β coefficient *(95% CIs)*	*p*	*n*	β coefficient *(95% CIs)*	*p*	*n*	β coefficient *(95% CIs)*	*p*	*n*	β coefficient *(95% CIs)*	*p*
IL-15	74	0.14 (-0.93, 1.20)	0.801	74	0.50 (-2.05, 3.05)	0.695	74	-0.30 (-0.95, 0.35)	0.364	73	-1.41 (-6.81, 3.98)	0.603
IL-16	74	0.01 (-0.00, 0.03)	0.063	74	0.04 (0.01, 0.07)	0.015	74	-0.00 (-0.01, 0.00)	0.350	73	0.00 (-0.07, 0.07)	0.988
IL-17A	74	0.50 (-0.11, 1.11)	0.106	74	1.37 (-0.09, 2.83)	0.065	74	-0.41 (-0.79, -0.04)	0.029	73	-1.88 (-4.98, 1.22)	0.230
IL-22	68	0.02 (-0.08, 0.12)	0.693	68	0.309 (-0.11, 0.35)	0.309	68	-0.03 (-0.09, 0.03)	0.350	67	-0.33 (-0.82, 0.15)	0.173
IL-27	74	-0.00 (-0.00, 0.00)	0.714	74	-0.00 (-0.00, 0.00)	0.982	74	-0.00 (-0.00, 0.00)	0.488	73	-0.00 (-0.01, 0.01)	0.449
IP-10	74	0.00 (-0.00, 0.01)	0.238	74	0.01 (-0.00, 0.02)	0.087	74	-0.00 (-0.00, 0.00)	0.793	73	-0.01 (-0.03, 0.02)	0.682
MCP-1	73	0.01 (0.00, 0.02)	0.035	74	0.01 (-0.01, 0.04)	0.330	**74**	**-0.01 (-0.02, -0.00)**	**0.002**	73	-0.05 (-0.10, 0.00)	0.062
MCP-4	74	-0.01 (-0.02, 0.01)	0.405	74	-0.03 (-0.06, 0.01)	0.111	74	-0.00 (-0.01, 0.01)	0.728	73	0.01 (-0.06, 0.09)	0.718
MDC	74	0.00 (-0.00, 0.00)	0.136	74	0.00 (-0.00, 0.01)	0.129	73	-0.00 (-0.00, -0.00)	0.033	73	-0.00 (-0.01, 0.01)	0.569
MIP-1α	68	0.01 (-0.00, 0.01)	0.244	68	0.02 (0.00, 0.04)	0.040	68	-0.01 (-0.01, -0.00)	0.035	67	-0.06 (-0.10, -0.01)	0.011
MIP-1β	74	0.01 (-0.00, 0.02)	0.088	73	0.03 (0.01, 0.06)	0.016	74	-0.01 (-0.02, -0.00)	0.022	73	-0.08 (-0.14, -0.02)	0.015
MIP-3α	73	0.02 (-0.03, 0.06)	0.396	73	0.07 (-0.03, 0.18)	0.162	73	-0.01 (-0.04, 0.02)	0.467	72	-0.10 (-0.31, 0.12)	0.373
TARC	74	0.00 (-0.00, 0.00)	0.980	74	-0.00 (-0.01, 0.01)	0.834	74	-0.00 (-0.00, 0.00)	0.711	73	0.01 (-0.02, 0.03)	0.545

Cytokine	Body mass index			Body fat percentage			EDE-Q global score			BDI-II total score		
	n	β coefficient *(95% CIs)*	*p*	*n*	β coefficient *(95% CIs)*	*p*	*n*	β coefficient *(95% CIs)*	*p*	*n*	β coefficient *(95% CIs)*	*p*
TNF-α	74	0.96 (-0.19, 2.10)	0.100	**72**	**4.84 (2.48, 7.19)**	**<0.001**	74	-0.39 (-1.10, 0.32)	0.279	73	-3.37 (-9.20, 2.46)	0.252
TNF-β	69	7.45 (0.59, 14.31)	0.034	69	7.91 (-8.85, 24.68)	0.349	69	-1.29 (-5.80, 3.21)	0.569	68	-20.01 (-55.96, 15.94)	0.270
VEGF	74	0.01 (-0.00, 0.02)	0.105	74	0.02 (-0.01, 0.05)	0.125	74	-0.00 (-0.01, 0.01)	0.481	73	-0.02 (-0.08, 0.05)	0.606

β coefficients and 95% confidence intervals were rounded to two decimal places. Results in bold text indicate that the cytokine was statistically significantly associated with the clinical characteristic, after controlling for multiple comparisons for regression models per clinical characteristic.

Abbreviations: *n* = number of observations; CI = confidence intervals; EDE-Q = Eating Disorder Examination Questionnaire; BDI-II = Beck's Depression Inventory-II; IFN = interferon; IL = interleukin; IP = interferon γ-induced protein; MCP = monocyte chemoattractant protein; MDC = macrophage-derived chemokine; MIP = macrophage inflammatory protein; TARC = thymus and activation-regulated chemokine; TNF = tumor necrosis factor; VEGF = vascular endothelial growth factor.

3.4.3 Discussion

In Study 2, we measured a broad range of cytokines and chemokines in an adequately powered sample of people with AN and HCs. Extending previous cytokine research in AN, the cross-sectional analyses included the covariates age, ethnicity and smoking status, factors which have been shown to influence cytokine concentrations (O'Connor et al., 2009; ter Horst et al., 2016). Following adjustment for multiple testing, the cytokine IL-7 and the chemokines MIP-1α and MIP-1β were found to be significantly lower in AN participants than in HCs.

IL-7 is an important immune-regulatory cytokine, involved in the development of B and T lymphocytes and induces the secretion of pro-inflammatory cytokines central to the inflammatory process e.g., IL-6 and TNF-α (Alderson, Tough, Ziegler, & Grabstein, 1991; Appasamy, 1999). Concentrations of IL-7 also drive the expansion of the pool of naïve T cells (Surh, Boyman, Purton, & Sprent, 2006; Surh & Sprent, 2008), which have been found to be reduced in AN (Allende et al., 1998). This is consistent with our findings of reduced IL-7 concentrations. IL-7 has been previously assessed in one small study: in ten patients with AN restricting type (AN-R) and five patients with AN binge-eating/purging type (AN-BP), 24-hour plasma IL-7 concentrations were found to be significantly lower compared to ten healthy-weight controls (Germain et al., 2016). IL-7 values were also significantly lower in AN-R compared to participants with constitutional thinness (Germain et al., 2016), an underweight state characterised by no change in feeding behaviour and normal menstruation (Estour et al., 2017). Indeed, we did find that lower IL-7 values were associated with increases in both ED and depression symptoms in our sample. While the current study only used one-point sampling, as opposed to a circadian analysis, the pattern of decreased IL-7 concentrations in AN participants was replicated.

The changes observed in IL-7 in this study may be related to the endocrine consequences of AN. Women with AN often display hypo-oestrogenism (i.e., oestrogen deficiency; Misra & Klibanski, 2014; Schorr & Miller, 2017; Warren, 2011) and reduced concentrations of IL-7 have also been found in women five years following the removal of the ovaries and the fallopian tubes, which induces surgical menopause (Tani et al., 2013). This would also account for why individuals with constitutional thinness do not differ from normal-weight individuals in concentrations of IL-7 (Germain et al., 2016). In relation to weight and adiposity, animal research focusing on obesity has reported that transgenic mice with over expression of IL-7, IL-7 receptor knockout mice, and mice administered IL-7 via injection exhibit significantly reduced body weight gain and visceral adiposity on both chow and high-fat diets (Lee, Song, Choi, Yu, & Park, 2015; Lucas et al., 2012). These findings would suggest we should expect elevated IL-7 in underweight AN. This is inconsistent with our

cross-sectional results and with our findings of positive associations between IL-7 with BMI and body fat percentage. However, it is well-established that findings in animal research do not always translate to humans (Bracken, 2009), which may explain these discrepancies. Ultimately, there has been little research on IL-7 and its potential role in weight, fat mass, feeding and appetite regulation, which precludes further speculation on the role of IL-7 in AN.

The MIP-1 proteins are chemokines produced by many cell types, in particular macrophages, dendritic cells, and lymphocytes (Maurer & von Stebut, 2004). MIP-1α and MIP-1β are highly related and both play a crucial role in the induction and modulation of inflammatory responses by recruiting macrophages to sites of infection/injury and attracting other pro-inflammatory cell types, and they are key regulators of tissue homeostasis (Maurer & von Stebut, 2004; Menten, Wuyts, & Van Damme, 2002). In the present study, both MIP-1α and MIP-1β were decreased in AN compared to HC participants. These chemokines have been associated with food intake and fat mass. It has been reported that MIP-1α and MIP-1β expression is elevated in the white adipose tissue of obese mice and humans (Huber et al., 2008; Jiao et al., 2009; Xu et al., 2003) and MIP-1α has been shown to positively correlate with central fat mass (Glintborg, Andersen, Richelsen, & Bruun, 2009) and waist circumference (Ognjanovic, Jacobs, Steinberger, Moran, & Sinaiko, 2013). From this, it may be hypothesised that the observed reductions in these chemokines may be associated with the decreased body fat mass in this underweight sample, which was supported by nominally significant associations between both MIP proteins and body fat percentage in the regression models. With respect to food intake, in rats, injection of MIP-1 proteins into the hypothalamus, the 'feeding centre' of the brain, has been shown to reduce short-term food intake (Miñano & Myers, 1991; Myers et al., 1994). Furthermore, in mice fed a high-fat diet, circulating concentrations of MIP-1β were decreased compared to mice on a control low-fat diet (Petersen et al., 2014). Therefore, we would potentially expect higher concentrations of these chemokines in the AN patients compared to the HCs, given the relatively low intake of fats reported in AN (e.g., Mayer, Schebendach, Bodell, Shingleton, & Walsh, 2012; Misra et al., 2006b). It may be that these chemokines are more strongly associated with body fat mass, rather than food intake. Our analyses also found nominally significant associations between the MIP-1 chemokines and psychopathology, in that reductions in MIP-1α and MIP-1β were related to increases in ED and depression symptoms. Few studies have assessed the relationship between these chemokines and depression; however, a meta-analysis of three studies suggests no alterations in MIP-1α in depressed participants (Köhler et al., 2017). This relationship needs to be further explored.

Due to the assumptions of factorial ANCOVAs, the analyses were repeated following the removal of outliers. These analyses further identified IL-12/IL-23p40, IL-12p70, IL-16, IL-17A, IP-10, MCP-1, and MDC as cytokines/chemokines that were significantly decreased in the AN group as compared to the HCs. However, as these analyses involved the exclusion of biologically plausible cytokine values, the association of these findings with ED-related phenotypes will only be briefly discussed. Firstly, IL-12p70 has not been associated with obesity and weight parameters in humans (Lichtenauer et al., 2015; Shelton et al., 2015) However, in our sample, IL-12p70 was associated with body fat percentage. This cytokine has also been little considered in research of related psychiatric disorders; however, a recent meta-analysis identified a significant elevation in IL-12 in patients with major depressive disorder (MDD; Köhler et al., 2017).

Secondly, IL-16 is considered a pro-inflammatory cytokine that stimulates the expression and production of other pro-inflammatory cytokines by human monocytes (Mathy et al., 2000). Positive correlations between IL-16 and parameters of obesity (weight, BMI, waist circumference) have been observed (Lichtenauer et al., 2015). Indeed, in this study, increases in IL-16 were associated with increases in body fat percentage. IL-16 has also been shown to be associated with current depressive episode in patients with MDD (Powell et al., 2018).

Third, IL-17A is an inflammatory Th17 cytokine that affects dopamine synthesis and has been associated with depression and depressive symptoms in humans and animals, although some of these findings in humans were mixed (Beurel & Lowell, 2018). For example, increases in IL-17A have been associated with depression symptoms (Davami et al., 2016). In contrast, IL-17A has also been negatively correlated with anxiety symptoms in females, such that lower IL-17A is associated with increased anxiety (Pallavi et al., 2015). Similarly, decreases in IL-17A were associated with increased ED symptom severity in this study. Together, this may suggest that IL-17A is particularly related to psychological aspects of the disorder, rather than physiological factors e.g., weight.

Fourth, IP-10 is secreted by several cell types, including adipocytes, monocytes, endothelial cells, and fibroblasts, in response to IFN-γ, and has been implicated in the stimulation of monocyte migration and modulation of adhesion molecule expression (Neville, Mathiak, & Bagasra, 1997). Elevated concentrations of IP-10 have been identified in people with morbid obesity when compared to normal-weight controls (Hueso et al., 2018) and in combination with our findings, may suggest a positive correlation between weight and IP-10 concentrations.

Fifth, MDC is highly regulated by inflammatory signals (e.g., IL-13) and has been implicated in a range of diverse pathologies, including allergies and human

immunodeficiency virus (Mantovani, Gray, Van Damme, & Sozzani, 2000). Recently, MDC has found to be a biomarker of pharmacological treatment response in depression (Milenkovic et al., 2017).

Sixth, MCP-1 is a chemokine that regulates migration and infiltration of monocytes (Deshmane, Kremlev, Amini, & Sawaya, 2009) and has been implicated in the induction of adipogenesis (Panee, 2012). Studies in obesity have shown that MCP-1 concentrations and expression are elevated in participants with obesity compared to normal-weight controls (Kim et al., 2006; Panee, 2012) and circulating concentrations of MCP-1 have been shown to be positively correlated with BMI and waist circumference (Kim et al., 2006). We similarly identified a positive association between MCP-1 and BMI.

To the best of our knowledge, except for Study 1 in this chapter, these cytokines have not been assessed in people with AN, and therefore, more research is needed to understand the influence of these cytokines in AN and their role in ED-related phenotypes e.g., weight, feeding behaviour, and comorbid psychiatric disorders.

3.5 General discussion

Given that the AN patients in this sample were seriously unwell, as evidenced by their low BMI, the loss of fat mass, and the presence of clinically significant ED and severe depression and anxiety symptoms, the finding that most inflammatory markers were unchanged in both studies may seem somewhat surprising. However, it has been proposed that the functioning of the immune system is relatively preserved in AN, despite malnutrition (Brambilla, 2001; Marcos, 1997). For example, extensive evidence suggests that patients with AN tend to be free from infectious diseases and rarely have colds and/or flu (at least until very advanced stages of the disease; Marcos, 1997; Slotwinska & Slotwinski, 2017), which is in contrast to the heightened risk of infection observed in those with typical malnutrition. A recent *in vitro* study of immune parameters in people with AN reported that despite reductions in some immune cell populations, AN is also associated with enhanced antioxidant potential and anti-inflammatory status, in comparison to HCs (Omodei et al., 2015). This may contribute to the preservation of immune system functioning reported in AN. However, it needs to be considered that data derived from *in vitro* methods may not reflect how cells would respond *in vivo*. An additional contributing factor could be a relatively sustained/unaffected gut barrier function in AN (Mörkl et al., 2018), in spite of the distinct alterations of the gut microbiome and low gut microbiome diversity reported in AN patients (Borgo et al., 2017). A 'leaky gut' is associated with low-grade inflammation, i.e., leaking of bacteria and/or their components from the gut into circulation is thought to elicit an inflammatory response (Alam et al., 2017; Żak-Gołąb et al., 2013); therefore, sustained gut permeability

will reduce the likelihood of a systemic inflammatory response. Finally, it may also be that competing pro-inflammatory and anti-inflammatory processes may leave cytokine concentrations relatively unchanged.

The findings of elevated IL-6 and IL-15 and reduced TNF-β and VEGF in AN, compared to healthy individuals, were not replicated in an independent adequately powered sample in Study 2. There are several potential explanations for this lack of reproducibility. Firstly, unlike Study 1 and many of the previously published studies in this area (see Chapter 2 for a review; Dalton et al., 2018a), in Study 2 several factors that have been shown to influence cytokine concentrations were controlled for in the statistical analyses. Indeed, group differences in cytokine concentrations in depression often disappear when mediating factors (e.g., sociodemographic variables) are taken into account (O'Connor et al., 2009; Palmos et al., 2019; Powell et al., 2018). Therefore, this may account for the lack of replication of associations between certain inflammatory markers and clinical characteristics from Study 1 in the latter study. Thus, Study 2 highlighted the importance of incorporating potentially confounding factors into research study design in terms of ensuring that they are assessed and reported, considered as potential exclusion criteria, and/or controlled for in analyses and study design (O'Connor et al., 2009).

Secondly, it is possible that there is a subset of patients with AN for whom inflammatory processes contribute to their clinical presentation. A similar hypothesis has been well-described in the depression literature (Raison & Miller, 2011). One point which supports this idea is that for any given inflammatory marker, there is typically an overlap between AN patients and HCs, regardless of differences in the mean/median values in each group. Thus, a proportion of patients with AN have similar cytokine values to the healthy comparison group, as seen in **Figure 3.1** and **Figure 3.2**. Then, there is another group of patients who have cytokine values that are clearly higher or lower than most of the HCs. These are the participants who contribute to the association between AN and inflammatory markers. From this perspective, it may be considered whether individuals with altered inflammatory markers represent a biologically pertinent subtype of AN for whom immune processes are particularly relevant to disease development and maintenance (Himmerich & Treasure, 2018).

In Study 2, the additional cytokine IL-7 and the chemokines MIP-1α and MIP-1β were found to be significantly lower in AN participants compared to HCs. It is unclear why these particular inflammatory markers were altered in people with AN, though these inflammatory markers have been associated with ED-related phenotypes. However, more research is needed to better understand why these cytokines in particular were altered in AN. There are a number of potential factors which may contribute to these alterations,

including stress and neuroendocrine functioning, genetics, the gut microbiota, early life stress, and negative health behaviours (e.g., disturbed sleep, altered diet, smoking; Bauer & Teixeira, 2018), as discussed in Chapter 1.

Across both Study 1 and 2, a broad range of inflammatory markers were measured to identify (a) novel associations between inflammatory markers and AN, and (b) patterns in inflammatory marker concentrations. Such a pattern could have been the consistent over-expression of pro-inflammatory cytokines, such as IL-1β, IL-6, and TNF-α, and down-regulation of anti-inflammatory cytokines, like IL-10. While alterations in the concentrations of inflammatory markers not previously reported in AN were observed (as described above), we did not find a consistent pattern. Thus, from these data it cannot be concluded that any of the main categories within the cytokine system – pro-inflammatory, anti-inflammatory, Th1, Th2 or the Th17 branches – play a particularly important role in AN.

The studies in this chapter also explored the associations between inflammatory markers and AN presentation, including illness duration and both physiological (BMI and body fat percentage) and psychological/cognitive factors (ED symptom severity and symptoms of general psychopathology, including depression). Few previous studies have investigated these relationships. Across both studies, no inflammatory markers were associated with illness duration, which is in line with previous research (e.g., Agnello et al., 2012; Brambilla et al., 2001). This suggests that chronicity of illness may not play a role in the extent to which inflammatory markers are altered in AN. Consistent with a previous study, that reported that cytokine concentrations did not differ between depressed and non-depressed ED patients (Ahrén-Moonga et al., 2011), inflammatory marker concentrations were, on the whole, not associated with depression symptomatology in either sample. This suggests that the alterations observed in inflammatory markers in AN are more likely related to the ED, rather than symptoms of comorbid depression. The results from the correlational analyses suggest that multiple inflammatory marker concentrations were associated with physiological aspects of the ED (i.e., BMI) more so than the psychological aspects (i.e., ED symptom severity). More research is needed to assess the replicability of these findings.

3.5.1 Strengths and limitations

These studies were the first to measure several inflammatory markers in patients with AN, including Th17 cytokines and chemokines, identifying several alterations in inflammatory markers in AN that warrant future research in larger samples. We also assessed illness severity, in terms of illness duration; ED symptoms, including psychological and anthropometric measures; and associated psychopathology (e.g., depression and anxiety),

in our participants. Few previous studies have included such variables, which in the current study have allowed us to explore the relationship between illness severity and inflammatory markers. In addition, for the HCs, the median values of a number of the assessed markers were similar to those observed in previous studies, suggesting that our HC groups were valid comparison groups. In both studies, participants reporting acute or chronic immune system related conditions, such as autoimmune diseases (e.g., psoriasis, rheumatoid arthritis, inflammatory bowel diseases) were excluded. While this may be stricter exclusion criteria than previous studies in this area (Chapter 2; Dalton et al., 2018a), as these conditions are associated with an increased risk of EDs (Zerwas et al., 2017), excluding these participants ensured that group differences were not artificially inflated.

Several limitations should be noted. The sample in Study 1 was small, which limits the power in this study, although Study 2 was adequately powered. In both studies, the sample was heterogeneous in terms of a number of factors, including age, ethnicity and smoking status, and factors specifically in the AN group varied, including weight, ED treatment status, ED psychopathology, illness duration, age of onset, AN subtype, recovery status and comorbidities. For example, while the AN and HC groups did not differ in mean age in either study, it must be mentioned that the AN group had a larger age range (Study 1: 18-67 years, Study 2: 18-53 years) than the HCs (Study 1: 20-36 years, Study 2: 18-31 years). Additionally, in Study 2, there was a significantly greater proportion of BAME participants in the HC group than in the AN group. Indeed, AN has been reported to be more common in Caucasian women compared to BAME women (Pike, Hoek, & Dunne, 2014; Striegel-Moore et al., 2003). Previous studies of cytokines in AN have tended to not report the ethnicity of their participants (e.g., Agnello et al., 2012; Terra et al., 2013; Víctor et al., 2015); however, ethnicity has been associated with concentrations of inflammatory markers (O'Connor et al., 2009). Therefore, two related strengths of Study 2 are (1) the assessment and reporting of ethnicity, and (2) that we were able to include age, ethnicity and smoking status as covariates in our analyses to account for sample heterogeneity in these factors that are known to influence inflammatory marker concentrations (O'Connor et al., 2009). The AN sample in both studies included inpatients and outpatients. Differences between these treatment settings in the opportunity to engage in ED behaviours, such as calorie restriction, self-induced vomiting, and excessive exercise, may affect cytokine concentrations (e.g., Canavan et al., 2005; Raschke & Eckel, 2013).

The two studies in this chapter used BIA to assess body fat percentage. This measure was chosen as, practically, it is an easy-to-perform, reproducible, non-invasive, and inexpensive approach to measuring body composition (Mattar et al., 2011). However, the

use of BIA to measure body composition has not been validated in AN (Marra et al., 2018; Mattar, Godart, Melchior, & Pichard, 2011). Research has suggested that the BIA measurement method for deriving body fat percentage may not be sensitive or reliable in groups with particularly low body weight, dehydration, or electrolyte imbalance and is sensitive to meal consumption and physical activity in the hours preceding a BIA measurement (Kyle et al., 2004; Mattar et al., 2011; Mialich, Sicchieri, & Junior, 2014). This is because BIA measurements of body composition are influenced by fluid and electrolyte status. Thus, the accuracy of BIA measurements of body composition may therefore be limited, particularly in the AN group where such features are often observed (Ackland et al., 2012; Kyle et al., 2004).

In Study 1, the duration for which the serum samples had been stored may have caused some degradation of inflammatory marker concentrations (Zhou et al., 2010). For practical reasons, it was not possible to ensure blood samples were drawn at a specific time of day and as cytokine production and release is reported to occur in a circadian manner, it is possible that some natural variations may have occurred (Labrecque & Cermakian, 2015). Also, in these studies we focussed on inflammatory molecules and did not measure cells of the immune system that produce cytokines (e.g., leukocytes, macrophages, monocytes) nor were we able to identify the sources of the inflammatory markers. Given that inflammatory markers are produced from multiple cell types throughout the body, knowledge about this may allow for greater understanding of the relationship between AN and markers of inflammation. It is also important to consider that many inflammatory parameters are physiologically present in the blood at low concentrations and poor assay sensitivity at these lower levels may result in a floor effect (Amsen, de Visser, & Town, 2009). Indeed, in the current and forthcoming studies, several cytokines had undetectable concentrations and these data were excluded from analyses. At present, there are no clear guidelines on the best method for handling cytokine data below the lower limit of detection (e.g., exclude/impute/substitute missing value). When interpreting the present results, it is important to consider that the method chosen may influence the findings e.g., over- or under-estimation of group differences.

As previously described, measurement of inflammatory markers can be affected by a number of pre-analytical factors (Dugué et al., 1996; O'Connor et al., 2009; ter Horst et al., 2016). Data on smoking status was not available for all participants in Study 1 and the limited power in this study precluded more complex analyses incorporating covariates. However, in Study 2, covariates were able to be included in the statistical analyses. Additionally, research has shown that antidepressant and antipsychotic medication can influence cytokine concentrations and production (Himmerich et al., 2011; Munzer et al.,

2013; Stapel et al., 2018). As these medications are prescribed to AN patients to target comorbid features of AN, such as depression and/or to induce weight gain (Himmerich & Treasure, 2018), it is important to report medication status (O'Connor et al., 2009). However, these data were not available in Study 1.

3.5.2 Conclusions

Study 1 was an exploratory investigation of a broad range of inflammatory markers, many of which had not been previously assessed in AN. IL-15, VEGF, and TNF-β, for the first time, were shown to be altered in people with AN in comparison to HCs. Previous findings regarding an elevation of IL-6 and VCAM-1 and a reduction in BDNF in AN participants were replicated.

Study two did not reproduce these findings when controlling for sociodemographic factors in the analyses. IL-7, MIP-1α and MIP-1β were significantly lower in AN participants than in HCs, after controlling for age, ethnicity, and smoking status and multiple testing. Exploratory regression analyses were also performed to provide a better understanding of the relationship between AN and inflammatory markers. IL-7 and the MIP-1 proteins were associated with both physiological and psychological aspects of AN. Indeed, these cytokines/chemokines have been associated with AN-related phenotypes, including weight, fat mass, and depression. These findings suggest that future research should include covariates in analyses of this relationship to explore whether this may account for some of the group differences in inflammatory markers observed in previous research.

Chapter 4. A longitudinal analysis of cytokines in anorexia nervosa

As published in:

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A copy of this article is shown in Appendix A Section 10.1.3.

The formatting of this publication has been amended here to ensure stylistic consistency throughout the thesis. The text remains largely unchanged, minor amendments have been made as follows: (a) references to work described in previous chapters have been added to the relevant citations for clarity, and (b) a brief statement, as signified by *italics*, was added specifically for the purposes of this thesis on Page 173 to ensure that the discussion is consistent with the findings reported in Study 2 of Chapter 3.

4.1 Abstract

Objective: Inflammation has been proposed to have a pathophysiological role in anorexia nervosa (AN) and to contribute to the maintenance of the disorder. Longitudinal cytokine research in AN has focussed on only a few pro-inflammatory cytokines. We assessed a broad range of cytokines over time in people undergoing specialised treatment for AN.

Method: We measured serum concentrations of 27 cytokines in people with AN (*n*=23). Body mass index (BMI), eating disorder (ED) symptoms and general psychopathology were assessed and blood samples were collected within four weeks of the commencement of specialised ED treatment (baseline) and at 12- and 24-week follow-ups.

Results: Both BMI and ED symptoms improved over the assessment period. Linear mixed models showed that log IL-6 decreased between baseline and week 12 assessments. By week 12, log IL-6 values were comparable to levels in healthy individuals. Log IL-7 increased from week 12 to week 24.

Conclusion: Initially elevated IL-6 serum concentrations appear to 'normalise' during the first 3-months of specialised treatment for AN and this co-occurs with improvements in ED symptoms. Therefore, IL-6 has the potential to be a state biomarker for AN.

4.2 Introduction

Anorexia nervosa (AN) is a serious psychiatric disorder characterised by restricted food intake and other inappropriate weight-loss behaviours (e.g., self-induced vomiting, laxative misuse, excessive exercise), accompanied by an intense fear of food and weight gain, and body dissatisfaction (American Psychiatric Association, 2013). AN has the highest standardised mortality rate of all psychiatric disorders, with a standardised mortality ratio between five and six (Himmerich et al., 2019b), and current treatment options for adults with AN are only moderately effective (Brockmeyer et al., 2018). The precise aetiology of AN is still unclear and without knowledge of these causal factors, opportunities for the development of alternative interventions, in particular biological-targeted treatments, are limited. Thus, investigation into the biological correlates of AN, that may contribute to the development and/or maintenance of the disorder, are crucial (Striegel-Moore & Bulik, 2007).

Cytokines, signalling molecules with particular importance in the immune system, have been suggested to play a pathophysiological role in AN (Chapter 2; Dalton et al., 2018a). Specifically, pro-inflammatory cytokines have been reported to be elevated in people with AN compared to healthy controls (HCs), including interleukin (IL)-1β, IL-6 and tumor necrosis factor (TNF)-α (Chapter 2; Dalton et al., 2018a; Solmi et al., 2015). A limited number of small studies have investigated longitudinal changes in these pro-inflammatory cytokines in patients receiving specialist treatment for AN. Some studies have reported a reduction in pro-inflammatory cytokines following refeeding and associated weight gain (e.g., Allende et al., 1998; Misra et al., 2006a; Pomeroy et al., 1994); whereas other studies did not find any longitudinal changes in pro-inflammatory cytokines, despite weight gain and/or psychological improvement (e.g., Brambilla et al., 1998; Nakai et al., 1999). As cytokines have been associated with overall body weight (Schmidt et al., 2015) and adipose tissue (Fantuzzi, 2005), stress (Ménard et al., 2017), and psychiatric disorders which are highly comorbid with AN e.g., depression (Köhler et al., 2017; Ulfvebrand et al., 2015), we would expect to see cytokine changes following weight gain and/or improvements in psychological eating disorder (ED) symptoms in patients with AN.

The symptom profile of AN comprises of both physiological (i.e., weight) and cognitive (e.g., fear of weight gain) features. However, an increase in body mass index (BMI), as a standardised measure of body weight change, has often been chosen as the main outcome of randomised controlled trials or meta-analyses of treatment efficacy in AN (e.g., Dold, Aigner, Klabunde, Treasure, & Kasper, 2015), and has been the most widely favoured index of recovery (Murray, Loeb, & Le Grange, 2018a). More recently, there has been a paradigm shift with research studies increasingly including analyses of both changes in

weight and psychological symptoms separately (e.g., Murray, Quintana, Loeb, Griffiths, & Le Grange, 2018b). An improvement in either dimension would be indicative of partial remission and an overall significant improvement in both body weight and the cognitive symptoms of AN would be a sign of full remission (Murray et al., 2018a). Generally, most studies in AN have focussed on cytokine changes as a function of weight gain, rather than improvements in the psychological state of the patient, with respect to both ED and general psychopathology symptoms (e.g., Misra et al., 2006a; Pomeroy et al., 1994). Therefore, it would be of scientific interest to explore whether cytokine changes during treatment would be associated with weight gain, improvements in AN psychopathology or with both.

There has been a strong focus on a few pro-inflammatory cytokines in the literature and given that pro-inflammatory cytokines function within a complex network of other cytokines, it would be important to consider whether longitudinal changes are observed in related cytokines. These include other cytokines with pro- (e.g., IL-8, IL-17) and anti-inflammatory (e.g., IL-10) functions, T-helper (Th) 1 (e.g., IL-2, IL-12, interferon [IFN]-γ) and Th2 (e.g., IL-4, IL-5, IL-13) cytokines, and chemokines, which are a subcategory of smaller cytokines (e.g., monocyte chemoattractant protein [MCP]). Therefore, the current study aimed to measure a broad range of cytokines over time in individuals undergoing specialised ED treatment for AN. To address this aim, we measured 27 cytokines in serum samples from AN patients at the start of treatment (baseline) and at 12- and 24-week follow-up assessments.

4.3 Methods

4.3.1 Participants and study design

Fifty-five people with AN were recruited as part of a larger study, comprising of a longitudinal naturalistic design (for full study details see: Keyes et al., 2015; Schmidt et al., 2017). Female adults with a primary diagnosis of AN and a BMI <17.5 kg/m^2 were recruited from Specialist Eating Disorder Services in and around London, UK. AN diagnosis was confirmed using the research version of the Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorders (DSM)-IV Axis I Disorders (First et al., 2002).

Participants were first assessed (baseline assessment) within the first four weeks of specialised ED treatment for AN. Typically, patients in the local services receive a combination of nutritional (e.g., dietetics, meal support) and psychological (e.g., individual psychotherapy, family therapy) intervention and general clinical management, with inpatients receiving additional input from physiotherapy and occupational therapy. Follow-up assessments were then conducted 12- and 24-weeks after the baseline

assessment. For the current study, only participants who provided blood samples at the baseline assessment and at least one follow-up assessment were included and participants reporting autoimmune and/or inflammatory diseases were also excluded. This resulted in a total of 23 participants to be included in the current analyses (*n*=19 completed 12-week follow-up; *n*=18 completed 24-week follow-up; *n*=14 completed all three assessments).

A healthy comparison group (HC) were also recruited as part of the larger study (Keyes et al., 2015; Schmidt et al., 2017). Normal-weight (BMI 18.5-24.5 kg/m^2) female adults with no physical illnesses and no history of or current mental health disorder were recruited via e-mail circulars to students and staff at King's College London (KCL). The absence of psychiatric disorders was confirmed using the research version of the Structured Clinical Interview for DSM-IV Axis I Disorders (First et al., 2002). HC participants who provided a blood sample and completed the measures described below on one occasion were included in the current study (*n*=13).

Informed consent was obtained from all participants.

4.3.2 Ethical approval

The study was conducted in accordance with the Declaration of Helsinki and the study received ethical approval from the South East London Research Ethics Committee (REC reference: 09/H0807/4).

4.3.3 Measures

At all assessments, height and body weight were measured, from which BMI (kg/m^2) was calculated. Body fat percentage and other body composition parameters (not presented here) were measured using a portable and non-invasive Inbody S10 machine (Inbody Co., Ltd., Seoul, South Korea), which uses the Bioelectrical Impedance Analysis (BIA) measurement method. ED symptoms over the previous 28 days were assessed using the Eating Disorder Examination-Questionnaire (EDE-Q; Fairburn, 2008) and general psychopathology over the previous seven days was measured using the Depression Anxiety and Stress Scales – 21 Version (DASS-21; Lovibond & Lovibond, 1995). As well as a total score, scores for the subscales depression, anxiety and stress can be calculated. Blood samples were also collected. A number of other measures were administered but data on these are presented elsewhere (Keyes et al., 2015; Schmidt et al., 2017).

4.3.4 Quantification of cytokine concentrations

Serum was stored at -80°C prior to use and thawed at room temperature. Concentrations of 40 inflammatory markers (including cytokines, cellular adhesion molecules, and growth factors) were quantified simultaneously using multiplex ELISA-based technology provided by the Meso Scale Discovery V-PLEX Human Biomarker 40-Plex Kit, following the

manufacturer's instructions (Meso Scale Discovery, Maryland, USA). Plates were scanned on the Meso Scale Discovery MESO Quickplex SQ 120 reader at the Social, Genetic, and Developmental Psychiatry (SGDP) Centre, KCL. We used the Meso Scale Discovery V-PLEX Human Biomarker 40-Plex Kit, as it quantifies TNF-α and IL-6, which were found to be elevated in AN in the latest meta-analysis (Chapter 2; Dalton et al., 2018a). It also measures other cytokines, such as IL-1β, IL-10, IL-12, IL-13 and interferon (IFN)-γ, which have been implicated in depression, anxiety disorders and obsessive-compulsive disorders (Himmerich et al., 2019d; Renna, O'Toole, Spaeth, Lekander, & Mennin, 2018), the major comorbidities of AN (Marucci et al., 2018). For the purpose of the current study, we limited our analyses to the following cytokines (*n*=27), rather than focusing broadly on all assayed inflammatory markers: Eotaxin, Eotaxin-3, granulocyte-macrophage colony-stimulating factor (GM-CSF), IFN-γ, IL-1α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12/IL-23p40, IL-12p70, IL-13, IL-15, IL-16, IL-17A, interferon γ-induced protein (IP)-10, macrophage inflammatory protein (MIP)-1α, MIP-1β, monocyte chemoattractant protein (MCP)-1, MCP-4, thymus and activation-regulated chemokine (TARC), TNF-α, and TNF-β. Cross-sectional comparisons of the baseline inflammatory marker data to HC participants are presented in Chapter 3.3 and Dalton et al. (2018b).

4.3.5 Statistical analyses

All statistical analyses were performed in Stata 15 (StataCorp, 2017). Standard curves were used to determine absolute quantities (pg/ml) of each cytokine. We transformed cytokine values by natural log to allow for parametric analyses.

Mixed model linear regressions fitted with maximum likelihood were used to identify changes over time in clinical characteristics and log-transformed cytokine values. Linear mixed models allow for all available outcome data to be included in the model simultaneously and provides estimates in the presence of missing data points (within-subjects; Gueorguieva & Krystal, 2004). With longitudinal data, measures within participants are correlated and linear mixed models take into account this within-subject and also between-subject variability. We constructed a model that specified log-transformed cytokine values or clinical characteristics (weight, body fat percentage, BMI, EDE-Q global score, and DASS-21 total score) with time point (baseline [week 0], week 12 and week 24) added as fixed effects and participant ID as a random effect. Bonferroni correction for pairwise comparisons was applied to each significant model post-hoc. We further applied a Bonferroni correction to account for multiple testing of cytokine values by dividing the *p* value (0.05) by the number of comparisons made (*n*=27). This gave a statistical significance threshold of $p = 0.002$.

For cytokines with significant longitudinal changes, we used between subjects t-tests to examine differences in log-transformed cytokine values between our sample of AN patients and the HC comparison group reported in in Chapter 3.3 and Dalton et al. (2018b). The level of significance was $p < 0.05$.

4.4 Results

Demographic and clinical characteristics are shown in **Table 4.1**. Body weight ($\chi^2(2) = 7.45$, $p = 0.024$) and BMI ($\chi^2(2) = 7.58$, $p = 0.023$) differed between time points; specifically, as expected, there was an increase in both variables between the baseline assessment and the 24-week follow-up ($z = 2.65$, $p = 0.024$). However, there were no changes in these variables between the baseline assessment and the intermediate week 12 timepoint. Body fat percentage ($\chi^2(2) = 3.92$, $p = 0.141$) did not differ between the three time points. ED symptoms showed an improvement ($\chi^2(2) = 14.23$, $p = 0.0008$) at both 12-weeks ($z = -3.04$, $p = 0.007$) and 24-weeks ($z = -3.28$, $p = 0.003$), compared to baseline. At both follow-up assessments, the mean EDE-Q global score was below the commonly used clinical cut-off score of four (e.g., Mond et al., 2006). No differences between time points were observed for general psychopathology ($\chi^2(2) = 0.01$, $p = 0.996$). DASS-21 severity cut-off scores indicated that participants reported severe depression, anxiety and stress at all three timepoints (Lovibond & Lovibond, 1995).

Table 4.1 Demographic, anthropometric and clinical characteristics at baseline and 12- and 24-weeks follow-up.

	Baseline (*n*=23)	**12-week follow-up (*n* =19)**	**24-week follow-up (*n*=18)**
Age [years] (mean ± SD)	32.57 ± 11.81		
Current smoker (*n*)	5	3	4
Treatment status [inpatient / outpatient] (*n*)	8 / 15		
AN subtype [AN-R / AN-BP] (*n*)	10 / 13		
Duration of diagnosis [years] (mean ± SD)	11.68 ± 12.20[a]		
Weight [kg] (mean ± SD)	42.75 ± 8.27	43.76 ± 9.48	46.95 ± 8.89*
BMI [kg/m^2] (mean ± SD)	15.45 ± 2.30	15.68 ± 2.65	16.76 ± 2.61*
Body fat [%] (mean ± SD)	8.20 ± 6.36	7.84 ± 5.76[a]	11.11 ± 7.80[b]
EDE-Q Global (mean ± SD)	4.39 ± 0.97	3.84 ± 1.36*	3.93 ± 1.00*
DASS-21 Total (mean ± SD)	72.78 ± 28.08	70.11 ± 32.45	72.22 ± 29.83
DASS-21 Depression (mean ± SD)	24.52 ± 12.99	24.74 ± 13.78	25.33 ± 10.98
DASS-21 Anxiety (mean ± SD)	19.30 ± 10.12	17.74 ± 11.28	20.00 ± 10.58
DASS-21 Stress (mean ± SD)	28.96 ± 8.48	27.63 ± 11.58	26.89 ± 10.50

[a]*n*=2 missing; [b]*n*=1 missing; * denotes significantly different from baseline at $p < 0.05$. Abbreviations: *n* = number of observations; SD = standard deviation; AN = anorexia nervosa; AN-R = anorexia nervosa restricting type; AN-BP = anorexia nervosa binge-eating/purging type; BMI = body mass index; EDE-Q = Eating Disorder Examination – Questionnaire; DASS-21 = Depression Anxiety and Stress Scales – 21 Version.

Log-transformed cytokine values for the three time points are presented in **Table 4.2**. The identified longitudinal changes in cytokines did not meet formal significance thresholds ($p = 0.002$), however, we present nominally significant findings here (i.e., without Bonferroni correction for multiple testing). The majority of cytokines were found not to differ between time points. Linear mixed models identified a difference between time points for log TNF-β ($\chi^2(2) = 6.66$, $p = 0.036$); however, these differences did not survive post-hoc correction. A difference in log IL-6 scores between time points ($\chi^2(2) = 9.42$, $p = 0.009$) was also identified. Bonferroni corrected pairwise comparisons showed that compared to baseline, log IL-6 was reduced at week 12 ($z = -3.03$, $p = 0.007$). Analyses were rerun after removal of two outliers, findings remained the same. Log IL-7 ($\chi^2(2) = 7.35$, $p = 0.025$) differed between time points, with an increase from week 12 to week 24 ($z = 2.66$, $p = 0.023$). **Figure 4.1** shows the serum concentration of IL-6 and IL-7 for AN patients at each time point with a comparison group of HCs.

Table 4.2 Mean (± standard deviation) log-transformed serum cytokine concentrations at baseline and 12- and 24-weeks follow-ups.

Log-transformed cytokines	Baseline		12-week follow-up		24-week follow-up	
	n	Mean ± SD	*n*	Mean ± SD	*n*	Mean ± SD
Eotaxin	23	2.29 ± 0.15	19	2.30 ± 0.17	18	2.35 ± 0.18
Eotaxin-3	23	1.19 ± 0.28	19	1.11 ± 0.26	18	1.18 ± 0.20
GM-CSF	18	-0.84 ± 0.38	18	-0.94 ± 0.54	16	0.76 ± 0.35
IFN-γ	23	0.76 ± 0.40	19	0.71 ± 0.27	18	0.80 ± 0.43
IL-1α	23	0.02 ± 0.27	19	0.02 ± 0.37	18	0.05 ± 0.31
IL-1β	18	-0.88 ± 0.70	14	-1.34 ± 0.99	11	-1.02 ± 0.49
IL-2	11	-0.93 ± 0.58	13	-1.07 ± 0.70	9	-0.78 ± 0.28
IL-4	21	-1.32 ± 0.77	14	-1.22 ± 0.72	15	-1.26 ± 0.67
IL-5	21	-0.02 ± 0.34	14	-0.07 ± 0.40	15	0.02 ± 0.18
IL-6	23	-0.22 ± 0.36	19	-0.42 ± 0.21*	18	-.035 ± 0.22
IL-7	23	1.10 ± 0.17	18	1.02 ± 0.19	18	1.14 ± 0.14†
IL-8	23	1.46 ± 0.57	19	1.40 ± 0.55	18	1.25 ± 0.33
IL-10	23	-0.65 ± 0.46	19	-0.84 ± 0.44	16	-0.86 ± 0.51
IL-12/IL-23p40	23	1.95 ± 0.19	19	1.83 ± 0.45	18	1.97 ± 0.14
IL-12p70	21	-0.80 ± 0.41	17	-0.78 ± 0.36	17	-0.79 ± 0.65
IL-13	13	0.33 ± 0.30	10	0.26 ± 0.35	13	0.30 ± 0.32
IL-15	23	0.47 ± 0.11	18	0.49 ± 0.14	18	0.44 ± 0.15
IL-16	23	2.31 ± 0.17	19	2.14 ± 0.43	18	2.25 ± 0.17
IL-17A	23	0.23 ± 0.26	18	0.27 ± 0.30	18	0.29 ± 0.37
IP-10	23	2.17 ± 0.25	19	2.05 ± 0.23	18	2.15 ± 0.22
MCP-1	23	2.28 ± 0.09	19	2.28 ± 0.12	18	2.33 ± 0.08
MCP-4	23	2.07 ± 0.20	19	2.05 ± 0.20	18	2.15 ± 0.12
MIP-1α	23	1.42 ± 0.17	19	1.39 ± 0.17	18	1.41 ± 0.12
MIP-1β	23	1.93 ± 0.19	19	1.92 ± 0.19	18	1.92 ± 0.17
TARC	23	2.60 ± 0.38	19	2.49 ± 0.27	18	2.57 ± 0.29
TNF-α	23	0.22 ± 0.17	19	0.19 ± 0.13	18	0.21 ± 0.14
TNF-β	23	-0.21 ± 0.10	19	-0.31 ± 0.24	18	-0.20 ± 0.12

* nominally significantly different from baseline at $p < 0.05$; † nominally significantly different from week-12 at $p < 0.05$. Abbreviations: *n* = number of observations; SD = standard deviation; GM-CSF = granulocyte-macrophage colony-stimulating factor; IFN-γ = interferon-γ; IL = interleukin; IP-10 = interferon γ-induced protein-10; MCP = monocyte chemoattractant protein; MIP = macrophage inflammatory protein; TARC = thymus and activation-regulated chemokine; TNF = tumor necrosis factor.

We used between subjects t-tests to compare log-transformed IL-6 and IL-7 values from the AN participants to a HC comparison group (n=13) from the larger study (see Chapter 3.3 and Dalton et al., 2018b for HC sample details), to determine whether cytokine values had 'normalised'. At baseline, the AN participants showed elevated log IL-6 values compared to the HCs ($t(34) = -2.67$, $p = 0.012$). At 12- and 24-weeks, log IL-6 for the AN participants no longer differed from HCs (12-weeks: $t(30) = -1.19$, $p = 0.242$; 24-weeks: $t(29) = -1.85$, $p = 0.075$). Log IL-7 did not differ between groups at any time point.

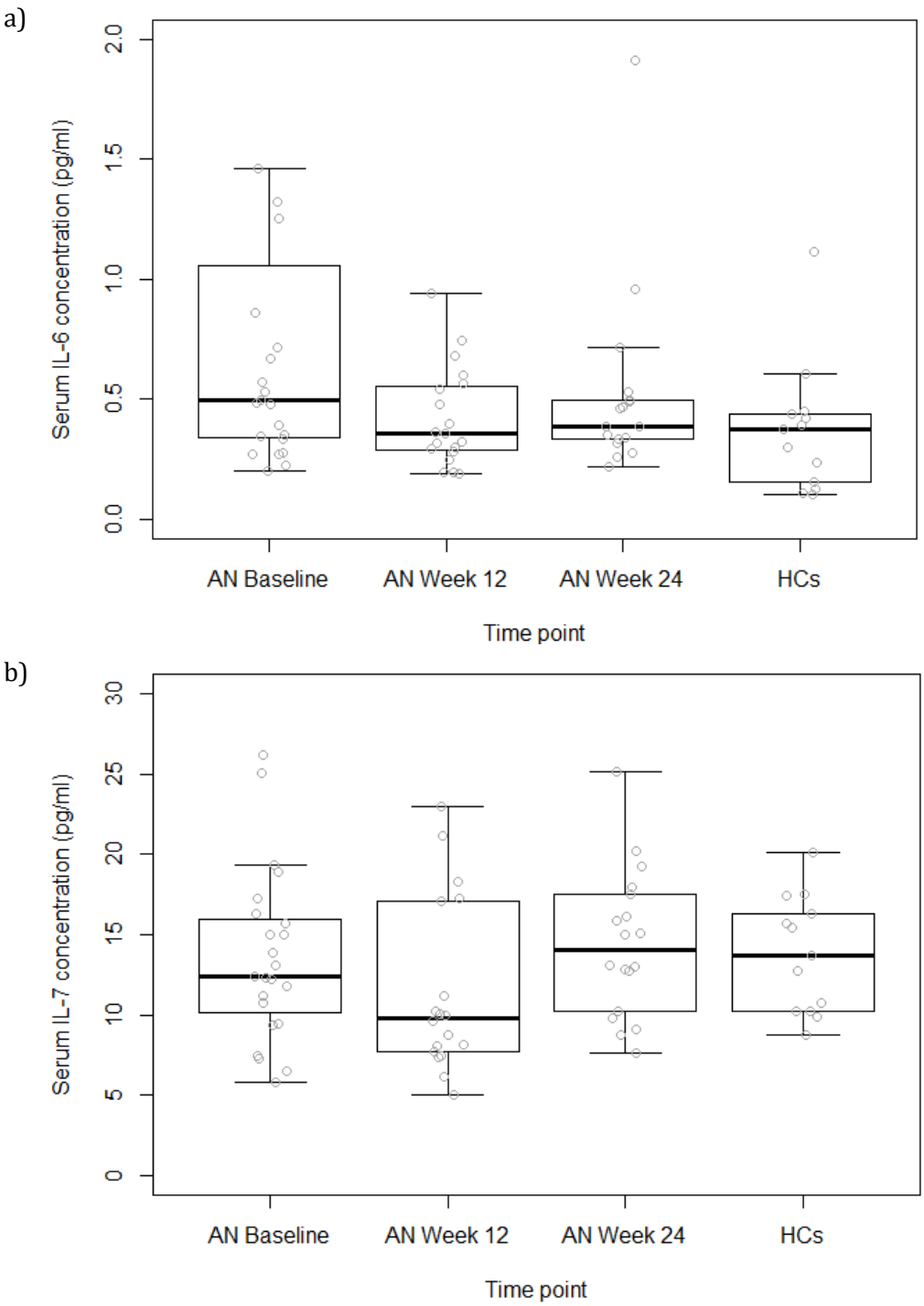

Figure 4.1 Box plots with individual data points of a) interleukin-6 serum concentrations (pg/ml), and b) interleukin-7 serum concentrations (pg/ml) at baseline, week 12 and week 24 in patients with anorexia nervosa. Our previously reported data for healthy control participants (Study 1 of Chapter 3; Dalton et al., 2018b) is provided for comparison (n=13). Log IL-6 significantly decreased between baseline and week 12 (z = -3.03, p = 0.007), with a mean reduction in serum IL-6 concentrations of approximately 26%. Log IL-7 significantly increased between week 12 to week 24 (z = 2.66, p = 0.023), with a mean increase in serum IL-7 concentrations of approximately 36%. Abbreviations: AN = anorexia nervosa; HC = healthy control; IL = interleukin.

4.5 Discussion

Our study is the first to measure a wide range of cytokines longitudinally in a sample of 23 AN patients receiving specialist ED treatment. Many of these cytokines have not previously been assessed over time in this patient group. Most existing studies only considered cytokine changes within the context of physical improvements, such as weight gain (e.g., Misra et al., 2006a). We assessed cytokine values in the context of both BMI and psychological ED symptoms. In our sample of patients with significantly low weight and body fat, and clinical levels of ED symptoms, depression, and anxiety, 25 cytokines were unchanged over the follow-up period, despite improvements in BMI and ED psychopathology. Two cytokines were found to change longitudinally: IL-6 decreased between baseline and week 12 assessments, and IL-7 increased between week 12 and week 24.

AN patients are metabolically different to healthy individuals, with significantly lower body weight and fat. Despite this, however, we previously reported that many cytokines were not altered in AN compared to HCs (Chapter 3.3; Dalton et al., 2018b). In the current study, we found that most cytokines remain unchanged by improvements in BMI and ED symptoms. This includes those cytokines shown to be previously altered in AN, such as TNF-β and IL-15 (Chapter 3.3; Dalton et al., 2018b), and those that can be hypothesised to have a role in weight, adipose tissue, appetite and feeding behaviour regulation such as IL-1β (a potently anorexigenic cytokine), IFN-γ, TNF-α, and MCP-1 (e.g., Alam, Ullah, Alam, & Ali, 2013; Chen et al., 2005; Labrecque et al., 2019; Peluso & Palmery, 2016; Rask-Andersen et al., 2010; Wong & Pinkney, 2004).

Not all cytokines shown to be involved in weight regulation have been found to be altered in AN e.g., IL-1β and IL-10 (Chapter 3.3; Dalton et al., 2018b). This suggests that AN may be a specific psychological and physical condition that may not involve all weight-regulating mechanisms. Additionally, the net serum concentration of a cytokine is the result of various physiological processes. For example, TNF-α has been shown to be elevated in AN in our recent meta-analysis (Chapter 2; Dalton et al., 2018a). It might be elevated in this patient group as it has been shown in animal experiments that psychological stress can lead to increased TNF-α production (Himmerich et al., 2013; Krügel et al., 2014). TNF-α has been shown to lead to weight, skeletal muscle and adipose tissue loss (Patel & Patel, 2017). However, TNF-α levels have also been shown to correlate with body weight (Himmerich et al., 2006b). If a patient with AN improves psychologically, there may be reduced production of TNF-α from stress-reactive cells. However, simultaneously, increases in body weight in AN recovery might lead to increased production of TNF-α in

other cell types. These two opposing processes might leave the net serum level relatively unchanged.

Despite improvement in the physical and mental states of patients with AN, it may also be that underlying biological processes contribute to the persistent alterations in these cytokines. For example, the gut microbiome could be relevant for alterations in cytokine production and the pathophysiology of AN (Bastiaanssen, Cowan, Claesson, Dinan, & Cryan, 2018; Herpertz-Dahlmann et al., 2017; Ruusunen, Rocks, Jacka, & Loughman, 2019) because gut dysbiosis and variation within the gut microbes has been associated with the stimulation of cytokine production (Schirmer et al., 2016). Furthermore, inflammatory responses within the gut can signal to the brain via the vagus nerve, which can subsequently affect brain function and behaviour (Bonaz, Bazin, & Pellissier, 2018; Sherwin et al., 2016). Another mechanism by which the microbiome can activate the immune system is through a 'leaky' gut (i.e., increased gut permeability): this allows antigens to cross the barrier into systemic circulation, which leads to an inflammatory response. In patients with AN, the gut barrier does not seem to be altered (Mörkl et al., 2019); however, to date, this has only been investigated in a single study with a small AN sample.

Specifically, people with AN have reduced but also altered diversity of bacterial species within the gut, and there is increased heterogeneity between subjects, as compared to HCs (Seitz et al., 2019). However, the connections between AN, the gut microbiota and the immune system are difficult to disentangle as it has shown that they influence each other. For example, a number of cytokines are involved in the regulation of intestinal microbiota, including TNF-β (Kruglov et al., 2013; Upadhyay & Fu, 2013), and bacteria from the gut microbiome can stimulate the production of cytokines, such as IL-1β, IL-6 and TNF-α (Schirmer et al., 2016; Sherwin et al., 2016).

In AN, longitudinal changes in the gut microbiome associated with nutritional rehabilitation and weight gain are unclear, given the limited research (Ruusunen et al., 2019). Results from two small studies show that differences in the gut microbiome composition in AN patients persist after refeeding (Kleiman et al., 2015; Mack et al., 2016). Taken together, biological processes associated with the microbiome may contribute to the cytokine disturbances seen in AN (Chapter 2; Dalton et al., 2018a), but it is unclear whether the gut microbiota changes significantly during the course of AN and whether these potential changes or the persistence of microbiota alterations have an influence on cytokine concentrations in the long-term.

Persistent cytokine disturbances may also be related to depression and anxiety, which were rated as severe by our sample at all time points. These disorders are highly comorbid

with AN (Marucci et al., 2018; Ulfvebrand et al., 2015) and have been associated with inflammation and cytokine alterations (Baldwin et al., 2017; Köhler et al., 2017). Furthermore, severe stress was present throughout assessment periods and may contribute to the persistent cytokine alterations. Indeed, acute and chronic social stress have been shown to promote cytokine production (Himmerich et al., 2013; Krügel et al., 2014). Therefore, these cytokines may be potentially more sensitive as a marker of general psychopathology, rather than of changes in ED symptoms.

We have previously reported that serum IL-6 levels are increased in people with AN compared to healthy individuals (Chapter 2; Dalton et al., 2018a). In the current study, we found that IL-6 decreased between the baseline and the 12-week assessment, with a mean reduction in serum IL-6 concentration of approximately 26%. At 12-weeks, IL-6 levels were comparable to those of HCs. There was no difference between baseline and 24-week IL-6 levels. Inspection of the box plot in **Figure 4.1a** also suggests a reduction in heterogeneity of IL-6 values over time in the AN patients. One explanation for this may be that there is a sub-group of AN patients with a pro-inflammatory profile that 'normalises' with improvement in ED symptoms.

We found that ED symptom severity improved during the first 12 weeks of treatment and the mean EDE-Q global score dropped below the typically used clinical cut-off (e.g., Mond et al., 2006). Thus, the 'normalisation' of IL-6 levels may not be strictly related to weight changes (e.g., Misra et al., 2006a), but also to psychological improvement. This suggests that IL-6 has the potential to be a state marker of AN. Similar findings have been reported in unmedicated patients with depression, where a reduction in IL-6 concentrations was associated with improvements in symptom severity following psychotherapy (Dahl et al., 2016; Del Grande da Silva et al., 2016); however, it is important to note that in these depressed patients, any weight changes were not reported.

IL-6 has been associated with psychological symptoms (including anxiety, social stress, compulsivity, and mood; e.g., Lichtblau et al., 2013; Niraula, Witcher, Sheridan, & Godbout, 2018), physical exercise (Lavebratt et al., 2017), and appetite regulation (Paulsen et al., 2017), all of which have been implicated in AN (Munro, Randell, & Lawrie, 2017). From a molecular perspective, peripheral cytokines, such as IL-6, have been shown to enter the brain and influence brain functioning and development, through humoral, neural and cellular pathways, and thus impact on psychological state (Capuron & Miller, 2011). Specifically, cytokines have been shown to affect neuroendocrine pathways and alter neurotransmitter production and signalling. For example, cytokines can activate the hypothalamic-pituitary-adrenal (HPA) axis and stimulate the expression and release of corticotropin-releasing hormone (CRH), adrenocorticotropic hormone (ACTH) and

cortisol (Capuron & Miller, 2011), all of which are reported to be elevated in patients with AN (Misra & Klibanski, 2014). Furthermore, relative hypercortisolaemia in AN has been associated with greater ED psychopathology (Lawson et al., 2011).

IL-6 is also reported to be involved in the regulation of adipose tissue (Wedell-Neergaard et al., 2018b) and food intake (Wong & Pinkney, 2004), and has been associated with gut microbiome composition (Cooper et al., 2016; Schirmer et al., 2016). Therefore, we may expect changes in IL-6 to coincide with changes in body fat mass and/or weight. Additionally, previous studies reported reductions in IL-6 concentrations, to the level of HCs, with weight gain in AN patients (Misra et al., 2006a; Pomeroy et al., 1994). In our sample, however, we did not observe a significant increase in weight, BMI or body fat percentage during this period from baseline to the 12-week assessment, in fact, there was only a 1.5% BMI increase (mean 0.23 kg/m^2) and a 2.0% weight increase (mean 1.0 kg). With respect to body fat, it has been suggested that the BIA measurement method for deriving body fat percentage used in the current study may not be sensitive or reliable in groups with particularly low body weight, dehydration, or electrolyte imbalance, features often seen in patients with AN (Kyle et al., 2004; Mattar et al., 2011; Mialich et al., 2014). A more reliable ultrasound method recently reported that subcutaneous adipose tissue differed significantly between AN individuals with the same BMI and that some AN participants had sufficiently high fat mass despite a low BMI (Lackner et al., 2019). As IL-6 is reported to be involved in the regulation and function of adipose tissue, including both visceral and subcutaneous fat (Wedell-Neergaard et al., 2018b), it may be that there is a sub-group of patients with different pro-inflammatory profiles associated with body composition. Taking together the described roles of IL-6 and that refeeding and weight restoration are key factors in specialised AN treatment, it would be important to consider the role of diet and both body and microbiome composition in longitudinal cytokine changes to more fully understand the longitudinal association between IL-6 and AN.

IL-7 has been less researched in relation to AN and more generally in psychiatry, in comparison to IL-6. IL-7 is predominantly produced by stromal and epithelial cells in various locations in the body periphery (Fry & Mackall, 2002), is required for lymphocyte homeostasis (Khaled & Durum, 2002), and has been shown to induce the production of the pro-inflammatory cytokines IL-1, IL-6, IL-8 and TNF-α (Alderson et al., 1991). IL-7 has also been associated with the regulation of body weight via hypothalamic and adipose tissue control (Lucas et al., 2012; Macia et al., 2010; Makki, Froguel, & Wolowczuk, 2013). For example, in three different models of experimentally-induced obesity, administration of IL-7 reduced white adipose tissue mass, protecting mice from obesity (Lucas et al., 2012; Macia et al., 2010). In our study, IL-7 differed between weeks 12 and 24, with a mean

increase of approximately 36%. No significant improvements in ED symptoms, BMI, or general psychopathology were observed during this time (12-week assessment to 24-week assessment). While this suggests that the increase in IL-7 was not associated with changes in illness state, it could be considered that the increase in IL-7 may be related to changes in diet and food intake (Germain et al., 2016). It is important to note that IL-7 values were not altered in comparison to HCs at any time point. This is *inconsistent with our findings of decreased IL-7 in 40 patients with AN in Study 2 of Chapter 3 and*[3] a previous study which found that in five patients with AN, already low levels of 24-hour plasma IL-7 levels (compared to HCs) further decreased following weight and menses recovery (Germain et al., 2016). However, these findings need to be considered in view of the small sample size in this study. Given the limited research of IL-7 in relation to weight regulation, food intake, and psychological symptoms in AN, it is unclear why IL-7 changes longitudinally in our sample and in combination with our data and study design, it does not allow for further conclusions.

It is important to consider that a further level of complexity is introduced by the fact that cytokines should be interpreted against the background of genetics, epigenetics and gene expression. It has been shown that cytokine production is also regulated at a genetic level (Li et al., 2016c). For example, a single nucleotide polymorphism in the promoter of the IL-6 gene (at position –174 G>C) is reported to regulate peripheral IL-6 concentrations, such that the CC genotype was associated with lower plasma IL-6 concentration (Fishman et al., 1998). Molecular genetics research has also suggested that certain IL-6 alleles and genotypes may decrease the risk for schizophrenia (Hudson & Miller, 2018) and depression (Khandaker, Zammit, Burgess, Lewis, & Jones, 2018). While IL-6 may be a promising biomarker of AN, genotyping studies in AN have focussed on TNF-α (Ando et al., 2001; Kanbur et al., 2008), a cytokine which has been less consistently associated with AN. Future research should therefore consider genotyping, and epigenetic and gene expression analyses of the IL-6 promotor gene to improve understanding of findings related to peripheral cytokine levels and individual differences in cytokines in patients with AN.

4.5.1 Strengths and limitations

This is the first study to assess a broad range of cytokines and chemokines longitudinally in patients with AN, using validated electrochemiluminescence methods, but as such, it has some limitations. We did not systematically document the various treatment elements the patients received nor recorded treatment status/engagement at the two follow-up

[3] The information in *italics* was not included in the original publication and has been added here specifically for the purpose of this thesis to ensure that the discussion is consistent with the findings reported in Study 2 in Chapter 3.

assessments. Also, anti-depressant and other psychotropic medication were not recorded. As anti-depressants are reported to be immune-modulating and hypothesised to exert an anti-inflammatory effect (Köhler et al., 2018), we cannot be sure that changes in cytokines are associated with symptom improvement rather than psychotropic medication. We did not conduct an *a priori* power analysis, as our intention was to perform an exploratory study with a broad range of cytokines that have not previously been measured in patients with AN. While we initially corrected for multiple testing of cytokine values, longitudinal changes in cytokines did not meet these formal significance thresholds and therefore, the findings presented have not been adjusted for multiple comparisons (i.e., they were only nominally significant). Due to the small sample size and limited power, we were unable to statistically assess the relationship between cytokine changes and changes in AN presentation, nor include confounding factors in our analyses that might influence cytokines (e.g., age, smoking, physical activity) and in turn, may have affected our results.

4.5.2 Conclusions

In patients with AN receiving specialist ED treatment, we measured 27 cytokines at three time points over a 24-week period. We found that IL-7 increased between weeks 12 and 24 in people with AN. Little research on this cytokine exists in AN and other related psychiatric disorders, therefore, research is needed to improve understanding of the role it may play in AN. Our findings also show that the IL-6 serum concentrations, which have been reported to be elevated in AN (Chapter 2; Dalton et al., 2018a), 'normalise' during the first three months of specialised treatment for AN. This reduction may be associated with an improvement in ED symptom severity. Therefore, IL-6 could be hypothesised as a state marker of AN psychopathology and may present a promising target for future biomarker research and treatment interventions. In our sample, after six months, only 20% of patients had reached a healthy weight (BMI >18.5 kg/m^2) and 33% still presented with clinically significant ED behaviours and symptoms. As such, more research will be needed in larger samples over a longer time frame to increase understanding of the role of IL-6 in AN and recovery.

The temporal sequence and direction of causality of the changes in IL-6 and IL-7 in relation to weight and ED symptom change are unclear from the current data and overall, the role of cytokines in AN is not well understood. Future research would benefit from including prospective longitudinal measurements of cytokines, along with ED-associated physical and mental presentation as well as genotyping, epigenetic characterisation of cytokines genes and the measurement of gene expression, to better understand the role of these cytokines in the development, maintenance and/or recovery from AN.

Chapter 5. Examining the potential role of stress in the relationship between inflammatory markers and eating disorders

5.1 Abstract

Objective: There is a bidirectional relationship between stress and the immune system. Research has shown that exposure to stressful experiences, including traumatic and stressful life events and a history of childhood maltreatment, has been associated with a pro-inflammatory profile. Across three studies, this chapter aimed to examine whether exposure to stressful life events, including traumatic experiences and childhood maltreatment, were associated with alterations in inflammatory markers in people with eating disorders (EDs).

Methods: Study 1 assessed the relationship between exposure to traumatic events and associated post-traumatic stress disorder (PTSD) symptoms with cytokine concentrations in a sample of females with anorexia nervosa (AN; *n*=40) and healthy controls (HC; *n*=34). Study 2 examined the association between trauma exposure in childhood and adulthood, and concentrations of C-reactive protein (CRP) in people with lifetime ED diagnoses (n=1049) and a HC comparison group (*n*=4196). Study 3 considered how the experience of childhood maltreatment and stressful life events was related to cytokine concentrations in emerging adults with current ED diagnoses (*n*=74).

Results: Findings were consistent across the three studies: exposure to stress, in the forms of trauma, stressful life events, and childhood maltreatment, were not significantly associated with concentrations of any inflammatory markers in people with EDs. Study 2 showed that in HCs, childhood trauma was associated with concentrations of CRP in midlife, suggesting differential effects of childhood trauma on inflammation in individuals with lifetime EDs and HCs.

Conclusion: These studies were the first to consider the role of event-related stress in the relationship between inflammatory markers and EDs. We did not replicate previous findings of elevated pro-inflammatory cytokines in people with a history of trauma exposure in individuals with EDs. The assessments of stress were unable to examine the stress response, timing of the event, and the severity and frequency of trauma exposure, which may have accounted for the findings. Future studies should perform detailed assessments of trauma exposure to further explore the relationship between stress and inflammation in people with EDs.

5.2 Introduction

Stress refers to an organism's response to a stressor e.g., an environmental condition. However, response to a stressor in humans is a cognitive process and depends on an individual's perception and interpretation of the stressor in the broad context in which it is presented (Selye, 1975). Therefore, response to a stressor may manifest differently in different individuals or vary depending on the stressor. To account for this, two types of stress defined by how an individual perceives a stressor have been described by Selye (1975). Distress occurs when a stressor is perceived negatively (e.g., seen as overwhelming or a negative threat or challenge), leading to a state of mental or emotional strain or tension. In contrast, eustress occurs primarily as a result of positive perception of the stressor (e.g., seen as a positive challenge or opportunity to grow) and may lead to a response characterised by a sense of meaning, feelings of fulfilment, hope, vigour or other positive feelings, and enhanced functioning. Ultimately, whether a stressor is perceived or interpreted as distressing or positive depends on a number of factors including an individual's resources, their mental and physical state, whether the stressor is perceived as within or beyond an individual's coping abilities, and the desirability and context of the stressor (e.g., timing, location). Typically, the term "stress" is used to refer to distress and therefore, will be used as such herein. Within this context, different types of stress have been categorised using a variety of approaches. For example, stressors can be classified based on the type of stressor: traumatic stress occurs in response to a traumatic event (e.g., a car accident), psychosocial stress in response to stressors in the social environment (e.g., relationship difficulties), and psychological stress in response to stressors for which a person feels unequipped to cope with a situation (e.g., financial problems). Stressors can be additionally defined based on the duration of the stressor; for example, acute stress occurs in response to a short-term time-limited stressor (e.g., a meeting at work), and chronic stress in response to an extended long-term stressor (e.g., caregiving).

A number of animal studies using both acute and chronic stress paradigms have induced hyper-production of cytokines (Himmerich et al., 2013; Krügel et al., 2014; Liu et al., 2012). In healthy humans, stress has been shown to modulate aspects of the immune system, including enhancing the release of inflammatory markers such as cytokines and C-reactive protein (CRP; Glaser & Kiecolt-Glaser, 2005; Herbert & Cohen, 1993; Ménard et al., 2017; Rohleder, 2014; Segerstrom & Miller, 2004; Steptoe et al., 2007; Takahashi, Flanigan, McEwen, & Russo, 2018). Elevated concentrations of circulating pro-inflammatory cytokines have been associated with various types of stress, including acute stress, both laboratory (Marsland et al., 2017; Slavish, Graham-Engeland, Smyth, & Engeland, 2015) and daily (Gouin, Glaser, Malarkey, Beversdorf, & Kiecolt-Glaser, 2012; Sin, Graham-Engeland, Ong, & Almeida, 2015) stressors; chronic stress e.g., caregiving

(Gouin, Hantsoo, & Kiecolt-Glaser, 2008; Potier, Degryse, & de Saint-Hubert, 2018); and event-related stress e.g., negative life events (Knowles, Ruiz, & O'Connor, 2019), adverse childhood experiences (Coelho, Viola, Walss-Bass, Brietzke, & Grassi-Oliveira, 2014; Danese & Lewis, 2017; Fagundes, Glaser, & Kiecolt-Glaser, 2013), and traumatic events (Passos et al., 2015).

The focus of the current chapter will be on adverse life experiences that are associated with acute and chronic stress. These include exposure to stressful and traumatic life events and childhood maltreatment trauma. The background for this chapter will first describe the literature on the association between these forms of stress and inflammation, before discussing the biological relationship between cytokines and the hypothalamic-pituitary-adrenal (HPA) axis.

5.2.1 Exposure to traumatic and stressful life events

Life events can be defined as discrete experiences that disrupt an individual's usual activities, causing substantial change and readjustment. It should be noted that while life events are discrete experiences, they can lead to ongoing chronic difficulties and stress.

5.2.1.1 Stressful life events

If life events are perceived as stressful, they can be termed stressful life events. Examples of stressful life events include marriage, separation or divorce, illness or injury, bereavement, moving house, and changing or losing a job. Stressful life events are relatively common, with approximately 25% of individuals having experienced one in the previous year (Brown & Harris, 1989) and over 80% having experienced one or more in their lifetime (Kendler, Neale, Kessler, Heath, & Eaves, 1993). Stressful life events have been linked to an increased risk of psychopathology, including the development of depression, anxiety and eating disorders (EDs), in later life (Brown & Harris, 1979, 1989; Horesh et al., 1995; Pike et al., 2006; Rojo, Conesa, Bermudez, & Livianos, 2006; Schmidt, Tiller, Blanchard, Andrews, & Treasure, 1997). Furthermore, research has reported a higher prevalence of experiencing stressful life events in people with current ED diagnoses, as compared to healthy and psychiatric controls (Rojo et al., 2006; Schmidt et al., 1997).

Stressful life events have been associated with alterations in cytokine expression and circulating concentrations of cytokines and CRP (e.g., Augustine et al., 2014; Cohen, Granger, & Fuller-Thomson, 2015; Di Nicola et al., 2013; Fagundes et al., 2018; Flouri, Francesconi, Papachristou, Midouhas, & Lewis, 2019; Hostinar, Lachman, Mroczek, Seeman, & Miller, 2015; Jones et al., 2017; Knowles et al., 2019; Osler, Bendix, Rask, & Rod, 2016). For example, the number of recent stressful life events were reported to be significantly positively correlated with messenger ribonucleic acid (mRNA) levels of

interleukin (IL)-1β and tumor necrosis factor (TNF)-α in first-episode psychosis patients (Di Nicola et al., 2013). Also, in relation to a specific stressful life event: higher concentrations of IL-6 have been found in bereaved adults compared to adults who have not experienced a bereavement (Knowles et al., 2019) and the number of recent bereavements has been shown to predict IL-6 concentrations (Cohen et al., 2015).

Further support for a role of stressful life events in inflammation comes from research into the course of inflammatory conditions. A vast range of stressful life events have been associated with the onset and exacerbations/relapses of inflammatory diseases including Multiple Sclerosis (Mitsonis, Potagas, Zervas, & Sfagos, 2009), psoriasis (Naldi, Peli, Parazzini, & Carrel, 2001), irritable bowel syndrome (Whitehead, Crowell, Robinson, Heller, & Schuster, 1992), and asthma (Oren, Gerald, Stern, Martinez, & Wright, 2017). It is thought that stressful life events activate the stress system in the body which leads to increases in inflammatory activity, as will be described in Section 5.2.3.

5.2.1.2 Traumatic life events

Traumatic events are defined as an experience in which a person or someone close to them is at risk of serious harm or death. These include events such as experiencing or witnessing a car accident or someone being injured, life-threatening illness, war and combat, sexual or physical assault, and natural disasters. It has been reported that up to 70% of the general population will experience a traumatic event during their lifetime (Keane, Marx, & Sloan, 2009), and that rates of trauma exposure are higher in urban environments and in samples of military personnel (Gates et al., 2012; Gillespie et al., 2009). In a United States based national comorbidity survey, the majority (90 to 100%) of individuals with a lifetime ED diagnosis reported having experienced a traumatic event (Mitchell, Mazzeo, Schlesinger, Brewerton, & Smith, 2012). Indeed, it has been suggested that experiencing a traumatic event precedes the development of EDs (Cachelin, Schug, Juarez, & Monreal, 2005; Collins, Fischer, Stojek, & Becker, 2014; Pike et al., 2006; Tagay et al., 2014).

Higher traumatic event exposure has been associated with elevated IL-6 and CRP concentrations and also a composite score of inflammation (CRP, IL-6, TNF-α, and Resistin), which remained significant following adjustments for sociodemographic factors and psychiatric disorders (O'Donovan, Neylan, Metzler, & Cohen, 2012). Considering specific traumatic events, exposure to lifetime intimate partner violence has been associated with increases in concentrations of IL-6 and TNF-α (Robertson Blackmore et al., 2016). However, findings have been inconsistent. For example, exposure to physical, sexual, or emotional violence in the preceding year was not associated with CRP or IL-6 concentrations in female sex workers (Heller et al., 2018). Nonetheless, a meta-analysis

including almost 15,000 participants concluded that trauma exposure was related to increased concentrations of CRP, IL-1β, IL-6, and TNF-α (Tursich et al., 2014).

In response to an exposure (direct or indirect) to a severe traumatic event, some people develop post-traumatic stress disorder (PTSD). PTSD is a chronic psychiatric disorder in which individuals re-experience the traumatic event (e.g., as intrusive recollections, nightmares or flashbacks) accompanied by severe emotional distress or physical reactivity, persistent avoidance of circumstances or thoughts related to the event, and negative emotions such as feelings of isolation, persecutory thoughts, or being unable to remember facts relating to the event (American Psychiatric Association, 2013). In the general population, it has been estimated that 7% of individuals who experience a traumatic event go on to develop PTSD (Keane et al., 2009). PTSD is variably associated with EDs, with a comorbidity estimate of between 4% and 62% (Brewerton, 2007; Swinbourne et al., 2012; Swinbourne & Touyz, 2007; Tagay et al., 2014; Vierling et al., 2015). In AN, differences have been reported between subtypes, with approximately 3% of patients with the restricting type of AN (AN-R) having a comorbid PTSD diagnosis and 7% of patients with the binge-eating/purging type of AN (AN-BP; Ulfvebrand et al., 2015).

Research has reported that in the immediate aftermath of trauma exposure lower concentrations of pro-inflammatory cytokines, specifically interferon (IFN)-γ and TNF-α, are associated with a greater risk for later developing PTSD (Michopoulos et al., 2019). Furthermore, resilience to a traumatic event (i.e., not developing later PTSD) has been associated with higher concentrations of anti-inflammatory cytokines e.g., IL-10 (Teche et al., 2017). Recent reviews and meta-analyses have identified elevated concentrations of the pro-inflammatory cytokines IL-1β, IL-6 and TNF-α in people with a diagnosis of PTSD (Hussein et al., 2017; Passos et al., 2015; Renna et al., 2018; Waheed et al., 2018). This elevation in cytokines remained when participants with comorbid depression were excluded from analyses (Passos et al., 2015). Higher concentrations of chemokines have also been associated with PTSD (Hoge et al., 2009; Ogłodek & Just, 2018). It has been suggested that pro-inflammatory markers could be a contributing factor to the pathophysiology of PTSD, and they may play a role in PTSD-related elevated risk for cardiovascular, autoimmune, and neurodegenerative diseases (O'Donovan et al., 2015; Song et al., 2019; Waheed et al., 2018; Yaffe et al., 2010). However, significant pro-inflammatory cytokine changes in patients with PTSD have not been consistently identified (e.g., Himmerich et al., 2015; Zhou et al., 2014). Furthermore, evidence for an association between PTSD symptom severity and concentrations of inflammatory markers has been mixed (Hori & Kim, 2019).

It is important to note that research on traumatic event exposure has historically been limited to using individuals with PTSD i.e., they experienced a traumatic event and subsequently developed PTSD (Tursich et al., 2014). Thus, some of the findings reported may be confounded by the presence of PTSD symptomology. Indeed, one research study assessing the cytokine concentrations in hurricane survivors found that concentrations of IL-2 and IL-6 were associated with psychiatric diagnoses (e.g., PTSD and depression) and demographic variables, rather than exposure to a natural disaster (Tucker et al., 2017).

5.2.2 Childhood maltreatment trauma

Childhood maltreatment trauma is a type of stressor that has been extensively researched in relation to inflammatory markers. Childhood maltreatment is usually defined as exposure to emotional abuse or neglect and physical and/or sexual abuse before the age of 18 years (World Health Organization, 2016). Worldwide, an estimated 25% of adults have reported childhood physical abuse, and 1 in 5 women and 1 in 13 men report having been sexually abused as a child (World Health Organization, 2016).

Childhood maltreatment has been associated with a range of adverse physical and mental health outcomes and behaviours (Rodgers et al., 2004; Walker et al., 1999). Exposure to childhood maltreatment can lead to greater susceptibility to the development of psychiatric disorders, including depression, anxiety, and EDs (Li, D'arcy, & Meng, 2016b; Norman et al., 2012). For example, childhood maltreatment has been associated with AN, BN, and BED (Afifi et al., 2017; Palmisano, Innamorati, & Vanderlinden, 2016) and the prevalence of childhood maltreatment is high in EDs (prevalence rates 21-59%) in comparison to healthy (prevalence rates 1-35%) and psychiatric (prevalence rates 5-46%) control groups (Molendijk, Hoek, Brewerton, & Elzinga, 2017). However, reported rates of childhood maltreatment do vary across diagnoses and in how this was ascertained (self-reported or observer-rated measures). For example, AN-R individuals report sexual, physical and emotional abuse less frequently than other diagnoses, a higher prevalence of sexual abuse is reported in BN and AN-BP, and people with BN report high rates (81%) of emotional abuse (Molendijk et al., 2017). In addition, ED patients reporting exposure to childhood maltreatment were more likely to have an earlier age of ED onset, to suffer a more severe form of the illness, to binge and purge more often, to be diagnosed with a comorbid psychiatric disorder and to be suicidal, relative to ED patients without exposure to childhood maltreatment. Together, this implies that childhood maltreatment may play a role in the development, maintenance, and course of EDs.

Animal research has shown that early life adversity can have prolonged effects on innate immune system function (Danese & Lewis, 2017; Ganguly & Brenhouse, 2015). Inflammation also has well-documented associations with early life stress in humans

(Fagundes et al., 2013). Childhood maltreatment has been found to predict adult CRP concentrations, independent of developmental and concurrent risk factors e.g., stress, health and health behaviours in adulthood (Danese et al., 2009; Danese, Pariante, Caspi, Taylor, & Poulton, 2007). Furthermore, in adults, childhood trauma, including physical and sexual abuse, has been consistently associated with elevated concentrations of pro-inflammatory cytokines IL-6 and TNF-α (Baumeister, Akhtar, Ciufolini, Pariante, & Mondelli, 2016; D'Elia et al., 2018), independent of psychiatric comorbidities (Coelho et al., 2014). In addition, adults with a history of childhood maltreatment have heightened inflammatory responses, e.g., increased IL-6 response to acute laboratory stress (Pace et al., 2006) and daily stressors (Gouin et al., 2012), which has been supported by similar findings from animal research (Danese & Lewis, 2017). The association between childhood maltreatment and increased inflammatory markers has been found to be particularly strong in individuals that develop depression later in life (Danese et al., 2008; de Punder et al., 2018; Lu et al., 2013). However, recent studies have not replicated the association between inflammatory markers and childhood maltreatment when taking into account a number of covariates such as age, body mass index (BMI), gender, ethnicity, and smoking status (Palmos et al., 2019).

5.2.3 The relationship between stress and inflammatory markers

The relationship between stress and inflammation is coordinated by the sympathetic nervous system, the HPA axis and the immune system. The bidirectional relationship between these biological systems has been well-described.

The sympathetic nervous system coordinates the body's response to acute stress (i.e., fight or flight response). Stress activation of this system leads to the release of catecholamines (e.g., adrenaline, noradrenaline). This, in turn, has been shown to enhance the production of pro-inflammatory cytokines (e.g., IL-1β and TNF-α) and other pro-inflammatory mediators (Lucas, Rothwell, & Gibson, 2006). Recent reviews have described the effect of acute stress on short-term cytokine concentrations and production. Reliable increases in circulating concentrations of cytokines (measured in blood and saliva), including IL-1β, IL-6, IL-10, and TNF-α (but not IFN-γ, IL-2 or the acute-phase protein CRP), following acute laboratory stress have been reported (Marsland et al., 2017; Slavish et al., 2015). Marsland et al. (2017) was also able to assess the time course of stress reactivity on IL-1β and IL-6, finding a peak concentration of IL-6 at 90 minutes post-stress and 40-50 minutes post-stress for IL-1β, highlighting the short-term effect of acute stress on cytokine concentrations. While a brief spike in pro-inflammatory cytokines in response to acute stress is physiologically appropriate, an exaggerated or prolonged immune response is maladaptive.

The HPA axis is the central response system to long-term stress, which is the focus of the current chapter. The activity of the HPA axis is controlled by the secretion of corticotrophin releasing hormone (CRH) and arginine–vasopressin (AVP) from neurons in the paraventricular nucleus (PVN) of the hypothalamus. These hormones, in turn, stimulate the anterior pituitary gland to produce and secrete adrenocorticotrophic hormone (ACTH) into circulation. ACTH then stimulates the synthesis and release of glucocorticoids (i.e., cortisol in humans) from the adrenal glands. These glucocorticoids interact with their receptors (in multiple target tissues) to terminate the stress response as part of a negative-feedback loop. In other words, secretion of CRH, AVP, and ACTH are, in part, controlled by the negative feedback exerted by cortisol when it binds with its receptor.

Cytokines are potent stimulators of the HPA axis. There is a broad literature describing how acute administration of cytokines can activate the HPA axis (Dunn, 2000) and research has shown that cytokines can stimulate the expression and release of CRH, ACTH and cortisol (Besedovsky & del Rey, 1996; Capuron & Miller, 2011). In turn, the activated HPA axis releases glucocorticoids (i.e., cortisol), which are considered to be anti-inflammatory (Rhen & Cidlowski, 2005), and modulates immune system activity in order to restore cytokine concentrations (Zen et al., 2011). For example, in healthy immune cells, when cortisol binds to glucocorticoid receptors, the production of pro-inflammatory cytokines are inhibited (Barnes, 1998; Waage, Slupphaug, & Shalaby, 1990). This reciprocal relationship maintains an appropriate allostatic load.

Chronic stress and thus prolonged activation of the inflammatory system can disrupt the typical functioning of and communication between the HPA axis and the immune system. Firstly, stress-induced changes in cytokine production can lead to changes in the function of the HPA axis. For example, chronic pro-inflammatory exposure (as in the case of chronic stress) has been proposed to impair glucocorticoid receptor function, which has been suggested to lead to decreased responsiveness of glucocorticoid receptors to glucocorticoids (glucocorticoid resistance; Capuron & Miller, 2011; Pace, Hu, & Miller, 2007; Pace & Miller, 2009; Zunszain, Anacker, Cattaneo, Carvalho, & Pariante, 2011). Research has indicated that pro-inflammatory cytokines and their signalling pathways do inhibit glucocorticoid receptor signalling and function (Miller, Pariante, & Pearce, 1999; Pace et al., 2007). This may then result in a failure to appropriately down-regulate pro-inflammatory responses (e.g., Cohen et al., 2012). As mentioned, childhood stressors, such as maltreatment, have been shown to lead to long-term changes in the regulation of the immune system and in cytokine expression in adolescence and adulthood (Cattaneo et al., 2015; Miller & Chen, 2007; Wieck, Grassi-Oliveira, Hartmann do Prado, Teixeira, & Bauer,

2014). It has been suggested that stress that occurs during sensitive periods when immune function is highly malleable may get entrenched in the functioning of cells that regulate inflammation, including those in the brain (Miller & Chen, 2007). Epigenetics may provide a potential mechanism that contributes to these effects (Cattaneo et al., 2015).

Secondly, stress can lead to alterations in the regulation and the signalling of the HPA axis and thus lead to altered cytokine production. For example, the circadian rhythm of glucocorticoids appears to be inversely related to the production of pro-inflammatory cytokines and the administration of glucocorticoids to healthy individuals has been reported to supress production of TNF-α (Petrovsky, McNair, & Harrison, 1998). Moreover, research has shown that chronic activation of the HPA axis can lead to defective immune system responses to an inflammatory challenge (Arzt et al., 2000; Besedovsky & del Rey, 2000; Reul, Labeur, Wiegers, & Linthorst, 1998; Schöbitz, Reul, & Holsboer, 1994). In depressed patients, elevated HPA axis activity has been associated with a suppression in TNF-α system activity and when HPA axis activity has normalised following successful antidepressant therapy, the TNF-α system appears to gain influence over the HPA axis (Himmerich et al., 2006a).

5.2.4 Aims of the current chapter

Taking together the reports which indicate that different types of stress may be related to cytokine concentrations in the blood and that stress exposure has been associated with ED development, it would be of interest to consider whether stressful experiences contribute to the associations we have observed between EDs and inflammatory markers. To the best of our knowledge, the role of chronic stress in the relationship between inflammatory marker concentrations and EDs has not been researched. Therefore, in this chapter, the role of stress in the relationship between EDs and inflammatory markers was investigated using data from three studies. The first study was exploratory and considered the effect of exposure to traumatic events and associated PTSD symptom severity on cytokine concentrations in patients with AN as compared to healthy controls (HCs). The second study aimed to determine the effects of exposure to trauma in childhood and adulthood on CRP concentrations in healthy individuals and those with a lifetime diagnosis of EDs, using the UK Biobank population-based cohort (www.ukbiobank.ac.uk; Sudlow et al., 2015). The third study investigated the effect of (a) childhood maltreatment trauma and (b) stressful life events on a broad range of cytokine concentrations in a sample of emerging adults with current AN and BN diagnoses.

5.3 Study 1: An exploratory study of the association between traumatic life events and related post-traumatic stress disorder symptom severity with cytokine concentrations in anorexia nervosa

As discussed, both the experience of a traumatic event and PTSD have been associated with alterations in circulating cytokine concentrations. However, previous research on cytokine concentrations in AN has not considered the role of these forms of stress. Therefore, this exploratory study aimed to investigate the association between traumatic life events and associated PTSD symptoms with cytokine concentrations in patients with a current diagnosis of AN as compared to a healthy comparison group, using data from the Cytokines in Anorexia Nervosa: CytAN study described in Study 2 of Chapter 3. As both exposure to traumatic life events and experiencing PTSD symptoms is typically associated with elevated pro-inflammatory markers, we may have expected a positive relationship between our assessments of stress and the pro-inflammatory cytokines measured in the present study.

5.3.1 Methods

5.3.1.1 Participants

Forty female adults with a current Diagnostic and Statistical Manual of Mental Disorders (DSM)-5 diagnosis (American Psychiatric Association, 2013) of AN and a BMI in the underweight range (<18.5 kg/m^2) took part in the study. Participants with AN were recruited from South London and Maudsley NHS Trust Foundation specialist inpatient and outpatient Eating Disorder Services via patient's clinicians, posters and circular emails at King's College London (KCL), and advertisement on Beat's website.

A comparison control group of 34 healthy-weight (BMI 18.5 to 24.5 kg/m^2) female adults were also recruited. Healthy control (HC) participants were recruited from posters and circular emails at KCL and had no history of or current psychiatric disorders, including EDs.

Exclusion criteria for all participants included any acute or chronic inflammatory condition (e.g., asthma, psoriasis, Crohn's Disease, inflammatory bowel disease, arthritis) and current pregnancy. All participants underwent a purposely designed screening (as described in Section 3.4.1.1 and see Appendix F Section 10.6) over the telephone to check eligibility against the criteria described above and provided informed consent prior to study participation.

5.3.1.2 Ethical approval

The study was conducted in accordance with the Declaration of Helsinki and the study received ethical approval from the London – City & East Research Ethics Committee (REC reference: 17/LO/2107).

5.3.1.3 Procedure

Eligible participants attended one research session at the Institute of Psychiatry, Psychology & Neuroscience (IoPPN), KCL lasting approximately one hour and 30 minutes. First, participants had blood samples collected, as detailed in Section 3.4.1.3 of Chapter 3. Participants then completed a questionnaire pack to obtain demographic information and assess mental health related symptoms. The revised Life Events Checklist for DSM-5 (LEC-5; Weathers et al., 2013a) and the PTSD Checklist for DSM-5 (PCL-5; Blevins, Weathers, Davis, Witte, & Domino, 2015; Weathers et al., 2013b) were completed as part of this questionnaire pack. Data from the other questionnaires was reported in Study 2 of Chapter 3. Participant's weight, height, and body composition, using the Inbody S10 machine (Inbody Co., Ltd., Seoul, Korea), were also measured.

5.3.1.4 Measures

Participants completed a number of questionnaires (see Section 3.4.1.3); however, for the purpose of the current chapter, only data from the LEC-5 and PCL-5 was used.

The LEC-5 is a self-report measure designed to screen for potentially traumatic events that participants may have experienced during their lifetime. It assessed their exposure to 16 events which may result in PTSD or distress (Weathers et al., 2013a). It is often used in combination with other measures (e.g., PCL-5) to establish exposure to a stressor that confers eligibility for a PTSD diagnosis. In the LEC-5, participants reported whether in their lifetime they had experienced any of the traumatic life events listed at varying levels of exposure: whether it happened to them personally, they witnessed it happen to someone else, they learned about it happening to a close family member or friend, or they experienced it as part of their job (e.g., paramedic, police, military, first responder). There is no formal scoring protocol or interpretation of the LEC-5. For the purpose of the current study, the number of traumatic event types that the participant reported being exposed to (i.e., happened to them personally, witnessed or learned about the event, including if experienced as part of job) were summed to provide a frequency of the type of traumatic events experienced. The LEC-5 has not been assessed for psychometric properties. However, as minimal revisions to the previous LEC version, which had adequate psychometric properties (Gray, Litz, Hsu, & Lombardo, 2004), were made to produce the LEC-5, we would expect similar psychometric properties.

For those participants that reported experiencing a traumatic event, the PCL-5 was completed. The PCL-5 is a 20-item self-report measure that assesses the 20 DSM-5 symptoms of PTSD and trauma exposure (Blevins et al., 2015; Weathers et al., 2013b). Participants were asked to keep their worst event experienced in mind and for each PTSD symptom, report how much they were bothered by the symptom in the past month. Items for each symptom were then rated on a 5-point scale, ranging from 0 ("Not at all") to 4 ("Extremely"). Items can be summed to provide a total severity score, which can range from 0 to 80. Preliminary research suggests that a clinical cut-off score of 33 may be reasonable (Weathers et al., 2013b). The PCL-5 is a psychometrically sound measure of PTSD symptomatology, with high internal consistency and test-retest reliability, and strong convergent and discriminant validity (Blevins et al., 2015; Bovin et al., 2016; Wortmann et al., 2016).

5.3.1.5 Cytokine quantification

Full details of blood collection and cytokine quantification can be found in Section 3.3.1.5. Briefly, serum was separated by centrifuge, stored at -80°C prior to use and was thawed at room temperature for use. The concentrations of 36 cytokines were quantified simultaneously using the Meso Scale Discovery V-PLEX Human Biomarker 36-Plex Kit, following the manufacturer's instructions (Meso Scale Diagnostics, LLC., Rockville, MD, USA). The plate was scanned on the Meso Scale Discovery MESO Quickplex SQ 120 reader at the Social, Genetic & Developmental Psychiatry (SGDP) Centre, IoPPN, KCL. Standard curves were used to determine absolute quantities (pg/ml) of each cytokine.

5.3.1.6 Statistical Analysis

This study uses the same sample from Study 2 in Chapter 3; therefore, demographic characteristics for the AN and HC groups are not shown here but are presented in **Table 3.5** in Section 3.4.2.1.

All statistical analyses were performed in Stata 15 (StataCorp, 2017). The median and the interquartile range for the frequency of type of traumatic event experienced and PCL-5 score is given due to the non-normal distribution of the data, which was determined by visual inspection of histograms and the Shapiro-Wilk test of normality. As such, Mann-Whitney U tests were used to assess group differences.

For the AN and HC groups separately, associations between (a) the total number of traumatic event types experienced and cytokine concentrations and (b) the PCL-5 total score and cytokine concentrations were assessed using Kendall's tau-b (τb) correlation coefficient. These analyses were performed for each cytokine concentration with detectable values in over 70% of samples as detailed in Section 3.4.2.2. To account for multiple testing, a Bonferroni correction was applied to each group separately for each

stress-related variable (total number of traumatic event types experienced and PCL-5 total score), accounting for tests on 27 cytokines. This resulted in a formal significance threshold of $p < 0.0018$.

5.3.2 Results

5.3.2.1 Association between cytokine concentrations and exposure to traumatic events

Groups did not differ in the median (with 25th and 75th percentile) total number of traumatic event types experienced (HC group: 3 [2, 5], AN group: 5 [3, 8]; $U = 539$, $z = -1.54$, $p = 0.1243$). Five participants in the AN and four participants in the HC group reported no traumatic event exposure. No formally significant associations between cytokine concentrations and total number of traumatic event types experienced were found in either group, as shown in **Table 5.1**. In the HC group, a nominally significant ($p<0.05$ i.e., did not meet formal significance thresholds given by correction for multiple testing) relationship was identified: increases in the total number of traumatic event types experienced was associated with lower concentrations of macrophage inflammatory protein (MIP)-1α ($\tau b = -0.27$). In the AN group, vascular endothelial growth factor (VEGF) and IL-15 were nominally associated with trauma exposure, such that the greater number of traumatic event types experienced the higher the concentration of IL-15 ($\tau b = 0.23$) and VEGF ($\tau b = 0.27$).

Table 5.1 Associations between traumatic event exposure and concentrations of cytokines in the healthy control and anorexia nervosa groups separately.

Cytokine	Healthy controls			Anorexia nervosa		
	n	**τb**	***p***	***n***	**τb**	***p***
Eotaxin	34	-0.08	0.5294	40	-0.05	0.6646
Eotaxin-3	34	0.04	0.7645	40	0.09	0.4462
IFN-γ	34	-0.21	0.0934	40	0.18	0.1218
IL-2	32	-0.17	0.2017	38	0.15	0.2105
IL-6	34	-0.17	0.1926	40	0.12	0.2861
IL-7	34	-0.17	0.1777	40	0.15	0.1934
IL-8	34	-0.11	0.4102	40	-0.03	0.8238
IL-10	34	-0.17	0.1683	40	-0.02	0.8421
IL-12/IL-23p40	34	-0.04	0.7418	40	0.01	0.9160
IL-12p70	27	0.08	0.5833	30	0.22	0.0981
IL-13	29	-0.04	0.7903	30	0.06	0.6793
IL-15	34	-0.07	0.5795	**40**	**0.23**	**0.0477**
IL-16	34	0.14	0.2877	40	-0.09	0.4604
IL-17A	34	0.03	0.8457	40	-0.07	0.5500
IL-22	31	-0.03	0.8637	37	-0.01	0.9580
IL-27	34	0.05	0.7305	40	0.04	0.7517
IP-10	34	-0.05	0.7305	40	-0.00	0.9907
MCP-1	34	-0.02	0.8928	40	0.06	0.6309
MCP-4	34	0.11	0.3934	40	-0.04	0.7517
MDC	34	0.23	0.0700	40	-0.06	0.6309
MIP-1α	**33**	**-0.27**	**0.0401**	35	0.16	0.1979
MIP-1β	34	-0.06	0.6641	40	0.01	0.9720
MIP-3α	34	-0.11	0.4102	39	-0.07	0.5509
TARC	34	0.05	0.7081	40	0.05	0.7696
TNF-α	34	-0.19	0.1304	40	-0.06	0.5817
TNF-β	30	-0.02	0.8856	39	-0.03	0.8265
VEGF	34	-0.17	0.1826	**40**	**0.27**	**0.0174**

τb were rounded to two decimal places. Findings that met nominal significance thresholds ($p < 0.05$) are highlighted in bold. Abbreviations: *n* = number of observations; τb = Kendall's tau-b; IFN = interferon; IL = interleukin; IP = interferon γ-induced protein; MCP = monocyte chemoattractant protein; MDC = macrophage-derived chemokine; MIP = macrophage inflammatory protein; TARC = thymus and activation-regulated chemokine; TNF = tumor necrosis factor; VEGF = vascular endothelial growth factor.

5.3.2.2 Association between cytokine concentrations and post-traumatic stress disorder symptoms

Sixty-five participants reported experiencing a traumatic life event and 64 of these participants completed the PCL-5. A Mann-Whitney U test showed that participants with AN had a significantly higher median (with 25th and 75th percentile) total score on the PCL-5 than the HC group (AN: 24.5 [8, 42]; HC: 3 [1, 7]; $U = 140$, $z = -4.99$, $p < 0.001$), indicating that the AN group had greater PTSD symptom severity than the HCs. There were no formally significant correlations between cytokine concentrations and PTSD symptom severity, as presented in **Table 5.2**. IL-27 and PTSD symptom severity were nominally significantly associated in the AN group, such that the greater the PTSD symptom severity, the lower the IL-27 concentration. There were no nominally significant findings in the HC group.

Table 5.2 Associations between post-traumatic stress disorder symptom severity and concentrations of cytokines in the healthy control and anorexia nervosa groups separately.

Cytokine	Healthy controls			Anorexia nervosa		
	n	**τb**	***p***	*n*	**τb**	***p***
Eotaxin	30	0.08	0.5768	34	-0.05	0.6777
Eotaxin-3	30	-0.17	0.2209	34	-0.14	0.2469
IFN-γ	30	0.19	0.1602	34	0.11	0.3892
IL-2	28	0.06	0.6605	32	0.14	0.2625
IL-6	30	0.03	0.8571	34	-0.01	0.9408
IL-7	30	0.15	0.2568	34	0.14	0.2720
IL-8	30	0.12	0.3682	34	-0.06	0.6138
IL-10	30	0.09	0.5287	34	-0.12	0.3420
IL-12/IL-23p40	30	-0.05	0.7460	34	0.12	0.3273
IL-12p70	24	-0.28	0.0714	24	0.11	0.4708
IL-13	27	0.10	0.5143	25	0.07	0.6230
IL-15	30	0.06	0.6526	34	-0.01	0.9290
IL-16	30	0.16	0.2349	34	0.10	0.4059
IL-17A	30	0.03	0.8572	34	0.09	0.4579
IL-22	27	0.05	0.7523	31	-0.16	0.2138
IL-27	30	-0.06	0.6921	**34**	**-0.30**	**0.0126**
IP-10	30	-0.07	0.6398	34	-0.13	0.2852
MCP-1	30	-0.09	0.5170	34	-0.05	0.6777
MCP-4	30	-0.18	0.1829	34	-0.02	0.8586
MDC	30	-0.14	0.2965	34	0.07	0.5931
MIP-1α	29	-0.01	0.9396	30	-0.13	0.3342
MIP-1β	30	0.26	0.0519	34	-0.22	0.0702
MIP-3α	30	-0.24	0.0778	33	0.08	0.5047
TARC	30	0.01	0.9426	34	0.21	0.0906
TNF-α	30	0.01	0.9713	34	0.00	1.0000
TNF-β	26	0.17	0.2467	34	-0.14	0.2589
VEGF	30	0.15	0.2802	34	0.14	0.2593

τb were rounded to two decimal places. Findings that met nominal significance thresholds ($p < 0.05$) are highlighted in bold. Abbreviations: *n* = number of observations; τb = Kendall's tau-b; IFN = interferon; IL = interleukin; IP = interferon γ-induced protein; MCP = monocyte chemoattractant protein; MDC = macrophage-derived chemokine; MIP = macrophage inflammatory protein; TARC = thymus and activation-regulated chemokine; TNF = tumor necrosis factor; VEGF = vascular endothelial growth factor.

5.3.3 Discussion

This exploratory study aimed to examine the association between trauma exposure and cytokine concentrations in people with AN and HCs. No formally significant associations were observed, although we did identify multiple nominally significant relationships. As such, it must be considered that these findings may be due to Type II error. In HCs, greater exposure to traumatic events was nominally associated with lower MIP-1α concentrations. In contrast, higher MIP-1α has been reported in trauma-exposed individuals, compared to HCs (Ogłodek & Just, 2018). However, these trauma-exposed participants had a diagnosis of PTSD, and therefore, the alteration in MIP-1α may be more associated with the pathogenesis of PTSD, rather than exposure to traumatic events.

In the AN group, exposure to a greater number of traumatic event types was nominally related to elevated concentrations of IL-15 and VEGF. Few studies have measured and reported detectable IL-15 in relation to trauma exposure. One study found that concentrations of IL-15 did not differ between individuals with and without trauma exposure (Zhou et al., 2014). However, the individuals with trauma exposure did have a diagnosis of PTSD, which may have confounded the findings. VEGF has previously been measured in two studies in relation to trauma exposure: in depressed patients, VEGF did not differ between those exposed and not exposed to trauma (Lu et al., 2013) and in individuals with first-episode psychosis, VEGF was lower in trauma exposed patients, compared to those without experience of trauma (Di Nicola et al., 2013). These findings contrast with those in the present study. However, this may be accounted for by the focus on childhood trauma in these previous studies.

As traumatic event exposure can lead to the development of PTSD, we also considered the association between PTSD symptom severity and cytokine concentrations. In AN patients reporting exposure to one or more traumatic events, higher levels of PTSD symptom severity were nominally associated with reduced concentrations of IL-27. To the best of our knowledge, IL-27 has not been assessed in individuals with PTSD. Generally, a pro-inflammatory profile has been associated with PTSD (e.g., elevated IL-6 and TNF-α); however, our data did not provide support for this.

It is unclear as to why these particular cytokines were associated with trauma exposure and PTSD symptom severity and why differential cytokines were associated with trauma exposure in AN and HCs. Given the exploratory nature of this study and the small sample sizes, we were unable to control for relevant sociodemographic factors that may influence cytokine concentrations (e.g., smoking; O'Connor et al., 2009, see **Box 1.1**). Therefore, associations identified in the current study may be attributable to confounding factors.

5.3.3.1 Strengths and limitations

This was the first study to consider the relationship between a broad range of cytokines, using validated electrochemiluminescence methods, with trauma exposure and associated PTSD symptomology in patients with AN. A variety of traumatic events were assessed at several levels of exposure. It has been reported that higher rates of PTSD are associated with more direct exposure to the traumatic event (i.e., directly experienced compared to witnessing or learning about the event; Hoge et al., 2004) so it may be expected that different levels of exposure may be associated with different cytokine concentrations. As most people had experienced traumatic events at multiple levels of exposure, we were unable to assess this in the current study. Also, information on the frequency of exposure to each traumatic event and the timing of the exposure cannot be determined from the questionnaire. This may be of particular relevance as traumatic events in childhood may lead to lasting changes in a number of biological systems, including the immune and stress systems (Baumeister et al., 2016).

While we assessed PTSD symptom severity, some of the PTSD symptoms listed in the PCL-5 relate to feelings experienced by people with AN (e.g., having strong negative feelings, loss of interest in previously enjoyed activities, feeling distant from other people, trouble experiencing positive feelings). This may contribute to the greater severity of PTSD symptoms in the AN group and may have affected our results. Also, we did not record whether any AN participants had received an official PTSD diagnosis.

5.3.3.2 Conclusion

Exposure to traumatic events was nominally associated with elevated IL-15 and VEGF concentrations in AN participants and with decreased MIP-1α in HCs. In the AN participants who had experienced a traumatic event, PTSD symptom severity was nominally related to lower IL-27. However, it may be that confounding sociodemographic factors (e.g., BMI, smoking status) account for these nominally significant associations.

5.4 Study 2: The association between traumatic event exposure and circulating concentrations of C-reactive protein in people with a lifetime eating disorder diagnosis

The current study aimed to investigate the relationship between self-reported lifetime exposure to traumatic events and CRP serum concentrations in patients with lifetime ED diagnoses. As discussed, exposure to traumatic events has been significantly associated with elevated CRP concentrations (Spitzer et al., 2010; Tursich et al., 2014). Elevated concentrations of CRP have also been consistently associated with the experience of childhood trauma, though with small effect sizes (Baumeister et al., 2016). Furthermore,

higher traumatic event exposure has been associated with higher concentrations of CRP (O'Donovan et al., 2012).

It has been suggested that the significant findings reported in previous studies may relate to limited covarying for confounding factors in analyses (Palmos et al., 2019). For example, experience of childhood maltreatment has been associated with a higher BMI (Mamun et al., 2007; Power, Pinto Pereira, & Li, 2015; Widom, Czaja, Bentley, & Johnson, 2012) and BMI has been reported to drive CRP concentrations (Timpson et al., 2011). Furthermore, structural equation modelling has shown that childhood trauma is associated with elevated CRP via increased BMI (Schrepf, Markon, & Lutgendorf, 2014). Indeed, when a broad range of covariates were controlled for, including BMI, childhood trauma was not associated with CRP or any other inflammatory markers in healthy individuals and people with depression (Palmos et al., 2019).

In the current study, the relationship between CRP concentrations and trauma exposure during childhood and adulthood was investigated, while controlling for a number of relevant sociodemographic factors, in people with lifetime ED diagnoses and HCs from a population-based cohort. Based on previous research, no relationship between trauma exposure and CRP concentrations may be expected.

5.4.1 Methods

5.4.1.1 Study design

The UK Biobank is a long-term prospective cohort study designed to collect data on individuals' health and lifestyle, with the overall aim of improving prevention, diagnosis and treatment of a range of illnesses (www.ukbiobank.ac.uk; Sudlow et al., 2015). Approximately 9.2 million residential addresses in the UK were mailed invitations to participate in the study, recruiting a total of 502,664 people aged between 37-73 years old between 2006 and 2010. Dedicated assessment centres were established at 22 sites across the UK where participants attended to provide informed consent and demographic information, complete an extensive battery of physical assessments, computerised questionnaires, and in-person interviews, and provide blood, urine and saliva samples (for full details, see Bycroft et al., 2018; Davis et al., 2018; Sudlow et al., 2015). The current study used a nested case-control design, in which cases and control were drawn from the UK Biobank cohort.

5.4.1.2 Sample and eligibility criteria

Males or females of any age and ethnicity with a lifetime diagnosis of an ED were eligible for inclusion. Potential participants were identified from linked patient hospital records and from their self-report response to a screening question (whether they have been diagnosed with mental health problems by a professional) on the UK Biobank's bespoke

Mental Health Questionnaire (Davis et al., 2018). Specifically, participants were eligible if they had a recorded primary or secondary International Classification of Diseases 10th revision (ICD-10; World Health Organization, 1992) diagnosis of AN (ICD-10 diagnosis F50.0) and/or BN (ICD-10 diagnosis F50.2) and/or if they self-reported a diagnosis of AN, BN, and/or psychological over-eating or binge eating on the mental health questionnaire. Males or females of any age and ethnicity with no self-reported or hospital-recorded diagnosis of psychiatric disorders and no use of psychiatric medication were included as a healthy comparison group. For all participants, a hospital-recorded ICD-10 diagnosis of an autoimmune or chronic inflammatory condition (as listed in **Table 10.1** of Appendix H Section 10.8) was an exclusion criterion.

5.4.1.3 Ethical approval

This study was covered under the generic ethical approval from the NHS Research Ethics Committee and ethics approval for the UK Biobank study was obtained from the North West Centre for Research Ethics Committee (REC reference: 11/NW/0382).

5.4.1.4 Assessment of demographic variables

Data on age, gender, and ethnicity were collected via computerised questionnaires at the UK Biobank testing centres. As a high proportion of the study population identified as Caucasian, ethnicity was dichotomised into Caucasian and black, Asian and minority ethnic (BAME). Weight and height data were collected at the initial assessment centre visit by a trained research assistant as part of the physical health assessments and were used to calculate BMI in line with standard procedures. Social deprivation was calculated using the Townsend Deprivation Index (Townsend, Phillimore, & Beattie, 1988), which uses data from an individual's postcode of their home address to provide an overall score of local deprivation on a continuous scale. Smoking status was examined in the last main-stage assessment centres, and was categorised as current, former or never.

5.4.1.5 Assessment of exposure to traumatic events

Exposure to traumatic events was assessed as part of the UK Biobank's online follow-up Mental Health Questionnaire (Davis et al., 2018). Participants reported whether they had experienced any of 16 traumatic experiences. These included five questions assessing traumatic experiences in childhood, using the Childhood Trauma Screener (a shortened version of the Childhood Trauma Questionnaire; Bernstein et al., 1994; Grabe et al., 2012), and five questions referring to traumatic experiences during adulthood, using an equivalent screener for adults developed by the UK Biobank Mental Health steering group to mirror the childhood items (Davis et al., 2018). The remaining questions asked participants whether they had experienced any of six catastrophic traumatic events.

For this study, data on traumatic events were limited to those which were enriched in a major depressive disorder (MDD) sample from Coleman et al. (2019). This left three experiences contributing to the childhood traumatic experiences variables: "I felt loved", "I felt that someone in my family hated me", and "Someone molested me (sexually)". Three experiences were included in the adulthood traumatic experiences variable: "A partner or ex-partner deliberately hit me or used violence in any other way", "A partner or ex-partner repeatedly belittled me to the extent that I felt worthless", and "A partner or ex-partner sexually interfered with me, or forced me to have sex against my wishes". Individuals who reported two or more types of traumatic experience were classified as reporters and those reporting no traumatic experiences as non-reporters. Individuals who reported only a single type of trauma were excluded in order to ensure that exposure to severe trauma was captured (Coleman et al., 2019).

5.4.1.6 Measurements of C-reactive protein

Blood samples were collected from participants on their visit to a UK Biobank assessment centre and serum was separated by centrifugation. Serum samples were stored at -80°C in a fully automated working archive (Owen & Woods, 2008) and thawed prior to use. Serum concentrations of CRP were quantified using high sensitivity immune-turbidimetric analysis methods (Beckman Coulter [UK], Ltd), using the Beckman Coulter AU5800 analytical platform (manufacturer's analytical range: 0.08–80 mg/l). Coefficients of variation were low indicating a well-controlled assay (Fry, Almond, Moffat, Gordon, & Singh, 2019). CRP values greater than 10 mg/l were excluded as this may indicate the presence of acute inflammation and infection (Pearson et al., 2003).

5.4.1.7 Statistical Analysis

All statistical analyses were performed in Stata 15 (StataCorp, 2017). Participants for the HC group were randomly sampled (using the sample command) from their respective groups at a ratio of 1 ED case to 4 controls to improve precision and reduce bias in estimates of effects (Linden & Samuels, 2013; Yamamoto, 2008). One thousand and forty-nine participants with a lifetime ED diagnosis had available data on trauma exposure and CRP concentrations. Thus, n=4196 HCs were randomly sampled for inclusion in the analyses from a sample of n=9514 participants (who were eligible for the current study and had the relevant data available).

For the ED and HC groups separately, we performed linear regressions with CRP concentration as the dependent variable and exposure to childhood or adulthood traumatic events (yes/no) as the independent variable, alongside gender, age, ethnicity, social deprivation, smoking status, and BMI as covariates. For sensitivity analyses, we repeated the regressions in each of the ED diagnoses separately (AN n=458, BN n=191,

BED *n*=276). For these sensitivity analyses, participants who reported more than one ED diagnosis were excluded. Analyses were repeated in five other randomly sampled groups of HC participants to check that findings in the initial sample were consistent across the wider sample.

5.4.2 Results

The demographic characteristics for the HC and ED groups are shown in **Table 5.3**..

Table 5.3 Characteristics of the eating disorder and healthy control groups.

		Healthy controls		Eating disorders	
		n		*n*	
Demographic characteristics					
Gender [*n* (%)]		4196		1049	
	Male		1597 (38.04)		68 (6.48)
	Female		2599 (61.94)		981 (93.52)
Age [years] [mean ± SD]		4196	54.83 ± 7.51	1049	52.02 ± 7.05
Ethnicity [*n* (%)]		4179		1043	
	Caucasian		4089 (97.85)		1011 (96.93)
	BAME		90 (2.15)		32 (3.07)
Smoking status [*n* (%)]		4182		1045	
	Current		625 (14.95)		97 (9.28)
	Former		1441 (34.46)		423 (40.48)
	Never		2116 (50.60)		525 (50.24)
Social deprivation [mean ± SD]		4185	-1.42 ± 3.01	1048	-0.86 ± 3.11
BMI [kg/m^2] [mean ± SD]		4130	27.06 ± 4.62	1025	25.24 ± 5.90
Inflammatory marker					
CRP [mg/l] [median (IQR[a])]		4196	1.2 (0.6, 2.46)	1049	0.71 (0.35, 1.65)
Experience of traumatic events					
During childhood [*n* (%)]		4196		1049	
	Reporters		2414 (57.53)		714 (68.06)
	Non-reporters		1782 (42.47)		335 (31.94)
During adulthood [*n* (%)]		4196		1049	
	Reporters		2707 (64.51)		804 (76.64)
	Non-reporters		1489 (35.49)		245 (23.36)

[a]25th and 75th percentile reported. Abbreviations: *n* = number of observations; SD = standard deviation; BAME = black, Asian and minority ethnic; BMI = body mass index; kg = kilogram; m = metre; CRP = C-reactive protein; mg = milligram; l = litre; IQR= interquartile range.

There were a greater proportion of males ($\chi^2(1) = 386.21$, $p < 0.0001$) and current smokers ($\chi^2(2) = 27.93$, $p < 0.0001$) in the HC group, compared to the ED group. The HC

group was also significantly older ($t(5243) = 10.97$, $p < 0.0001$), more affluent ($t(5231) = -5.35$, $p < 0.0001$), and had a higher BMI ($t(5153) = 10.64$, $p < 0.0001$), than the ED group. ED participants had significantly lower CRP concentrations than the HCs (U = 1681827, z = 11.83, p < 0.0001). A greater proportion of ED participants reported childhood ($\chi^2(1) = 38.69$, $p < 0.0001$) and adulthood trauma ($\chi^2(1) = 55.80$, $p < 0.0001$), compared to HCs.

We found no significant associations between childhood or adulthood traumatic events and concentrations of serum CRP in ED patients (see **Table 5.4**). Sensitivity analyses examining each ED diagnosis separately were consistent with this finding. In HC participants, experience of childhood traumatic events was significantly associated with adult CRP concentrations ($F(8, 4082) = 129.74$, $p < 0.0001$, 20.12% variance explained), such that exposure to traumatic events as a child was related to higher CRP concentrations as an adult (see **Table 5.4**). This pattern of findings was not replicated in four out of the five additionally randomly sampled HC groups. Exposure to traumatic events as an adult was not significantly associated with increased CRP concentrations in HCs (see **Table 5.4**) and this was replicated in four out of the five additionally randomly sampled HC groups.

Table 5.4 Analysis of associations between exposure to traumatic events and concentrations of C-reactive protein in the healthy control and eating disorder groups (controlling for gender, age, ethnicity, social deprivation, smoking status and body mass index).

	Exposure to traumatic events as a child			**Exposure to traumatic events as an adult**		
	β	95% CIs	*p*	β	95% CIs	*p*
Healthy control	0.11	0.00 to 0.21	0.0445	0.07	-0.04 to 0.18	0.1905
Eating disorder	0.06	-0.13 to 0.25	0.5413	-0.03	-0.24 to 0.18	0.7564

β coefficients and 95% confidence intervals were rounded to two decimal places.
Abbreviations: CIs = confidence intervals.

5.4.3 Discussion

This study sought to investigate whether traumatic events have differential effects on the concentration of CRP between participants with EDs and HCs, while ensuring that relevant confounding variables were controlled for in analyses. We found no significant associations between traumatic events (experienced during childhood or adulthood) and CRP concentrations in the ED group. Our findings do not support the idea of a specific ED subtype that is closely related to or clearly defined by childhood trauma and elevated CRP. This contrasts with research from the depression literature, in which depressed patients

with a history of childhood maltreatment have higher CRP concentrations than depressed patients without childhood trauma exposure (Danese et al., 2008; de Punder et al., 2018).

In contrast to findings in the ED group, we found a significant association between exposure to traumatic events during childhood and increases in CRP concentrations as an adult in the control participants. This is consistent with existing studies in which higher concentrations of inflammatory markers have been reported in individuals exposed to childhood maltreatment (Baumeister et al., 2016; Danese et al., 2009; Danese et al., 2007), independent of developmental and concurrent risk factors, including psychiatric disorders (Coelho et al., 2014; Danese et al., 2009; Danese et al., 2007). Our findings differ from Palmos et al. (2019) potentially due to the larger sample and different method of defining and measuring childhood trauma used in the current study. It must be mentioned that our findings were, however, not consistent across other randomly sampled HC groups.

Exposure to traumatic events as an adult was not associated with CRP concentrations in either group. Previous research corroborates this finding; for example, intimate partner violence was not associated with CRP concentrations when relevant confounding variables were controlled for (Newton et al., 2011). Furthermore, experience of interpersonal violence has not been associated with CRP concentrations, rather CRP concentrations were related to the presence of probable PTSD (Heath et al., 2013). As mentioned, within the field of trauma research, studies of adult trauma exposure have historically been limited to the PTSD literature (Tursich et al., 2014). A meta-analysis reported that CRP concentrations did not differ between individuals with and without PTSD (Passos et al., 2015). However, for the purpose of interpreting data on exposure to traumatic events, this does not simply reflect trauma exposure as cytokine concentrations may be confounded by PTSD and other psychiatric diagnoses. Indeed, it has been reported that individuals with PTSD have higher CRP concentrations than trauma-exposed controls (Bersani et al., 2016) and when controlling for trauma exposure, higher concentrations of CRP are observed in PTSD patients compared to non-PTSD individuals (Spitzer et al., 2010). However, no differences between PTSD and trauma-exposed individuals in CRP concentrations have also been reported (von Känel et al., 2007). Taken together, the reliability of our findings is unclear.

5.4.3.1 Strengths and limitations

This was the first study to assess CRP concentrations in relation to trauma exposure in individuals with EDs. This study had a large sample size and assessed both childhood and adulthood trauma exposure across individuals with different ED diagnoses.

Defining reported trauma exposure is complex as evidenced by the multiple approaches that have been used in the field. There were several limitations associated with this in the

present study which need to be considered. Firstly, the data available for the current study was limited to traumatic events that were most associated with MDD (Coleman et al., 2019). As such, we cannot be sure that the included items were relevant events in the development of EDs nor that the two childhood and two adulthood excluded traumatic experiences were not relevant. Although, it was reported that the items included in the analyses were more enriched in females than in males (Coleman et al., 2019), which is pertinent to the current sample, as EDs are more prevalent in females than males (Hoek, 2006; Striegel-Moore & Bulik, 2007).

Secondly, due to the necessary limitations of obtaining data on reported trauma exposure in a biobank-scale dataset, we were unable to consider the severity of the trauma. However, using multiple reports of trauma (as opposed to a report of a single type of trauma) was determined to be the best proxy available for trauma severity i.e., the more types of trauma experienced, the more severe the trauma. However, there may still be relevant effects on CRP when only a single type of trauma has been experienced, which we were unable to explore.

Third, prior to exclusion of items, the assessment of trauma was already based on a limited number of events. Therefore, individuals in both groups may have experienced other traumatic events that contribute to alterations in inflammatory markers but were not assessed in the present study.

Fourth, given the cross-sectional design of the study and limited information available, we were unable to infer the temporal association between ED diagnosis and exposure to traumatic events and consider how this relates to alterations in concentrations of CRP.

Fifth, there may be a number of other factors that influence circulating concentrations of CRP that we were unable to include as covariates in our analyses (e.g., time of blood collection, medication, alcohol use) and that may have affected our results.

5.4.3.2 Conclusion

In our sample of over one thousand participants with a lifetime diagnosis of EDs, exposure to traumatic events during childhood and adulthood was not associated with adult CRP serum concentrations. Data from the HCs provides some support for previous research of an association between exposure to childhood traumatic events and higher concentrations of CRP in adulthood. Differential associations between trauma exposure and CRP concentrations across the cases and controls suggest that trauma may impact biological systems differently in these groups. Alternatively, it may be that there are a variety of factors (e.g., diet, psychiatric comorbidity) that blur the association observed in HCs, in patients with EDs.

5.5 Study 3: Association between cytokine concentrations and childhood maltreatment trauma and stressful life events in emerging adults with eating disorders

Inflammation and activation of pro-inflammatory cytokines is a biological mechanism that has been associated with both childhood maltreatment trauma and stressful life events. The present study aimed to further examine the association between childhood maltreatment trauma and cytokine concentrations in a sample of emerging adults (aged 18-25 years) with a current ED diagnosis. In Study 2, we found that childhood trauma was not associated with the pro-inflammatory marker, CRP, in people with lifetime EDs. The current study sought to expand upon this previous study by using a more comprehensive assessment of childhood maltreatment trauma, measuring a broader range of pro-inflammatory markers (e.g., IL-6, TNF-α), and investigating these in individuals who were currently unwell with an ED.

This study also aimed to explore the relationship between stressful life events and cytokine concentrations in EDs. As mentioned previously, stressful life events have been implicated in the development of EDs (Rojo et al., 2006; Schmidt et al., 1997) and the interaction between stress and the immune system may be a mechanism by which stressful life events contribute to the onset of EDs. This was the first study to assess the possible role of stressful life events in cytokine concentrations in people with EDs. Based on previous research, it may be expected that the experience of stressful life events is related to a pro-inflammatory profile.

5.5.1 Methods

5.5.1.1 Participants

Community-dwelling young adults aged 18-25 years old with a current diagnosis of AN with a BMI below 18.5 kg/m^2 or BN were eligible for the study. Participants were also required to be of a Caucasian ethnic background, not be currently pregnant, not be taking any medication for serious health issues (aside from mental health) and have no contraindications to magnetic resonance imaging (MRI). Eligibility was assessed using a purposely-designed screening questionnaire and ED diagnosis was confirmed using the Eating Disorder Diagnostic Scale (EDDS; Stice et al., 2000). Participants were recruited from a specialist Eating Disorder Outpatient Service in London and via online advertisements on social media, Call for Participants and Beat, and posters and email circulars at KCL.

The current study used a subsample of participants (*n*=75) from the Brain Activation in Eating Disorders (ESTRA) branch of the Brain Network Based Stratification of

Reinforcement-Related Disorders (STRATIFY) study. All participants provided informed consent.

5.5.1.2 Ethical approval

The study was conducted in accordance with the Declaration of Helsinki and the study received ethical approval from the London – Westminster Research Ethics Committee (REC reference: 17/LO/0552).

5.5.1.3 Procedure

The ESTRA study consisted of two parts. In the first part, participants were asked to complete questionnaires online about themselves and their lifestyle. These questionnaires took approximately 1.5-2 hours to complete. The second part of the study involved a visit to the IoPPN at KCL lasting approximately 5 hours. In this research session, participants completed additional questionnaires assessing emotions, behaviours, lifestyle and personality; neuropsychological computer tasks; a breathalyser test; an MRI scan; and had blood and urine samples, and height and weight measurements collected. In the 24 hours prior to the session, participants were asked not to drink alcohol or use drugs in order to not affect task performance or the quality of the MRI data.

5.5.1.4 Measures

For the purpose of the current study, data from the following measures were used: the Life Events Questionnaire (LEQ; completed during the online assessment), the Alcohol Timeline Followback (TLFB) - Drug, Cigarette, and Marijuana (Sobell et al., 1996; completed during the research session), and the Childhood Trauma Questionnaire (CTQ; Bernstein, Ahluvalia, Pogge, & Handelsman, 1997; Bernstein et al., 1994; completed during the research session). Weight and height measurements collected during the research session were used to calculate BMI.

Information about smoking status was collected from the Alcohol Timeline Followback (TLFB) - Drug, Cigarette, and Marijuana (Sobell et al., 1996). Individuals estimated the number of cigarettes used per day in the previous one month. Participants were categorised as a current smoker if they reported smoking within the last 30 days. Medication use was also recorded on this measure, from which information regarding current antidepressant use was extracted.

The LEQ, which is an adaptation of the Stressful Life-Events Questionnaire (Newcomb, Huba, & Bentler, 1981), was used to measure the lifetime occurrence of stressful life events, using Psytools software (Delosis Ltd, London, UK) via its internet-based platform. Participants indicated whether they had or had not experienced any of the 39 stressful life events (see Appendix G Section 10.7.7). Participants also rated how the event did or would

make them feel to assess the valence of the stressful life event. Participants could select a number on the following scale: -2 Very Unhappy, -1 Unhappy, 0 Neutral, 1 Happy, and 2 Very Happy. A stressful life events score was calculated by summing the number of life events the participants experienced during their lifetime. The questionnaire was administered in such a way that events that had occurred could only be counted once.

The CTQ (Bernstein et al., 1997; Bernstein et al., 1994) was used to assess childhood maltreatment across childhood and adolescence. It consists of 25 items pertaining to five domains: emotional abuse, emotional neglect, physical abuse, physical neglect, and sexual abuse. During reproduction of this measure for the wider study, the items on the sexual abuse subscale were not fully and correctly reproduced. Due to this error, we were therefore unable to assess sexual abuse and analyses of childhood maltreatment were based on the remaining four domains only. Participants who endorsed childhood maltreatment on any subscale, regardless of level of severity (low to extreme) were classed as childhood maltreatment reporters. Those who consistently did not report childhood maltreatment across the four remaining domains (scored eight or less on the physical abuse or neglect subscale, nine or less on the emotional abuse subscale, or ten or less on the emotional neglect subscale) were categorised as experiencing no childhood maltreatment. The measure has been reported to be a reliable and valid measure of abuse and neglect in healthy and psychiatric adolescent and young adult populations (Bernstein et al., 1997; Paivio & Cramer, 2004). Although in females, the validity of the physical abuse factor has been questioned (Wright et al., 2001).

5.5.1.5 Cytokine quantification

Two tubes of blood (with ethylenediaminetetraacetic acid [EDTA]) were obtained by venepuncture, blood was processed and four 500µl aliquots of plasma were stored at -80°C prior to use. Plasma was thawed at room temperature for use. The concentrations of 36 inflammatory markers were quantified simultaneously using the Meso Scale Discovery V-PLEX Human Biomarker 36-Plex Kit, following the manufacturer's instructions (Meso Scale Diagnostics, LLC., Rockville, MD, USA). This kit was also used in Study 2 of Chapter 3 and Study 1 of Chapter 5. The inflammatory markers measured in the 36-plex array were listed in Section 3.3.1.5. The position of each case was randomised on the plate. The plate was scanned on the Meso Scale Discovery MESO Quickplex SQ 120 reader at the SGDP Centre, IoPPN, KCL. Measurement of IL-8 and IL-17A was duplicated across two plates. Analyses were performed on the duplicate with the lowest proportion of samples with undetectable cytokine concentrations.

5.5.1.6 Statistical Analysis

All statistical analyses were performed in Stata 15 (StataCorp, 2017). To ensure a larger sample, analyses were performed on the whole group and were not conducted on each ED diagnosis (AN or BN) separately. One participant was excluded as they were taking antibiotic medication at the time of participation, giving a total of 74 participants.

To examine whether childhood maltreatment trauma and stressful life events were associated with cytokine concentrations, linear regressions were performed with childhood maltreatment trauma (dichotomous variable: presence or absence of maltreatment) or stressful life events (continuous variable: LEQ score) as the independent variable and cytokine concentrations as the dependent variable, with age and smoking status included as covariates. Separate regression models were performed for each cytokine. Bonferroni adjustment was applied to each independent variable to account for multiple testing ($p < 0.05$ divided by the number of cytokines with a sufficient number of detectable samples).

5.5.2 Results

5.5.2.1 Demographic characteristics

Participant characteristics of the ED sample are presented in **Table 5.5**. All participants were female. Within the sample, *n*=40 participants met the criteria for AN and *n*=34 for BN.

Table 5.5 Participant characteristics of the whole sample (*n*=74).

Demographic characteristics	
Age [years] [median (IQR[a])]	21 (20, 23)
Current smoker [*n* (%)]	26 (35.13)
BMI [kg/m^2] [median (IQR[a])]	18.65 (16.30, 22.20)[b]
Current antidepressants [*n* (%)]	25 (33.78)
No child maltreatment trauma [*n* (%)]	21 (28.77)[c]
Childhood maltreatment trauma [*n* (%)]	52 (71.23)[c]
LEQ Score [median (IQR[a])]	4 (1, 9)

[a]25th and 75th percentile reported; [b]*n*=64; [c]*n*=73. Abbreviations: IQR = interquartile range; *n* = number of observations; BMI = body mass index; kg = kilograms; m = metres; LEQ = Life Events Questionnaire.

5.5.2.2 Cytokine metrics

Greater than 30% of the sample had undetectable concentrations of the following five cytokines: granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-1β, IL-4, IL-13, and IL-21 (see **Table 5.6**), and were excluded from subsequent analyses. Thus, *n*=30

cytokines were available for statistical analysis. The Bonferroni correction for multiple comparisons was based on this figure, giving a formal significance threshold of $p < 0.0016$. Known quantities within the standard curves correlated highly with quantities predicted by fluorescence intensity ($r^2 > 0.99$).

Table 5.6 Cytokine metrics and median (with interquartile range[a]) cytokine concentrations for the whole sample.

Cytokine	Undetectable[b] [*n* (%)]	Lowest detectable concentration (pg/ml)	Highest detectable concentration (pg/ml)	Median cytokine concentration
Lowly expressed cytokines (<1 pg/ml)				
GM-CSF	24 (32.00)	0.130	1070	0.16 (0.08, 0.27)
IL-1β	27 (36.00)	0.0675	574	0.06 (0.04, 0.14)
IL-2	6 (8.00)	0.0762	1400	0.15 (0.07, 0.19)
IL-4	29 (38.67)	0.0314	218	0.02 (0.01, 0.04)
IL-5	1 (1.33)	0.308	830	0.95 (0.72, 1.31)
IL-6	0	0.0620	748	0.32 (0.21, 0.46)
IL-10	0	0.101	376	0.25 (0.19, 0.37)
IL-12p70	2 (2.67)	0.0827	551	0.16 (0.08, 0.25)
IL-13	32 (42.67)	0.690	503	0.63 (0.30, 1.21)
IL-31	0	0.0751	962	0.89 (0.61, 1.21)
TNF-β	10 (13.33)	0.131	711	0.21 (0.13, 0.30)
Low to moderately expressed cytokines (1-20 pg/ml)				
Eotaxin-3	0	0.847	6240	12.37 (8.62, 15.81)
IFN-γ	0	0.710	1270	2.71 (1.91, 4.17)
IL-1α	0	0.205	454	7.17 (5.44, 12.39)
IL-7	0	0.209	846	3.61 (1.74, 7.04)
IL-8	0	0.0692	553	3.12 (2.20, 4.43)
IL-15	0	0.220	829	3.04 (2.61, 3.43)
IL-17A	0	0.320	6240	1.81 (1.31, 2.67)
IL-21	25 (33.33)	0.875	842	5.84 (2.99, 8.27)
IL-22	1 (1.33)	0.379	551	1.58 (1.04, 2.10)
IL-23	2 (2.67)	0.533	4600	5.88 (4.26, 7.84)
MIP-3α	0	0.244	566	9.46 (7.34, 12.05)
TNF-α	0	0.0760	323	1.60 (1.32, 1.92)
Moderate to highly expressed cytokines (21-400 pg/ml)				
Eotaxin	0	3.92	1720	110.72 (92.61, 142.40)
IL-12/IL-23p40	0	0.387	3650	125.44 (84.83, 168.87)
IL-16	0	0.265	2640	103.40 (90.87, 120.27)
IP-10	0	0.0984	2360	208.17 (152.94, 301.16)
MCP-1	0	0.0479	562	65.90 (55.59, 78.27)
MCP-4	0	5.84	724	90.13 (75.64, 106.45)
MIP-1α	0	5.35	1140	24.37 (20.73, 29.63)
MIP-1β	0	1.24	1180	49.52 (42.19, 60.33)

Cytokine	Undetectable[b] [*n* (%)]	Lowest detectable concentration (pg/ml)	Highest detectable concentration (pg/ml)	Median cytokine concentration
TARC	0	2.35	1780	69.21 (43.64, 190.63)
VEGF	0	0.953	1120	35.16 (29.35, 51.10)
Highly expressed cytokines (401-25,000 pg/ml)				
IL-27	0	14.5	16100	1281.38 (1006.14, 1525.50)
MDC	0	3.97	11800	1005.36 (864.59, 1185.97)

[a]25th and 75th percentile reported. [b]Below fit curve range. Abbreviations: *n* = number of observations; pg = picogram; ml = millilitre; GM-CSF = granulocyte-macrophage colony-stimulating factor; IL = interleukin; TNF = tumor necrosis factor; IFN = interferon; MIP = macrophage inflammatory protein; IP = interferon γ-induced protein; MCP = monocyte chemoattractant protein; TARC = thymus and activation-regulated chemokine; VEGF = vascular endothelial growth factor; MDC = macrophage-derived chemokine.

5.5.2.3 Childhood maltreatment

Childhood maltreatment was assessed in *n*=74 participants and descriptive statistics on the presence of maltreatment can be seen in **Table 5.5**. There were no formally significant associations between cytokines and childhood maltreatment status (i.e., after controlling for multiple testing; see **Table 5.7**). IL-23 was nominally significantly associated with childhood maltreatment trauma: the presence of childhood maltreatment was related to higher concentrations of IL-23.

5.5.2.4 Stressful life events

The median number of stressful life events experienced are reported in **Table 5.5**. Seventeen participants (22.67%) reported experiencing no life events. No cytokines were significantly associated with stressful life events at the formal significance threshold (i.e., after application of Bonferroni correction; see **Table 5.7**). TNF-β was nominally significantly associated with experience of stressful life events, such that the greater number of stressful life events experienced, the lower the plasma concentration of TNF-β.

Table 5.7 Analysis of associations between childhood maltreatment trauma or stressful life events and cytokine concentrations, controlling for age and smoking status.

Cytokine	Childhood maltreatment trauma				Stressful life events			
	n	β	95% CIs	*p*	*n*	β	95% CIs	*p*
Eotaxin	73	19.04	-3.01, 41.09	0.0894	74	0.02	-0.01, 0.04	0.2627
Eotaxin-3	73	3.18	-3.08, 9.44	0.3141	74	-0.04	-0.14, 0.06	0.3965
IFN-γ	73	-0.33	-3.77, 3.11	0.8499	74	-0.01	-0.19, 0.18	0.9445
IL-1α	73	0.49	-4.20, 5.18	0.8353	74	-0.07	-0.20, 0.06	0.3046
IL-2	67	0.06	-0.03, 0.14	0.1677	68	-2.38	-11.05, 6.29	0.5852
IL-5	72	0.12	-0.51, 0.74	0.7043	73	0.07	-0.95, 1.10	0.8887
IL-6	73	0.06	-0.11, 0.24	0.4830	74	-1.87	-5.33, 1.60	0.2858
IL-7	73	0.63	-3.09, 4.36	0.7350	74	-0.08	-0.25, 0.09	0.3431
IL-8	73	0.25	-0.81, 1.31	0.6450	74	-0.10	-0.70, 0.49	0.7275
IL-10	73	0.03	-0.13, 0.19	0.7204	74	-1.83	-5.83, 2.16	0.3635
IL-12/IL-23p40	73	4.18	-24.62, 32.98	0.7730	74	-0.00	-0.02, 0.02	0.9948
IL-12p70	71	-0.06	-0.17, 0.06	0.3413	72	0.98	-4.72, 6.69	0.7326
IL-15	73	0.16	-0.14, 0.45	0.2862	74	-0.18	-2.28, 1.92	0.8645
IL-16	73	17.79	-15.20, 50.78	0.2859	74	0.01	-0.01, 0.03	0.2808
IL-17A	73	0.07	-0.60, 0.73	0.8463	74	-0.39	-1.32, 0.55	0.4155
IL-22	72	-0.57	-1.49, 0.35	0.2205	73	-0.06	-0.76, 0.64	0.8644
IL-23	**71**	**2.39**	**0.66, 4.12**	**0.0075**	72	-0.10	-0.45, 0.25	0.5687
IL-27	73	22.88	-177.35, 223.10	0.8204	74	0.00	-0.00, 0.01	0.1571
IL-31	73	0.05	-0.16, 0.25	0.6593	74	-0.96	-3.88, 1.97	0.5155
IP-10	73	20.14	-63.23, 103.52	0.6313	74	-0.00	-0.01, 0.01	0.6779
MCP-1	73	3.66	-5.04, 12.37	0.4042	74	0.02	-0.05, 0.09	0.6393
MCP-4	73	10.53	-6.74, 27.80	0.2281	74	-0.01	-0.05, 0.02	0.4730
MDC	73	25.20	-114.16, 164.56	0.7194	74	-0.00	-0.01, 0.00	0.1880
MIP-1α	73	2.58	-1.57, 6.72	0.2194	74	-0.02	-0.17, 0.13	0.7684
MIP-1β	73	4.29	-5.04, 13.61	0.3621	74	0.03	-0.04, 0.09	0.4663
MIP-3α	73	-1.91	-4.48, 0.65	0.1416	74	-0.04	-0.28, 0.21	0.7730
TARC	73	54.70	-17.25, 126.64	0.1339	74	-0.00	-0.01, 0.01	0.6919
TNF-α	73	0.21	-0.02, 0.43	0.0733	74	0.32	-2.38, 3.02	0.8123
TNF-β	64	-0.04	-0.13, 0.05	0.3805	**65**	**-9.01**	**-16.78, -1.23**	**0.0239**
VEGF	73	5.09	-12.30, 22.48	0.5612	74	-0.01	-0.05, 0.02	0.4512

β coefficients and 95% CIs were rounded to two decimal places. Findings that met nominal significance thresholds ($p < 0.05$) are highlighted in bold. Abbreviations: *n* = number of observations; CIs = confidence intervals; IFN = interferon; IL = interleukin; IP = interferon γ-induced protein; MCP = monocyte chemoattractant protein; MDC = macrophage-derived chemokine; MIP = macrophage inflammatory protein; TARC = thymus and activation-

regulated chemokine; TNF = tumor necrosis factor; VEGF = vascular endothelial growth factor.

5.5.3 Discussion

In this sample of participants with a current ED diagnosis, childhood maltreatment trauma was not associated with any cytokine concentrations after correction for multiple comparisons. These findings differ from more common reports of a pro-inflammatory state in individuals with a history of childhood maltreatment (Baumeister et al., 2016). However, this is consistent with our findings from Study 2, in which childhood trauma was not associated with concentrations of CRP.

After adjustment for multiple testing, stressful life events were also not associated with any cytokine concentrations. This is in contrast to previous literature (e.g., Hostinar et al., 2015). Much existing research has focussed on negatively valenced stressful life events (e.g., bereavement; Knowles et al., 2019). However, the stressful life events questionnaire used in the current study also included positively valenced stressful life events (e.g., marriage, outstanding personal achievement), which may account for the differing findings. Thirteen of the stressful life events were consistently rated as negative (mean valence score of between -2 and -1; e.g., divorce, trouble with in-laws, death of a close family member). Exposure to these events was low in the present sample, likely due to the age of our participants. This prohibited the opportunity to run analyses with only these negative life events to further explore the relationship between stressful life events and cytokines. Future research should ensure life events assessed are age appropriate.

Prior to correction for multiple comparisons, presence of childhood maltreatment was associated with higher IL-23 concentrations and the experience of a greater number of stressful life events was related to lower TNF-β concentrations. To the best of our knowledge, these cytokines have not previously been researched in relation to trauma and stress. Therefore, it is not completely clear why these specific cytokines showed an association. However, as these findings became non-significant after adjustment for multiple testing, they may be false negatives and due to Type II error.

5.5.3.1 Strengths and limitations

We assessed a broad range of cytokines and chemokines in people with current EDs. In comparison to Study 2, a comprehensive assessment of exposure to childhood trauma was performed, using a validated and well-characterised assessment for childhood maltreatment (Bernstein et al., 1997; Bernstein et al., 1994). However, the errors with reproduction of the sexual abuse subscale questions and subsequent removal of this subscale from analyses may mean that participants were miscategorised as not reporting childhood trauma. Furthermore, it must be noted that this method of assessing childhood

maltreatment is associated with retrospective biases. Indeed, a recent meta-analysis found poor agreement between prospective and retrospective measures of childhood maltreatment and reported that these two methods of ascertainment identify largely different groups of individuals (Baldwin, Reuben, Newbury, & Danese, 2019). Using maltreatment as a binary variable in which those reporting low childhood trauma are grouped with those reporting moderate or severe childhood maltreatment trauma is perhaps a lenient definition of childhood trauma and may misidentify as positive, a proportion of non-maltreatment cases (Bernstein & Fink, 1998). This may account for the seemingly high proportion of individuals reporting childhood maltreatment (71%) in the current study. Dichotomising the maltreatment variable also meant that we were unable to consider the effects of the severity of maltreatment (higher scores on the CTQ indicate greater severity of maltreatment). Few participants in our sample experienced severe maltreatment, with most participants who reported trauma experiencing mild to moderate maltreatment. Therefore, it may be that the biological effects of childhood maltreatment related stress were less pervasive in our sample, which may account for why we did not observe an association between cytokines and childhood maltreatment. We were also unable to consider the severity of the stressful life events and response to either type of stress. Finally, we did not have available data for a HC comparison group. Therefore, we were unable to examine whether cytokines are differentially associated with childhood maltreatment trauma or stressful life events in healthy individuals, compared to ED participants, as was found in Study 1 and Study 2.

5.5.3.2 Conclusions

In a sample of emerging adults with a current ED diagnosis, cytokine concentrations were not found to be associated with the experience of stressful life events and childhood maltreatment. This was inconsistent with previous research which tends to indicate that inflammation is associated with exposure to these forms of stress. Future research in EDs should aim to assess the severity of these stress types in relation to cytokine concentrations.

5.6 General discussion

To date, the role of stress in the relationship between cytokines and EDs has not been studied. As such, these were the first studies to consider how event-related stress, in the form of childhood maltreatment, stressful life events and traumatic events (with related PTSD symptoms), may be associated with inflammatory marker concentrations in EDs. Across three studies, we did not identify any significant relationships between stress and inflammatory marker concentrations in people with EDs. Given the findings were inconsistent with previous literature in healthy individuals and those with depression, this

may suggest that in EDs, stress has differential effects on circulating markers of inflammation. More broadly, it may also suggest a difference in the biological foundation of depression symptoms between people with AN and comorbid depression and people with a diagnosis of MDD only. This question as to whether the depression in AN is the same as in MDD warrants further investigation; however, is beyond the purpose of the present thesis.

There are several methodological factors to be considered that may have contributed to our findings. Firstly, it is important to acknowledge that people respond differently to stress. Some people develop continued symptoms, such as PTSD, following stress exposure and others do not. For example, not all military personnel exposed to combat-related trauma develop PTSD. Indeed, there is an area of research that has assessed potential moderating factors in the relationship between stress and inflammatory marker concentrations that may account for individual differences in cytokine-related stress response e.g., vulnerability versus resilience (Ménard et al., 2017). For example, it has been reported that individuals with greater trauma exposure had higher concentrations of CRP only when they reported lower levels of mastery, a form of perceived control which refers to an individual's beliefs in their ability to perform specific behaviours to attain goals (Elliot, Mooney, Infurna, & Chapman, 2017). Another example is perceived relationship support which has been reported to moderate the relationship between stress and cytokines (Beach et al., 2019). Given these individual differences in psychological response, individuals may also have differential biological responses to stress (e.g., cortisol, adrenaline, noradrenaline). For example, glucocorticoid resistance, which refers to the decreased responsiveness of glucocorticoid receptors to glucocorticoids, has been reported to be a requisite for increased inflammatory markers in individuals with depression (Capuron & Miller, 2011; Pace et al., 2007; Perrin, Horowitz, Roelofs, Zunszain, & Pariante, 2019; Zunszain et al., 2011). Research has reliably reported glucocorticoid resistance in individuals with EDs (e.g., Connan et al., 2007; Díaz-Marsá et al., 2008; Lo Sauro et al., 2008), which suggests a possible mechanism via which cytokines may be altered in EDs. Taken together, this is an important limitation of the studies within this chapter: we measured exposure to the event rather than an individual's response. Thus, these observations underscore the importance of measuring biomarkers of the stress system, in particular glucocorticoid concentrations (Kuhlman, Horn, Chiang, & Bower, 2019), and subjective measures of event-related stress/emotional response in addition to inflammatory markers in future research to better understand the relationship between these markers and stress in EDs.

Second, aspects associated with the timing of the event may also be of relevance. In Study 1 and 3, lifetime exposure to traumatic/stressful life events was assessed. However, it may be that more recent life events have a more pervasive effect on inflammation. For example, it has been suggested that following a bereavement there is an acute enhancement of pro-inflammatory markers, but this normalises over time in most individuals (Knowles et al., 2019). Also, findings in Study 2 further suggest that the timing of the exposure to trauma is relevant: childhood, but not adulthood, trauma exposure was associated with elevations in CRP. These results suggest that early life trauma may lead to a vulnerability to immune dysregulation in later life (e.g., increased pro-inflammatory markers or increased sensitivity to inflammatory responses). It has been proposed that exposure to stress during a sensitive developmental period when neuronal mechanisms and the function of the immune system are malleable, such as in childhood, can become embedded in the functioning of cells that contribute to the regulation of inflammation (Kuhlman, Chiang, Horn, & Bower, 2017; Miller & Chen, 2007). For example, immune cells within the brain (e.g., microglia, dendritic cells) may become hypersensitive due to an up-regulation of pro-inflammatory and a down-regulation of anti-inflammatory signals, which subsequently leads to a chronic pro-inflammatory state that may contribute to the development of adverse physical or mental health consequences.

One biological mechanism that may contribute to this is epigenetics (Cattaneo et al., 2015; Vaiserman & Koliada, 2017), which refers to long-lasting modifications to gene expression without alterations to the deoxyribonucleic acid (DNA) sequence itself. Trauma exposure during early life may activate epigenetic mechanisms that produce stable and enduring changes in the expression of immune/inflammation related genes that may persist into adulthood. Indeed, DNA methylation (addition of methyl groups to the DNA molecule that can change genetic expression) in immune system genes, that were related to childhood adversity, has been associated with the expression of circulating concentrations of inflammatory markers in adulthood (McDade et al., 2017). Furthermore, research has reported that greater exposure to childhood trauma was associated with lower levels of DNA methylation of the IL-6 gene promoter and in turn, elevated concentrations of IL-6 in response to an acute stress test (Janusek, Tell, Gaylord-Harden, & Mathews, 2017). This area of research is in its infancy and therefore, more research is needed to further examine this possible mechanism linking childhood maltreatment trauma to adult dysregulation of the immune system. Despite no evidence of this in Study 2, trauma during adulthood can also lead to alterations in circulating inflammatory markers (e.g., Woods et al., 2005); although, this has been a lesser focus in the literature, without the confounding of PTSD diagnoses. Therefore, it would be important to ensure age of exposure is reported, which

is likely to play an important role in the relationship between trauma exposure and inflammation.

Third, different specific types of trauma/life events may be differentially associated with inflammation. For example, in a cohort study of children, divorce/separation of the parents was significantly associated with increased IL-4 concentrations, however, death of a family member and severe disease of a family member were not associated with IL-4 (Herberth et al., 2008). Furthermore, specific types of childhood trauma have been associated with specific inflammatory profiles (Baumeister et al., 2016). In the studies described in this chapter, we focussed on overall exposure to traumatic events, rather than considering specific types of traumatic event. While previous findings suggest value in exploring inflammatory responses to specific traumatic events, the focus on the presence or absence of a single type of life event limits the extent to which conclusions can be drawn about the effects of life event exposure more generally. Therefore, our studies do expand on findings from previous research by assessing cumulative exposure to different categories of traumatic events across the lifespan. Indeed, research has shown that different types of life events (traumatic and/or stressful) may have additive effects on inflammation (O'Donovan et al., 2012). For example, adverse childhood experiences with recent life events have been shown to have an additive effect on inflammation in midlife (Hostinar et al., 2015).

Fourth, the number/frequency of exposures to each specific type of traumatic event (i.e., how many times an individual had been exposed to each trauma), which we did not assess, likely contributes to the trauma exposure-inflammation association. Given that the effects of stress often appear to be cumulative (Lampert et al., 2016), it may be expected that greater frequency of trauma experience is associated with higher circulating concentrations of inflammatory markers. Indeed, research has suggested that cumulative exposure to a traumatic event is related to higher concentrations of inflammatory markers. For example, individuals who had experienced two or more types of severe childhood maltreatment, but not those with one experience of maltreatment, had higher CRP concentrations than those this no childhood maltreatment history (Danese et al., 2007).

As has been highlighted here, there are multiple factors that need to be taken into consideration when assessing exposure to traumatic and stressful life events, including stress response to event exposure (e.g., development of PTSD symptoms), type of event, number of exposures to each type of event, and timing of event. Severity of the traumatic or stressful life event and the duration of event may also play a role in the relationship between life event experience and inflammatory markers. Indeed, acute and chronic stress

have been reported to have differential effects on the immune system (Segerstrom & Miller, 2004). Taken together, this shows that exposure to life events can be difficult to measure, particularly in large samples, and suggests that future research should incorporate comprehensive assessments of event exposure.

This chapter has focussed on the prolonged alterations stress exposure may have on inflammatory markers. While we did not find evidence for this in EDs, it should also be considered that exposure to stress may alternatively affect one's inflammatory response to acute stress. For example, individuals with a history of childhood maltreatment and adversity had greater acute release of IL-6 in response to laboratory stress, than people with no history of childhood maltreatment (Carpenter et al., 2010; Pace et al., 2006). This demonstrated a hyper-responsive inflammatory response to acute stress and highlights individual differences in responsivity to environmental stressors that may be clinically significant and associated with future health risk (Marsland et al., 2017). As such, it may be of value to assess acute inflammatory responses in stressful situations in future research in EDs.

5.6.1 Conclusions

Concentrations of inflammatory markers were not found to be associated with childhood maltreatment trauma or exposure to stressful or traumatic life events in people with EDs. Several methodological considerations may account for these findings, including the lack of assessment of stress response, trauma severity, and timing of the event. Addressing these factors in future research will be of importance.

Chapter 6. Investigating the role of dietary inflammation in eating disorders

6.1 Abstract

Objective: Diet plays a central role in the modulation and regulation of inflammatory markers. Dietary intake and patterns are significantly altered in eating and weight-related disorders. The studies in this chapter aimed to assess dietary inflammation, which can be quantified using the Dietary Inflammatory Index (DII), in people with obesity, individuals with lifetime diagnoses of anorexia nervosa (AN), bulimia nervosa (BN) or binge eating disorder (BED; Study 1), and people with current AN (Study 2), relative to healthy controls (HCs).

Methods: Dietary intake was assessed using multiple 24-hour dietary recalls in the UK Biobank, a population-based sample, in Study 1 and by a Food Frequency Questionnaire in a purposive sample of females with AN in Study 2. Nutrient intake from these dietary assessments was estimated and from this, the DII was calculated. Group differences in DII score were examined and logistic regressions were used to determine whether the DII was associated with group membership. The association between DII score and C-reactive protein (CRP) in Study 1 and cytokine concentrations (n=27) in Study 2 was also investigated.

Results: Study 1 reported that obesity was associated with a higher DII score when relevant sociodemographic factors were controlled for. In both studies, eating disorder (ED) groups did not differ from HCs in DII score. In Study 1, DII score was significantly associated with CRP; however, when examined in only the ED sample, this finding was no longer significant. Cytokine concentrations were not associated with DII score in Study 2, after adjusting for multiple comparisons.

Conclusion: Dietary inflammation may partially contribute to the chronic low-grade inflammation observed in obesity. These studies were the first to assess dietary inflammation in EDs. Findings suggested that other factors aside from eating behaviour may contribute to the regulation of cytokines in EDs. The DII score was validated against CRP in Study 1; however, the DII was not associated with any cytokine concentrations in Study 2. These findings need to be explored further in larger samples of currently unwell ED patients. Future studies should also assess dietary intake more comprehensively when calculating the DII.

6.2 Introduction

In eating disorders (EDs), dietary patterns differ from the general population: all EDs are characterised by disrupted eating behaviours (Forbush & Hunt, 2014). Indeed, these are detailed within the Diagnostic and Statistical Manual of Mental Disorders 5th Edition (DSM-5; American Psychiatric Association, 2013) diagnostic criteria for EDs. Restrictive energy intake (relative to an individual's requirements) is needed for an anorexia nervosa (AN) diagnosis and recurrent episodes of binge eating (eating an amount of food that is larger than what most individuals would eat in a similar discrete period of time under similar circumstances) are required for the diagnosis of both bulimia nervosa (BN) and binge eating disorder (BED; American Psychiatric Association, 2013). Research in patients with AN has shown that individuals severely restrict the frequency and variety of food intake leading to decreased energy and nutrient intakes. For example, community-dwelling adolescents with AN reported consuming significantly fewer calories than healthy adolescents and consumed significantly fewer calories from fat (Baskaran et al., 2017; Mayer et al., 2012; Misra et al., 2006b). In BN, binge eating associated nutrient intake was significantly higher in sugar- and fat-derived energy and lower in protein, compared to non-binge episodes (Gendall, Sullivan, Joyce, Carter, & Bulik, 1997). Similarly, on days of binge eating episodes, people with BED were reported to consume significantly more carbohydrate, fat, and protein, compared to days with no binge eating episode (Raymond, Neumeyer, Warren, Lee, & Peterson, 2003; Reeves et al., 2001). Certain dietary patterns have also been suggested to be protective against the development of EDs. For example, adherence to a Mediterranean diet (emphasises eating primarily plant-based foods, such as fruits and vegetables, whole grains, legumes and nuts, and using healthy fats, such as olive oil) has been shown to be inversely associated with risk for ED development (Bertoli et al., 2015; Leone et al., 2018). Relatedly, a pro-inflammatory diet has been associated with increased risk of depression (Oddy et al., 2018; Tolkien, Bradburn, & Murgatroyd, 2018), a disorder that is highly prevalent in EDs (Ulfvebrand et al., 2015).

It has been established that diet plays a central role in the modulation and regulation of inflammation (Cavicchia et al., 2009; Giugliano, Ceriello, & Esposito, 2006). For example, longitudinal structural equation modelling has shown that diet at age 14 years is associated with C-reactive protein (CRP) concentrations later on in adolescence (aged 17 years): a Western-type diet (high in red and processed meat, high-fat dairy products, refined grains, and simple carbohydrates) was positively associated with, and a 'healthy' dietary pattern (high in fruit, vegetables, fish, and whole-grains) was negatively correlated with CRP concentrations at aged 17 years (Oddy et al., 2018). Indeed, adherence to certain dietary models has been shown to be associated with inflammatory markers in a number

of reviews (e.g., Smidowicz & Regula, 2015). For example, a Western-type diet has been associated with higher concentrations of inflammatory markers like interleukin (IL)-6 and CRP (Giugliano et al., 2006). In contrast, adherence to a Mediterranean diet has been associated with better health outcomes (Sofi, Macchi, Abbate, Gensini, & Casini, 2014) and reduced inflammation, including concentrations of CRP and IL-6 (Schwingshackl & Hoffmann, 2014). However, it is important to consider that the Mediterranean diets implemented in research included in these reviews were highly heterogenous; for example, some dietary interventions focussed on changes to the whole diet, whereas others altered individual dietary components. It is also unclear whether the reviewed research controlled for other health behaviours during the study, such as smoking, drugs, alcohol, and exercise, which may additionally influence health outcomes and inflammation (O'Connor et al., 2009).

Given disruption and alterations in dietary intake are fundamental characteristics of EDs, it would be of interest to determine whether diet may contribute to the inflammatory disturbances observed in these conditions (e.g., Chapter 2; Dalton et al., 2018a; Shank et al., 2017; Succurro et al., 2015). A tool for calculating the inflammatory potential of an individual's diet (i.e., dietary inflammation) is the Dietary Inflammatory Index (DII; Shivappa et al., 2014a). The DII is a literature-derived, population-based index that was designed to compare populations on the inflammatory potential of their diets. Its creation was based on literature linking any aspect of diet to one or more of six inflammatory markers (IL-1β, IL-4, IL-6, IL-10, tumor necrosis factor [TNF]-α, and CRP). The DII has been validated against several peripheral markers of inflammation, including CRP (Corley, Shivappa, Hebert, Starr, & Deary, 2019; Shivappa et al., 2014b), IL-6 (Corley et al., 2019; Shivappa et al., 2015) and TNF-α (Shivappa et al., 2017b). The DII has recently been applied to dietary data in participants with serious mental illnesses (including schizophrenia, bipolar disorder, and major depressive disorder [MDD]) finding that these participants had more dietary inflammation than their healthy counterparts (Firth et al., 2018; Firth et al., 2019; Tolkien et al., 2018). In previous research, a high DII score, which is indicative of a pro-inflammatory diet, has been closely linked to adverse health outcomes, including a number of different cancers (Liu et al., 2019; Moradi, Issah, Mohammadi, & Mirzaei, 2018; Namazi, Larijani, & Azadbakht, 2018), cardiovascular disease (Shivappa et al., 2018a), general obesity (Ruiz-Canela et al., 2015), and all-cause mortality (Garcia-Arellano et al., 2019) in various global populations.

In this chapter, the relationship between the inflammatory potential of an individual's diet and EDs was examined using data from two studies. The first study applied the DII to dietary intake data from people with lifetime AN, BN, and/or BED, people with obesity and

healthy individuals from the UK Biobank (Bycroft et al., 2018; Sudlow et al., 2015) and aimed to validate the DII against serum CRP values. The second study applied the DII to dietary intake data from a sample of currently unwell AN individuals and a healthy comparison group (from the Cytokines in Anorexia Nervosa: CytAN sample previously described in Chapter 3.4 and Chapter 5.3) and aimed to validate the DII against serum concentrations of multiple cytokines.

6.3 Study 1: Association between dietary inflammation and C-reactive protein in lifetime eating disorders and obesity

The DII has been previously applied to investigations of obesity, a weight-related metabolic condition characterised by overeating which leads to weight gain. Obesity is highly comorbid with EDs, with the prevalence of lifetime obesity in ED cases averaging at 28.8% (ranging from 5% in AN to 87% in BED; Villarejo et al., 2012). Obesity and EDs share overlapping aetiology, and a number of psychosocial and medical comorbidities and risk factors (Rancourt & McCullough, 2015; Striegel-Moore & Bulik, 2007). An immunological component of particular interest that has been implicated in both obesity and EDs is inflammation. Indeed, obesity has also been associated with a chronic pro-inflammatory state (Brooks, Blaha, & Blumenthal, 2010; Ellulu, Patimah, Khaza'ai, Rahmat, & Abed, 2017), as has been shown in AN (Chapter 2; Dalton et al., 2018a). Studies assessing dietary inflammation in obesity have reported that a higher DII score (i.e., a pro-inflammatory diet) has been associated with indices of obesity (higher average body mass index [BMI], waist circumference, and waist to height ratio), risk of developing obesity, and higher annual weight gain (Ramallal et al., 2017; Ruiz-Canela et al., 2015).

Dietary inflammation has yet to be investigated in EDs. Therefore, in this study we aimed to assess the inflammatory potential of participant's diet in people with lifetime ED diagnoses and current obesity in comparison to healthy controls (HCs) using the DII (Shivappa et al., 2014a), in a population-scale dataset from the UK Biobank (Bycroft et al., 2018; Davis et al., 2018; Sudlow et al., 2015). In line with previous research (e.g., Ramallal et al., 2017; Ruiz-Canela et al., 2015), people with current obesity would be expected to have a higher DII score than HC participants.

With respect to the DII in ED groups, this was an exploratory study; therefore, we refrained from making specific hypotheses. Binge eating behaviour and bulimic symptoms have been found to be positively associated with concentrations of pro-inflammatory markers, such as IL-6 and TNF-α (Lofrano-Prado et al., 2017; Lofrano-Prado et al., 2011) and it could be proposed that dietary behaviours may contribute to this relationship. Research on nutrient intake between obesity and people with BED on non-binge days is mixed but generally suggests that groups do not differ in food intake (Raymond et al.,

2003) or that BED have increased caloric intake to non-BED obese participants (Engel et al., 2009). Therefore, a similarly high DII score in people with BED, as seen in obesity, may be expected. Binge episodes in both BED and BN have been associated with elevated intake of calories from fats and sugars (Gendall et al., 1997; Raymond et al., 2003), which may suggest a pro-inflammatory diet and a higher DII score. However, typical dietary intake in BN has been found to be highly heterogenous with some studies reporting active food restriction and others suggesting over-eating (Forbush & Hunt, 2014), so it is unclear as to what may be expected in BN participants.

In contrast, research has suggested that people with AN show a preference for vegetables and consume less fatty and fried foods (Jauregui Lobera & Bolanos Rios, 2009; Kanayama et al., 2019), which may be suggestive of an anti-inflammatory dietary profile and lower DII score. Furthermore, an anti-inflammatory diet consists mainly of plant-based foods (Hodge et al., 2018) and it has been reported that people with AN are significantly more likely to be and or have a history of being vegetarian, as compared to HCs (Bardone-Cone et al., 2012; Marzola, Nasser, Hashim, Shih, & Kaye, 2013). In contrast, a reduction in energy intake has been associated with a more pro-inflammatory DII score (Hodge et al., 2018). Additionally, data report elevated pro-inflammatory cytokine concentrations (IL-6 and TNF-α) in AN compared to HCs (Chapter 2; Dalton et al., 2018a); therefore, it may be that different or specific dietary patterns in patients with AN are high in inflammatory potential which may contribute to inflammatory processes and associated over-expression of pro-inflammatory cytokines.

We also investigated the association between the DII score and serum CRP concentrations, aiming to validate the DII in our sample. CRP is an acute-phase protein that is produced by the liver in response to inflammation and as previously mentioned, has been associated with the DII score (Corley et al., 2019; Shivappa et al., 2014b). Therefore, we would anticipate that a higher DII score is related to higher concentrations of CRP.

6.3.1 Methods

6.3.1.1 Study design

The study design of the UK Biobank has been described in Section 5.4.1.1 of Chapter 5. Briefly, the UK Biobank is a long-term prospective cohort study designed to study common diseases of middle and old age, providing data on over 500,000 individuals across the UK (www.ukbiobank.ac.uk; Sudlow et al., 2015). A range of health-related phenotypes and biological measures were assessed through physical assessments, computerised questionnaires, in-person interviews, and blood, urine and saliva samples (for full details, see Bycroft et al., 2018; Davis et al., 2018; Sudlow et al., 2015). The current study used a

nested case-control design, in which cases and control were drawn from the UK Biobank cohort.

6.3.1.2 Sample and eligibility criteria

Males or females of any age and ethnicity with a lifetime diagnosis of an ED were eligible for inclusion. Potential participants were identified from linked patient hospital records and from their self-report response to a screening question (whether they have been diagnosed with mental health problems by a professional) on the UK Biobank's bespoke Mental Health Questionnaire (Davis et al., 2018). Specifically, participants were eligible if they had a recorded primary or secondary International Classification of Diseases 10th revision (ICD-10; World Health Organization, 1992) diagnosis of AN (ICD-10 diagnosis F50.0) and/or BN (ICD-10 diagnosis F50.2) and/or if they self-reported a diagnosis of AN, BN, and/or psychological over-eating or binge eating on the mental health questionnaire. Following identification, participants were then divided based on their reported diagnosis into those with lifetime AN, BN, or psychological over-eating or binge eating (i.e., BED). For the current study, participants who reported more than one ED diagnosis were excluded from analyses. Males or females of any age and ethnicity with current obesity (BMI >30 kg/m^2) and no self-reported or hospital-recorded diagnosis of EDs or psychiatric disorders were also selected. For a comparison group, healthy-weight (BMI 18.5 to 24.5 kg/m^2) control participants (HC) with no reported diagnosis (self-reported and/or hospital records) of a psychiatric condition, including an ED diagnosis, were also sampled.

For all participants, further exclusion criteria included a diagnosis of a neurological condition known to affect memory (and thus impede dietary recall) based on self-report and ICD-10 diagnoses[4].

6.3.1.3 Ethical approval

This study was covered under the generic ethical approval from the NHS Research Ethics Committee and ethics approval for the UK Biobank study was obtained from the North West Centre for Research Ethics Committee (REC reference: 11/NW/0382).

6.3.1.4 Assessment of dietary intake

The Oxford WebQ, a computerised 24-hour dietary recall questionnaire, was used to assess dietary food intake (for full details, see Liu et al., 2011). It is a self-administered

[4] Neurological conditions included as exclusion criteria were: brain cancer/primary malignant tumour, brain haemorrhage, brain/intracranial abscess, cerebral aneurysm, cerebral palsy, chronic/degenerative neurological problem, dementia/Alzheimer's disease/cognitive impairment, encephalitis, epilepsy, head injury, infection of nervous system, ischaemic stroke, meningeal cancer/malignant meningioma, meningioma (benign), meningitis, motor neurone disease, multiple sclerosis, neurological injury/trauma, neuroma (benign), other demyelinating condition, other neurological problem, Parkinson's disease, spina bifida, stroke, subarachnoid haemorrhage, subdural haematoma, and transient ischaemic attack.

measure that takes approximately 12.5 minutes to complete. The questionnaire records information about foods (and portion size) consumed on the previous day (i.e., during the preceding 24 hours) from each of 21 food groups, consisting of 206 foods and 32 beverages. Nutrient intake is automatically calculated using standardised nutritional composition values for each food/drink, providing estimates of daily intake of total energy, macronutrients, and micronutrients. The current study used data on intake of the following: alcohol, carbohydrate (total), carotene, energy, englyst dietary fibre, fat (total), folate, iron, magnesium, polyunsaturated fat, protein, retinol, saturated fat, vitamin B12, vitamin B6, vitamin C, vitamin D, and vitamin E.

The Oxford WebQ was administered on up to five separate occasions, three to four months apart, over a 16-month period, to capture both day-to-day and seasonal variation in food intake. The first assessment was administered on-site at the UK Biobank assessment centre, with all subsequent questionnaires completed online. Participants who completed a minimum of one dietary recall assessment with sufficient detail to average daily intake of parameters required to calculate the DII (listed above) were included. As participants provided dietary recall data on up to five occasions, mean nutrient intake was calculated across all available data points.

The Oxford WebQ appears to be an acceptable method of recording dietary intake (Galante et al., 2016) and has been validated against an interviewer-administered 24-hour dietary recall (Liu et al., 2011). However, self-reported dietary intake data always contains some degree of measurement error and therefore, when using this data, the biological plausibility of dietary intake needs to be considered (Banna, McCrory, Fialkowski, & Boushey, 2017). We applied a crude method to account for the plausibility of data by excluding participants reporting energy intakes > 4780.12 kcal per day for total energy intake (the threshold deemed as non-credible by UK Biobank). Extreme values of the nutrients listed above were also excluded. Upper cut-off values were decided in collaboration with a dietician, using recommended and average daily intake data from the British Nutrition Foundation (https://www.nutrition.org.uk/) and with visual inspection of the data. Upper limits were applied as follows: alcohol 150g, carbohydrate – total 1000g, carotene 25,000ug, englyst dietary fibre 60g, fat – total 300g, folate 1000ug, iron 40mg, magnesium 1000mg, polyunsaturated fat 60g, protein 300g, retinol 1000ug, saturated fat 120g, vitamin B12 50ug, vitamin B6 6mg, vitamin C 1000mg, vitamin D 25ug, and vitamin E 50mg. If participants had any of their energy or nutrient values excluded, the remaining data points at that time point were not included in analyses.

6.3.1.5 Assessment of dietary inflammatory potential

The DII was used to estimate the overall inflammatory potential of participants' diets (for full details on the development of the DII, see Shivappa et al., 2014a). The DII is literature-derived using a large-scale meta-analytic strategy to compute averaged inflammatory/anti-inflammatory effects for individual nutrient parameters that have sufficient evidence to ascertain the effect on inflammatory markers (Shivappa et al., 2014a). The inflammatory effects of dietary parameters were created by scoring them according to whether they increased (+1), decreased (−1) or had no effect (0) on six markers of inflammation (IL-1β, IL-4, IL-6, IL-10, TNF-α and CRP). An overall food parameter-specific inflammatory effect score was then calculated (weighted by study design) for 45 food and nutrient parameters (for full details, see Shivappa et al., 2014a). The sequence of steps in creating the DII is shown in **Figure 6.1**.

In the current study, the DII was computed from the Oxford WebQ data on the 18 micronutrients and macronutrients listed above. The steps to calculate the DII are summarised in **Figure 6.1** and are described in full elsewhere (Shivappa et al., 2014a). Briefly, values of the nutrients listed above are standardised into a z score by subtracting the mean from the global database and dividing this by the standard deviation from the global database (global daily mean intakes and standard deviations listed in Shivappa et al., 2014a for each nutrient). The global database was based on actual human consumption of foods and nutrients in eleven populations from different parts of the world and it provided a robust estimate of a mean and standard deviation for each parameter, which could then be used to express an individual's exposure, relative to the 'standard global mean'. To minimise the effect of 'right skewing' this value is converted into a percentile score. To achieve a symmetrical distribution with values centred on 0 (null) and bounded between −1 (maximally anti-inflammatory) and +1 (maximally pro-inflammatory), each percentile score is then doubled and '1' is subtracted. The centred percentile value for each food parameter is then multiplied by its respective 'overall food parameter-specific inflammatory effect score', listed in Shivappa et al. (2014a), to obtain the 'food parameter-specific DII score'. Finally, all of the 'food parameter-specific DII scores' for the available nutrients are summed to create the 'overall DII score' for an individual. A higher DII score indicates greater inflammatory potential of an individual's diet. The DII score could range from +7.98 (maximally pro-inflammatory) to -8.87 (maximally anti-inflammatory) when calculated from all 45 food parameters for which the creators of the DII calculated an inflammatory score (Hébert, Shivappa, Wirth, Hussey, & Hurley, 2019).

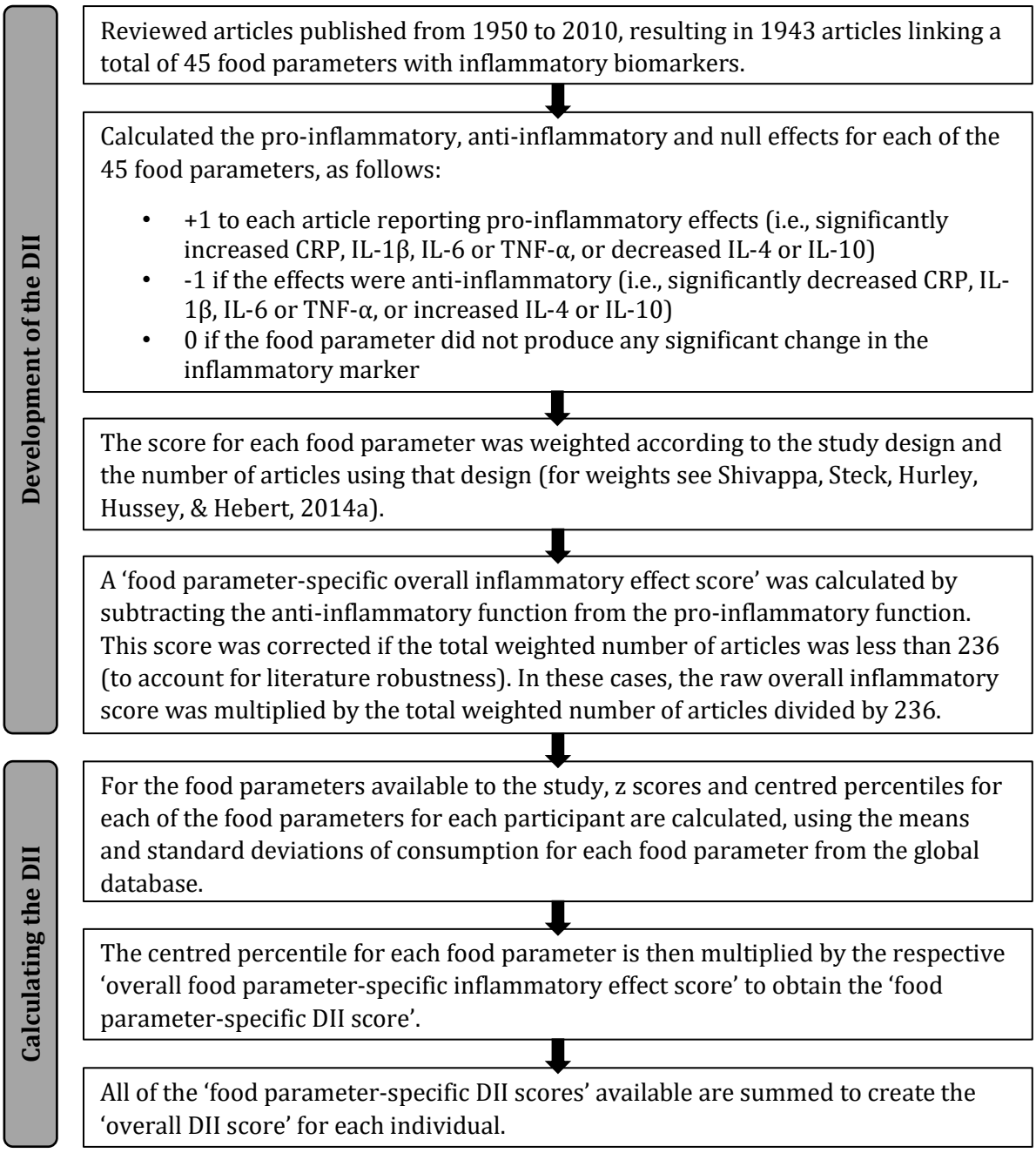

Figure 6.1 Sequence of steps in developing and calculating the Dietary Inflammatory Index, adapted from Shivappa et al. (2017b) and Shivappa et al. (2014a). Abbreviations: DII = Dietary Inflammatory Index; CRP = C-reactive protein; IL = interleukin; TNF = tumor necrosis factor.

6.3.1.6 Measurements of C-reactive protein

Blood samples were collected from participants on their visit to a UK Biobank assessment centre and serum was separated by centrifugation. Serum samples were stored at -80°C in a fully automated working archive (Owen & Woods, 2008) and thawed prior to use. Serum concentrations of CRP were quantified using high sensitivity immune-turbidimetric analysis methods (Beckman Coulter [UK], Ltd) using the Beckman Coulter AU5800 analytical platform (see Section 5.4.1.6 in Chapter 5 for further details). CRP values greater

than 10 mg/l were excluded as this may indicate the presence of acute inflammation and infection (Pearson et al., 2003).

6.3.1.7 Statistical Analysis

All statistical analyses were performed in Stata 15 (StataCorp, 2017). A total of 53,741 HC participants, 32,707 individuals with obesity, and 1,140 people with EDs (*n*=546 AN, *n*=206 BN, *n*=388 BED) had complete estimates of energy and nutrient intake from the Oxford WebQ for at least one time point. Participants for the HC and obesity groups were randomly sampled (using the sample command) from their respective groups at a ratio of 1 ED case to 4 controls to improve precision and reduce bias in estimates of effects (Linden & Samuels, 2013; Yamamoto, 2008). Thus, *n*=4560 HC and *n*=4560 participants with obesity were randomly sampled for inclusion in the analyses.

Demographics and nutrient intake data. Separate analyses were performed for each obesity or ED group in comparison to HCs (i.e., HC versus obesity, HC versus AN, HC versus BN, HC versus BED). Median and interquartile ranges (25^{th} and 75^{th} percentile) are given for non-normally distributed data or data with outliers and Mann Whitney U-tests were used to assess group differences in demographic characteristics, nutrient values, DII scores, and CRP concentrations. For normally distributed data, means and standard deviations are given and between subjects t-tests were used to compare group means of demographic characteristics, nutrient values, DII scores, and CRP concentrations. For categorical variables (e.g., gender, smoking status, ethnicity, and level of highest qualification), the chi-square test of homogeneity was used to determine if the proportion of the categories of these variables differed between HCs and each remaining group. To account for multiple comparisons a Bonferroni correction was applied to each set of group comparisons of nutrient intake by dividing the *p* value (0.05) by the number of comparisons made (*n*=18). This gave a statistical significance threshold for group comparisons of dietary intake data of $p = 0.0027$.

Association between the Dietary Inflammatory Index and case/control status. For groups with significantly different DII scores to HCs, the relationship between the DII score and group status was further explored using logistic regressions. In a first model, age and gender were included as covariates, as these factors alter the recommended daily intakes of total energy and macronutrients according to international dietary guidelines (World Health Organization, 2018). A fully adjusted model aiming to determine how the DII score differs across groups independently of other sociodemographic factors was also performed. In this, age, gender, ethnicity, social deprivation, highest qualification, and BMI were included as covariates. However, BMI was not included in any regression model

between normal-weight controls and the obesity group as this covariate would perfectly predict group status and we wanted to look for BMI-driven changes in DII score.

Association between the Dietary Inflammatory Index and anthropometric parameters. Exploratory linear regression analyses were performed to examine the association between DII score and BMI and waist to hip ratio (WHR), given the findings from previous research (e.g., Ramallal et al., 2017; Ruiz-Canela et al., 2015). A linear regression with DII score as the independent variable and BMI or WHR as the dependent variable were conducted. A second model then included sociodemographic factors as covariates (gender, age, ethnicity, social deprivation, and highest qualification). Finally, a fully adjusted model additionally included the alternate anthropometric parameter as a covariate.

Association between C-reactive protein and case/control status. A total of 10,179 participants had CRP data available. A total of *n*=420 participants (4.13% of sample) had CRP concentrations greater than 10mg/l and were therefore excluded from analyses. Logistic regressions were performed to examine whether CRP serum concentration (as a continuous variable) was associated with each ED/obesity group relative to being a HC, if a significant group difference was identified. Gender, age, ethnicity, smoking status, social deprivation, BMI and presence of an autoimmune or inflammatory condition (dichotomised as present or absent from ICD-10 diagnoses in hospital records, list of conditions shown in **Table 10.1** of Appendix H Section 10.8) were included as covariates. As before, BMI was not used as a covariate in the model including obesity participants.

Association between the Dietary Inflammatory Index and C-reactive protein. For those participants with both a DII score and CRP measurement available, a multivariate logistic regression was used to determine whether DII score was associated with CRP serum concentration (as a continuous variable). This model was adjusted for relevant sociodemographic characteristics (gender, age, ethnicity, smoking status, social deprivation, highest qualification, presence of an autoimmune or inflammatory condition). The regression model was repeated with the additional inclusion of BMI as a covariate. To further explore the relationship between DII and CRP in EDs, these regression models were repeated in only participants reporting a lifetime ED diagnosis.

All regression analyses including HCs or obesity participants were repeated five times with a different sample of randomly selected HCs and participants with obesity to ensure results were consistent across the wider sample.

6.3.2 Results

6.3.2.1 Demographic characteristics

The demographic characteristics for the HC, obesity, and ED groups (total n=10,260) are presented in **Table 6.1**. The proportion of males was significantly lower in the ED groups and significantly higher in the obesity group, compared to HCs ($p < 0.0001$). Each ED group was significantly younger, and the obesity group was significantly older than the HC group ($p < 0.0001$). Only the BED group significantly differed from HCs in ethnicity, with the BED group having a greater proportion of BAME participants than the HCs ($p < 0.0001$). ED groups had a significantly higher proportion of current smokers than the HC group ($p < 0.0001$) and the obesity group had a significantly greater proportion of former smokers than the HCs ($p < 0.0001$). The BN group had a significantly higher and the obesity and BED groups had a significantly lower proportion of participants who had completed degrees than the HCs ($p < 0.05$). All ED groups and the obesity group were significantly less affluent than the HCs ($p < 0.0006$). The obesity, BN and BED participants had a significantly higher BMI and the AN group significantly lower than the HCs ($p < 0.0007$). Obesity and BED groups also had significantly higher WHR than HCs ($p < 0.0001$) and the BN and AN participants had significantly lower WHR compared to HCs ($p < 0.0017$).

Table 6.1 Demographic characteristics for healthy control, obesity and eating disorder groups.

	Healthy control		Obesity			Anorexia nervosa			Bulimia nervosa			Binge eating disorder		
	n		***n***			***n***			***n***			***n***		
Gender [*n* (%)]	4560		4560			546			206			388		
Male		1476 (32.37)		2183 (47.87)	*		25 (4.58)	*		5 (2.43)	*		55 (14.18)	*
Female		3084 (67.63)		2377 (52.13)			521 (95.42)			201 (97.57)			333 (85.82)	
Age [years] [mean ± SD]	4560	54.91 ± 7.88	4560	56.30 ± 7.54	*	546	53.31 ± 6.93	*	206	49.92 ± 6.87	*	388	53.16 ± 7.46	*
Ethnicity [*n* (%)]	4544		4541			540			205			386		
Caucasian		4424 (97.36)		4403 (96.96)			529 (97.96)			197 (96.10)			361 (93.52)	
BAME		120 (2.64)		138 (3.04)			11 (2.04)			8 (3.90)			25 (6.48)*	
Smoking status [*n* (%)]	4556		4554			531			203			388		
Current		268 (5.88)		258 (5.68)			48 (9.04)			22 (10.84)			31 (7.99)	
Former		1334 (29.28)		1894 (41.68)	*		193 (36.35)	*		80 (39.41)	*		168 (43.30)	*
Never		2954 (64.84)		2392 (52.64)			290 (54.61)			101 (49.75)			189 (48.71)	
Highest qualification	4543		4538			531			203			388		
Degree		2527 (55.62)		1804 (39.75)	*		304 (57.25)			126 (62.07)	*		183 (47.16)	*
A Levels or below		2016 (44.38)		2734 (60.25)			227 (57.25)			77 (37.93)			205 (52.84)	
Social deprivation [median (IQR[a])]	4552	-2.44 (-3.82, -0.14)	4557	-2.22 (-3.82, 0.26)	*	545	-2.03 (-3.66, 0.84)	*	206	-1.11 (-3.10, 1.61)	*	388	0.89 (-2.99, 1.96)	*
BMI [kg/m^2] [median (IQR[a])]	4560	22.70 (21.60, 23.60)	4560	32.50 (31.10, 34.80)	*	534	22.10 (20.20, 24.20)	*	197	23.40 (21.80, 25.70)	*	378	31.70 (26.60, 37.80)	*
WHR [mean ± SD]	4560	0.81 ± 0.07	4560	0.91 ± 0.09	*	546	0.79 ± 0.07	*	206	0.79 ± 0.06	*	384	0.85 ± 0.10	*
DII [mean ± SD]	4560	0.56 ± 1.35	4560	0.85 ± 1.37	*	546	0.55 ± 1.48		206	0.51 ± 1.45		388	0.69 ± 1.60	

	Healthy control		Obesity			Anorexia nervosa			Bulimia nervosa			Binge eating disorder		
	n		***n***			***n***			***n***			***n***		
CRP [mg/l] [median (IQR[a])]	4487	0.66 (0.37, 1.22)	4261	2.12 (1.21, 3.70)	*	500	0.55 (0.30, 1.21)	*	187	0.66 (0.33, 1.62)		324	1.58 (0.71, 3.50)	*

*indicates significantly different from HCs at $p < 0.05$. [a]25th and 75th percentile reported. Abbreviations: *n* = number of observations; SD = standard deviation; BAME = black, Asian and minority ethnic; IQR = interquartile range; BMI = body mass index; kg = kilogram; m = metre; WHR = waist to hip ratio; DII = Dietary Inflammatory Index; CRP = C-reactive protein; mg = micrograms; l = litre.

6.3.2.2 Nutrient intake

The mean number of dietary recalls completed by participants was 2.57 ± 1.19. Median energy and nutrient intake for each group is shown in **Table 6.2**. After correction for multiple comparisons, median intake of protein, total fat, saturated fat, and vitamin B6 were significantly higher, and consumption of carbohydrate, englyst dietary fibre, iron, folate, vitamin C, magnesium, carotene, and vitamin E significantly lower in the obesity group compared to HCs. The AN group had significantly lower median alcohol, energy, protein, fat (polyunsaturated, saturated, and total), total carbohydrate, iron, retinol, vitamin B6, vitamin B12, and vitamin D intake than the HC group. The BN group reported consuming significantly less energy and carbohydrates than the HCs. Elevated intake of protein, saturated fat and reduced consumption of alcohol was observed in the BED group compared to HCs.

Table 6.2 Median (and interquartile range[a]) nutrient intake for the healthy, obesity and eating disorder groups.

	Healthy control (*n*=4560)	**Obesity (*n*=4560)**	**Anorexia nervosa (*n*=546)**	**Bulimia nervosa (*n*=206)**	**Binge eating disorder (*n*=388)**
Energy [kcal]	2024.92 (1730.32, 2356.65)	2053.95 (1723.81, 2422.59)	1887.35 (1552.92, 2221.55)*	1882.92 (1646.49, 2195.14)*	2069.24 (1654.13, 2481.08)
Protein [g]	78.04 (65.98, 91.36)	82.74 (68.68, 97.32)*	71.74 (56.85, 86.58)*	75.23 (62.08, 88.68)	82.70 (68.62, 99.08)*
Fat - total [g]	73.74 (58.25, 90.94)	75.78 (59.60, 94.98)*	65.71 (50.54, 83.00)*	69.41 (52.96, 89.26)	78.93 (58.56, 99.49)
Carbohydrate - total [g]	247.43 (205.52, 293.39)	239.62 (197.04, 289.96)*	234.06 (186.88, 282.55)*	233.26 (179.13, 272.28)*	246.85 (192.19, 299.87)
Saturated fat [g]	27.56 (21.14, 35.01)	29.30 (22.21, 37.05)*	24.87 (18.65, 33.26)*	25.47 (20.04, 34.66)	29.49 (21.97, 39.00)*
Polyunsaturated fat [g]	13.07 (9.51, 17.43)	13.43 (9.48, 18.06)	11.32 (7.92, 15.28)*	12.32 (8.43, 15.89)	13.68 (9.28, 18.22)
Englyst dietary fibre [g]	16.15 (12.77, 20.11)	15.30 (11.81, 19.31)*	16.33 (12.75, 20.66)	15.86 (12.43, 21.06)	16.37 (12.12, 21.47)
Iron [mg]	13.51 (11.26, 16.08)	12.95 (10.62, 15.54)*	12.50 (10.08, 15.41)*	12.76 (10.66, 15.81)	12.85 (9.94, 16.20)
Vitamin B6 [mg]	2.07 (1.69, 2.48)	2.15 (1.74, 2.61)*	1.92 (1.54, 2.30)*	2.02 (1.62, 2.36)	2.15 (1.65, 2.64)

	Healthy control (*n*=4560)	Obesity (*n*=4560)	Anorexia nervosa (*n*=546)	Bulimia nervosa (*n*=206)	Binge eating disorder (*n*=388)
Vitamin B12 [µg]	5.39 (3.61, 7.93)	5.39 (3.70, 7.85)	4.51 (2.90, 6.69)*	5.41 (3.40, 8.16)	5.38 (3.71, 7.97)
Folate [µg]	288.98 (234.37, 354.07)	284.74 (227.55, 352.25)*	279.78 (218.00, 351.90)	268.38 (219.48, 360.90)	279.88 (218.53, 364.27)
Vitamin C [mg]	141.05 (90.99, 204.33)	126.77 (77.96, 187.90)*	138.82 (90.85, 213.70)	139.97 (85.09, 211.42)	134.42 (77.92, 206.03)
Magnesium [mg]	344.94 (291.51, 404.41)	330.16 (275.08, 390.01)*	338.19 (276.35, 399.63)	331.07 (277.74, 404.54)	335.68 (274.39, 416.25)
Retinol [µg]	312.07 (215.85, 428.45)	313.30 (217.46, 433.75)	268.86 (187.17, 399.85)*	292.04 (186.82, 391.70)	315.96 (211.91, 444.14)
Carotene [µg]	2741.96 (1593.17, 4289.67)	2449.84 (1235.19, 4098.80)*	2917.31 (1671.20, 4849.77)	2856.49 (1369.92, 4731.88)	2738.20 (1376.30, 4600.63)
Vitamin D [µg]	2.07 (1.15, 3.83)	2.11 (1.28, 3.58)	1.54 (0.76, 3.00)*	1.99 (0.97, 3.74)	2.15 (1.21, 3.88)
Alcohol [g]	9.94 (0, 22.17)	8.16 (0, 26.02)	7.05 (0, 20.66)*	6.94 (0, 22.55)	0.07 (0, 15.55)*
Vitamin E [mg]	9.06 (6.87, 11.68)	8.34 (6.28, 10.83)*	8.85 (6.86, 11.62)	9.33 (7.00, 11.65)	9.46 (6.90, 12.19)

*indicates significantly different from HCs at $p < 0.0027$. [a]25th and 75th percentile reported. Abbreviations: kcal = kilocalorie; g = grams; mg = milligram; µg = microgram.

6.3.2.3 Dietary Inflammatory Index

In the whole sample, the mean DII score was 0.69 ± 1.39 and DII scores ranged from a maximum anti-inflammatory value of -3.67 to a maximum pro-inflammatory value of +4.35. The mean DII score for each group is shown in **Table 6.1**. Between subjects t-tests showed that the obesity group had a significantly higher DII score than the HC group ($t(9118) = -10.31$, $p < 0.0001$). None of the ED groups significantly differed from HCs on DII score (AN versus HC: $t(5104) = 0.15$, $p = 0.8800$; BN versus HC: $t(4764) = 0.49$, $p = 0.6237$; BED versus HC: $t(4946) = -1.78$, $p = 0.0750$).

A logistic regression was then used to determine if DII score was associated with membership of the obesity group relative to HCs. A higher DII score was associated with being in the obese group, relative to the HC group ($p < 0.0001$) in a model adjusted for gender and age ($\chi^2(3) = 394.41$, $p < 0.0001$; odds ratio [OR] 1.18, 95% confidence intervals [CIs] 1.14 to 1.22, $p < 0.0001$). These findings remained significant in a fully adjusted model where age, gender, ethnicity, social deprivation, and highest qualification were included as covariates ($\chi^2(9) = 650.34$, $p < 0.0001$), such that a higher DII score was associated with being in the obesity group relative to the HCs (OR 1.16, 95% CIs 1.12 to 1.19, $p < 0.0001$). Analyses were repeated with a further five random samples and results remained the same.

Association between the Dietary Inflammatory Index and anthropometric parameters.
Exploratory analyses showed that the DII score was significantly associated with BMI in unadjusted ($F(1, 10227) = 134.23$, $p < 0.0001$) and fully adjusted models with ($F(7, 10155) = 912.49$, $p < 0.0001$) and without ($F(6, 10156) = 109.07$, $p < 0.0001$) WHR as a covariate. Increases in DII score were associated with increases in BMI in the fully adjusted model with WHR ($\beta = 0.24$, 95% CIs 0.17 to 0.31, $p < 0.0001$). Results remained the same when repeated with additional random samples of HC and obesity participants. DII score was significantly associated with WHR in unadjusted ($F(1, 10254) = 83.65$, $p < 0.0001$) and fully adjusted models with ($F(7, 10155) = 2625.53$, $p < 0.0001$) and without BMI ($F(6, 10180) = 1414.13$, $p < 0.0001$) as a covariate, such that increases in WHR were associated with increases in DII score (when BMI was included as a covariate; $\beta = 0.001$, 95% CIs 0.00 to 0.00, $p = 0.0165$). When repeated with different random samples of HCs and obesity participants, results generally remained the same, except for two out of five fully adjusted models including BMI as a covariate, which were non-significant.

6.3.2.4 C-reactive protein

The median serum CRP concentrations (mg/l) for each group are shown in **Table 6.1**. The obesity group ($U = 3409034.50$, $z = -52.10$, $p < 0.0001$) and the BED group ($U = 388388.50$, $z = -14.02$, $p < 0.0001$) had significantly higher CRP concentrations than the HCs. People

with AN had significantly lower CRP than the HC group (U = 1042506.50, z = 2.60, p = 0.0095). BN and HC participants did not differ (U = 409230.00, z = -0.57, p = 0.5687). The significant group comparisons were further explored using logistic regressions to examine if CRP concentrations were associated with group membership. A higher CRP concentration was associated with increased odds of being in the obesity group relative to the HC group ($\chi^2(9) = 2816.49$, $p < 0.0001$; OR 2.30, 95% CIs 2.20 to 2.40, $p < 0.0001$). CRP concentration was not able to differentiate between AN and HC groups (p = 0.5890) and BED and HC groups (p = 0.6133). Analyses were repeated with a further five random samples and results remained the same.

6.3.2.5 Association between the Dietary Inflammatory Index and concentrations of C-reactive protein

DII score was significantly associated with serum CRP concentrations in an unadjusted linear regression (β = 0.12, 95% CIs 0.10 to 0.15, $p < 0.0001$) and a linear regression, controlling for sociodemographic factors. This showed that DII score was associated with CRP serum concentrations, such that a more pro-inflammatory DII score was related to higher concentrations of CRP (β = 0.12, 95% CIs 0.09 to 0.14, $p < 0.0001$). This association remained significant when additionally covarying for BMI (β = 0.04, 95% CIs 0.02 to 0.07, p = 0.0002). Findings were replicated in other samples including different randomly selected HCs and obesity participants (except for one out of five fully adjusted models, including BMI, which was non-significant).

In the ED participants, in an unadjusted and adjusted regression model (not including BMI as a covariate), DII was significantly associated with CRP in a similar pattern to in the whole sample. However, when BMI was controlled for, CRP concentrations were no longer associated with DII score in the ED sample (p = 0.0971).

6.3.3 Discussion

We found that having obesity (as defined by a BMI greater than 30 kg/m^2), relative to being a HC, was associated with a higher DII score. However, the ED groups did not differ in DII score to HCs. DII score was found to be significantly and positively associated with the anthropometric parameters of BMI and WHR.

The finding that people with current obesity have a higher DII score than HCs is consistent with previous research that found a positive association between indicators of obesity (Cantero et al., 2018; Ruiz-Canela et al., 2015), independent of established risk factors for obesity (e.g., age, smoking status, marital status, educational level, physical activity, diabetes, and hypertension; Ruiz-Canela et al., 2015). A high DII score (compared to a medium or low DII score) has also been associated with higher annual weight gain and a higher risk of developing overweight or obesity (Ramallal et al., 2017). Dietary

inflammation may contribute to obesity and may partially explain the low-grade inflammation observed in people with obesity (Gregor & Hotamisligil, 2011). An important consideration is that people with obesity have been consistently shown to under-report energy intake (Briefel, Sempos, McDowell, Chien, & Alaimo, 1997; Johnson, 2002; Prentice et al., 1986; Wehling & Lusher, 2017) and consequently, other nutrients. Therefore, it may be that the real group differences in DII are even greater than seen here.

It was perhaps to be expected that, as in obesity, people with BED would have a higher DII score than HCs, as research has reported comparable or increased dietary intake in BED to obesity (Raymond et al., 2003). While the DII score was higher in BED participants compared to the HC group, similar to in the obesity group, this difference was not significant. To date, there is little research characterising daily nutrient intake in people with BED (Forbush & Hunt, 2014). However, existing research has typically used small samples and has mainly focussed on binge versus non-binge days. In our sample, nutrient intake was comparable between HCs and BED participants, with the only differences being that the BED group consumed more protein and saturated fat and less alcohol. However, research has suggested that people with obesity and BED tend to underreport food intake to a significantly greater extent than people with obesity without BED (Bartholome, Peterson, Raatz & Raymond, 2013). This may therefore account for the lack of group differences observed in nutrient intake and DII score.

The participants with lifetime AN reported a significantly altered diet with lower intake than HCs of energy, the macronutrients carbohydrate, protein and fat (including, total, saturated and polyunsaturated), and several micronutrients. This reduced consumption of total calories and calories from fat in AN has been previously reported (Mayer et al., 2012; Misra et al., 2006b), as has reduced intake of several micronutrients e.g., vitamin B6 and vitamin D (Chiurazzi et al., 2017). Furthermore, in line with our findings, research has shown that even in weight-restored AN patients, altered eating behaviours often persist (Mayer et al., 2012). Given the observed alterations in dietary intake, it is unclear as to why the DII score did not significantly differ between the AN group and HC participants. Therefore, further research is needed to explore dietary inflammation in AN.

Relatively little research has characterised diet in BN on non-binge days compared to HCs. One study assessing nutrient intake in women with BN on non-binge days found significantly lower energy, protein, carbohydrates, sucrose, fat and fatty acids (saturated, monounsaturated and polyunsaturated), cholesterol, zinc, and vitamin B12, but significantly greater alcohol consumption, compared to population medians (Gendall et al., 1997). Also, some studies have reported food restriction on non-binge days in BN, although nutrient intake is likely highly heterogenous in BN (Forbush & Hunt, 2014).

However, in the current sample, few differences in nutrient intake were observed between BN and HC groups. It is therefore not surprising that the DII score also did not differ in this group when compared to HCs. It is important to note that from the available data it is unclear whether dietary recall was recorded on binge eating days, and as participants in this study had a lifetime diagnosis of BN, they may not have been currently unwell with the disorder and actively engaging in binge eating behaviour.

We also found that serum concentrations of CRP were significantly higher in the obese and BED groups and significantly lower in the AN group as compared to the HCs, which is consistent with previous research. However, logistic regressions (controlling for relevant sociodemographic factors) only identified a significant association between CRP concentrations and current obesity status: participants were at an increased odds of being in the obesity group, relative to the HC group, if they had a higher CRP concentration. Indeed, reviews have concluded that obesity is associated with higher concentrations of CRP (Choi, Joseph, & Pilote, 2013). In the whole sample, we found that a higher DII score was significantly associated with a higher serum CRP concentration, even after controlling for sociodemographic factors, including BMI. Our findings corroborate the results from several previous studies, validating the DII against inflammatory markers (as reviewed in Yang et al., 2019) and provide further evidence for the potential role of diet in modulating inflammation within the body. However, when this relationship was further explored in the ED sample only, including all covariates in the analysis, DII score was not associated with CRP. This suggests that in the ED group, factors other than dietary inflammatory potential may contribute to CRP concentrations.

6.3.3.1 Strengths and limitations

This study was the first to assess dietary inflammation in EDs and compare this to participants with current obesity and participants of a healthy-weight, using a large-scale population-based sample. It is well-established that dietary recall data becomes more reliable at providing an insight into an individual's diet the more times it is completed, given large day-to-day variation in nutrient and food intake for a variety of reasons e.g., appetite, physical activity, personal economic conditions (Institute of Medicine Committee on Dietary Risk Assessment in the Women, Infants, and Children Program, 2002). Therefore, a particular strength of this study was the administration of dietary recall questionnaires on five separate occasions and at different times of year to capture seasonal variation. It has been suggested that energy intake is under-reported in the first dietary recall questionnaire and three dietary recall questionnaires is optimal for estimating energy intake (Ma et al., 2009). In our study, the mean number of questionnaires completed by participants was 2.57 with 75.84% of participants

completing two or more and 25.81% of participants completing the 'optimum' three dietary recall questionnaires.

The findings need to be viewed in light of several limitations. Firstly, data on EDs was limited to a lifetime ED diagnosis. As such, it cannot be confirmed whether participants have a current ED and information on more detailed aspects of EDs, including ED severity, illness duration and engagement in treatment, cannot be determined. Second, we used a crude method to manage extreme and implausible values of energy and nutrient intake. This method is not individualised and may not identify all implausible reports (Banna et al., 2017). However, this method does provide a consistent protocol for dietary report instruments (including dietary recalls and food frequency questionnaires) that do not allow for an accurate estimation of energy and nutrient intake (Banna et al., 2017). Third, blood samples were not collected at the same time as dietary data (as this was collected over several time points) and this study used a cross-sectional design, therefore, we were unable to infer temporal associations between the DII score and CRP concentrations.

6.3.3.2 Conclusion

Taken together, people with lifetime ED diagnoses do not differ from HCs in the inflammatory potential of their diet. This may be due to the use of lifetime diagnoses and thus, the potential absence of current ED symptoms and behaviours in this sample. It may also be that diet does not play a substantial role in the pro-inflammatory state observed in EDs. Current obesity was found to be significantly associated with a more pro-inflammatory diet, as indicated by a higher DII score. Thus, diet may account for some of the chronic inflammation observed in people with obesity (Ellulu et al., 2017). DII score was significantly associated with serum CRP concentrations, such that a more pro-inflammatory diet was predictive of higher CRP concentrations. However, this finding was not upheld when examined in a sample of ED only participants.

6.4 Study 2: Association between inflammatory potential of diet and cytokine concentrations in people with anorexia nervosa

This study was the first to assess the inflammatory potential of diet in a purposively sampled group of females with current AN and HCs. In Study 1, participants reporting lifetime AN had comparable dietary inflammation to HCs. Following on from this, in the current study, the DII was calculated based on data from a Food Frequency Questionnaire (FFQ) assessing average diet over the last year in a sample of females currently unwell with AN. By using the FFQ, a greater number of nutrients (*n*=25) were available to be included in the calculation of the DII than when using the Oxford WebQ 24-hour dietary recall in Study 1 (*n*=18 nutrients). This study used the Cytokine in Anorexia Nervosa: CytAN sample which was previously described in Chapter 3.4 and Chapter 5.3.

The main aim of this study was to examine DII scores in females with current AN. Based on the findings from Study 1, it was expected that participants with AN would have a similar DII score and thus similar levels of dietary-related inflammation to HCs. It was also predicted that energy intake and intake of certain nutrients (e.g., fats) would be lower in the AN group, as compared to the HC group, in line with Study 1 and previous research (e.g., Misra et al., 2006b). This study also aimed to determine if the DII was associated with cytokine concentrations in this sample. Given the findings from Study 2, it was unclear whether previous validations of the DII against inflammatory marker concentrations would be replicated in the current ED sample.

6.4.1 Methods

6.4.1.1 Participants

Adult (>18 years old) females with a DSM-5 (American Psychiatric Association, 2013) diagnosis of AN and a BMI <18.5 kg/m^2 were recruited from Specialist Eating Disorders in South London and Maudsley NHS Trust Foundation, the Beat research recruitment webpage, and from poster and email circular advertisements at King's College London (KCL). Adult females with a healthy BMI (18.5-24.5 kg/m^2) and no current or history of any psychiatric disorders were recruited from email circulars and poster advertisements at KCL. Exclusion criteria for all participants included an autoimmune or chronic inflammatory disease/condition and current pregnancy. To determine eligibility, all potential participants underwent a short telephone screening as described fully in Section 3.4.1.1 in Chapter 3. All participants provided informed consent before study participation.

6.4.1.2 Ethical approval

The study was conducted in accordance with the Declaration of Helsinki and the study received ethical approval from the London - City & East Research Ethics Committee (REC reference: 17/LO/2107).

6.4.1.3 Procedure

Full details of the methods of this study were described in Chapter 3.4.1. Briefly, participants attended a research session (lasting approximately one and a half hours) at the Institute of Psychiatry, Psychology & Neuroscience (IoPPN), KCL. In this session, participants had their blood collected and completed a purposely designed record form to collect demographic data and a questionnaire pack. For the purpose of the current study, only data from the European Prospective Investigation of Cancer - Norfolk Food Frequency Questionnaire (EPIC-Norfolk FFQ; Bingham et al., 1994; Bingham et al., 2001) for the assessment of dietary intake was used. Finally, height, body weight and body composition (using Inbody S10 machine, Biospace Co., Ltd) were measured.

6.4.1.4 Assessment of dietary intake

The EPIC-Norfolk FFQ (Bingham et al., 1994; Bingham et al., 2001) is a semi-quantitative self-report questionnaire designed to record the average intake of foods and beverages during the previous year. The food list in the EPIC-Norfolk FFQ is based on items from an FFQ widely used in the US (Bingham et al., 1994; Rimm et al., 1992), but it was modified to reflect differences in American versus UK food items and brand names. Specifically, the EPIC-Norfolk FFQ uses 290 foods. The questionnaire consists of two parts. Part 1 is a food list of 130 lines and the lines are either individual foods, combinations of individual foods or food types. Each line also has a portion size attached to it, which is a medium serving, standard unit or household measure. Respondents select an appropriate frequency of consumption for their average use over the last year for each line. They can select from nine frequency categories ranging from "Never or less than once/month" to "6+ per day". Part 2 consists of a number of questions that ask for more detailed information about the food lines in Part 1. For example, further information about breakfast cereals and fats is requested. The EPIC-Norfolk FFQ has been widely used to assess dietary intake in large populations (Bingham et al., 2001) and extensively validated (Bingham et al., 1997; McKeown et al., 2001).

FFQ EPIC Tool for Analysis (FETA) is an open source, cross-platform software tool designed to convert EPIC-Norfolk FFQ data into nutrient and food group values (Mulligan et al., 2014). Data is entered into a purposively designed comma-separated values input file following coding instructions (http://www.srl.cam.ac.uk/epic/epicffq /websitedocumentation.shtml), which is then uploaded to FETA. The output from FETA provides an average daily nutrient and food group intake for an individual from all FFQ foods consumed; specifically, intake data for 46 nutrients and 14 basic food groups. This software produces similar nutrient and food group values to a previously validated, but less accessible tool (Compositional Analyses from Frequency Estimates [CAFÉ]) designed for converting EPIC-Norfolk FFQ data (Welch, Luben, Khaw, & Bingham, 2005).

6.4.1.5 Assessment of dietary inflammatory potential

The DII (Shivappa et al., 2014a) has been fully described in Study 1 of this Chapter. In order to calculate the DII, nutrient intake data from the EPIC-Norfolk FFQ, as calculated by FETA (Mulligan et al., 2014), was used. Specifically the following 25 nutrients (for which an overall inflammatory effect score was calculated by Shivappa et al., 2014a) were used: alcohol, β-carotene, carbohydrate, cholesterol, energy (kcal), fat, englyst fibre, folate, iron, magnesium, monounsaturated fatty acids (MUFA), niacin, protein, polyunsaturated fatty acids (PUFA), riboflavin, saturated fat, selenium, thiamine, retinol, vitamin B6, vitamin

B12, vitamin C, vitamin D, vitamin E, and zinc. The steps to calculate the DII are fully detailed in Section 6.3.1.5 and **Figure 6.1** (Shivappa et al., 2014a).

6.4.1.6 Quantification of cytokine concentrations

Full details of blood collection and the measurement of cytokines was described in Section 3.3.1.5 in Chapter 3. Briefly, serum was stored at -80°C prior to use and thawed at room temperature for use. The concentrations of 36 cytokines were quantified simultaneously using the Meso Scale Discovery V-PLEX Plus Human Biomarker 36-Plex Kit, following the manufacturer's instructions (Meso Scale Discovery, Maryland, USA). Cases and controls were randomised across the assay, and plates were scanned on a Meso Scale Discovery MESO Quickplex SQ 120 reader at the IoPPN, KCL. Standard curves were used to determine absolute quantities (pg/ml) of each inflammatory marker. The following cytokines were detectable in greater than 70% of the sample and were used in the current analyses (see Section 3.4.2.2): Eotaxin, Eotaxin-3, interferon (IFN)-γ, IL-2, IL-6, IL-7, IL-8, IL-10, IL-12/IL-23p40, IL-12p70, IL-13, IL-15, IL-16, IL-17A, IL-22, IL-27, interferon gamma-induced protein (IP)-10, monocyte chemoattractant protein (MCP)-1, MCP-4, macrophage-derived chemokine (MDC), macrophage inflammatory protein (MIP)-1α, MIP-1β, MIP-3α, thymus and activation regulated chemokine (TARC), TNF-α, TNF-β, and vascular endothelial growth factor (VEGF).

6.4.1.7 Statistical Analysis

All statistical analyses were performed in Stata 15 (StataCorp, 2017). The distribution of data was examined through visual inspection of histograms and the Shapiro-Wilk test for normality. Median and interquartile ranges (25^{th} and 75^{th} percentile) are given for non-normally distributed data and Mann Whitney U-tests were used to assess group differences in demographic characteristics and nutrient values. For normally distributed data, means and standard deviations are presented and between subjects t-tests were used to compare group means of demographic characteristics and nutrient values. For analyses of demographic data, the level of significance was set at $p < 0.05$. For nutrient data, Benjamini-Hochberg procedure with a false discovery rate (FDR) of 0.1 was applied to account for multiple comparisons.

The DII was calculated for each participant using the steps described previously (Section 6.3.1.5). A logistic regression was then performed to determine whether DII score (independent variable) was associated with group status (dependent variable i.e., AN versus HC). Following this, an adjusted logistic regression was performed, including age, ethnicity and smoking status (yes/no) as covariates. For the unadjusted and adjusted logistic regressions, influential points/outliers (if studentised residuals greater than ±3 standard deviations and/or Cook's distance was greater than 0.5) were removed to

address assumptions. Exploratory linear regression analyses were also performed to examine the association between BMI and DII score. An unadjusted linear regression with BMI as the independent variable and DII score as the dependent variable was first performed. Then, in a multivariate linear regression, DII score was regressed on BMI with age, ethnicity and smoking status as covariates. The level of significance was set at $p < 0.05$ for these analyses.

To examine whether DII score was associated with cytokine concentrations, multivariate linear regressions were performed with DII score as the dependent variable and the cytokine value as the independent variable, with age, ethnicity, and smoking status included as covariates. Separate regression models were performed for each cytokine. Studentised residuals greater than ±3 standard deviations were deemed to be outliers and were removed, and assumptions were tested and met. The Benjamini-Hochberg procedure with an FDR of 0.1 was applied to the regression models as a correction for multiple testing.

6.4.2 Results

Participant demographic and clinical characteristics are presented in **Table 6.3**. Thirty-five HCs and 33 AN participants had FFQ data available and were included in this study. Groups did not differ in age or proportion of current smokers. There were a higher proportion of Caucasian participants in the AN group. BMI and body fat percentage were significantly lower in AN participants compared to HCs.

Table 6.3 Participant characteristics for healthy control and anorexia nervosa participants, with group comparisons.

	Healthy Controls (*n*=35)	**Anorexia Nervosa (*n*=33)**	**Group comparison**
Age [years] [median (IQR[a])]	24.00 (21.00, 25.00)	24.00 (21.00, 27.00)	$U = 496.00$ $z = -1.00$ $p = 0.3150$
Current smoker [*n*]	5	5	χ^2 (1) = 0.01 $p = 0.9200$
Ethnicity [Caucasian/BAME] [*n*]	16 / 19	28 / 5	χ^2 (1) = 11.39 **$p = 0.001$**
BMI [kg/m^2] [mean ± SD]	21.23 ± 1.93[b]	16.00 ± 1.31	$t(65) = 12.97$ **$p < 0.0001$**
Body fat [%] [median (IQR[a])]	24.15 (21.60, 29.10) [b]	10.80 (8.70, 14.20)	$U = 42.50$ $z = 6.50$ **$p < 0.0001$**

AN subtype [AN-R/AN-BP] [*n*]		27/6	
Current treatment [none/outpatient/inpatient] [*n*]		5/27/1	
DII [median (IQR[a])]	0.25 (-0.79, 1.02)	0.30 (-1.70, 1.67)	*U* = 569.00 *z* = 0.10 *p* = 0.9169

Statistically significant group comparisons at $p < 0.05$ are highlighted in bold. [a]25th and 75th percentile reported. [b]*n*=34. Abbreviations: *n* = number of observations; IQR = interquartile range; BAME = black, Asian, and minority ethnic; BMI = body mass index; AN = anorexia nervosa; AN-R = anorexia nervosa restricting type; AN-BP = anorexia nervosa binge-eating/purging type.

Median nutrient values for the AN and HC groups are shown in **Table 6.4**. HC participants had greater alcohol, cholesterol and vitamin D intake than the AN participants. However, estimated habitual nutrient intakes over the previous year did not appear to differ between groups after controlling for multiple testing.

Table 6.4 Median and interquartile ranges[a] of the nutrient values for the healthy control and anorexia nervosa groups.

Nutrient	Healthy Controls (*n*=35)	Anorexia Nervosa (*n*=33)	Group comparison
Alcohol [g]	2.07 (0.76, 4.13)	0 (0, 2.54)	U = 400, z = 2.23, p = 0.026
Vitamin B12 [µg]	5.08 (2.68, 7.92)	3.05 (1.91, 5.48)	U = 447, z = 1.60, p = 0.109
Vitamin B6 [mg]	2.04 (1.53, 2.82)	1.76 (1.38, 2.37)	U = 472, z = 1.30, p = 0.196
Beta carotene [µg]	3850.87 (2255.52, 6707.06)	4052.33 (2526.90, 4922.35)	U = 575, z = -0.03, p = 0.976
Carbohydrate - total [g]	206.13 (126.65, 269.35)	211.32 (140.08, 278.75)	U = 562, z = -0.19, p = 0.849
Cholesterol [mg]	269.31 (107.63, 394.78)	184.69 (68.65, 255.55)	U = 404, z = 2.13, p = 0.033
Energy [kcal]	1652.95 (1063.16, 2256.72)	1486.45 (1149.15, 1962.66)	U = 534, z = 0.53, p = 0.594
Fat - total [g]	64.47 (36.06, 90.45)	60.44 (43.40, 74.13)	U = 505, z = 0.89, p = 0.374
Englyst fibre [g]	16.27 (11.35, 25.86)	16.69 (14.68, 21.47)	U = 554, z = -0.29, p = 0.773
Total folate [µg]	286.35 (208.04, 408.71)	275.86 (198.99, 366.01)	U = 574, z = -0.04, p = 0.966
Iron [mg]	10.16 (8.09, 16.46)	9.97 (7.48, 13.38)	U = 522, z = 0.68, p = 0.496
Magnesium [mg]	300.93 (231.92, 454.23)	320.68 (233.91, 406.84)	U = 577, z = -0.01, p = 0.995
MUFA - total [g]	26.29 (16.53, 34.29)	24.30 (13.42, 30.48)	U = 512, z = 0.80, p = 0.422
Niacin [mg]	20.04 (15.01, 25.65)	18.46 (15.43, 24.15)	U = 567, z = 0.13, p = 0.898
Protein [g]	74.97 (54.78, 110.35)	59.86 (47.51, 90.33)	U = 465, z = 1.38, p = 0.167
PUFA - total [g]	11.12 (7.78, 15.81)	12.09 (8.59, 15.10)	U = 555, z = -0.28, p = 0.783
Riboflavin (Vitamin B2) [mg]	1.69 (1.27, 2.14)	1.61 (1.30, 2.36)	U = 552, z = -0.31, p = 0.754
Saturated fatty acids (SFA - total) [g]	21.84 (12.24, 31.30)	16.97 (11.34, 28.37)	U = 502, z = 0.93, p = 0.354

Nutrient	Healthy Controls (*n*=35)	Anorexia Nervosa (*n*=33)	Group comparison
Selenium [µg]	52.66 (41.68, 81.95)	46.55 (33.97, 63.19)	U = 449, z = 1.58, p = 0.115
Thiamin (Vitamin B1) [mg]	1.40 (1.01, 1.97)	1.34 (0.95, 2.05)	U = 572, z = -0.07, p = 0.946
Retinol equivalents (Vitamin A) [µg]	1035.18 (657.73, 1668.48)	1043.89 (744.88, 1300.30)	U = 555, z = 0.28, p = 0.783
Vitamin C [mg]	119.34 (66.78, 179.78)	123.24 (96.51, 159.48)	U = 503, z = -0.91, p = 0.361
Vitamin D [µg]	2.57 (1.44, 3.46)	1.35 (0.69, 2.26)	U = 353, z = 2.76, p = 0.006
Vitamin E [mg]	10.84 (7.82, 17.31)	10.91 (10.05, 14.83)	U = 510, z = -0.83, p = 0.408
Zinc [mg]	8.64 (5.66, 12.78)	6.81 (5.21, 10.06)	U = 460, z = 1.44, p = 0.149

[a]25th percentile and 75th percentile reported. Abbreviations: *n* = number of observations; kcal = kilocalorie; g = grams; mg = milligram; µg = microgram; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids.

In the whole sample, the median DII score was 0.29 ranging from a maximum anti-inflammatory value of −3.73 to a maximum pro-inflammatory value of +3.26. Median DII scores were lower in AN compared to HC participants, as shown in **Table 6.3**, suggesting less dietary inflammation in AN. However, this group difference was not significant (p = 0.9169). There was greater variance in DII scores in the HC group, as compared to the AN group (see **Figure 6.2**). Both unadjusted ($\chi^2(1) = 0.05$, p = 0.8184) and adjusted (NB., one influential point was removed from this analysis; $\chi^2(4) = 13.14$, p = 0.0106) logistic regressions showed that DII score was not associated with group membership (p = 0.818 and p = 0.940, respectively). BMI was not associated with DII score in this sample in an unadjusted regression (β = 0.07, 95% CIs -0.08 to 0.21, p = 0.374) nor in a multivariate linear regression adjusted for sociodemographic characteristics (β = 0.07, 95% CIs -0.09 to 0.22, p = 0.402).

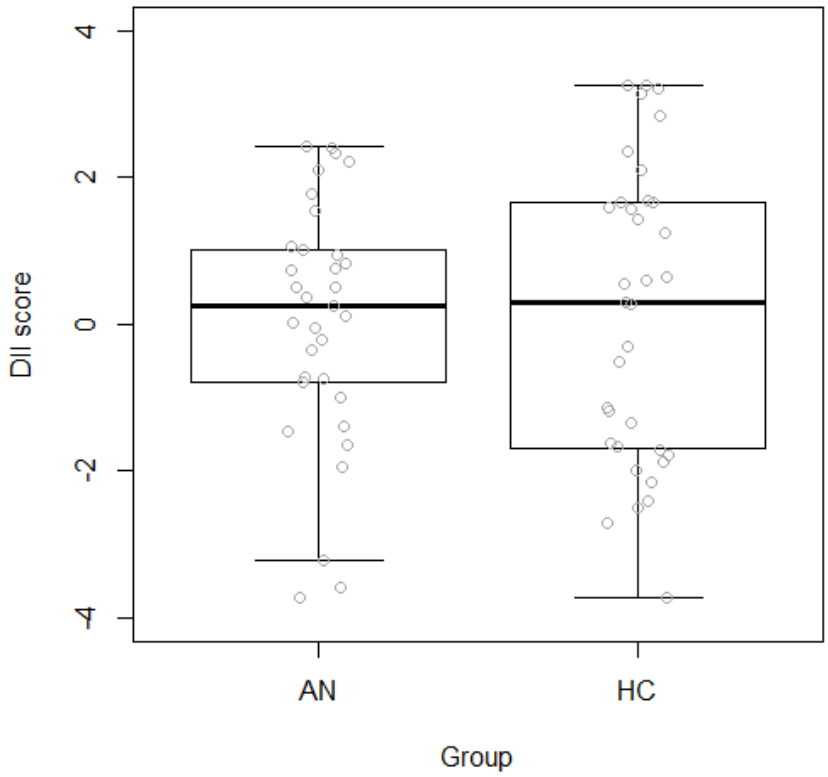

Figure 6.2 Box plot of median Dietary Inflammatory Index score for the healthy control and anorexia nervosa groups. Individual data points are shown in grey. Abbreviations: DII = Dietary Inflammatory Index; HC = healthy control; AN = anorexia nervosa.

Exploratory multivariate linear regressions of DII score on cytokine concentrations (see **Table 6.5**), while controlling for sociodemographic factors and multiple comparisons, did not identify any significant associations. Two cytokines were nominally significantly associated (i.e., significance did not survive correction for multiple testing) with DII score, such that a higher DII score was associated with higher concentrations of IL-2 and VEGF.

Table 6.5 Analysis of associations between Dietary Inflammatory Index score and cytokine concentrations, controlling for age, ethnicity and smoking status.

Cytokine	*n*	β	*95% CIs*	*p*
Eotaxin	67	-0.00	-0.01, 0.00	0.411
Eotaxin-3	67	-0.00	-0.01, 0.01	0.962
IFN-γ	67	0.01	-0.01, 0.03	0.499
IL-2	63	1.55	0.21, 2.89	0.024
IL-6	67	-0.07	-0.20, 0.06	0.298
IL-7	67	-0.01	-0.08, 0.08	0.909
IL-8	67	0.08	-0.05, 0.20	0.221
IL-10	67	-0.28	-1.08, 0.51	0.479
IL-12/IL-23p40	67	0.00	-0.00, 0.01	0.303
IL-12p70	54	-0.18	-1.56, 1.19	0.790
IL-13	55	-0.01	-0.17, 0.15	0.903
IL-15	67	-0.23	-0.97, 0.50	0.526
IL-16	67	0.01	-0.00, 0.01	0.163
IL-17A	67	0.02	-0.38, 0.42	0.911
IL-22	62	0.01	-0.05, 0.06	0.748
IL-27	67	-0.00	-0.00, 0.00	0.150
IP-10	67	-0.00	-0.01, 0.00	0.353
MCP-1	67	-0.00	-0.01, 0.00	0.307
MCP-4	67	-0.00	-0.01, 0.01	0.473
MDC	67	-0.00	-0.00, 0.00	0.846
MIP-1α	63	0.00	-0.00, 0.01	0.174
MIP-1β	67	0.00	-0.01, 0.01	0.434
MIP-3α	67	-0.00	-0.03, 0.03	0.924
TARC	67	0.00	-0.00, 0.00	0.944
TNF-α	67	0.26	-0.47, 1.00	0.478
TNF-β	63	-0.93	-5.45, 3.59	0.683
VEGF	67	0.01	0.00, 0.02	0.028

β coefficients and 95% confidence intervals were rounded to two decimal places. Abbreviations: *n* = number of observations; CI = confidence intervals; IFN = interferon; IL = interleukin; IP = interferon γ-induced protein; MCP = monocyte chemoattractant protein; MDC = macrophage-derived chemokine; MIP = macrophage inflammatory protein; TARC = thymus and activation-regulated chemokine; TNF = tumor necrosis factor; VEGF = vascular endothelial growth factor.

6.4.3 Discussion

In line with the previous study, we did not find differences between HC and AN participants in the inflammatory potential of their diet: while the AN group did have a slightly lower DII score than HCs, it was not significantly different. In contrast to Study 1, nutrient intake on the whole did not differ between groups. This may be because a high proportion of the AN participants were receiving specialist ED treatment (85%) and typical treatment for AN often involves aspects of weight restoration and nutritional rehabilitation, which encourages an escalation in caloric intake (Brockmeyer et al., 2018; Marzola et al., 2013). Alternatively, it may be that memory of diet over the previous year as assessed in our study can be biased by individual's present diet. Furthermore, it has been reported that people with AN tend to overestimate energy intake perhaps due to over-reporting of caloric intake; in contrast, HCs tend to consistently under-report caloric intake (Forbush & Hunt, 2014; Hadigan et al., 2000; Schebendach, Porter, Wolper, Walsh, & Mayer, 2012). As the FFQ completed in this study used standard measures of food and beverages (e.g., medium serving, one slice, one fruit) with no option for amendment of this portion size, it is possible that estimations of food intake may have been incorrectly reported in both AN and HC participants.

BMI was not associated with DII score in this sample, although the association was in the expected direction. While several previous studies have found a significant association with BMI, such that a higher BMI is associated with a higher DII score (Aslani et al., 2018; Ramallal et al., 2017; Ruiz-Canela et al., 2015) as indeed we did in Study 1, these studies have been performed in samples of thousands of participants. Therefore, the small sample in the current study may account for the lack of findings. Similarly, a study using a smaller sample than previous research (n=430) did not identify a significant association between BMI and DII score (Wirth et al., 2016).

Many cytokines not previously assessed in relation to DII score were included in this study. We found that cytokine concentrations were not associated with DII score, after controlling for relevant sociodemographic covariates and multiple testing. This may be due to the small sample, although it may suggest that factors other than dietary intake may regulate cytokines in AN and be responsible for the alterations in cytokine concentrations observed in AN (Chapter 2; Dalton et al., 2018a). Alternative factors could include stress, genetics, and comorbid psychiatric disorders, as well as specifically AN-related factors like current recovery and refeeding status, recent weight gain, and current ED behaviours (e.g., self-starvation and compensatory mechanisms).

6.4.3.1 Strengths and limitations

This study was the first to assess dietary inflammation in currently unwell AN patients. However, the sample was relatively small, exclusively female and heterogenous in several aspects e.g., age, ethnicity, illness duration, current treatment. The number of nutrients (*n*=25) available from the FFQ used in the current study exceeded the 18 nutrients used to calculate the DII in Study 1, providing a more detailed picture of dietary intake in this sample.

There are also some inherent strengths and limitations associated with the use of FFQs. Generally, they are less burdensome to participants than other more intensive dietary assessment measures (e.g., 24-hour dietary recalls performed on multiple occasions) and are able to assess long-term, typical diet (Shim, Oh, & Kim, 2014). However, FFQs are subject to systematic biases; for example, recall bias, as mentioned previously, and they require some ability to perform cognitively complex memory and averaging tasks (Pérez Rodrigo, Aranceta, Salvador, & Varela-Moreiras, 2015). FFQs are also restricted to items listed in the instrument. As the FFQ used in this study was designed approximately 20 years ago (Bingham et al., 1994; Bingham et al., 2001), there has likely been changes in typical dietary consumption across the UK and these changes may not be reflected in the instrument. For example, many of our participants reported consumption of non-dairy milk alternatives (e.g., almond milk, coconut milk), which could not be fully captured. Furthermore, as diet is heavily influenced by ethnicity and culture, participants' diet may not be appropriately captured by the measure (Pérez Rodrigo et al., 2015; Shim et al., 2014).

6.4.3.2 Conclusions

The findings from this study suggested that it is unlikely that dietary inflammation accounts for the increased concentrations of pro-inflammatory cytokines nor the other cytokine alterations that have been observed in people with current AN.

6.5 General discussion

Study 1 showed that the DII score did not differ between ED groups and HCs. However, participants with current obesity had a significantly higher DII score than HCs. Thus, the inflammatory potential of an individual's diet may be associated with obesity outcomes and the inflammation observed in people with obesity (Brooks et al., 2010; Ellulu et al., 2017; Gregor & Hotamisligil, 2011).

Adipose tissue is a producer of pro-inflammatory cytokines and a pro-inflammatory diet can increase or maintain adiposity. In addition, the pro-inflammatory nature of certain foods may lead to alterations in the gut microbiota, e.g., increases in pro-inflammatory bacteria and a leaky gut (the leakage of bacterial components across the intestinal barrier)

as described in Section 1.3.2.2 in Chapter 1, which consequently may lead to low-grade systemic inflammation (Araújo, Tomas, Brenner, & Sansonetti, 2017; Cani et al., 2007). For example, a recent study showed that among women with higher serum zonulin (a biomarker of gut permeability), energy and macronutrient intake, measures of adiposity and markers of inflammation were increased, compared to people with lower zonulin levels (Mörkl et al., 2018). Furthermore, alterations in the gut microbiome in people with obesity has been associated with local and systemic inflammation (Verdam et al., 2013). Indeed, certain foods (e.g., processed foods) and dietary patterns (e.g., high-fat diets) associated with the development of obesity have been shown to induce gut dysbiosis and related pro-inflammatory processes (Araújo et al., 2017; Cani et al., 2007; Hall et al., 2019; Smidowicz & Regula, 2015). For instance, animal research has shown that changes in the gut microbiota as a result of consuming a high-fat diet increases intestinal inflammation and subsequently leads to increases in circulating concentrations of inflammatory markers (Guo et al., 2017).

Study 1 found that DII score was not related to lifetime diagnoses of BN or BED. Given the use of lifetime diagnoses in this study, future research should assess nutrient intake and related dietary inflammation (using the DII) in currently unwell patients. Across the two studies in this chapter, the DII score was consistently found to not be associated with AN. This was in both people with a lifetime diagnosis of AN and those with current AN, the majority of which were in specialist ED treatment. Furthermore, this was in spite of significant differences in energy and nutrient intake between AN participants and HCs in Study 1. In the AN sample in Study 2, there were no differences in dietary intake between groups, which may be accounted for by the type of dietary assessment used and/or because many of the AN sample were in specialist ED treatment, of which nutritional restoration is a key aspect. While all methods of dietary assessment are limited to some extent (Shim et al., 2014), it may be of use for future studies to assess nutrient intake using 24-hour dietary recalls in currently ill ED patients to avoid problems associated with averaging intake over a long period of time (e.g., as in Study 2), particularly if they are in treatment as dietary habits may have changed substantially in a shorter period than the response period given by the FFQ e.g., one year. Multiple dietary recalls may also better capture the consistently reported altered energy and food intake in AN, including after weight restoration (e.g., Mayer et al., 2012; Misra et al., 2006b).

In both studies, we aimed to validate the DII against serum concentrations of inflammatory markers. While previous research has associated a higher DII score with higher concentrations of pro-inflammatory markers (Shivappa et al., 2017b; Shivappa et al., 2015; Shivappa et al., 2014b), not all studies have consistently validated the DII against

all inflammatory parameters (e.g., Shivappa et al., 2017b; Shivappa et al., 2015). Previous studies have tended to provide limited information regarding the variance in concentrations of inflammatory markers that can be explained by DII score, and therefore, the clinical relevance of these findings is unclear. In Study 1, DII score was indeed positively associated with CRP serum concentrations. However, when assessed in the ED sample only, DII score was not significantly related to circulating CRP, suggesting that the findings obtained from healthy populations may not apply to ED samples. Furthermore, in Study 2, there were no formally significant relationships between any of the 27 cytokines measured and DII score. Unlike some previous studies (e.g., Shivappa et al., 2017b), these non-significant associations were not necessarily in the expected direction i.e., positive correlation for pro-inflammatory cytokines. These findings may be accounted for by the small heterogenous sample in Study 2, the potential under- or over-reporting of nutrient intake in the ED groups, or it may be that in samples of currently unwell ED participants there is likely a combination of factors that contribute to the observed altered concentrations of inflammatory markers. Indeed, a number of independent factors such as stress, genetics, immune-related diseases and infections, and psychiatric disorders (e.g., depression) influence both the development of EDs and circulating concentrations of cytokines.

6.5.1 Dietary Inflammatory Index considerations

The DII has been used across a range of populations and cultures, with varying sociodemographic and anthropometric characteristics and in a range of illness states, including cancer, cardiovascular disease, depression and other mental health outcomes (Hébert et al., 2019). The process of standardising the nutrient intake values to the means and standard deviations from the global database when calculating the DII allows for results to be compared across studies (Hébert et al., 2019). The ranges of DII scores in both studies were comparable to previous findings in other UK, European, Australian and American based populations (Hodge et al., 2018; Ramallal et al., 2017; Ruiz-Canela et al., 2015; Shivappa, Hebert, Kivimaki, & Akbaraly, 2017a; Shivappa et al., 2018b; Shivappa et al., 2014b; Veronese et al., 2018). However, the DII score was perhaps slightly higher in the Study 1 sample than observed in these previous studies.

The DII has been calculated using multiple types of dietary assessments including 24-hour and 7-day dietary recalls, and FFQs. A validation study found that DII scores calculated from 24-hour dietary recalls were comparable to DII scores from 7-day dietary recalls (Shivappa et al., 2014b). However, to date the use of FFQs for calculating the DII has not been validated. The validation study also showed that DII scores were similar when the DII was calculated from a different number of nutrient and food parameters (27 versus 44

nutrient and food components; Shivappa et al., 2014b). Of the 45 possible items in the DII, Study 1 used 18 and Study 2 used 25, which is slightly lower than that of the validation study. Thought, in previous studies, the number of food parameters used for the calculation of the DII ranged between 17 and 44 (Yang et al., 2019) and therefore, the number of food parameters used in both studies was likely sufficient. Although, future studies in EDs should use dietary assessments that provide more varied nutrient information to ensure the DII is calculated with a more comprehensive picture of dietary intake.

Finally, while the DII assesses the inflammatory potential of the whole diet, the index was created based on articles that assessed the effect of specific food and nutrient parameters on markers of inflammation. As nutrients and whole foods are not consumed in isolation, the inflammatory scores given to the nutrient parameters may not correctly reflect the inflammatory potential of nutrients consumed simultaneously.

6.5.2 Conclusions

A higher DII score was associated with current obesity in a population based-sample, suggesting dietary inflammation may account for some of the chronic low-grade inflammation observed in people with obesity (Ellulu et al., 2017). In contrast, relative to HCs, lifetime diagnoses of AN, BN and BED, and also current AN were not associated with DII score. This suggests that other factors aside from eating behaviour may contribute to the regulation of cytokines in AN and potentially other EDs. As these were the first studies to assess dietary inflammation in EDs, future research should further explore the use of the DII in samples with current EDs, using multiple 24-hour dietary recalls.

Chapter 7. Using polygenic risk scores to examine the relationship between C-reactive protein and eating disorders

7.1 Abstract

Objective: Production of inflammatory markers is regulated at a genetic level and molecular genetics findings point towards inflammatory processes playing a role in the aetiology of anorexia nervosa (AN). Therefore, the current study aimed to further explore the relationship between eating disorders (EDs) and inflammatory markers using polygenic risk scores (PRSs) in a genotyped population-based sample.

Methods: Participants were *n*=4492 healthy controls (HCs) and *n*=1193 ED participants, who self-reported a clinical diagnosis of an ED or who had a diagnosis from medical records, from the UK Biobank. Concentrations of C-reactive protein (CRP) were measured in serum samples. PRSs were calculated for circulating concentrations of CRP, AN, and body mass index (BMI) using summary statistics from the largest available genome-wide association studies (GWAS). We tested the associations between (a) CRP PRS and circulating concentrations of CRP, (b) AN PRS and circulating concentrations of CRP, (c) CRP PRS and ED case status, and (d) BMI PRS and circulating concentrations of CRP.

Results: Linear regression models (adjusted for the first six principal components and relevant demographic factors) showed a positive correlation between the CRP PRS and circulating concentrations of CRP ($p < 0.0001$). AN and CRP were not associated when considering the relationship between either the CRP PRS with AN ($p = 0.7655$) or the AN PRS with CRP ($p = 0.2722$). The CRP PRS was not related to bulimia nervosa (BN) case status ($p = 0.3076$). A higher CRP PRS was associated with binge eating disorder (BED) caseness, relative to control status, at several *p*-thresholds. The BMI PRS was significantly associated with CRP concentrations ($p < 0.0001$); however, this became non-significant when current BMI was included in the analyses.

Conclusion: The current findings do not replicate previous research reporting a negative genetic correlation between AN and CRP. This may be due to methodological considerations related to polygenic scoring, but also it should be noted that the ED phenotypes in the UK Biobank rely on a single self-report item or diagnoses from medical records. Our results suggest that a self-reported diagnosis of BED may be associated with a genetic propensity towards elevated CRP. Future research needs to explore this relationship using more detailed assessments of ED presentation and other types of research methods, such as Mendelian randomisation which assesses the direction of causation between two phenotypes.

7.2 Introduction

The causes of eating disorders (EDs) are multifactorial and genetic factors are deemed to play a significant role. Indeed, EDs are highly heritable (Hübel, Leppä, Breen, & Bulik, 2018). Heritability refers to the proportion of phenotypic variance that can be explained by inherited (additive genetic, non-environmental) factors. Heritability estimates range from 0 (i.e., no effect of genes on phenotypic variation) to 1.0 (i.e., genes are the only contributor to phenotypic variation). Estimates of heritability from family and twin studies range from 0.48 to 0.74 in anorexia nervosa (AN), from 0.55 to 0.62 in bulimia nervosa (BN), and between 0.39 and 0.45 in binge eating disorder (BED; Himmerich et al., 2019a; Hübel et al., 2018; Yilmaz, Hardaway, & Bulik, 2015). Genome-wide studies of heritability have only been performed in AN (Bulik, Blake, & Austin, 2019) due to the large sample sizes required, identifying a more conservative heritability estimate of approximately 0.20 (Duncan et al., 2017). It is important to note here that it is well-established that environmental factors also contribute to the development and maintenance of EDs; however, to what extent and how this plays out in each individual is unknown (Breithaupt, Hübel, & Bulik, 2018).

As mentioned in Chapter 4, it is important for circulating concentrations of inflammatory markers to be considered against the background of genetics. Production of inflammatory markers is regulated at a genetic, epigenetic and gene-expression level (Li et al., 2016c) and thus, individuals may differ in their capacity to produce inflammatory markers due to their genotype and the epigenetically regulated accessibility of their genes (Fishman et al., 1998; Louis et al., 1998; Warle et al., 2003; Yilmaz et al., 2005). Molecular genetic research studies in AN have provided insights into the possible role of inflammatory markers in the pathophysiology of AN (Himmerich et al., 2019a). However, research has not yet assessed the role of inflammatory markers in BN and BED, and genome-wide association studies (GWAS) in these EDs are yet to be conducted (Bulik et al., 2019). Thus, the current chapter will first provide a brief review of molecular genetics research to date that has described findings relevant to inflammatory markers in AN, before introducing the present study investigating the association between polygenic risk of circulating CRP and EDs.

7.2.1 Review of molecular genetics research of inflammatory markers in anorexia nervosa

7.2.1.1 Gene expression studies

Gene expression is the process by which genetic instructions coded on the deoxyribonucleic acid (DNA) are used in the synthesis of a functional gene product, such as messenger ribonucleic acid (mRNA). mRNA expression levels of interleukin (IL)-6 and tumor necrosis factor (TNF)-α have been shown to be elevated in a small sample (*n*=11) of

AN patients, when compared to healthy individuals (Kahl, Kruse, Rieckmann, & Schmidt, 2004). Expression of TNF-α mRNA also remained elevated following weight restoration in the AN patients; however, expression of IL-6 mRNA decreased with weight gain.

7.2.1.2 Candidate gene association studies

Candidate gene association studies examine specific variants of a particular gene in a hypothesis-driven manner. Most often candidate genes are selected based on a biological hypothesis. Genetic association studies in AN have focussed on TNF-α genes (Rask-Andersen et al., 2010). Research has suggested that a TNF-α gene promoter polymorphism at position −308 (G to A substitution), which increases transcription of TNF-α *in vitro*, may be associated with AN (Kanbur et al., 2008). It was found that lifetime minimum body mass index (BMI) values were significantly higher in those AN patients that had a TNF-α −308 G to A polymorphism. Also, there was a significantly higher ratio of TNF-α −308 G/A genotype in the AN group (31%) compared to healthy controls (HCs; 7%). However, the sample size for this study was only 16 patients and findings are inconsistent: a larger study of this TNF-α gene polymorphism found no significant differences between AN and HC groups in genotype frequency (Slopien et al., 2014). Another study investigating three other polymorphisms in the promotor gene encoding TNF-α (at positions −1031 [T to C substitution], −863 [C to A substitution] and −857 [C to T substitution]) also found no group differences in genotype frequency (Ando et al., 2001).

Given that TNF-α has been less consistently associated with AN than IL-6, for example, genotyping of IL-6 associated genes and alleles will be important in future research to better understand the role genetic factors may play in altered IL-6 concentrations (Chapter 2; Dalton et al., 2018a; Study 1 of Chapter 3; Dalton et al., 2018b). This would also be of interest given that certain IL-6 alleles and genotypes may decrease the risk for schizophrenia (Hudson & Miller, 2018) and depression (Khandaker et al., 2018). However, it must be noted that no candidate gene study of AN has produced robust and reproducible findings (Yilmaz et al., 2015), and thus, genome-wide approaches should be the initial step in genetic analyses with candidate gene studies being used to replicate and further explore the findings from GWAS.

7.2.1.3 Genome-wide association studies

GWAS are observational studies of millions of markers across the genome used to determine whether any genetic variant is associated with a trait (e.g., AN status, BMI, body composition, etc.). GWAS take a hypothesis-free approach and require large samples sizes, usually tens or hundreds of thousands non-genetically related individuals. One of the initial GWAS of AN provided evidence for the relevance of cytokines (Boraska et al., 2014):

of the four most important signals, two genes (CUL3 and PPP3CA) were closely linked to cytokine signalling (Lee et al., 2012; Wang et al., 1996). In a GWAS comprising of 3,495 AN cases and 10,982 controls, only one genome-wide significant locus, in a region that has been associated with a range of autoimmune disorders, was identified (Duncan et al., 2017). In focussed secondary single nucleotide polymorphism (SNP)-based analyses, FAM19A2, which is proposed to function as a chemokine/cytokine, was found to be the fourth locus most associated with AN. However, in the most recent analysis including 16,992 AN cases and 55,525 controls, this finding was not replicated (Watson et al., 2019). As with most psychiatric disorders, findings from GWAS suggest that AN is polygenic in nature, that is, several genetic variants, both common and rare, of small effect are involved. As with AN and all psychiatric disorders, BN and BED are also complex traits and thus it would be expected that the contributions of multiple individual loci are small.

Recently, Tylee et al. (2018) investigated the genetic correlations among psychiatric and immune-related phenotypes using summary statistics from AN (Duncan et al., 2017) and CRP (Dehghan et al., 2011) GWAS, identifying a significant inverse genetic correlation (r_g = -0.30) between AN and CRP. Using a meta-analysis of GWAS of circulating CRP and published GWAS summary statistics for AN (Bulik-Sullivan et al., 2015), Ligthart et al. (2018) replicated this inverse genetic correlation between AN and CRP (r_g = -0.22). Indeed, these findings are consistent with a recent meta-analysis reporting reduced circulating concentrations of CRP in AN (Solmi et al., 2015), which may reflect immunosuppression associated with severe weight loss.

7.2.1.4 Epigenetics studies

Epigenetic studies examine changes in gene expression that do not involve changes in the DNA sequence. Most studies of epigenetic alterations in EDs have focussed on DNA methylation (Thaler & Steiger, 2017), in which methyl is added to specific genomic regions (where C is followed by G). Generally, when gene promotors are methylated, gene expression tends to be reduced. DNA methylation is influenced by many environmental exposures e.g., dietary factors, early life stressors (Thaler & Steiger, 2017).

Genome-wide epigenetics studies in AN have reported hypermethylation in areas associated with inflammatory responses and immune function. Booij et al. (2015) and Kesselmeier et al. (2018) have reported hypermethylated regions at the TNXB gene in AN patients compared to healthy-weight and lean (without AN) females. This gene is located on chromosome 6 within a region that contains genes of immunological relevance, including cytokine genes e.g., TNF-α (Weissensteiner & Lanchbury, 1997). Steiger et al. (2019) also performed a genome-wide study of DNA methylation in AN, finding differentially methylated sites between AN and non-ED participants, and active AN versus

remitted AN participants, which included genes associated with immune function and inflammation (e.g., FCGR2A, RELT TNF receptor). These sites had higher methylation in the AN group in comparison to both HCs and remitted AN participants, suggesting reduced expression of the implicated genes in AN. Furthermore, longitudinal data showed that increases in BMI coincided with increases in methylation-level changes at genes associated with immune and inflammatory processes. It is important to note that how DNA methylation on these genes is associated with gene expression and circulating cytokine concentrations has yet to be explored in AN.

7.2.2 The current study

The genome-wide findings reported above point toward immune function and inflammatory processes playing a role in the aetiology of AN. New methods to analyse data from GWAS have been developed to address a number of questions about psychiatric disorders. One such method used to predict genetic risk in individuals is the generation of a polygenic risk score (PRS; Maier, Visscher, Robinson, & Wray, 2018). A PRS is a composite score that aims to quantify the cumulative effects of several SNPs, which are most strongly associated with the trait but may individually have a very small effect on genetic risk. PRSs therefore aim to capture the full polygenic signal, that is, both SNPs that reached genome-wide significance in GWAS and those that were below the stringent genome-wide significance threshold, but may still carry valuable genetic information, are included (Hübel et al., 2018). The method for calculating a PRS first uses summary statistics from a GWAS conducted in a 'discovery' sample. In an independent 'target' sample, the PRS is calculated for each individual as the sum count of the genetic risk alleles carried by an individual, weighted by the effect size in the 'discovery' sample (Hübel et al., 2018; Wray et al., 2014). The higher the PRS, the greater number of risk variants an individual carries.

PRSs have been used in AN, for example to determine whether polygenic risk for AN predicts neuroticism (Gale et al., 2016) and facial emotion recognition (Coleman et al., 2017). Aside from being incorporated into regression analyses as predictors for a number of outcomes (e.g., case status, disease course, treatment outcome), PRSs can also be used to determine associations within traits (i.e., if a genome-wide identified PRS associated with the same trait in a second sample) and across complex traits (i.e., assess the genetic overlap between disorders and traits; Breithaupt et al., 2018). Thus, PRSs can be used to replicate genetic correlations between traits and phenotypes (cross-trait association; Breithaupt et al., 2018). As cited above, a significant inverse genetic correlation between AN and CRP has been reported (Ligthart et al., 2018; Tylee et al., 2018). Therefore, it would be of interest to assess this relationship further using PRSs.

Like AN, circulating CRP is a complex trait and an important contributor to CRP concentrations are genetic variants. Serum CRP concentrations have a reported family- and twin-heritability of between 0.25 and 0.52 (Dupuis et al., 2005; Pankow et al., 2001; Sas et al., 2017) and GWAS have identified over 50 distinct loci associated with circulating CRP, including loci implicated in metabolic and immune pathways (Dehghan et al., 2011; Ligthart et al., 2018). Therefore, using CRP GWAS summary statistics from Ligthart et al. (2018), we created a CRP PRS to address three main aims described below. In this context, using PRSs will provide the opportunity to index an individual's genetic susceptibility to systemic inflammation. The first aim of this study was to determine whether the CRP PRS was associated with serum concentrations of CRP in a population-scale dataset from the UK Biobank (Bycroft et al., 2018; Sudlow et al., 2015). The second aim was to explore the relationship between AN and CRP and assess the replicability of the reported genetic correlations by (a) examining whether the CRP PRS is associated with self-reported AN diagnosis, (b) using a PRS calculated from summary statistics from the most recent GWAS of AN (Watson et al., 2019) to assess the association between an AN PRS and circulating concentrations of CRP, and (c) correlating the CRP PRS and AN PRS. The third aim was to expand the literature by performing exploratory analyses examining the association between the CRP PRS and other self-reported ED diagnoses (BN and BED). The associations we aimed to explore in this study are presented schematically in **Figure 7.1**.

Given the genetic correlation findings between AN and CRP (Ligthart et al., 2018; Tylee et al., 2018), we may expect a lower PRS for CRP to predict case status in AN and for a higher AN PRS to be associated with lower circulating CRP concentrations. There is little data on CRP concentrations in other EDs. Although, two recent studies, one in youth with or without loss of control eating (Shank et al., 2017) and one comparing obese people with BED with non-BED obese people (Succurro et al., 2015), found that the participants exhibiting binge/loss of control eating had higher concentrations of CRP than control participants. Additionally, in Study 1 of Chapter 6, concentrations of CRP were found to be significantly higher in people with BED, compared to HCs, in the UK Biobank sample. Thus, we may expect that a higher CRP PRS predicts BED case status. CRP concentrations have not been assessed in BN except for in the current thesis, which found no differences between BN and HC participants (Chapter 6). However, as binge eating has been associated with elevated CRP (Shank et al., 2017; Succurro et al., 2015), we may also expect a higher CRP PRS to be associated with BN case status.

Finally, BMI is a major determinant of CRP (Timpson et al., 2011) and is associated with ED diagnosis. It has also been suggested that the identified low concentrations of CRP in AN patients may be related to low weight (Solmi et al., 2015). Therefore, we also assessed

the relationship between BMI and CRP (see **Figure 7.1**), using a BMI PRS calculated from data on the largest available GWAS (Locke et al., 2015). We would expect the BMI PRS to be highly associated with CRP, such that BMI PRS would predict circulating concentrations of CRP in this sample.

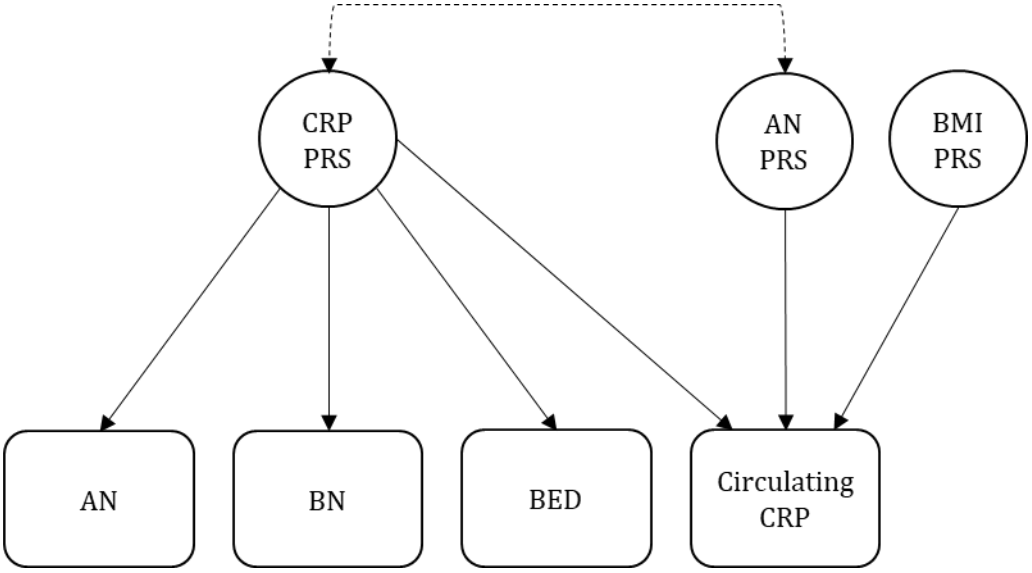

Figure 7.1 Schematic representation of the aims and associations tested in the current study. Abbreviations: CRP = C-reactive protein; PRS = polygenic risk score; AN = anorexia nervosa; BMI = body mass index; BN = bulimia nervosa; BED = binge eating disorder.

7.3 Methods

7.3.1 Study design and sample

The UK Biobank measured a wide range of health-related phenotypes and biological variables including genome-wide genotype data in a population-based prospective cohort of approximately 500,000 individuals in the UK (Allen, Sudlow, Peakman, & Collins, 2014). A more detailed description of the UK Biobank can be seen in Section 5.4.1.1 in Chapter 5. Within this sample, 157,366 participants also completed an online follow-up questionnaire (http://www.ukbiobank.ac.uk/wp-content/uploads/2017/09/ MentalHealthQuestionnaire_for_ Website-1.pdf) designed to assess common mental health disorders, including lifetime history of ED diagnoses (Davis et al., 2018).

For the current study, cases were defined as participants who self-reported a previous physician diagnosis of an ED on the mental health questionnaire and/or had a hospital-recorded primary or secondary International Classification of Diseases 10th revision (ICD-10; World Health Organization, 1992) diagnosis of AN (ICD-10 diagnosis F50.0) and/or BN (ICD-10 diagnosis F50.2). Following identification, ED participants were then subdivided into groups based on their self-reported diagnosis into those with lifetime AN, BN, or psychological over-eating or binge eating (i.e., BED). Control participants were defined as

individuals who did not self-report any previous physician diagnosis of a psychiatric disorder on the mental health questionnaire, had no hospital-recorded ICD-10 diagnosis of psychiatric disorders and who were not receiving any psychotropic medication, including antidepressants, antipsychotics, and anxiolytics.

Any participant with an ICD-10 diagnosis of an autoimmune, inflammatory or immunodeficiency condition (see **Table 10.1** in Section 10.8 Appendix H for list of conditions) and/or reported taking corticosteroids or antiretroviral medication were excluded. Participants of non-European ancestry were also excluded as the PRS were calculated from GWAS of individuals with European ancestry. We identified individuals with non-European ancestry by *k*-means clustering ($k = 4$) on the first two principal components derived from the genotype data, and related individuals were excluded (KING relatedness metric > 0.088, equivalent to a relatedness value of 0.25; n=7765).

7.3.2 Ethical approval

This study was covered under the generic ethical approval from the NHS Research Ethics Committee and ethics approval for the UK Biobank study was obtained from the North West Centre for Research Ethics Committee (REC reference: 11/NW/0382).

7.3.3 Measurements of C-reactive protein

Blood samples were collected from participants on their visit to a UK Biobank assessment centre and serum was separated by centrifugation. Serum samples were stored at -80°C in a fully automated working archive (Owen & Woods, 2008) and thawed prior to use. Serum concentrations of CRP were quantified using high sensitivity immune-turbidimetric analysis methods (Beckman Coulter [UK], Ltd), using the Beckman Coulter AU5800 analytical platform. Additional assay details can be seen in Section 5.4.1.6 of Chapter 5. CRP values greater than 10 mg/l were excluded as this may indicate the presence of acute inflammation and infection (Pearson et al., 2003).

7.3.4 Polygenic risk scores

All PRSs were calculated from the largest available GWAS available that did not contain UK Biobank participants, to ensure that the target sample was independent of the discovery sample.

7.3.4.1 C-reactive protein polygenic risk score

The CRP PRS was calculated from the summary statistics (detailing the association between circulating CRP and each of the imputed SNPs) of the recent CRP GWAS meta-analysis conducted by the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Inflammation Working Group (Ligthart et al., 2018; Psaty et al., 2009). The meta-analysis was performed on data from 88 studies comprising of a sample of 204,402

individuals of European ancestry and controlled for age, sex, and BMI (Ligthart et al., 2018). Participants taking immune-modulating agents, with autoimmune diseases and/or CRP values greater than four standard deviations from the mean were excluded from this meta-analysis. The UK Biobank cohort was not included in the discovery sample as reported in the supplementary files (Ligthart et al., 2018).

7.3.4.2 Anorexia nervosa polygenic risk score

The AN PRS was calculated from the summary statistics of the recent AN GWAS meta-analysis (Watson et al., 2019). This meta-analysis was performed on data from 16,992 AN cases and 55,525 controls of European ancestry from 17 countries. The UK Biobank cohort was originally included in the discovery sample as reported in the supplementary tables (Watson et al., 2019); however, these participants were removed prior to the calculation of the PRS to ensure independence between the discovery and target samples.

7.3.4.3 Body mass index polygenic risk score

The BMI PRS was calculated using summary statistics from Locke et al. (2015), which meta-analysed data from up to 339,224 individuals from 125 studies, controlling for age. The UK Biobank cohort was not included in the discovery sample as detailed in the supplementary files (Locke et al., 2015).

7.3.4.4 Calculating the polygenic risk scores

The three PRSs were calculated in the same way using the default option in PRSice version 2.2.2 (https://github.com/choishingwan/PRSice; Choi & O'Reilly, 2019; Euesden, Lewis, & O'Reilly, 2015). *P*-value-informed ($p < 1.0$) clumping was performed with a cut-off of $r^2 = 0.1$ using a 250-kb window. The process of clumping removes SNPs that are in linkage disequilibrium with each other (Wray et al., 2014), where linkage disequilibrium refers to non-random associations between alleles at different loci in a given population. Following clumping, the PRSs were calculated based on the following number of SNPs that were present in both the discovery sample GWAS and the UK Biobank genotyped data: CRP PRS 167,395 SNPs, AN PRS 122,956 SNPs, and BMI PRS 82,991 SNPs.

The PRSs were then calculated as the sum of associated alleles, weighted by the reported effect sizes in their original GWAS discovery sample. PRSs were calculated for each individual at multiple *p*-thresholds ($5 \times 10^{(-8)}$, $1 \times 10^{(-5)}$, 0.001, 0.01, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 1.0) and were standardised (Choi, Heng Mak, & O'Reilly, 2018). The *p*-threshold determines the selection of SNPs from the discovery sample that are included in the PRS calculation (Wray et al., 2014) and is based on the *p*-value of the SNPs association with circulating CRP, AN, or BMI, as reported in their respective GWAS. As this *p* value increases, the number of SNPs included in the PRS also increases. We assumed an omnigenic model (the whole genome contributes to a trait) and as such, PRSs at the *p*-

value threshold of $p < 1.0$ were used for primary analyses (Boyle, Li, & Pritchard, 2017). PRSs at stricter *p*-thresholds ($5 \times 10^{(-8)}$, $1 \times 10^{(-5)}$, 0.001, 0.01, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5) were used in sensitivity analyses.

7.3.5 Statistical Analysis

All statistical analyses were performed in Stata 15 (StataCorp, 2017) and PRSice-2 (Choi & O'Reilly, 2019). A total of 76,886 control participants and 1,084 ED participants (*n*=513 AN, *n*=275 BN, and *n*=296 BED) met the inclusion criteria and had genetic data available. For these analyses, the control group was randomly sampled at a ratio of one case to four controls (Linden & Samuels, 2013; Yamamoto, 2008), giving a final HC group sample of *n*=4336.

The distribution of continuous demographic data was examined through visual inspection of histograms. For continuous demographic variables, group comparisons were assessed using t-tests or Mann-Whitney U tests depending on the distribution of the data. For categorical variables (e.g., gender and smoking status), the chi-square test of homogeneity was used to determine if the proportion of the categories of these variables differed between groups. For each analysis, each ED diagnosis was separately compared to the HC sample, due to an overlap in participants between ED groups (i.e., some participants reported more than one lifetime ED diagnosis). The level of significance was set at $p < 0.05$.

7.3.5.1 Association between anorexia nervosa and C-reactive protein polygenic risk scores

A partial correlation was performed between the AN PRS and CRP PRS with the first six principal components to examine whether the same genetic variants play a role in both traits. This correlation was conducted only at the primary *p*-threshold (1.0).

7.3.5.2 Association between polygenic risk scores and circulating concentrations of C-reactive protein

In the whole sample, we evaluated the association between CRP PRS, AN PRS and BMI PRS (independent variable) and the serum concentration of CRP (dependent variable) using linear regressions. For each PRS, a linear regression including the first six principal components, to account for underlying population stratification in the UK Biobank sample, was performed. Then, a fully adjusted linear regression for each PRS was run controlling for gender, age, smoking status, social deprivation (as measured by the Townsend index; Townsend et al., 1988), BMI and the first six principal components. For the analysis using BMI PRS, sensitivity analyses removing BMI as a covariate were performed. As mentioned, PRSs at the *p*-value threshold of $p < 1.0$ were used for primary analyses. To account for multiple testing, a Bonferroni correction was applied (correcting for both the adjusted and

fully adjusted linear regressions), giving a formal threshold for significance of $p < 0.0083$. Sensitivity analyses repeated these analyses using PRSs at stricter *p*-thresholds.

7.3.5.3 Association between C-reactive protein polygenic risk score and eating disorder diagnosis

Binomial logistic regressions were performed to determine whether CRP PRS (independent variable) was associated with case/control status (dependent variable) including six principal components as covariates. Separate logistic regressions were run for each ED diagnosis. Fully adjusted logistic regressions were then performed, additionally including the covariates of gender, age, social deprivation, smoking status and BMI. As described, primary analyses used the CRP PRS at the *p*-threshold 1.00. A conservative Bonferroni adjustment for multiple testing was used, correcting for six tests (two analyses per ED diagnosis), giving a formal threshold for significance of $p < 0.0083$. Additional sensitivity analyses repeated the analyses at the ten remaining stricter p-thresholds and repeated primary analyses in ED participants who only reported one lifetime ED diagnosis.

All analyses using PRSs at the primary *p*-threshold were repeated in five other randomly sampled groups of HC participants to check that findings in the initial sample were consistent across the wider sample.

7.4 Results

Participant demographics for the HC and ED groups are presented in **Table 7.1**. There were a significantly greater proportion of females in the ED groups, as compared to the HC group (AN vs HC: $\chi^2(1) = 308.78$, $p < 0.0001$; BN vs HC: $\chi^2(1) = 183.38$, $p < 0.0001$; BED vs HC: $\chi^2(1) = 127.16$, $p < 0.0001$). Participants in the ED groups were significantly younger than the HCs (AN vs HC: $t(4847) = 7.90$, $p < 0.0001$; BN vs HC: $t(4609) = 10.78$, $p < 0.0001$; BED vs HC: $t(4630) = 5.52$, $p < 0.0001$). There were a significantly greater proportion of current/former smokers in the ED groups than in the HC group (AN vs HC: $\chi^2(2) = 34.60$, $p < 0.0001$; BN vs HC: $\chi^2(2) = 17.54$, $p < 0.0001$; BED vs HC: $\chi^2(2) = 14.10$, $p < 0.0001$). HCs were significantly more affluent than each of the ED groups (AN vs HC: $t(4841) = -6.13$, $p < 0.0001$; BN vs HC: $t(4604) = -5.43$, $p < 0.0001$; BED vs HC: $t(4625) = -7.99$, $p < 0.0001$). The AN ($t(4786) = 20.20$, $p < 0.0001$) and BN ($t(4555) = 7.69$, $p < 0.0001$) groups had a significantly lower BMI relative to the HCs; in contrast, the BED group had a significantly higher BMI than the HCs ($t(4575) = -12.46$, $p < 0.0001$). AN ($U = 752425$, $z = 12.00$, $p < 0.0001$) and BN ($U = 471883$, $z = 5.81$, $p < 0.0001$) participants had significantly lower and BED ($U = 530273$, $z = -5.01$, $p < 0.0001$) significantly higher serum concentrations of CRP than the HCs.

Table 7.1 Demographic characteristics for the healthy control (*n*=4336) and eating disorder (*n*=1084) groups from the UK Biobank. NB., cases may have reported more than one eating disorder diagnosis and therefore, be represented in more than one eating disorder category.

	Healthy controls		**Anorexia nervosa**			**Bulimia nervosa**			**Binge eating disorder**		
	n		*n*			*n*			*n*		
Gender [*n* (%)]	4336		513			275			296		
Male		2000 (46.13)		29 (5.65)	*		12 (4.36)	*		37 (12.50)	*
Female		2336 (53.87)		484 (94.35)			263 (95.64)			259 (87.50)	
Age [years] [mean ± SD]	4336	55.38 ± 7.73	513	52.56 ± 7.02	*	275	50.24 ± 6.83	*	296	52.73 ± 7.50	*
Smoking status [*n* (%)]	4330		494			266			294		
Current		290 (6.70)		53 (10.73)			18 (6.77)			23 (7.82)	
Former		1484 (34.27)		189 (44.47)	*		124 (46.62)	*		130 (44.22)	*
Never		2556 (59.03)		205 (48.24)			124 (46.62)			141 (47.96)	
Social deprivation [mean ± SD]	4331	-1.81 ± 2.74	512	-1.02 ± 3.11	*	275	-0.88 ± 3.27	*	296	-0.49 ± 2.99	*
BMI [kg/m^2] [mean ± SD]	4287	26.41 ± 4.16	501	22.50 ± 3.56	*	270	24.39 ± 4.76	*	290	29.72 ± 6.77	*
CRP [mg/l] [median (IQR[a])]	4336	1.00 (0.52, 1.99)	513	0.52 (0.30, 1.07)	*	275	0.65 (0.33, 1.49)	*	296	1.45 (0.65, 2.99)	*

[a]25th and 75th percentile reported. * denotes significantly different from HC participants at $p < 0.05$. Abbreviations: *n* = number of observations; SD = standard deviation; BMI = body mass index; kg = kilogram; m = metre; CRP = C-reactive protein; mg = milligram; l = litre; IQR = interquartile range.

7.4.1 Association between anorexia nervosa and C-reactive protein polygenic risk scores

The AN PRS and the CRP PRS were inversely correlated (when including the first six principal components in the analysis, r = -0.03) at the 1.00 p-threshold, though this did not reach formal significance thresholds (p =0.0551). However, in four other random samples a significant inverse correlation between AN PRS and CRP PRS was found ($p < 0.05$).

7.4.2 Association between polygenic risk scores and circulating concentrations of C-reactive protein

7.4.2.1 C-reactive protein polygenic risk score and C-reactive protein

In the whole sample, the standardised CRP PRS was significantly associated with serum concentrations of CRP, both in a partially adjusted regression (only the first six principal components included as covariates; $F(7, 5275) = 12.56$, $p < 0.0001$, adjusted $R^2 = 1.51\%$) and in the fully adjusted model controlling for gender, age, smoking status, social deprivation, BMI, and six principal components ($F(13, 5161) = 97.45$, $p < 0.0001$, adjusted $R^2 = 19.51\%$). In the adjusted model, for every 1 mg/l increase in serum CRP, the standardised CRP PRS increased by β = 0.13 (95% confidence intervals [CIs] 0.09 to 0.17, $p < 0.0001$; see **Figure 7.2**). Sensitivity analyses at the remaining ten p-thresholds were consistent with these findings (see **Figure 7.2**). Furthermore, these results were replicated across five additional random samples.

7.4.2.2 Anorexia nervosa polygenic risk score and C-reactive protein

A linear regression model covarying for six principal components ($F(7, 5275) = 2.20$, p = 0.0315, adjusted $R^2 = 0.16\%$) did not identify a formally significant association between the standardised AN PRS and CRP concentrations (β = -0.05, 95% CIs -0.10 to 0.01, p = 0.0275). When demographic factors were additionally controlled for ($F(13, 5161) = 93.74$, $p < 0.0001$, adjusted $R^2 = 18.90\%$), the association between the standardised AN PRS and CRP remained non-significant (β = -0.02, 95% CIs -0.06 to 0.02, p = 0.2722; see **Figure 7.2**). This pattern of findings remained consistent across the sensitivity analyses at different p-thresholds (see **Figure 7.2**) and in the additional random samples. However, a formally significant negative association (only covarying for the first six principal components) was observed in one random sample, suggesting that for every 1 mg/l increase in serum CRP, the standardised AN PRS decreased by β = 0.06 (95% CIs -0.10 to -0.02, p = 0.008).

7.4.2.3 Body mass index polygenic risk score and C-reactive protein

BMI PRS was significantly associated with serum CRP concentration in a regression model covarying for the first six principal components only ($F(7, 5275) = 5.22$, p = 0.0001, adjusted $R^2 = 0.56\%$) across all samples. However, when controlling for demographic factors there was no significant association between BMI PRS and CRP (see **Figure 7.2**),

except a nominally significant association (p = 0.021; i.e., did not meet formal significance thresholds that account for multiple testing) was observed in one of the additional samples. Sensitivity analyses reproduced these patterns for the fully adjusted models at all p-thresholds, except for at 0.01 where BMI PRS and CRP were significantly associated (see **Figure 7.2**; β = 0.06, 95% CIs -0.10 to -0.02, p = 0.0043). Sensitivity analyses excluding BMI as a covariate showed a significant association between BMI PRS and CRP across all samples and at all p-thresholds (at p-threshold 1.00: $F(12, 5230) = 6.57$, $p < 0.0001$, adjusted R^2 = 1.26%; β = 0.011, 95% CIs 0.07 to 0.16, $p < 0.0001$), except for $1 \times 10^{(-5)}$. Given the apparent role of current BMI in these analyses, we performed an unadjusted linear regression with CRP as the dependent variable and BMI as the independent variable to examine the variance in CRP explained by BMI. BMI explained 18.23% of the variance in CRP ($F(1, 5211) = 1162.80$, $p < 0.0001$; β = 0.15, 95% CIs 0.14 to 0.16, $p < 0.0001$).

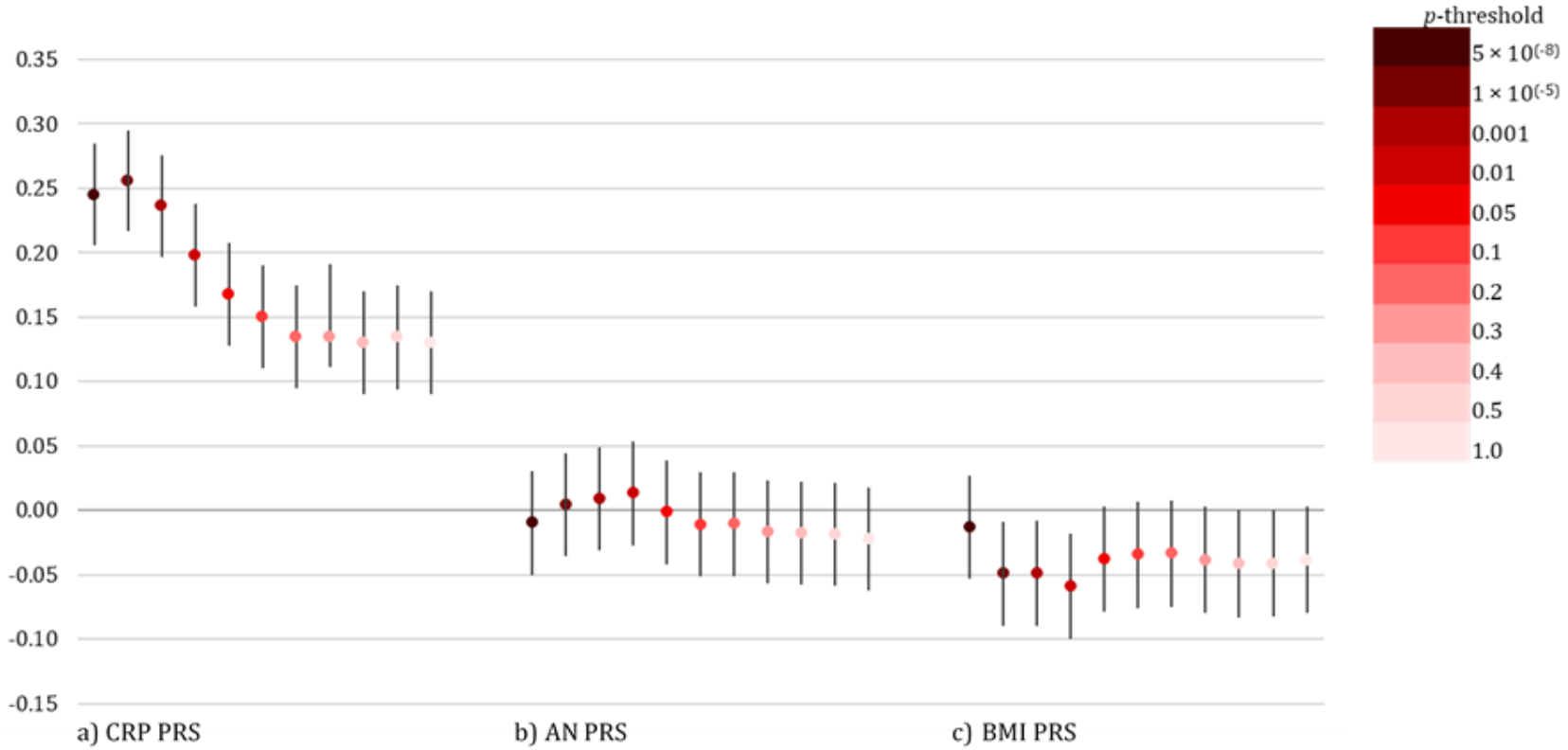

Figure 7.2 Regression coefficient (β) with 95% confidence intervals for the association between circulating serum concentrations of C-reactive protein with (a) C-reactive protein polygenic risk score, (b) anorexia nervosa polygenic risk score, and (c) body mass index polygenic risk score, controlling for relevant socio-demographic factors and six principal components. Polygenic risk scores at threshold $p < 1.00$ were used for the main analysis and polygenic risk scores at other p-thresholds were used for sensitivity analyses.

7.4.3 Association between C-reactive protein polygenic risk score and eating disorder diagnosis

7.4.3.1 Lifetime anorexia nervosa diagnosis versus healthy controls

The binomial logistic regression model (covarying for six principal components) showed that CRP PRS was not significantly associated with AN versus HC status (odds ratio [OR] 0.94, 95% CIs 0.85 to 1.03, $p = 0.1635$), except for a nominally significant association in one random sample ($p = 0.049$). When relevant demographic factors were additionally included as covariates, CRP PRS as a predictor of case/control status remained non-significant (OR 0.98, 95% CIs 0.89 to 1.10, $p = 0.7655$). Sensitivity analyses with alternative p-thresholds, the 'pure' AN diagnosis group and across additional random samples in the fully adjusted models produced consistent findings.

7.4.3.2 Lifetime bulimia nervosa diagnosis versus healthy controls

The CRP PRS was not associated with group status in BN when controlling for six principal components (OR 1.05, 95% CIs 0.93 to 1.19, $p = 0.4486$) and when additionally covarying for demographic variables (OR 1.07, 95% CIs 0.94 to 1.23, $p = 0.3076$) across all random samples. Findings were consistent in sensitivity analyses with the people only reporting a BN diagnosis and at the remaining *p*-thresholds, except for the association between CRP PRS and pure BN diagnosis at $p = 0.05$, which was nominally significant (OR = 1.16, 95% CIs 1.01 to 1.33, $p = 0.0327$).

7.4.3.3 Lifetime binge eating disorder diagnosis versus healthy controls

CRP PRS was a nominally significant predictor of group status (OR 1.17, 95% CIs 1.04 to 1.32, $p = 0.0115$) across all random samples, such that a higher CRP PRS was associated with a lifetime BED diagnosis relative to being a HC. This finding did not remain significant when demographic variables were included as covariates (OR 1.09, 95% CIs 0.95 to 1.24, $p = 0.2222$). Sensitivity analyses showed that CRP PRS in the model adjusted for six principal components met formal significance thresholds ($p < 0.0083$) as a predictor of group status at the following *p*-thresholds: $5 \times 10^{(-8)}$, $1 \times 10^{(-5)}$, 0.001, 0.05, 0.1, 0.2, and 0.3. Fully adjusted models were nominally significant at 0.001, 0.01, 0.05, and 0.1, and formally significant at $5 \times 10^{(-8)}$ and $1 \times 10^{(-5)}$. When primary analyses were repeated with the 'pure' BED diagnosis group, the partially adjusted regression model was nominally significant ($p < 0.05$) and the fully adjusted model was non-significant ($p = 0.4156$).

7.5 Discussion

Little is known about the role of genetic factors in the relationship between inflammatory markers and EDs. This study explored this using CRP, AN, and BMI PRSs, in a population-scale dataset from the UK Biobank (Bycroft et al., 2018; Sudlow et al., 2015). A summary of the results in relation to our aims is shown in **Figure 7.3**.

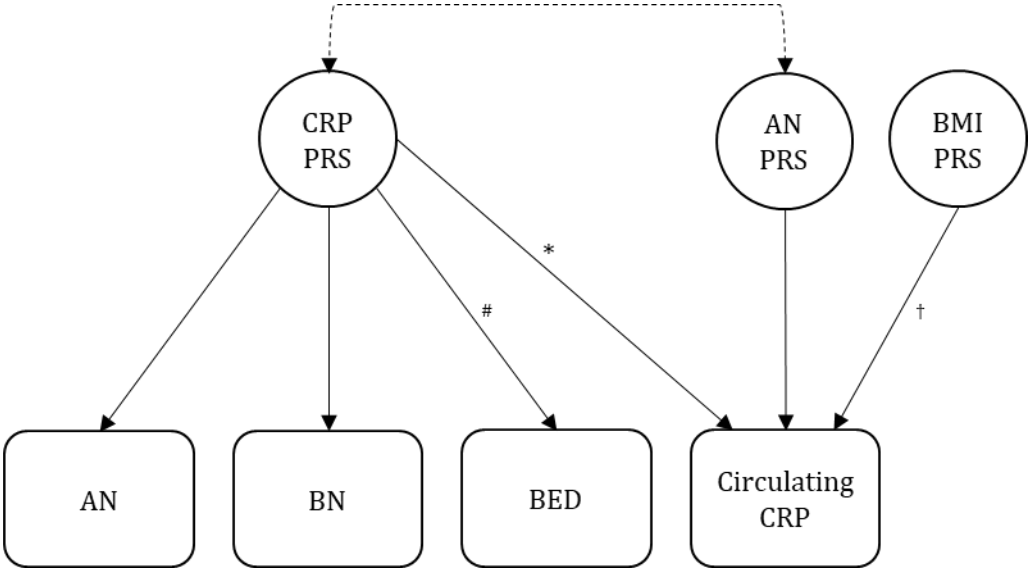

Figure 7.3 Summary of the findings in the primary sample at a polygenic risk score *p*-threshold of 1.0. * indicates a formally significant association in the fully adjusted regression model; † specifies a formally significant association in the partially adjusted regression model; and # denotes a nominally significant association ($p < 0.05$) in the partially adjusted regression model. Abbreviations: CRP = C-reactive protein; PRS = polygenic risk score; AN = anorexia nervosa; BMI = body mass index; BN = bulimia nervosa; BED = binge eating disorder.

7.5.1 Association between C-reactive protein polygenic risk score and C-reactive protein

The CRP PRS was significantly associated with circulating concentrations of serum CRP in this sample. This shows that common genetic variants are implicated in the concentrations of circulating CRP. The variance explained can only be as high as the trait variance explained by common genetic variants (i.e., heritability) in the CRP GWAS, which was ≈13% (Ligthart et al., 2018). In the current study, across all *p*-thresholds, the CRP PRS explained approximately 0.2% to 2.9% of the variance in serum CRP concentrations. However, as mentioned throughout this thesis, CRP concentrations are highly influenced by state factors including BMI, diet and lifestyle factors (e.g., physical activity), smoking status, and infections or injury (Kathiresan et al., 2006; O'Connor et al., 2009; Shen & Ordovas, 2009; Sproston & Ashworth, 2018). Indeed, BMI, as the main non-genetic determinant of circulating CRP concentrations, has been reported to explain up to 15% of variation in CRP concentrations (Kathiresan et al., 2006). In our study, addition of relevant sociodemographic factors known to influence concentrations of inflammatory markers, including BMI, to the regression models accounted for up to 21% of the variance in circulating CRP concentrations. Therefore, we further explored the role of BMI in CRP concentrations, finding that BMI explained approximately 18% of the variance in

circulating CRP. This highlights the importance of taking into account BMI in analyses of inflammatory markers.

7.5.2 Association between eating disorders and C-reactive protein

Anorexia nervosa. Previous research has reported an inverse genetic correlation between AN and CRP (Ligthart et al., 2018; Tylee et al., 2018), which is consistent with the lower circulating concentrations of CRP observed in AN, compared to heathy individuals (Solmi et al., 2015). While we were unable to carry out genetic correlations, we were able to analyse correlations between the AN PRS and CRP PRS. We identified an inverse correlation between the AN PRS and the CRP PRS in four out of six samples, which suggests that the genetic variants associated with lifetime AN may be correlated with the genetic variants associated with lower CRP concentrations.

Our study did not replicate the pattern identified by previous genetic correlations (Ligthart et al., 2018; Tylee et al., 2018) when examining the association between AN PRS and CRP and between CRP PRS and lifetime AN status. It has been proposed that alterations in the immune system and related inflammatory markers contribute to the pathogenesis and course of AN (Holden & Pakula, 1996) and are also evident in those with lifetime AN diagnoses (as shown in Study 1 of Chapter 6). Therefore, it is reasonable to assume that some of the SNPs associated with AN are also involved in the regulation of immune function. However, at all *p*-thresholds, the AN PRS was not associated with circulating concentrations of CRP and the CRP PRS was not associated with AN case status, even when relevant covariates were included in the analyses. The discrepancy between the previously reported inverse AN-CRP genetic correlation (Ligthart et al., 2018; Tylee et al., 2018) and our findings of a non-significant genetic association between AN and CRP may be explained by several factors. Firstly, the PRSs (both AN and CRP) are based on tagged SNPs; however, they are not necessarily causal. Secondly, polygenic measures may not reflect the more complex genetic architecture of these traits (Manolio et al., 2009). Thirdly, the effect of each genetic variant is estimated with error, which may be improved with increasing size of the discovery sample. These considerations may also account for the fact that the CRP PRS was statistically significantly associated with circulating CRP but with small effects.

Bulimia nervosa. Assessing the association between BN and CRP PRS was an exploratory step, given the minimal data on CRP and other inflammatory markers in BN. We stated a preliminary hypothesis of a higher CRP PRS being associated with BN case status on the basis that previous research reported an association between binge eating and elevated CRP (Shank et al., 2017; Succurro et al., 2015). However, we did not find that the CRP PRS

was significantly associated with lifetime BN case status. This may suggest that CRP does not play a role in the pathophysiology of BN.

To the best of our knowledge, concentrations of CRP have not yet been measured in people with BN, aside from in the current thesis. We may have expected CRP to be elevated in BN given the mentioned link between binge eating and CRP, and also given that self-induced vomiting can increase systemic inflammation due to related medical complications (Westmoreland et al., 2016). However, in contrast to Study 1 of Chapter 6, in which CRP concentrations did not differ between individuals with a lifetime diagnosis of 'pure' BN (i.e., have not received any other ED diagnoses) and HCs, the current study found reduced concentrations of CRP in BN participants (who may have also reported other ED diagnoses). There is significant diagnostic crossover between EDs (e.g., Fichter & Quadflieg, 2007) and as people with AN show lower concentrations of CRP, a history of AN may be a possible explanation for the lower CRP seen in people with lifetime BN. In addition, the difference in findings may be explained by the lack of BMI exclusion criteria in the current study: HCs in Study 1 of Chapter 6 were required to be within the normal-weight range. Furthermore, to date, only a few research studies have investigated inflammatory markers in BN (Ahrén-Moonga et al., 2011; Brambilla et al., 1998; Nagata et al., 2006; Nakai et al., 2000) with mixed findings (Chapter 2; Dalton et al., 2018a). At present there is no GWAS for BN available and therefore, we were unable to further explore the relationship using a BN PRS. More research is needed to better understand our observed findings and examine their replicability.

Binge eating disorder. A nominally significant association between the CRP PRS and lifetime BED case status was identified in our partially adjusted regression models, such that a higher CRP PRS and therefore a greater susceptibility to systemic inflammation was related to having BED, relative to being a HC. This direction of effect is consistent with our findings of elevated CRP concentrations in people with lifetime BED in the current study, in Study 1 of Chapter 6 and with previous findings in which higher CRP concentrations were associated with loss of control eating and BED status (Study 1 in Chapter 6; Shank et al., 2017; Succurro et al., 2015). Furthermore, higher concentrations of IL-6, the principal regulator of CRP synthesis, have been shown to be related to binge eating behaviour (Lofrano-Prado et al., 2017). The pattern of results was also observed when assessing participants who only reported a lifetime diagnosis of BED and no other ED diagnoses (i.e., AN or BN).

However, in our fully adjusted models, this relationship was not consistently reported across samples at the highest *p*-threshold, which assumes an omnigenic model. However, a positive significant association between CRP PRS and BED was observed at other *p*-

thresholds. A possible explanation for identifying the relationship only at more stringent thresholds is that the CRP PRS probably contains more noise when generated at higher *p*-value thresholds. In the fully adjusted regression models, we controlled for BMI. Therefore, the identified relationship between the CRP PRS and BED at the more conservative *p*-thresholds was independent of BMI. This suggests that the observed association is specific to BED status rather than related to obesity status, a metabolic state which is commonly associated with BED (Villarejo et al., 2012). Furthermore, it implies that there are characteristics related to BED that are also associated with genetic propensity for elevated CRP. These characteristics may include binge eating behaviours, psychiatric comorbidities (e.g., depression), and emotional distress. However, these characteristics are also present in BN and as such, it may be expected that CRP would be altered in BN, which was not seen in the current study. Finally, it may also be that the genetic variants associated with the regulation of CRP may confer risk for BED; however, our study design and analyses were unable to evaluate this and determine the direction of effect. It will be of value to further explore this relationship using different study designs (e.g., longitudinal) and/or analyses (e.g., genetic correlations, Mendelian Randomisation) when a BED GWAS becomes available.

7.5.3 Association between body mass index polygenic risk score and C-reactive protein

A positive genetic correlation between BMI and CRP has been reported (Ligthart et al., 2018). In our sensitivity analyses, not including BMI as a covariate, we replicated this correlation, showing that a higher BMI PRS was associated with higher serum concentrations of CRP. However, when current BMI was included in the analyses, this relationship became non-significant at the more liberal *p*-thresholds (although a significant association was seen at the more conservative *p*-thresholds below 0.05). These findings may be accounted for by the PRSs at more liberal *p*-thresholds including many SNPs that are not necessarily causal. However, this may also indicate that the relationship between BMI PRS and CRP is mediated by current BMI. As we did not have longitudinal data available, we were unable to perform a formal mediation analysis to further explore this association. However, a study using Mendelian Randomisation, a type of analysis in which the causal effect of traits can be assessed (Davies, Holmes, & Davey Smith, 2018), showed that the associations consistently observed between circulating concentrations of CRP and BMI are likely driven by BMI, with CRP being a marker of adiposity (Timpson et al., 2011). Indeed, investigations of biological processes support this: adipocytes produce a substantial proportion of circulating concentrations of IL-6, which regulates the production of CRP (Kathiresan et al., 2006). Therefore, as BMI and adiposity increase, as does the ability to produce IL-6 and subsequent CRP.

7.5.4 Considerations relating to the use of polygenic risk scores

There are several considerations that need to be discussed in relation to the use and interpretation of PRSs.

PRSs are not a diagnostic tool; they provide an indication of risk. This means that they do not provide evidence of causation, but rather provide a useful tool for investigating the correlations between genotypes and phenotypes (Martin, Daly, Robinson, Hyman, & Neale, 2019). One reason for this is that many SNPs are highly pleiotropic, influencing multiple different biological processes, which may be indirectly related to the outcome of interest. Given the large number of SNPs used to calculate PRSs, this can make them particularly sensitive to the effect of pleiotropy (Martin et al., 2019). Therefore, it is often useful to complement PRSs with analyses that allow for the investigation of causal relationships between phenotypes (e.g., Mendelian randomisation or related modelling methods; O'Connor & Price, 2018). This may be a useful next step in furthering our understanding of the genetic relationship between inflammatory markers and EDs.

Another reason for this is that the interaction between an individual's genes and the environment contribute to the aetiology and pathophysiology of psychiatric disorders, including EDs (Striegel-Moore & Bulik, 2007). A number of environmental factors are thought to be involved in the development of EDs (e.g., internalisation of the thin ideal, personality factors such as perfectionism and neuroticism, weight concerns; Keel & Forney, 2013; Striegel-Moore & Bulik, 2007) and environmental factors also influence circulating concentrations of inflammatory markers (e.g., seasonality, BMI, smoking status; Kathiresan et al., 2006; O'Connor et al., 2009; ter Horst et al., 2016). This is highlighted by the additional variance explained in the regression models when environmental aspects were included. However, it is unknown how the contribution of environmental factors plays out in each individual. Thus, a high PRS does not signify that the individual will necessarily develop a psychiatric disorder or have high/low concentrations of circulating inflammatory markers, and a low PRS does not indicate that an individual will never develop a psychiatric disorder or have altered inflammatory markers. Rather, the outcome may depend on environmental factors (e.g., protective factors, absence or presence of exposure to environmental triggers) or other genetic factors (e.g., contribute to resilience or vulnerability; Hübel et al., 2018). Furthermore, for these reasons, if two individuals have a similarly high PRS for the same psychiatric disorder, this also does not necessarily correspond to homogenous biological dysregulation across the individuals; rather, biological pathways relevant to the disorder may be affected in different ways in these individuals.

PRSs only capture common genetic variants. While most genetic variation within a population is explained by common genetic variants, for many traits, risk is conferred by the additive effect of genetic variants across the frequency spectrum, including de novo and rare variants, which can have larger effects than common variants (Martin et al., 2019). This may mean that individuals with phenotypic extremes are more likely to have an average PRS than the expected given the individuals' deviation from the mean, due to the contribution of rare genetic variants or environmental factors. However, a recent GWAS of low-frequency and rare genetic variants in AN did not report any significant associations, despite being sufficiently powered to detect these low-frequency genetic variants with large effect size (Huckins et al., 2018). Nevertheless, integrating information from both common and rare genetic variants will strengthen our understanding of genetic risk for AN.

There is a Eurocentric bias and lack of ancestral diversity in GWAS studies. GWAS are often limited to individuals with a shared ancestry to avoid population stratification, where systemic differences exist in allele frequencies between subpopulations within a population. Indeed, the majority of GWAS have been performed in individuals with European ancestry (Gurdasani, Barroso, Zeggini, & Sandhu, 2019). This consequently limits the application of PRSs to diverse populations and can introduce biases in the direction of associations across populations of different ancestry (Martin et al., 2019; Martin et al., 2017).

7.5.5 Strengths and limitations

This study used the largest and most recently available GWAS in order to calculate the CRP (Ligthart et al., 2018), AN (Watson et al., 2019), and BMI (Locke et al., 2015) PRSs while avoiding sample overlap. As a number of biobehavioural factors influence concentrations of CRP, a strength of this study is the use of objective measures of genetic risk for circulating CRP, which is perhaps a more stable indicator of propensity to systemic inflammation.

This study needs to be viewed in light of several limitations. Our analyses were limited to individuals from European ancestry, as the PRSs were restricted to studies from samples with European ancestry (Ligthart et al., 2018; Locke et al., 2015; Watson et al., 2019). Therefore, these findings may not be generalisable to individuals of other ancestries (Martin et al., 2019), which may be particularly relevant given that the prevalence of EDs are increasing in other countries and cultures worldwide (Pike et al., 2014).

We used the largest GWAS available in the present study. Indeed, the scale of AN GWAS has rapidly expanded over the last decade: ≈16,000 AN cases in the most recent GWAS (Watson et al., 2019) compared to 3,495 AN cases in the previous GWAS (Duncan et al.,

2017). However, the AN discovery sample was still small in comparison to GWAS in other psychiatric disorders (>246,000 cases in depression GWAS, Howard et al., 2019; ≈37,000 cases in schizophrenia GWAS, Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014). This may limit the power of PRSs. It is anticipated that larger discovery sample sizes will improve the robustness of PRSs as predictors of risk as a greater proportion of phenotypic variance may be explained by PRSs and PRSs may be better able to capture causal genetic effects (Dudbridge, 2013). Unfortunately, GWAS in BN or BED have yet to be performed (Hübel et al., 2018). As such, we were unable to further explore the genetic relationship between these EDs and CRP.

The target samples for each ED were relatively small. Additionally, the phenotype data available for this study was limited to lifetime diagnoses of EDs, with the majority being self-reported and not corroborated by medical records. As data on current ED status was not available, we were unable to take into consideration current ED behaviours (e.g., self-induced vomiting, binge eating) that may influence CRP concentrations in our analyses. Future studies should use more detailed phenotypic assessments (e.g., include current ED symptom severity) to provide more informative data on the relationship between inflammatory markers and EDs.

The current study's analyses were limited to CRP. However, a number of other inflammatory markers, in particular pro-inflammatory cytokines, have been associated with EDs. For example, elevated IL-6 concentrations have been reported in individuals with AN, relative to HCs (Chapter 2; Dalton et al., 2018a). There is evidence that concentrations of circulating IL-6 and other cytokines are also under significant genetic influence (de Craen et al., 2005; Li et al., 2016c) and have been reported to be highly heritable e.g., a heritability estimate of IL-6 based on twin studies was reported to be 0.57 (de Craen et al., 2005). Therefore, studies assessing the genetic association between these additional inflammatory markers and EDs are warranted and will provide a greater understanding of this relationship. Furthermore, our analyses assume that circulating CRP concentrations provide a proxy for inflammatory processes that confer risk for EDs. However, CRP concentrations may also represent consequences of ED pathology.

Finally, some of the environmental variables included as covariates in the analyses also have a genetic component (e.g., smoking behaviour; Erzurumluoglu et al., 2019) and are partially heritable. Therefore, it would be important to additionally control for the genetic components of these variables in future analyses.

7.5.6 Conclusions

We observed a significant positive association between CRP PRS and serum CRP concentrations, highlighting the role genetics play in the regulation of circulating

concentrations of CRP. Analyses on BMI PRS and CRP suggest that this relationship is mediated by current BMI. AN and BN case status were not associated with CRP PRS, nor was the AN PRS with CRP concentrations. There are several methodological factors relating to PRS that may account for the non-significant findings between CRP and AN, in light of previously reported significant genetic correlations (Ligthart et al., 2018; Tylee et al., 2018). BED case status was positively associated with CRP PRS across multiple stricter *p*-thresholds. When a BED GWAS becomes available, it would be of value to further explore the relationship between BED and CRP using a BED PRS and using Mendelian Randomisation, in which the risk for BED conferred by the SNPs associated with CRP concentrations could be assessed.

Chapter 8. General overview

8.1 Summary of the main findings

The overall aim of the work described in this thesis was to improve our understanding of the relationship between inflammatory markers (such as cytokines, chemokines and acute-phase proteins) and eating disorders (EDs). This was with the broader aim of increasing our knowledge of the biological correlates involved in the development and maintenance of EDs. The complexity of the systems (i.e., the network of inflammatory markers) involved and how they interact with a number of other physiological systems (e.g., the hypothalamic–pituitary–adrenal [HPA] axis) has necessitated that many of the studies in this thesis were exploratory. The main findings were as follows:

a) Alterations in inflammatory markers occur in anorexia nervosa (AN; e.g., increased concentrations of interleukin [IL]-6, IL-15 and tumor necrosis factor [TNF]-α, and reductions in C-reactive protein [CRP], IL-7, macrophage inflammatory protein [MIP]-1α, MIP-1β, and TNF-β) and other EDs (e.g., elevated CRP in binge eating disorder [BED]). However, findings were not consistent across all samples studied (Chapters 2, 3, and 6).

b) During specialist treatment for EDs, concentrations of IL-6 increase and IL-7 decrease and this co-occurs with clinical improvements in AN e.g., reduced ED symptom severity and weight gain (Chapter 4).

c) In people with EDs, concentrations of inflammatory markers do not appear to be associated with stress (Chapter 5) and diet (Chapter 6), i.e., factors that typically influence cytokine production in healthy and other clinical populations. However, genetic factors may play a role (Chapter 7): a higher polygenic risk for circulating CRP was associated with BED.

A schematic representation of the primary findings are shown in **Figure 8.1** and **Figure 8.2** and these are discussed below.

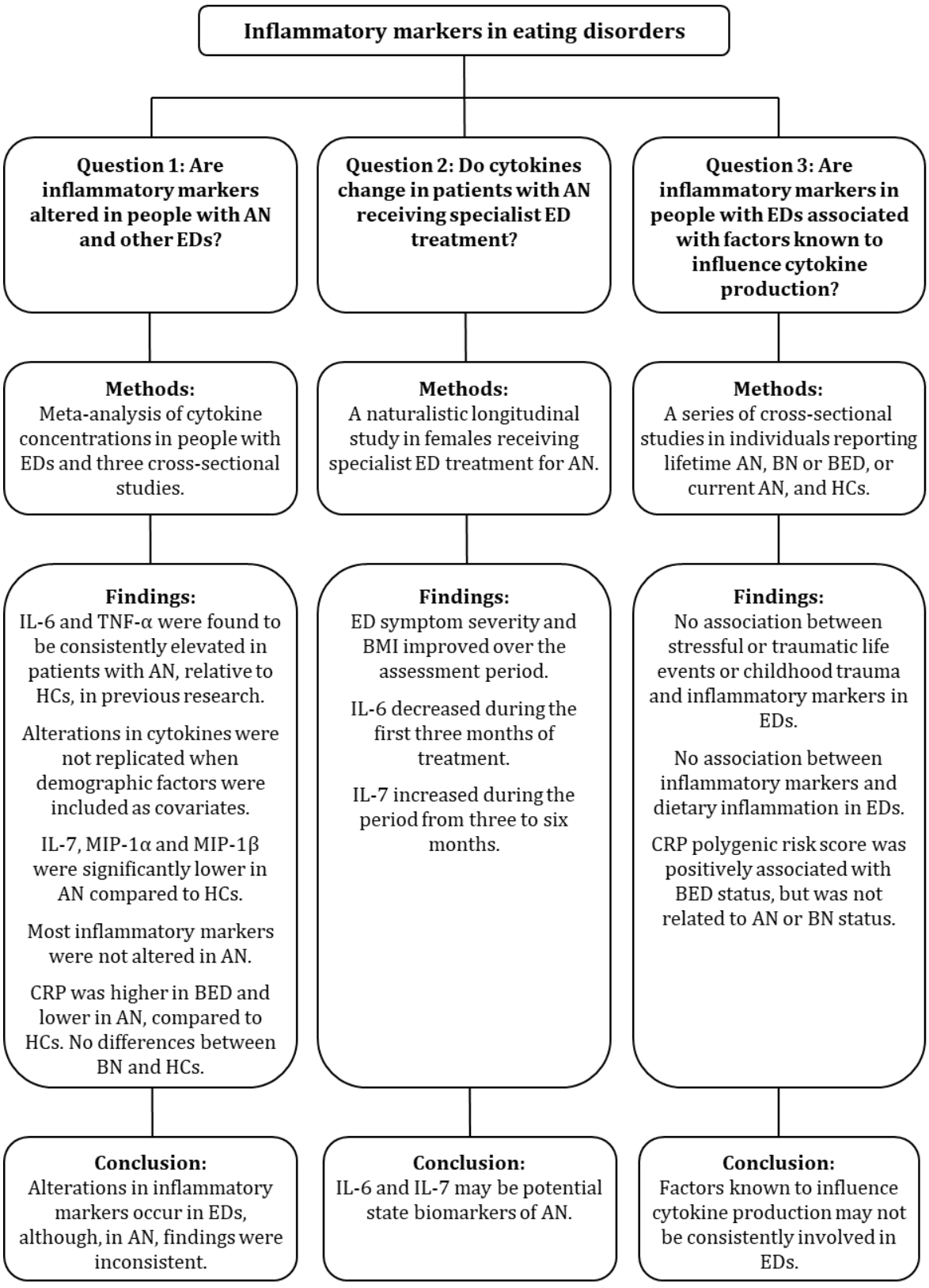

Figure 8.1 Summary of the primary findings. Abbreviations: AN = anorexia nervosa; ED = eating disorder; IL = interleukin; TNF = tumor necrosis factor; HC = healthy control; MIP = macrophage inflammatory protein; CRP = C-reactive protein; BED = binge eating disorder; BN = bulimia nervosa; BMI = body mass index.

8.1.1 Inflammatory markers were inconsistently altered in anorexia nervosa and other eating disorders

The studies presented in Chapters 2, 3, and 6 investigated cross-sectional differences in cytokine concentrations between people with EDs and healthy controls (HCs) to identify novel associations between inflammatory markers and EDs, and potential patterns in the alterations of inflammatory markers. These studies provided mixed evidence for alterations in inflammatory markers between these groups.

Given the heterogeneity in previous research on cytokines in EDs, Chapter 2 systematically reviewed and meta-analysed the existing evidence on cytokine concentrations in EDs. This review concluded that few cytokines had been consistently assessed in AN. Elevated concentrations of the pro-inflammatory cytokines IL-6 and TNF-α were reported in patients with AN, compared to control participants; thus, providing support for the association between alterations in inflammatory markers and AN. No alterations in cytokines were consistently associated with bulimia nervosa (BN) and research investigating cytokine concentrations in people with BED was not available. This review also identified inconsistencies across previous studies regarding the management of known confounding factors of circulating inflammatory marker concentrations (e.g., age, smoking status; O'Connor et al., 2009) i.e., whether these factors were accounted for in the study design (e.g., exclusion criteria) and/or analyses (e.g., included as a covariate).

Given the limited scope of previous research (as described in Chapter 2), the two studies presented in Chapter 3 explored alterations in a broad range of inflammatory markers, many of which had not been previously measured in EDs, in participants with AN and HCs. Study 1 of Chapter 3 identified nominally significant elevations in concentrations of IL-6, IL-15, and vascular cell adhesion molecule (VCAM)-1 and reductions in concentrations of TNF-β and vascular endothelial growth factor (VEGF) in AN patients, compared to HCs. This was the first study to identify alterations in IL-15, TNF-β and VEGF in AN, which led to the conception of a confirmatory study (Study 2 of Chapter 3).

Study 2 of Chapter 3 aimed to assess the replicability of these novel alterations in inflammatory markers in a larger sample of individuals with AN. On the basis of the conclusions from Chapter 2, I ensured that relevant factors (e.g., age, ethnicity, smoking status, presence of immune-related conditions) were accounted for in the study design and statistical analyses of this study. I did not replicate the findings from the first study in Chapter 3 nor the consistent alterations in IL-6 and TNF-α identified in Chapter 2. The absence of reliable patient and control group differences in these cytokines and other inflammatory markers may be due to lifestyle and demographic factors. In Study 2 of Chapter 3, IL-7 and the chemokines MIP-1α and MIP-1β were found to be significantly

lower in AN participants relative to HCs. Lower concentrations of IL-7 were associated with AN presentation and status, in terms of lower BMI and body fat percentage, and higher ED and depression symptom severity. Consistent with this, lower concentrations of IL-7 have been reported in patients with depression (Grosse et al., 2016; Lehto et al., 2010); though findings have been mixed (e.g., Dahl et al., 2014).

In Study 1 of Chapter 6, circulating concentrations of serum CRP were measured in people with lifetime ED diagnoses in the UK Biobank, a population-based cohort (Allen et al., 2014). CRP concentrations were found to be lower in individuals with current and/or a history of AN, compared to HCs. This was in contrast to findings from Study 1 of Chapter 3, in which no group differences in circulating CRP were identified (Dalton et al., 2018b). However, lower concentrations of CRP in AN was consistent with results from a meta-analysis (Solmi et al., 2015). In line with previous reports (Succurro et al., 2015), individuals with current and/or a history of BED had elevated CRP in comparison to HC participants. This was the first study to assess CRP in people with BN, finding that altered CRP concentrations were not associated with a lifetime BN diagnosis. As discussed in Chapter 7, BMI is a key determinant of CRP concentrations (Timpson et al., 2011). BED often co-occurs with obesity (Villarejo et al., 2012), AN is associated with low weight (American Psychiatric Association, 2013), and people with BN are typically of normal weight. Therefore, because demographic factors were not controlled for in this study, we cannot be sure that the altered CRP concentrations observed were not related to weight.

Across both studies in Chapter 3, in the majority of inflammatory markers, group differences were not apparent, suggesting that aberrant functioning of the inflammatory system is not widespread in AN. It has been reported that the immune system in AN is better preserved than may be expected given the physical and nutritional status of AN patients (Brambilla, 2001). This is in contrast to individuals with malnutrition (but are otherwise healthy and have no history of or current ED) who show significantly altered concentrations of multiple cytokines and inflammatory mediators (Chandra, 2002; Takele et al., 2016). It has been suggested that specific nutritional feeding habits of AN patients may account for this difference. Alternatively, changes in other biological parameters (e.g., neuroendocrine) related to the pathophysiology of AN may interfere with the immunological dysfunction associated with malnutrition (Brambilla, 2001).

An important observation related to these findings is that the alterations in inflammatory markers observed in patients with EDs in this thesis were modest in comparison to the alterations seen in people with autoimmune or infectious diseases. While this may suggest that these findings are physiologically irrelevant, this would arguably be an incorrect interpretation. Small physiological differences in inflammatory markers can have a

considerable effect if they are chronically altered in a consistent direction (e.g., persistent elevation of pro-inflammatory cytokines), and are therefore of clinical relevance. Indeed, even small increases in inflammatory markers, which indicate chronic subthreshold inflammation, have been shown to predict the development of serious disease states, such as cardiovascular disease (Ridker, Hennekens, Buring, & Rifai, 2000), diabetes (Spranger et al., 2003), and cognitive decline (Laurin, David Curb, Masaki, White, & Launer, 2009), in healthy individuals and clinical populations.

Taken together, there were several inconsistencies within our cross-sectional findings, and it is unclear why the specific inflammatory markers were found to be altered. As may have been expected from previous research (Solmi et al., 2015), I did not identify a uniformly pro-inflammatory profile in individuals with AN. Relatedly, a study assessing other pro-inflammatory parameters (e.g., inflammatory enzymes), in addition to cytokines, did not find a consistent pro-inflammatory status across biomarkers in patients with EDs (MacDowell et al., 2013). This emphasises the complexity of this biological system, demonstrating that circulating concentrations of inflammatory markers are affected by many factors and individual differences that are not fully understood and that different aspects of the inflammatory response are likely to be differentially regulated.

8.1.2 Concentrations of certain cytokines change during specialist eating disorder treatment

Chapter 4 used a naturalistic longitudinal approach to explore whether concentrations of multiple cytokines (n=27) changed over time in individuals receiving specialist ED treatment for AN. Over the 6-month assessment period, improvements in BMI and ED symptoms were observed. These changes in AN presentation co-occurred firstly with reductions in initially elevated IL-6 serum concentrations (baseline to 3 months) and then with increases in IL-7 concentrations (3 months to 6 months). While these participants did not have altered IL-7 concentrations compared to HCs, these findings were in line with decreased concentrations of IL-7 observed in AN participants in Study 2 of Chapter 3 and one previous study (Germain et al., 2016). The reductions observed in IL-6 were similar to findings from the depression and schizophrenia literature reporting treatment-associated changes in cytokine concentrations (Goldsmith et al., 2016; Köhler et al., 2018). However, an additional complicating factor with our research in AN is that cytokines are influenced by body weight/BMI and body fat (ter Horst et al., 2016; Wedell-Neergaard et al., 2018b). Therefore, from this study it cannot be ascertained whether changes in concentrations of IL-6 and IL-7 were due to improvements in psychopathology or changes in physical state. Indeed, IL-7 concentrations in Study 2 of Chapter 3 were shown to be significantly associated with both BMI and percentage body fat, but also ED symptom severity. Given

the associations with physical condition, directions of causality are difficult i.e., whether the observed cytokine changes were influenced by a change in anthropometric parameters (e.g., weight gain) or whether shifts in cytokines concentrations caused changes in the physical state. Ultimately, the findings from Chapter 4 demonstrated that cytokine changes co-occurred with changes in AN presentation; however, from our data it is unclear which factors regulate cytokines in AN. Therefore, future research should further investigate IL-6 and IL-7 as potential state biomarkers of AN and treatment response.

8.1.3 Genetic factors, but not diet or stress, play a role in inflammatory markers in eating disorders

The work described in the latter part of this thesis (Chapters 5 to 7) aimed to investigate factors that may influence cytokine concentrations in both healthy individuals and people with EDs. Specifically, I explored the association between stressful events and trauma (Chapter 5), the inflammatory potential of an individual's diet (Chapter 6), and polygenic risk scores (PRSs) for CRP (Chapter 7) with inflammatory markers in people with EDs. Given that clinical associations with inflammatory markers are likely mediated or moderated by constructs relating to behaviours, experiences, lifestyle, and biological predisposition, these chapters explored some of these concepts for the first time in ED samples.

Stress. It has been suggested that exposure to stressful life events (Rojo et al., 2006; Schmidt et al., 1997) and/or childhood trauma (Molendijk et al., 2017) increases vulnerability to EDs. In addition, experience of childhood maltreatment has been associated with more unfavourable psychiatric outcomes in ED patients (Molendijk et al., 2017). Furthermore, stress-related disorders (e.g., post-traumatic stress disorder [PTSD]) have been linked to cytokine alterations (Passos et al., 2015). Across three studies, the findings from Chapter 5 led to the conclusion that exposure to lifetime stressful or traumatic events, adult trauma, and childhood maltreatment trauma were not associated with concentrations of inflammatory markers in EDs. This was in contrast to the literature in both healthy and other clinical populations (Baumeister et al., 2016; Passos et al., 2015). The findings in Chapter 5 may indicate that dysregulation of inflammatory markers may not be a mechanism that supports the association between stress/trauma and EDs. It may be that diet, psychiatric comorbidities and other associated factors may obscure the association in people with EDs. However, in this research, I was unable to measure several important variables associated with life events and trauma, including chronicity and context of the stressor, age of exposure, and the duration and frequency of exposure, which may offer greater insights into the association between EDs, inflammatory markers and stressful experiences.

While there were no significant relationships between inflammatory markers and stress in our ED samples after controlling for multiple testing, several markers were nominally associated with measures of stress. Specifically, in relation to life events, IL-15 and VEGF were associated with exposure to traumatic events in a clinical AN sample, and TNF-β was negatively associated with stressful life events in community-dwelling young adults with AN and BN. These inflammatory markers were also reported to be altered in patients with AN in Study 1 of Chapter 3. Additionally, IL-23 was positively related to the experience of childhood maltreatment in young adults with current ED diagnoses. These associations merit further examination in larger samples with more well-defined and comprehensive assessments of stress and trauma.

Given that stress is a broad topic area within which there are multiple types of stressor, it cannot be ruled out that the investigation of alternative stressors (e.g., acute laboratory stress, daily hassles) may identify a role for stress in inflammatory marker concentrations in people with EDs. Furthermore, inflammatory markers do not function in isolation: they interact extensively with many other physiological systems in the brain and body that are also believed to be involved in the pathophysiology of EDs, such as the HPA axis (Lo Sauro et al., 2008). This thesis did not explore how cytokines were associated with parameters of the HPA axis. While an association between the stress response, inflammation and EDs has been proposed (Brambilla, 2001), little research has investigated this. For example, in a small group of AN patients, the cell-mediated immune response (T-lymphocyte responses to mitogens) following intravenous administration of corticotropin-releasing hormone (CRH; which is thought to mirror the effects of acute psychological stress and is known to inhibit T-lymphocyte responses) was assessed (Brambilla et al., 1993). Despite basal hypersecretion of CRH in the AN patients, further CRH administration and the associated acute stress reaction did not modify the specific immune parameter differently to what was seen in HCs. It was concluded that the immune response to acute stress was not impaired in AN and it may be that alterations in neuroendocrine hormones are not responsible for the normal immune function seen in AN (Brambilla et al., 2001). In view of the limited research, as discussed in Chapter 5, experimental studies are needed to better understand the relationship between stress and the inflammatory response in patients with EDs.

Diet. The relationship between dietary inflammation, EDs and inflammatory markers was explored in Chapter 6. The inflammatory potential of an individual's diet was not associated with ED status across two studies, one using participants from the UK Biobank with lifetime ED diagnoses (AN, BN or BED; Study 1) and the other in females with current AN (Study 2). Based on the established disturbances in dietary intake in EDs (Forbush &

Hunt, 2014), this was unexpected. However, this may be accounted for by the limited assessment of participants' nutrient intake or by the fact that the sample in Study 1 consisted of people with lifetime, rather than current, ED diagnoses. Dietary inflammation was also not associated with inflammatory marker concentrations in people with EDs in either study. While diet is an important modulator of cytokine production (Chandra, 2002; Grimble, 1996), it may be that there are additional more potent factors that contribute to the regulation of inflammatory markers in EDs. An alternative possible explanation for the lack of association between dietary inflammation and inflammatory marker concentrations in our AN participants may be related to gut permeability. Lower consumption of total calories, protein, fat, carbohydrates, and vitamin B12, as was observed in AN participants in Study 1, has been associated with lower zonulin concentrations (Mörkl et al., 2018). Zonulin is a biomarker of gut permeability and higher concentrations are associated with a leaky gut. Lower zonulin has also been associated with lower BMI and body fat mass (Mörkl et al., 2018), as seen in AN. Therefore, it may be that in our AN participants, the bacteria within the gut, which is affected by nutrient intake, are unable to translocate into circulation and have a subsequent influence on peripheral concentrations of inflammatory markers. In addition, women with lower zonulin concentrations, as compared to women with higher, had lower concentrations of the pro-inflammatory markers CRP and IL-6 (Mörkl et al., 2018). Thus, the incorporation of measures of the microbiome and gut permeability may be useful in better understanding the relationship between dietary inflammation and circulating inflammatory markers in EDs.

Genetic factors. Finally, in Chapter 7, it was highlighted that a PRS for circulating CRP was not related to lifetime AN or BN status. Previous research reported pleiotropy between CRP and AN: shared genetic variants influence AN and circulating CRP, though in opposite directions (Ligthart et al., 2018; Tylee et al., 2018). Our findings did not replicate the pattern of this inverse genetic correlation when analysing the relationship between polygenic scores for CRP and AN, nor when assessing the association between CRP PRS and AN status, and AN PRS and circulating CRP. However, there were multiple methodological considerations that may account for this. These include PRS-related limitations and sample limitations, such as lifetime ED diagnoses, diagnostic transition across ED presentations, and poor phenotype characterisation (e.g., self-reported diagnosis by a professional, rather than a clinical interview with a trained clinician). However, it was found that a higher CRP PRS was associated with having current and/or a history of BED, independent of BMI, at several *p*-thresholds. This is consistent with evidence of a higher CRP concentration in people with BED and those who report binge eating behaviours (Study 1 of Chapter 6; Chapter 7; Shank et al., 2017; Succurro et al.,

2015). This novel finding suggests shared genetic aetiology between CRP and BED, and that the role of inflammatory markers in BED warrants further investigation. For example, the genes predisposing to higher CRP may also be associated with broader phenotypes relating to BED. Sample collection to contribute to a BED genome-wide association study (GWAS) is underway (Hübel et al., 2018) and this will provide the opportunity to further explore this relationship using follow-on approaches such as PRSs and genetic correlations.

EDs are complex phenotypes (i.e., binge eating behaviours, restricting behaviours, compensatory behaviours). Further exploring the associations assessed here with more comprehensive phenotyping would be of interest, as would incorporating possible environmental interactions into analyses of genetic variants, given the important role of the environment in the aetiology of EDs (Striegel-Moore & Bulik, 2007). For example, possible mediating/moderating factors discussed in this thesis, such as childhood maltreatment, could be examined. Future studies should also consider the association between EDs and genetic predisposition for the production of other inflammatory markers, such as cytokines that have been associated with EDs. This would improve our understanding of how cytokines may contribute to the pathogenesis of EDs.

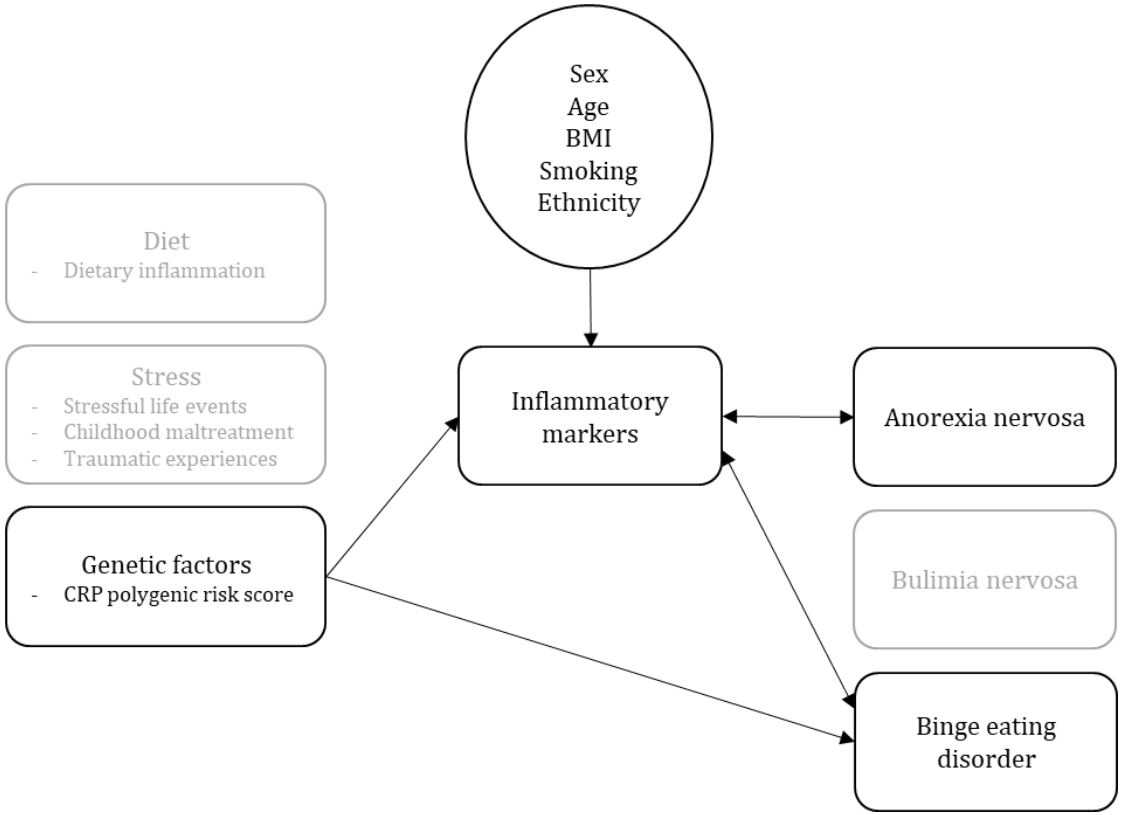

Figure 8.2 Schematic representation of the primary findings of this thesis. Boxes in grey indicate no related significant findings and boxes in black indicate significant associations, as briefly described here. Demographic factors influenced inflammatory marker concentrations (Chapter 3). Altered concentrations of inflammatory markers were associated with anorexia nervosa (Chapter 2, 3, 4 and 6) and binge eating disorder (Chapter 6), but not bulimia nervosa (Chapter 2 and 6). Polygenic risk score for C-reactive protein was associated with concentrations of circulating C-reactive protein and with binge eating disorder status, but not with anorexia nervosa or bulimia nervosa status (Chapter 7). Stress (Chapter 5) and diet (Chapter 6) were not associated with inflammatory marker concentrations in people with eating disorders. Abbreviations: BMI = body mass index, CRP = C-reactive protein.

8.2 General limitations

This research has a number of general limitations, both in the overall approach and in the methodologies used. There is no generally accepted method of measuring, processing and analysing cytokine data, which may account for the observed heterogeneity in cytokine research. Despite the reported sensitivity of the assays used (Dabitao et al., 2011), a number of inflammatory markers were not detected in the samples, including granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-1β, IL-4, and IL-21, due to their detection limit. This resulted in a reduced sample of cytokines and other inflammatory markers that could be examined across studies, limiting the scope within which circulating inflammatory markers in EDs could be examined. In most studies, the inflammatory markers were measured only at a single time point in the periphery (serum or plasma), representing a snapshot of inflammatory marker concentrations at a given time. Furthermore, measurement of circulating (in plasma or serum) inflammatory markers only represents one aspect of the inflammatory response. However, assessing gene expression of the cytokines (e.g., Kahl et al., 2004) or neuroinflammatory measures (e.g., in the cerebrospinal fluid) may have provided a more comprehensive and clinically relevant picture of alterations in the inflammatory response in people with EDs. As each cytokine does not function independently, it would be worthwhile to measure overall patterns of immune activation by calculating a composite score. While a broad range of cytokines were measured, the sample sizes did not permit a sufficiently powered component or factor analysis, and thus, I was unable to analyse a composite score of inflammatory markers.

As discussed throughout this thesis, there are various methodological considerations in the measurement of inflammatory markers and there are several factors that may affect their concentration (Dugué et al., 1996; O'Connor et al., 2009; ter Horst et al., 2016), which I was unable to assess and/or incorporate into our study design and analyses. For example, cytokines show diurnal and temporal variability; however, time of blood draw was not standardised across participants. Blood samples were also non-fasting, and I did not instruct participants to refrain from vigorous exercise prior to taking part in the research session. Furthermore, measurement of inflammatory markers in the complete absence of medication was not possible. It is also important to note that in all but one of the samples used in my studies, participants were exclusively female. It is likely that gender differences exist with respect to inflammatory marker concentrations (ter Horst et al., 2016). Therefore, these findings are not generalisable to male populations. Menstrual cycle phase at the time of research participation was not taken into account despite research reporting that cytokine concentrations vary across the menstrual cycle (Sahin Aydınyurt, Yuncu, Tekin, & Ertugrul, 2018; Whitcomb et al., 2014). Given that many

women with AN experience amenorrhea, it would have been important to assess normally menstruating females in the phase of their menstrual cycle which is most similar to the state of amenorrhea (the early follicular phase). However, basing research participation on timing of the menstrual cycle would have been complicated by the use of hormonal contraception in our participants. Taken together, this may mean that the relationship between inflammatory markers and the different factors explored in this thesis may be under- or over-estimated.

Across my studies, sample sizes were generally small and limited to female participants, and samples were heterogenous in many aspects of ED-related characteristics (e.g., illness duration, AN subtype, current or lifetime diagnosis, etc.). This limits the generalisability of our findings. All studies, except for Chapter 4, were cross-sectional in design. Therefore, the direction of effect in the associations identified cannot be established. Similarly, I did not experimentally assess whether cytokine concentrations can be changed with treatment. Rather, the longitudinal study in Chapter 4 had a naturalistic observational study design in which participants were receiving different treatment combinations (e.g., inpatient or outpatient treatment, psychopharmacology). This limits our ability to make conclusions about the direction of effects between changes in cytokine concentrations and treatment-related changes in psychopathology. Therefore, the studies in this thesis cannot provide any empirical information regarding the role of cytokines as a correlate or biomarker of treatment response.

8.3 General strengths

Several strengths exist across the studies included in this thesis. The thesis presents a comprehensive exploration of the relationship between inflammatory markers and EDs, in particular AN, through nine studies (using both cross-sectional and longitudinal approaches) across four samples (with a variety of ED diagnoses) and one meta-analysis. The main strength of this thesis relates to the novelty of the research performed. Firstly, a broad range of inflammatory markers were quantified, which has provided the opportunity to identify novel associations between inflammatory markers and EDs, and examine possible patterns in the alterations of these markers. Inflammatory markers were measured simultaneously using highly sensitive, reliable and validated assays (Dabitao et al., 2011). Secondly, this research was the first to explore the role of possible explanatory factors (stress, diet, and polygenic risk) in the relationship between inflammatory markers and EDs. Furthermore, inflammatory markers were considered in relation to the severity of ED and general psychopathology symptoms, providing an extension of previous work. Third, many previous studies investigating cytokines in AN have not considered the role of confounding factors (Chapter 2; Dalton et al., 2018a). Therefore, where possible (due to

sample size and availability of data), I controlled for various factors that have been shown to influence cytokine concentrations, such as age and ethnicity (Dugué et al., 1996; O'Connor et al., 2009; ter Horst et al., 2016). Relatedly, efforts were made to exclude individuals with conditions known to significantly affect the immune system (e.g., autoimmune disorders, inflammatory conditions).

8.4 Directions for future research

Reflecting on the studies within this thesis, there are several avenues of research that may be promising for answering further questions on the role of inflammatory markers in EDs. Focusing on these questions may provide opportunities for new therapeutic and preventative interventions. For example, preliminary research of cytokine blockers as a treatment for depression and bipolar disorder has shown promise for a subgroup of patients (e.g., those with a history of childhood maltreatment or high baseline inflammatory marker levels; McIntyre et al., 2019; Raison et al., 2013). However, given the observed inconsistent alterations in cytokine concentrations, it is premature to suggest investigation of cytokine blockers or inflammatory-reducing interventions for the treatment of EDs e.g., omega-3 supplementation. The questions that should be addressed in future research will now be discussed.

8.4.1 Is there a subgroup of individuals with eating disorders who show inflammatory marker alterations?

We have shown that certain immune parameters were altered in AN (Chapters 2-3) and BED (Chapter 6). However, it is unclear whether these alterations are homogenous across patients with AN or other EDs, or whether heterogeneity exists, consistent with the hypothesis that there is a specific subgroup of patients with EDs in which immune alterations can be observed. The greater variability in cytokine concentrations in AN, relative to HCs, seen in Study 1 of Chapter 3, suggests that there indeed may be a subgroup of patients with EDs who show alterations in immune parameters. A first step in addressing this research question more formally would be to perform a meta-analysis of the variability in cytokine concentrations between ED patients and HCs, as has been done in patients with psychosis (Pillinger et al., 2018). The meta-analysis works on the basis that if there is an 'immune' subgroup of patients (i.e., a subgroup defined by altered inflammatory markers), then greater variability in immune parameters in patients compared to controls would be expected i.e., reflecting heterogeneity in cytokine dysregulation. Alternatively, if immune dysregulation is a core component of ED pathophysiology, we would expect less variability in immune parameters i.e., reflecting homogeneity in cytokine dysregulation. A further step would be to examine cytokine concentrations in larger samples of ED patients to determine whether there is an 'immune'

subgroup of ED patients, defined by and/or closely related to altered inflammatory markers.

8.4.2 Could inflammatory markers be a risk factor for the development of eating disorders?

It is unknown whether cytokines and other inflammatory markers constitute a risk factor for the development of EDs. Given the complex interconnections between the immune system and other systems and individual differences in responses within these pathways, people may vary considerably in their sensitivity to the behavioural effects of inflammatory signalling (Raison & Miller, 2011). For example, inflammatory markers may be protective against or increase vulnerability for the development of EDs and other psychiatric disorders. Therefore, understanding whether inflammation is causally relevant to the aetiology of EDs will advance our knowledge of these conditions. For such studies, the use of data from pre-existing epidemiological and prospective studies may be of use in determining whether inflammatory markers in childhood are associated with later ED onset and behaviours (e.g., in adolescence). Longitudinal assessments of cytokine concentrations in EDs will also provide the opportunity to explore the mediating and moderating factors of this relationship, using more advanced statistical techniques e.g., mediation analysis and structural equation modelling. Relatedly, it would be important to consider and assess factors preceding and contributing to the inflammation e.g., neuroendocrine regulation, metabolism, diet and the gut microbiome, and negative health behaviours (Bauer & Teixeira, 2018).

Similarly, use of statistical genetics methods, such as Mendelian randomisation, should be used to assess the causal relationship between inflammatory markers and risk for EDs. Mendelian randomisation works on the basis that during human gamete formation, the alleles of any given single-nucleotide polymorphism (SNP) are randomly allocated to egg/sperm cells. This means that inherited genetic variants are independent of potentially confounding environmental exposures as they are 'randomly' allocated to individuals before any exposure or outcome, which has always presented as a problem in epidemiological studies. They are also not subject to reverse causation. Thus, if genetic variants that alter the level of a modifiable biomarker that is causal in disease, then these genetic variants should also be associated with disease risk to the extent predicted by the effect of the genetic variant with the biomarker (Sekula, Del Greco M, Pattaro, & Köttgen, 2016). Research using Mendelian randomisation in related psychiatric disorders has shown that CRP and IL-6 are likely causal risk factors for depression (Khandaker et al., 2019) and that CRP has a protective effect for schizophrenia risk (Hartwig, Borges, Horta, Bowden, & Davey Smith, 2017).

8.4.3 Could inflammatory markers be used as a biomarker or predictor of treatment response in eating disorders?

Previous research, as well as the findings from Chapter 4, suggest that concentrations of certain cytokines do change in patients undergoing treatment for EDs and they may be associated with weight gain and improvements in psychopathology. However, it is unclear whether cytokines may be a biomarker of treatment outcomes. Determining this would be of benefit in the future for personalising treatment. In the depression literature, several studies have shown that different measurements of cytokine and inflammatory markers levels, for example, mRNA expression levels (Cattaneo et al., 2016), in serum (Uher et al., 2014), in plasma (Jha et al., 2017a; Jha et al., 2017b), and using PRS (Zwicker et al., 2018), have promise in predicting response to different antidepressant medications. For example, a review of the literature concluded that a higher baseline pro-inflammatory state was associated with treatment resistance in people with depression (Strawbridge et al., 2015). Therefore, future longitudinal treatment studies in EDs should consider measuring baseline inflammatory markers to establish biomarkers and assess the possible relationship between inflammatory markers and treatment response and outcome.

8.4.4 Should inflammatory markers be researched in relation to specific symptoms, rather than overall diagnosis?

The present research relied on a categorical diagnostic approach to EDs, which considers the illness as being present or absent. This model of diagnostic classification has facilitated reliable clinical diagnosis and psychiatric research for many years. However, these diagnostic categories (based upon presenting signs and symptoms) do not tend to align with findings emerging from clinical neuroscience and genetics research, to be predictive of treatment response, nor capture fundamental underlying mechanisms of dysfunction (Insel et al., 2010). Thus, the US National Institute of Mental Health launched the Research Domain Criteria (RDoC) project to create a framework for research on pathophysiology, which encourages a dimensional approach to the study of psychopathology (Morris & Cuthbert, 2012). Within the RDoC framework, the assessment of inflammatory molecules has been highlighted as a unit of analysis in the study of the construct of loss within the negative valence systems domain (National Institute of Mental Health, 2019). Indeed, the alterations in inflammatory markers investigated in this thesis have been implicated not only in EDs, but also in several other psychiatric disorders, including depression, schizophrenia and PTSD, as discussed in Section 1.3.1.4, suggesting inflammatory dysregulation may be non-specific. Also, certain symptoms are shared across EDs (e.g., binge eating) and other psychiatric disorders (e.g., low mood). Relatedly, it has been highlighted that the investigation of inflammatory profiles using diagnostic labels, rather than focusing on specific symptoms, makes the interpretation and extrapolation of

findings difficult (Baumeister et al., 2014). Consequently, future research in EDs may benefit from assessing cytokines concentrations in relation to specific symptoms, rather than purely based on the diagnostic category, which ultimately may serve to identify subgroups for treatment and to inform evidence-based clinical decision making in future (Insel et al., 2010).

It would be of importance for all future research to measure a broad range of cytokines and inflammatory markers, using sensitive assay kits, in larger samples. Much previous research in EDs has focussed on systemic inflammatory marker concentrations sampled from the blood. Measuring inflammatory markers in different biological samples (e.g., cerebrospinal fluid, whole blood, adipose tissue), in relation to other cells of the immune system (e.g., lymphocytes, monocytes) and at different biological levels (e.g., genetics) may uncover networks and pathways that connect ED-related behaviour and the inflammatory response, and ultimately improve our understanding of the role of inflammatory markers in EDs.

8.5 Overall conclusions

The studies in this thesis investigated inflammatory markers in EDs. Overall, it was found that inflammatory markers were inconsistently altered in EDs and it is unclear as to what factors influence the observed alterations. The research had three main aims: to assess alterations in inflammatory markers in EDs, to examine whether cytokine concentrations change during specialist ED treatment for AN, and to explore whether factors consistently reported to influence cytokine concentrations in healthy populations were associated with inflammatory markers in people with EDs. The first aim was achieved through a meta-analysis and several cross-sectional studies. Elevations of pro-inflammatory cytokines reported in previous studies were not fully replicated in our samples. Measurements of a broad range of inflammatory markers identified novel associations between cytokines and EDs, though these findings were not supported when relevant sociodemographic factors were covaried for. IL-7, MIP-1α, and MIP-1β appear to be promising correlates of AN and were related to patients physical and mental status. Research is needed to confirm these novel findings. The second aim was addressed in an observational longitudinal study. Changes in IL-6 and IL-7 concentrations co-occurred with improvements in AN presentation. This study lends support for the potential role of these cytokines as state biomarkers of AN and treatment response. The third aim was achieved across multiple cross-sectional studies, assessing the association between stress, diet and genetic factors with inflammatory markers in people with EDs. Both traumatic and stressful experiences, and dietary inflammation was not related to concentrations of inflammatory markers in

people with EDs. As these were the first studies to explore the role of additional factors in the relationship between inflammatory markers and EDs, further research is needed to examine these findings. A higher PRS for circulating CRP was associated with BED, although was not related to AN or BN. This finding was supported by elevated concentrations of circulating CRP in people with lifetime BED. This finding highlights a promising avenue for future research. In addition, future research should explore the possible 'immune' subtype in EDs, the potential role of inflammatory markers as a risk factor for EDs and biomarker of treatment response in EDs, and whether altered inflammatory markers are related to specific symptom profiles, rather than diagnoses. The findings from this thesis have advanced our knowledge on the relationship between inflammatory markers and EDs and have provided a foundation for future research.

Chapter 9. References

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Chapter 10. Appendices

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