The effects of obesity on asthma and a randomised controlled trial assessing a total diet replacement programme in people with difficult-to-treat asthma and obesity

A thesis by

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Abstract

In this thesis, three studies are reported in patients with difficult-to-treat asthma. The primary focus is a randomised controlled study assessing a weight management programme in participants with difficult-to-treat asthma and obesity compared to usual care. The other two are retrospective analyses: one, a study to assess if any correlation or relationship exists between obesity and markers of type 2 inflammation in asthma, the other assessing if there is any difference in sleep parameters using wearable technology between participants with mild and difficult-to-treat asthma.

Obesity-associated asthma is a complex phenotype on the rise often characterised by more difficult-to-treat disease, increased morbidity and mortality and great economic burden. Previous studies assessing weight loss in asthma suggest a benefit in asthma control and quality of life but have issues with heterogeneity in populations and interventions studied, lack controls or robustness. In excess, adipose organ dysfunction results in an imbalance in adipokine-mediated pro-inflammatory and anti-inflammatory markers favouring airway inflammation and hyperresponsiveness. Additionally, a few studies have suggested an inverse effect of increasing obesity on markers of type 2 inflammatory markers potentially limiting their use in this cohort. Complicating matters, sleep breathing disorders, common in obesity, such as obstructive sleep apnoea syndrome are known to increase asthma exacerbation severity. Robust and well-constructed trials assessing conservative options for weight management in people with obesity and asthma are needed. The Counterweight-Plus weight management programme is one such option, with an evidence base in type 2 diabetes mellitus. We hypothesised that use of this dietitian-supported total diet replacement weight management programme would result in improved asthma control and quality of life in people with difficult-to-treat asthma and obesity.

We performed a single-centre, open-label randomised controlled trial assessing the Counterweight-Plus programme (CWP) against usual care (UC) in people with difficult-to-treat asthma and obesity. This programme entails a 12-week c850 calorie/day total diet replacement phase followed by a food re-introduction and then weight maintenance phase up to one-year with dietitian input. Follow-up visits occurred at 16-weeks (the primary outcome) and 52-weeks. Primary

outcome assessed the change in Asthma Control Questionnaire (ACQ6) scores at 16-weeks between groups and key secondary outcomes included change in Asthma Quality of Life (AQLQ) scores at 16-weeks, comparing proportions of people experiencing minimal clinically important difference (MCID) in ACQ6 and AQLQ, and assessing these outcomes again at one-year.

Thirty-three participants attended at 16-weeks for primary outcome assessment. Weight-loss was greater with CWP compared to UC (mean difference –12kg [95%CI –17, -7kg]; p < 0.001). ACQ6 and AQLQ scores improved with CWP compared to UC (mean difference –0.7 [95%CI –1.4, 0.0], p = 0.048; mean difference 0.8 [95%CI 0.2, 1.3], p = 0.013 respectively) and a greater proportion of participants achieved MCID in ACQ6 with CWP and UC (53 vs 19%, p = 0.041) at 16-weeks.

Twenty-nine participants attended at 52-weeks with weight-loss sustained with CWP (median weight change –14kg [IQR –15, -9kg]) compared to UC (median 2kg [IQR –7, 8]; p = 0.015). The 53% achieving MCID in ACQ6 at 16-weeks sustained this at 52-weeks (compared to UC 25%, p = 0.101). A higher proportion of participants achieved MCID in AQLQ with CWP compared to UC (71 vs 6%, p < 0.001), including AQLQ symptom domain (71 vs 31%; p = 0.024), activity domain (53 vs 19%; p = 0.041) and environmental domain (65 vs 19%; p = 0.008). Furthermore, CWP resulted in a reduction in number of prednisolone courses from 4 (IQR 2, 5) at baseline to 0 (0, 2) at 52-weeks (p<0.001).

Interpretations from this data are limited by considerable missing data, primarily because of the COVID-19 pandemic restrictions. No significant conclusions can therefore be drawn regarding effects of weight loss on lung function, markers of inflammation or activity levels. The study was underpowered at the one-year time-point limiting conclusions about effects of weight loss on asthma control and quality of life.

Additionally, we performed a retrospective analysis assessing the effects of obesity on type 2 biomarkers in mild (n = 51) and difficult-to-treat (n = 102) asthma from two datasets of recent in-house trials. We assessed body mass index (BMI) against fractional exhaled nitric oxide (FeNO) and peripheral eosinophil count. When stratified by BMI tertile, we observed reduced FeNO levels in the highest BMI tertile compared to the lowest (18 vs 25ppb respectively, p = 0.014)

and, within the difficult-to-treat group only, reduced eosinophils in the highest BMI tertile compared to the lowest ($0.2x10^9$/L vs $0.3x10^9$/L respectively; $p = 0.020$). Adjusted linear regression (corrected for age, sex, smoking status, atopic status, rhinitis and both inhaled and oral corticosteroid use) showed BMI was a predictor of FeNO ($\beta = -2.848$, $p = 0.019$). Interpretation of these results must be with caution as numerous limitations must be acknowledged including unequal weighting of groups, possible effects of both confounder and collider bias, and the retrospective nature of this study. A dedicated prospective trial is warranted based on these findings.

Accelerometers have been validated against polysomnography to assess sleep metrics in general and asthma populations. We hypothesised that accelerometer-derived sleep parameters would differ between healthy BMI mild and obesity-associated difficult-to-treat asthma groups and conducted a retrospective analysis on overnight accelerometer data derived from two recent in-house trials. Participants in these trials wore accelerometer devices twenty-four hours a day for seven days. Data from 124 participants (80 difficult-to-treat, 24 mild asthma) showed broadly comparable results in sleep window time, sleep time, sleep efficiency and wake onset time with a clinically unclear difference of roughly forty minutes in median sleep onset time. Results were also similar to general population results from previous studies. This retrospective study also had unequal weighting of groups, lacked corroboration from sleep diaries or objective assessments of sleep quality or sleep-disordered breathing, limiting its clinical effectiveness.

In summary, effects of obesity on asthma are not fully understood, in particular with relevance to airway inflammation and type 2 biomarkers. Use of a total diet replacement weight management programme results in improved asthma control and quality of life in the short term with encouraging longer-term signals in asthma quality of life and frequency of exacerbation. Further research is needed to elucidate mechanisms underlying obesity-mediated asthma and to assess effects of this weight management programme on lung function and inflammation. A larger sample size is needed to definitively assess effects of the Counterweight Plus programme on asthma outcomes at one-year.

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V Sharma, HC Ricketts, F Steffensen, A Goodfellow and DC Cowan. **Obesity affects type 2 biomarker levels in asthma**. *Journal of Asthma* 2022, 60(5):1-11

V Sharma, HC Ricketts, L McCombie, N Brosnahan, L Crawford, L Slaughter, A Goodfellow, F Steffensen, DS Buchan, R Chaudhuri, MEJ Lean and DC Cowan. **A total diet replacement weight management program for difficult-to-treat asthma associated with obesity: a randomized controlled feasibility trial**. *CHEST* 2023, 163(5):1026-1037

V Sharma, HC Ricketts, F Steffensen, A Goodfellow, DS Buchan and DC Cowan. **Accelerometer-derived sleep metrics in mild and difficult-to-treat asthma**. *Allergy, Asthma and Clinical Immunology* 2024, 20(1):5

V Sharma, HC Ricketts, L McCombie, N Brosnahan, L Crawford, L Slaughter, A Goodfellow, F Steffensen, R Chaudhuri, MEJ Lean and DC Cowan. **A one-year weight management programme for difficult-to-treat asthma and obesity: a randomised controlled study**. Submitted for publication 2024

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V Sharma, HC Ricketts, F Steffensen, A Goodfellow and DC Cowan. **P208 Does obesity affect fractional exhaled nitric oxide interpretation in difficult asthma?** *British Thoracic Society Winter Meeting*, Virtual 2021

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V Sharma, HC Ricketts, L McCombie, N Brosnahan, L Crawford, L Slaughter, A Goodfellow, F Steffensen, R Chaudhuri, MEJ Lean and DC Cowan. **A randomised controlled trial of the effects of a total diet replacement programme in uncontrolled asthma associated with obesity**. *European Respiratory Society International Congress*, Barcelona 2022

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I have been very fortunate during my period of research and have many people to thank for this. I am indebted to Dr Douglas Cowan who has acted as supervisor and mentor. Beyond asthma and airways disease, I have learnt more about respiratory medicine from him than anywhere else and am grateful to him for his logical and pragmatic approach that has helped give me focus and confidence in my own practice. Grateful I am also to Dr Clare Ricketts who aided in patient recruitment during the initial phase as my predecessor, helping lay the groundwork for my project.

This project would not exist without the pioneering work of Professor Mike Lean who has championed the intervention in this trial benefitting so many patients. Beyond this, his expert guidance and wealth of experience has been invaluable throughout.

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I am forever grateful to the participants of the trial themselves, who have trusted us and given up their time and effort in the name of clinical science.

The significance of the time period in which this research was conducted should be acknowledged. Effects of the COVID-19 pandemic pulsed through every aspect of our society and unexpected, and frequently difficult challenges arose as a result. I appreciate how fortunate I am to have come through this time with all my loved ones.

I am grateful to my friends. My second sisters. They know who they are.

I am eternally thankful to my family who have been my biggest supporters from putting me through school and then university and keeping me grounded. My niece Aaradhya provided welcome humour. My brother-in-law Rajeev always welcoming and providing comfort. My brother Gaurav and sister Sonia have acted as second parents, guiding me throughout my life. My father Prabhu has instilled in me a strong work ethic and sense of independence. My mother Janak who has a quiet strength that I fail to aspire to every day.

My son Fintan was born two weeks before my research period began. He was born in a time of national restrictions and lockdowns. I thank him especially for distracting me during some of the most difficult times I have encountered and for helping me understand my priorities. And for his infectious laugh.

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Declaration

I am the sole author of this thesis and have consulted all references personally. The primary trial for this thesis was devised and set-up by Dr Douglas Cowan with input from Prof Mike Lean and Prof Rekha Chaudhuri. I recruited most participants for the trial in this thesis, with early assistance from Dr Douglas Cowan and Dr Clare Ricketts. Study visits and data collection were conducted by myself in conjunction with specialist research nurses at the Glasgow Royal Infirmary Clinical Research Facility, Femke Steffensen and Anna Goodfellow. I managed the day-to-day running of the trial. Dr Duncan Buchan manipulated the accelerometery data into tangible variables for analysis by myself, a process described in detail in the relevant chapters. A team of trained dietitians (Louise McCombie, Naomi Brosnahan, Luisa Crawford, and Lesley Slaughter) delivered the intervention package with input as needed from Prof Mike Lean, Dr Douglas Cowan and myself where clinical or trial conduct queries arose. Data for the retrospective analyses described in Chapters Five and Six were obtained from two in-house trials; firstly, from some of the weight management participants described in this thesis, and secondly from a recent trial of pulmonary rehabilitation in patients with difficult-to-treat asthma (alongside a sub-study of activity levels in mild and difficult-to-treat asthma participants). The latter trial was the focus of my colleague Dr Clare Ricketts' research. The analyses throughout this thesis were conducted personally.

This thesis has not previously been submitted for a higher degree.

Abbreviations

6MWD	Six-minute walk distance
6MWT	Six-minute walk test
95%CI	95% confidence interval
ACAT-1	Acyl-coenzyme A: cholesterol acyltransferase 1
ACQ	Asthma control questionnaire
AdipoR1	Adiponectin receptor 1
ADMA	Asymmetric di-methyl arginine
AE	Adverse event
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
AQLQ	Asthma quality of life questionnaire
ATS	American Thoracic Society
BAT	Brown adipose tissue
BD	Bronchodilator
BDP	Beclomethasone dipropionate
BMI	Body mass index
BTS	British Thoracic Society
CCL2	CC-chemokine ligand 2
COPD	Chronic obstructive pulmonary disease
COVID-19	Severe acute respiratory syndrome coronavirus 2 disease
CRF	Case report form
CRP	C-reactive protein
CRT	Clinical research team
CWP	Counterweight-Plus programme
CXCL	Chemokine (C-X-C motif) ligand
EAT	Epicardial adipose tissue
ED	Emergency department
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
ENMO	Euclidean Norm Minus One

ERS	European Respiratory Society
ERV	Expiratory reserve volume
FeNO	Fractional exhaled nitric oxide
FEV_1	Forced expiratory volume in 1 second
FRC	Functional residual capacity
FVC	Forced vital capacity
GINA	Global Initiative for Asthma
GLP-1	Glucagon-like peptide 1
GM-CSF	Granulocyte-macrophage colony-stimulating factor
GORD	Gastro-oesophageal reflux disease
GP	General Practitioner
GRI	Glasgow Royal Infirmary
HADS	Hospital anxiety and depression scale
HRA	Health Research Authority
ICS	Inhaled corticosteroids
ICU	Intensive care unit
IFN-γ	Interferon-gamma
IgE	Immunoglobulin-E
IL	Interleukin
ILC	Innate lymphoid cells
iNOS	Inducible nitric oxide synthase
LABA	Long-acting anti-muscarinic
LPA	Light physical activity
LRA	Leukotriene receptor antagonist
MAb	Monoclonal antibody
MAR	Missing at random
MCAR	Missing completely at random
MCID	Minimal clinically important difference
MCP-1	Monocyte chemoattractant protein 1
MDI	Metered dose inhaler
MKP-1	mitogen-activated protein kinase phosphatase 1

MMP	Matrix metalloprotease
MRC	Medical Research Council
MVPA	Moderate-vigorous physical activity
NFκB	Nuclear factor kappa B
NLRP3	Nucleotide-binding domain leucine-rich repeat and pyrin domain containing receptor 3
NNT	Number needed to treat
NO	Nitric oxide
OAA	Obesity-associated asthma
OCS	Oral corticosteroids
OHS	Obesity hypoventilation syndrome
OR	Odds ratio
OSAS	Obstructive sleep apnoea syndrome
PEFR	Peak expiratory flow rate
PIS	Patient information sheet
PVAT	Peri-vascular adipose tissue
RAST	Radioallergosorbent test
REC	Research Ethics Committee
SABA	Short-acting beta-2 agonist
SAE	Serious adverse event
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SIGN	Scottish Intercollegiate Guidelines Network
SST	Serum separator clot activator
T2	Type 2 inflammation
TDR	Total diet replacement
Th2	T helper 2 cell
TNF	Tumour necrosis factor
TSLP	Thymic stromal lymphopoeitin
UC	Usual care
UKSAR	UK Severe Asthma Registry
VCAM-1	Vascular cell adhesion molecule 1
WAT	White adipose tissue

WC	Waist circumference
WHO	World Health Organisation
WtH	Waist-to-hip ratio
WtHt	Waist-to-height ratio

Chapter One: Introduction

1.1 Asthma

1.1.1 Introduction

Despite advances in technology with the digital revolution and deeper understanding of the clinical heterogeneity of airway diseases, there remains no gold standard test to diagnose asthma and incomplete appreciation of this syndrome. Highlighting this, a previous study showed that a third of patients with physician-diagnosed asthma did not have evidence of current asthma on full assessment [1]. Among the severe asthma patients, and indeed increasingly prominent, are the obesity-associated asthma (OAA) population that have become a large proportion of any secondary/tertiary asthma specialist clinic burden in the Western world. Furthermore, whilst asthma-related co-morbidities such as chronic rhinitis and gastro-oesophageal reflux form part of the so-called "treatable traits" management plan in order to improve asthma-specific outcomes, in most specialist asthma centres around the UK there remains inadequate treatment of the treatable trait of obesity. In the US, among patients with severe asthma, 58% have obesity [2], and 11% of patients with obesity have asthma [3].

The mystery surrounding asthma is deepened by ever-changing definitions of the disease aimed at characterising as well as laying the framework for diagnosis of asthma. Current guideline definitions vary but include the presence of characteristic variable symptoms and evidence of either reversible airflow obstruction or bronchial hyper-reactivity. These definitions are of limited value as they do not offer insight into asthma phenotypes or endotypes nor allow for individualisation of asthma therapy based on presence of co-morbidities or biomarkers; neither do they address disease overlap with other airway diseases such as chronic obstructive airway disease (COPD) or bronchiectasis. On a pathological level, there is evidence of chronic airway inflammation which, in its severest form, can be refractory to treatment. Management of this often-debilitating condition, is aimed at reducing hospital admissions and length-of-stay, reducing acute asthma exacerbations, controlling symptomology and/or improving quality of life.

For the purposes of this thesis, definitions from the Global Initiative for Asthma (GINA) [4, 5] and British Thoracic Society (BTS)/Scottish Intercollegiate Guidelines Network (SIGN) [6] guidelines have been used:

- Asthma – "Asthma is a heterogeneous disease, usually characterized by chronic airway inflammation. It is defined by the history of respiratory symptoms such as wheeze, shortness of breath, chest tightness and cough that vary over time and in intensity, together with variable expiratory airflow limitation."
- Uncontrolled asthma – Either poor symptom control (characterised by recurrent reliever inhaler use, activity limitation or nocturnal awakening due to symptoms) or excessive acute asthma exacerbations (two or more per year treated with oral corticosteroids (OCS), or one or more per year leading to hospital admission). Reliever use more than twice in one week is considered excessive and is indicative of poor symptom control.
- Difficult-to-treat/difficult asthma – uncontrolled asthma whilst prescribed step 4 or above asthma treatment as per GINA guidelines.
- Severe asthma – Uncontrolled asthma whilst adherent to optimal therapy and appropriate concurrent asthma-related co-morbidity treatment.

The majority of disease can be controlled by adherence and correct use of mainstay treatments such as inhaled corticosteroids (ICS) and only a small proportion of patients fit the severe asthma category. The exact prevalence of difficult-to-treat asthma is unclear but may be as high as 17% of all people with asthma, with severe asthma around 5% [5].

Asthma remains a global health concern with an estimated 339 million people affected worldwide in 2016 [7]. The UK performs poorly with regards to asthma mortality rates when compared to other wealthy European countries [8] and a recent national review of asthma deaths revealed poor all-round asthma care and major avoidable factors in the majority of deaths [9]. The economic burden is also significant with estimated costs of one billion pounds per year in the UK, of which around eighty percent is spent on those with severest disease [10].

1.1.2 Pathophysiology

1.1.2.1 Progression of inflammatory changes in asthma

Pathologically, asthma is characterised by airway inflammation caused by inhalation of either allergens or noxious insults, affecting large but more so distal airways [11]. As a result, there is mucosal oedema, mucus hypersecretion and airway smooth muscle constriction resulting in expiratory airflow disruption that classically clinically manifests as variable wheeze, cough, chest tightness and dyspnoea. In mild or early disease this can be completely reversible with appropriate treatment however can progress and develop into a chronic inflammatory state that may result in fixed airflow limitation on spirometry. This chronic phase is characterised by airway remodelling resulting from bronchial smooth muscle thickening, difficulty with mucus clearance, a more complex luminal and sub-luminal inflammatory milieu, and sub-epithelial fibrotic changes [12]. Figures 1.1 (adapted from Doeing and Solway [13]) and 1.2 (adapted from King et al [14]) summarise the pathological changes.

Figure 1. 1 - Diagrammatic comparison of a normal airway (left) with an airway in chronic asthma (right) showing airflow obstruction from intraluminal mucus, inflammation and airway remodelling

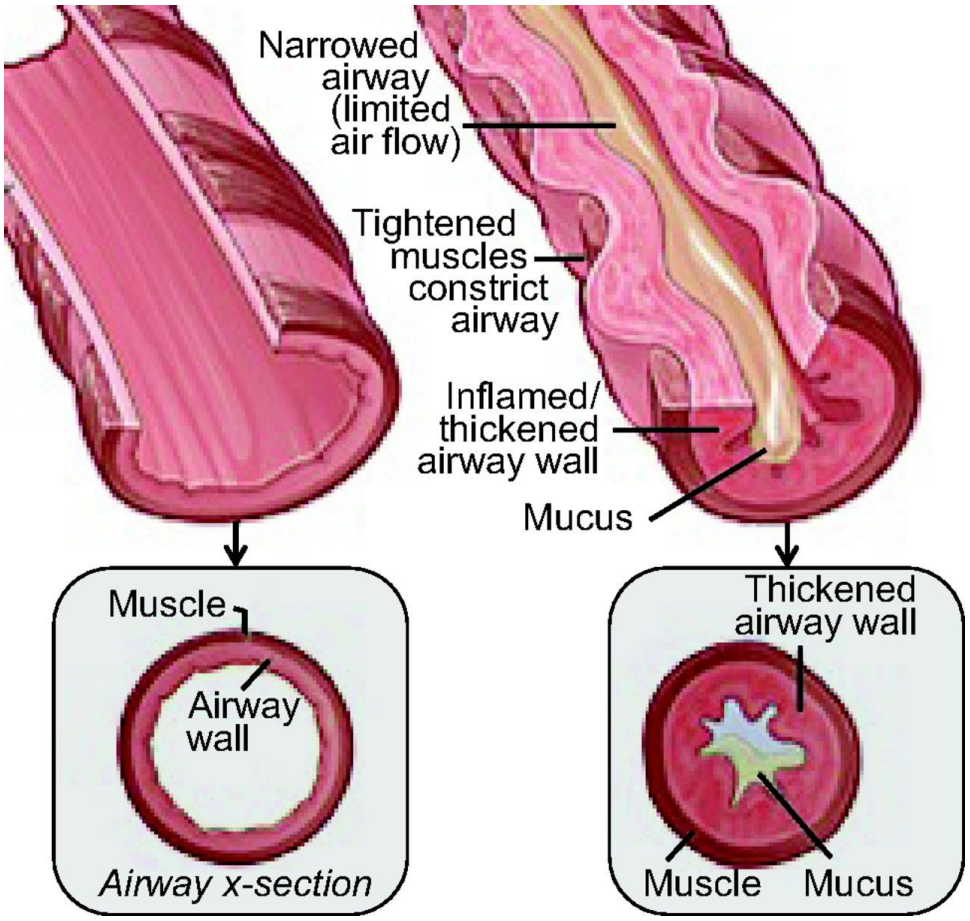
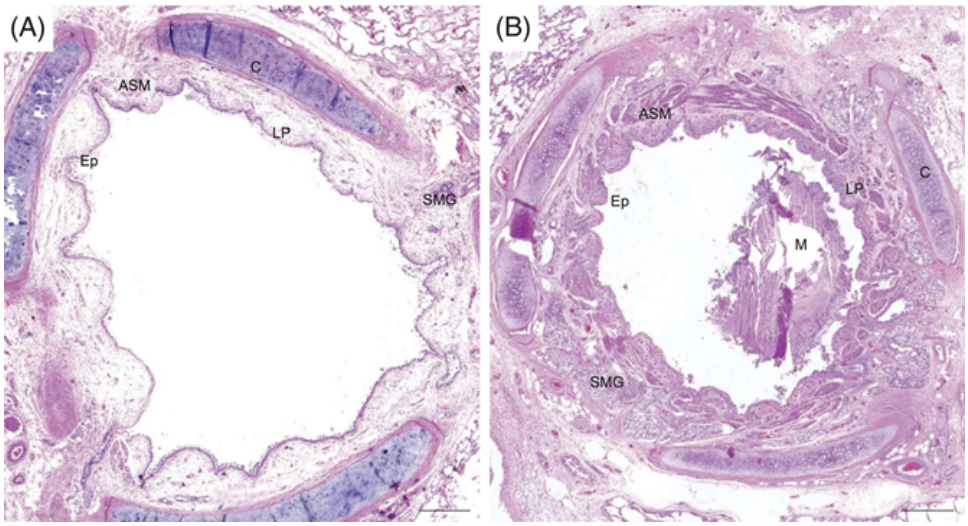


Figure 1. 2 - Pathology slides from large airways from a healthy subject without asthma (A) and post-mortem from severe asthma (B), showing enlarged airway smooth muscle (ASM), submucosal mucous glands (SMG), lamina propria (LP) inflammation and significant mucus (M)



Acute exacerbations with bronchoconstriction result in even narrower airway lumens and resultant obstructive lung disease that can be severe or life-threatening.

This is a somewhat simplified summary of inflammatory airway changes associated with asthma however more recent insights into phenotypes and endotypes have provided a greater level of detail.

1.1.2.2 Phenotype versus endotype

The term “asthma” was previously used to describe a singular clinical condition however this way of thinking is now obsolete, and asthma is considered an umbrella term used to describe a heterogeneous collection of airway conditions. Until recently, these were divided by observable clinical characteristics – phenotypes. Initially this comprised primarily of two main categories, atopic and non-atopic asthma with further delineation by attributes such as frequency of exacerbations, age of onset, airflow limitation, triggers (including drugs or exercise), inflammatory cell profile and indeed, presence of obesity. Substantial overlap exists using this method and asthma populations cannot be subcategorised effectively in this manner.

To characterise patients further, underlying molecular processes have been studied, particularly using cluster cohort methodology, to identify distinguishable pathophysiological mechanisms separating asthma into endotypes. By doing so, our collective understanding of the intricate nature of asthma grows allowing meaningful change to treatment with a focus on precision medicine. Targeted treatments have resulted from this imparting a clinical and socioeconomic benefit to asthma sufferers worldwide. Two main endotypes are currently appreciated: type 2 (T2)-high and T2-low (or non-T2).

1.1.2.3 T2-high asthma

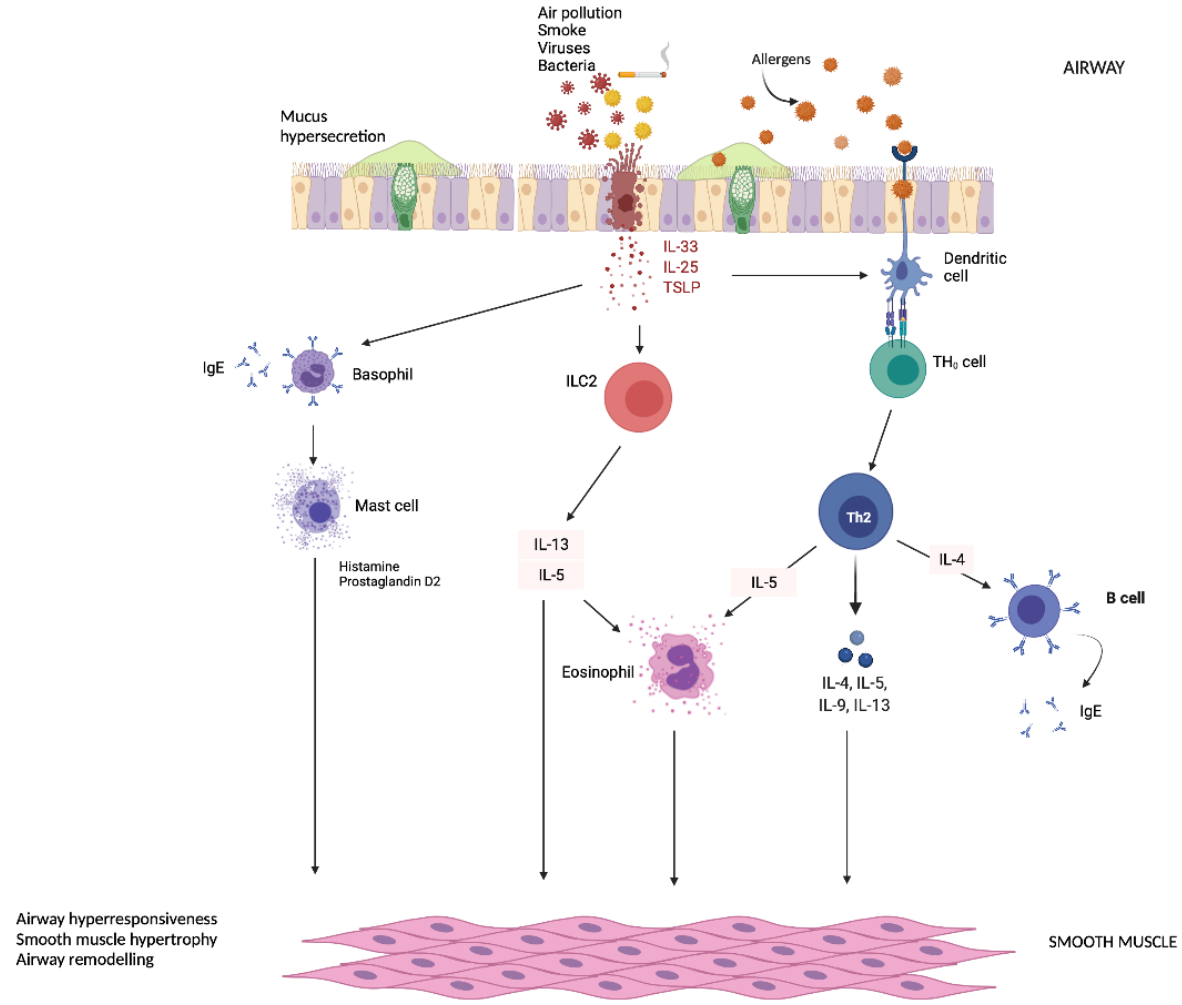
The T2-high endotype is characterised by overlapping adaptive and innate responses to an external stimulus, classically an allergen but may also be an air contaminant or pathogen (Figure 1.3). Clinically, biomarkers are used to

categorise patients as T2-high (e.g., eosinophils >0.15x10^9/L, FeNO >25 ppb). The inhaled exogenous trigger provokes an inflammatory cascade in the airway epithelium either by causing cytokine secretion (so-called “alarmins” - Interleukin (IL)-25, IL-33 and thymic stromal lymphopoeitin, TSLP), or via direct stimulation of dendritic cells (antigen-presenting cell type); note that TSLP release can also indirectly engage dendritic cells [15]. From here there is stimulation of basophils and mast cells, innate lymphoid type 2 (ILC2) cells, T-helper 2 (Th2) and B cells with subsequent IgE and downstream cytokine production promoting a histamine-mediated and/or eosinophilic inflammatory response in the bronchioles.

Key T2-high cytokines released in response to alarmin-mediated cell stimulation include IL-4, IL-13, IL-5 and IL-9. IL-4 and IL-13 are structurally similar and act on IL-4Rα receptors causing numerous effects in T2-inflammation such as IgE class-switching of plasma cells, promoting eosinophil chemotaxis and adhesion, inducing goblet cell hyperplasia, stimulating inducible nitric oxide synthase (iNOS) in airway epithelium (producing NO), increasing bronchial hyperresponsiveness, enhancing smooth muscle hyperplasia and increasing myofibroblast-mediated collagen deposition [16, 17]. IL-5 is incriminated in eosinophil transit and survival [18], and IL-9 results in increased airway hyperresponsiveness, goblet cell proliferation, mast cell survival and fibroblast stimulation [19, 20].

The majority of asthma is T2-high and tends to be steroid-responsive.

Figure 1. 3 - Summary of T2-high inflammatory pathways



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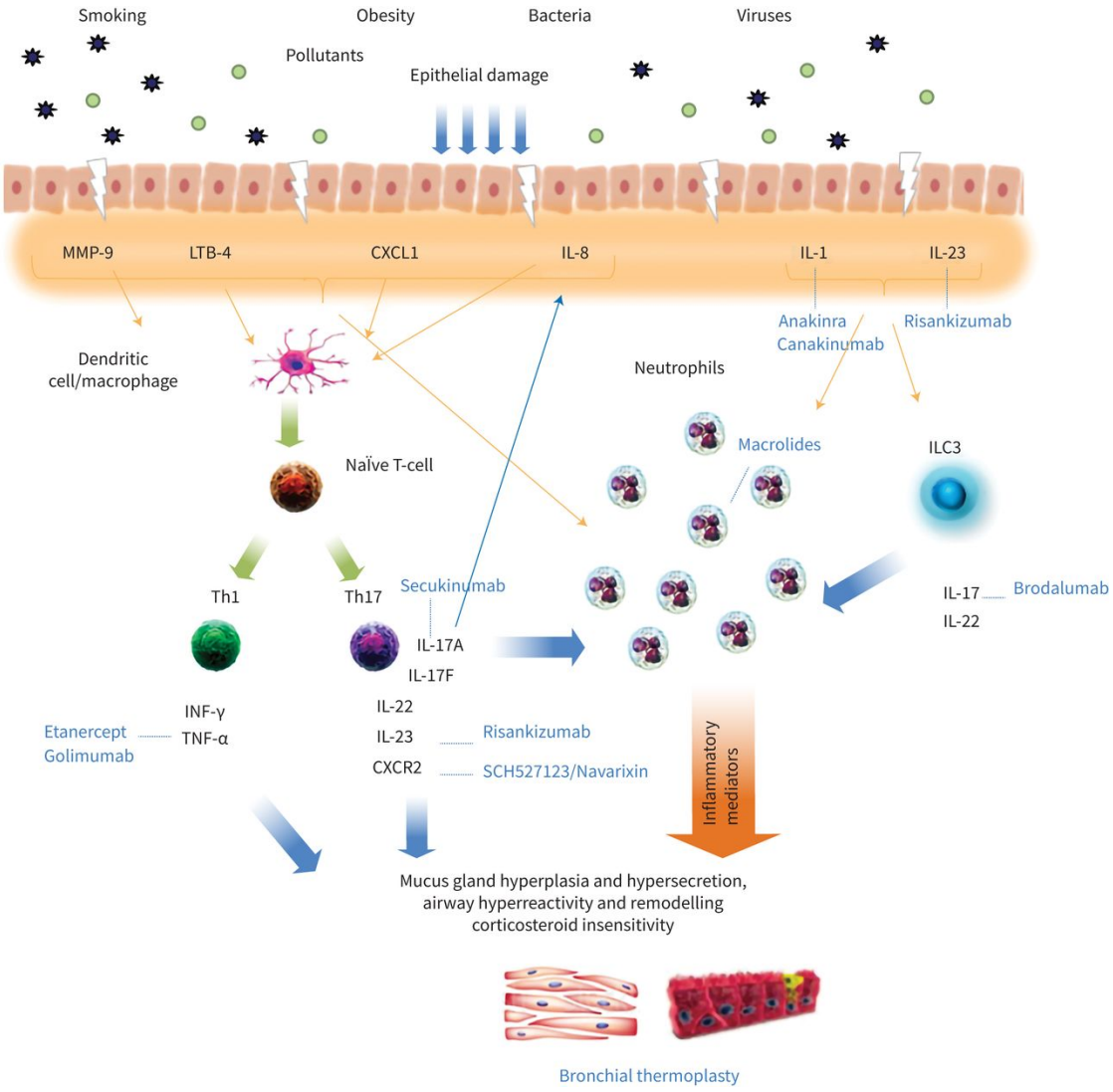
1.1.2.4 T2-low asthma

Formal definition of T2-low asthma remains elusive complicating our ability to formally quantify prevalence in a given population. It is widely appreciated to be an endotype that lacks any biomarkers of T2-high inflammation (I.e. FeNO <25 ppb, eosinophils $<0.15x10^9/L$), though this methodology is flawed as biomarkers are influenced by other variables, such as glucocorticoid use (the biggest contributor to FeNO and eosinophil suppression), and overlap can exist between T2-high and T2-low pathways. There is ongoing study into the T2-low endotype, but current understanding is summarised in Figure 1.4 (Adapted from Kyriakopoulos et al [21]).

Figure 1. 4 - T2-low inflammatory pathways and potential targeted therapies (highlighted in light blue)



T2-low asthma is associated with a neutrophilic or paucigranulocytic inflammatory profile, as well as some forms of the OAA phenotype [21]. In health, sputum analysis has shown neutrophils account for 30-40% of airway cells [22], whilst a neutrophilic asthma phenotype has been identified using similar methods displaying neutrophil percentages >60% [23]. The prevalence of neutrophilic asthma remains controversial with approximate values varying from 20 - 46% reported [22, 24]. This disparity may in part be due to the known effects of factors such as smoking, corticosteroid use and pollution leading to airway neutrophilia [25-27]. Neutrophilic asthma has been linked with more severe disease, higher treatment burden and reduced response to corticosteroids [28, 29]. Furthermore, OAA has been linked with higher counts of airway neutrophils and this link may account for the poorer response to treatment often seen in OAA [30, 31].

As well as phagocytosis, neutrophils act by degranulation of reactive oxygen species and cytotoxic proteases (including neutrophil elastase, myeloperoxidase and MMPs) which stimulate localised inflammation [32, 33]. These effects are upregulated in neutrophilic asthma with increased neutrophil migration and activation seen in the lung [34], alongside increased secretion of pro-inflammatory peptides [35]. The upshot is increased bronchial inflammation, bronchoconstriction and airway hyperresponsiveness [36, 37].

The stimuli for T2-low pathways are not typically allergens but more noxious insults including environmental factors such as tobacco smoker, air pollutants and pathogens. Activation of the nucleotide-binding domain leucine-rich repeat and pyrin domain containing receptor 3 (NLRP3) intracellular epithelial inflammasome has been implicated, leading to pro-inflammatory cytokine release (e.g., IL-1ß) [38]. Additionally, epithelial release of potent proteins such as matrix metalloprotease (MMP)-9, CXCL1 (a key chemokine), IL-8 and IL-23 result in either macrophage-mediated differentiation of T-cells into Th1 and Th17 cells and/or ILC3 cell stimulation [21]. These cells directly upregulate production of inflammatory modulators such as Tumour necrosis factor (TNF) α, interferon-gamma (IFN-γ), IL-17 and IL-22 and recruit and stimulate neutrophil-

mediated inflammation [39]. Th17 cells are stimulated by IL-23, itself produced by airway dendritic cell stimulation [40]. Subsequently, Th17 cells secrete IL-17 which has a basis in neutrophil recruitment, smooth muscle contraction, airway remodelling and possibly steroid-resistance [40-42]. T2-low asthma is therefore typically non-atopic, non-eosinophilic and poorly steroid-responsive [28]. Whilst the alarmins stimulate dendritic cells, ILC2 cells, macrophages, eosinophils and mast cells potentiating a T2 inflammatory response, research suggests that TSLP is implicated in T2-low disease. Evidence has shown Th17 cell differentiation in response to TSLP [43]. Furthermore, trials of anti-TSLP Mab therapy (tezepelumab) have shown efficacy in patients with low eosinophil counts, as well as T2-high disease, though this benefit appears minimal compared to T2-high disease [44].

1.1.3 Management strategies in chronic asthma

1.1.3.1 Precision medicine

Traditionally, management strategies in asthma were centred around patient symptom burden, lung function and reliever use [45]. However, this method alone is flawed as clinical judgement and lung function poorly predict the presence of active airway inflammation [46, 47] and therefore, identifying those at risk of severe asthma exacerbation, or even a fatal attack, proved difficult. A wealth of asthma research over the last two decades has led to significant changes in our approach to investigation and management with more onus on individualised care based on asthma endotypes, and the availability of MAb therapy. Much more emphasis now is placed on precision medicine in airways disease with the recognition that asthma is heterogeneous and managing aspects of the disease rather than sticking to a “one size fits all” ideology provides better care [48]. This is the basis of identifying and modifying asthma-related co-morbidities and treatable traits.

1.1.3.2 Treatable traits

The identification and modification of separate traits in airways disease is a novel approach to asthma management and one that moves towards delivering tailored treatments to the right subgroups. These traits can be comprised of

either specific phenotypes (e.g., recurrent infective exacerbators, exercise-induced disease), endotypes (e.g., type 2 high inflammatory profile) or asthma-related comorbidities (such as nasal polyps). Uncontrolled presence of these traits increases asthma-related morbidity.

Treatable traits can be thought of as pulmonary (including airflow limitation, cough hypersensitivity, recurrent infections, presence of eosinophilic airway inflammation, mucus hypersecretion), extra-pulmonary (outlined in Table 1.1, adapted from Pavord et al [48]) and behavioural or environmental (e.g., smoking, occupational exposure, treatment adherence).

Table 1. 1 - Extra-pulmonary treatable traits

Comorbidity	***Suggested treatment***
Rhinitis/rhinosinusitis	Nasal steroids, antihistamines
Nasal polyps	Polypectomy
Gastro-oesophageal reflux	Proton pump inhibitors, H2-receptor antagonists
Anxiety/depression	Counselling, anti-depressants
Dysfunctional breathing or inducible laryngeal obstruction	Physiotherapy, speech therapy, breathing pattern retraining
ACE-inhibitors	Removal of treatment or alternative (e.g., ARB)
Obesity	Weight loss

Satisfactory treatment of these traits, alongside asthma-specific management, improves asthma outcomes. By effectively deconstructing the label of asthma into these traits, appropriate treatments and lack of inappropriate treatments can be assured, and dedicated research can evolve to tackle areas where effective treatments are lacking.

1.1.4 Discussion

Asthma is complicated. Incomplete understanding of inflammatory pathways, disease heterogeneity, paucity of specific biomarkers and presence of numerous treatable traits continue to make difficult-to-treat or severe asthma a challenge to the specialist. Recent advances identifying new endotypes have led to a variety of advanced therapies but their ability to control disease is not unlimited nor without cost. Addressing treatable traits may curtail the need to consider advanced therapy altogether however these traits themselves are not fully understood, nor their relationship to asthma fully elucidated, and treatment options may be limited. Obesity is one such trait. Arguably the biggest threat to wealthy healthcare systems, the management of obesity remains elusive. The relationship between obesity and asthma is explored in the remainder of this thesis.

1.2 Obesity

1.2.1 Epidemiology and burden

In 2021, the WHO published a report stating that, globally, 650 million adults are obese [49]. The obesity epidemic threatens becoming a pandemic, as obesity prevalence continues to rise worldwide including in Europe, North America, Australia and Korea. Moreover, an extensive simulation published in the Lancet [50] predicts a profound and deepening impact on health consequences in the UK/USA by 2030 with projected numbers of an extra 11 million obese adults in the UK and estimates of £2 billion annual costs for healthcare. Obesity penetrates all aspects of our society causing a substantial economic burden, reducing life expectancy and increasing loss of productivity, preventable diseases, disability, mental health disorders, fertility and congenital abnormalities amongst others [50-52]. These preventable diseases include cardiovascular disease, type 2 diabetes mellitus, malignant disease, non-alcoholic fatty liver disease and indeed, asthma [50, 53]. Finally, obesity is independently associated with increased morbidity and mortality in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) disease, COVID-19 [54-57]. It is the relationship between obesity and difficult-to-treat asthma that is explored in this thesis.

1.2.2 Adipose “tissue”

1.2.2.1 Composition

Adipose tissue is complex and not fully understood. It is more than a passive energy source and behaves more like an organ than a tissue; the adipose organ has intricate paracrine, endocrine and autocrine functions and a role in nutrition and inflammation homeostasis. For simplicity, despite the misnomer, the rest of this thesis will refer to this organ as adipose tissue. Adipose tissue varies within the body and can comprise of one or more different types of adipocytes [58]:

- Brown adipocytes, so-called due to their microscopic brown appearance caused by a large number of intracellular mitochondria interspersed by several small lipid droplets. A collection of brown adipocytes forms brown adipose tissue (BAT)

- White adipocytes which appear microscopically pale due to the presence of a singular large lipid droplet and sporadic mitochondria. These accumulate to form white adipose tissue (WAT)
- Beige adipocytes which have a microscopic appearance midway between brown and white adipocytes. They contain more mitochondria and lipid droplets than white adipocytes, though fewer than brown cells. Beige adipocytes form beige adipose tissue.

Study into adipose tissue continues and recently discovered forms of adipose tissue have been identified including peri-vascular adipose tissue (PVAT) and epicardial adipose tissue (EAT), both of which can appear similar to both BAT and WAT for reasons that are yet be fully elucidated [59, 60]. As well as adipocytes, adipose tissue is made up of mesenchymal cells, pre-adipocytes, blood and lymph vessels, nerves and inflammatory cells including macrophages and lymphocytes [61]. The inflammatory cellular makeup of adipose tissue appears to vary based on weight, for example, a greater abundance of macrophages has been observed in adipose tissue of individuals with obesity than in their lean counterparts [62].

1.2.2.2 Brown adipose tissue

Within the human body, BAT is found in various locations including supraclavicular, cervical, mediastinal, peri-renal, interscapular and sub-scapular regions [60]. BAT has auto- and paracrine effects such as secretion of cytokines (called adipokines) promoting vascular and nerve growth and promoting brown adipocyte recruitment. As well as this, BAT has local roles with non-shivering thermogenesis, glucose and lipoprotein metabolism, and systemic effects including possible skeletal muscle metabolic effects and metabolic cytokine secretion, for example insulin-like growth factor 1 [59, 63]. Non-shivering thermogenesis is thought to be the primary role of BAT and is a vital anthropological mechanism for adapting to cold environs.

1.2.2.3 White adipose tissue

WAT forms the bulk of fatty deposits in adults and is primarily found in subcutaneous and visceral fat [60]. Its primary role is in energy conservation, the large lipid droplet acting as an energy repository that can be called upon during high levels of energy consumption, but it also serves as insulation in lower temperatures. However, like BAT, it plays a role in inflammation homeostasis, with secretion of key adipokines as described in detail below.

1.2.2.4 Beige adipose tissue

The role of beige adipose tissue remains incompletely understood. It may represent heterogeneity of adipose tissue in general, lying in a spectrum between WAT and BAT. It has been found primarily within WAT deposits and has both thermogenic and energy reserve functions at the least [60, 63]. In response to stimuli, such as cold temperature or cytokine effects, WAT composition can change to form beige adipocytes in a process called “browning” of white fat. This is presumably another homeostatic mechanism designed to shift the balance of white to brown fat in response to the current stimulus. Further study is ongoing in this area.

1.2.2.5 Peri-vascular (PVAT) and epicardial adipose tissue (EAT)

As the name suggests, PVAT surrounds blood vessels and has a role in vascular tone homeostasis, which may have profound implications for cardiovascular disease, particularly hypertension. Composition of PVAT is variable and has been found to be similar to both BAT and WAT and has a role in thermogenesis and metabolism also [59, 60, 64].

EAT covers most of the surface of the heart and is situated between the peri- and myocardium. Like PVAT, it has been found to have both BAT- and WAT-like properties, but its endocrine function appears to play a cardioprotective role directly, though it also acts as a mechanical shield against trauma [59].

1.2.3 Adipose tissue and inflammation

All adipose tissue plays a role in either local or systemic inflammation, through release of adipokines. In health, these adipokines should be in balance and harmonious in function, but in obesity this balance is disturbed with in favour of pro-inflammatory states. Key adipokines involved in this process include [65-73]:

- Adiponectin – an anti-inflammatory cytokine with immunomodulatory and metabolic effects. Adiponectin levels are reduced in obesity,
- Leptin – involved in hypothalamus-mediated satiety control, however this is dysfunctional in obesity. It causes a pro-inflammatory cascade by encouraging cytokine production (including secretion of IL-6, IL-12, IL-18 and TNF α) and monocyte and macrophage chemoattraction and activation. It also increases eosinophil chemotaxis and survival,
- IL-6 - metabolic effects in healthy individuals and has displayed both pro- and anti-inflammatory effects, however in obesity there is enhanced pro-inflammatory effects, and it is secreted in excess,
- IL-10 – main role as an anti-inflammatory immunomodulator. It reduces macrophage and T cell activity, as well as cytokine and reactive oxygen species release. There are controversial reports of both high and low levels seen in obesity, however low levels are seen in patients with metabolic syndrome,
- TNFα - pro-inflammatory cytokine, with increased levels seen in obesity,
- Resistin – pro-inflammatory cytokine, with increased levels seen in obesity, and is also associated with increased pulmonary inflammation,
- CC-chemokine ligand 2 (CCL2) - enhanced pro-inflammatory effects in obesity
- Chemerin - enhanced pro-inflammatory effects in obesity,
- Monocyte chemoattractant protein 1 (MCP-1) - aids monocyte recruitment; increased levels in obesity,
- Retinol binding protein 4 – found in adipose and hepatic cells as well as macrophages. Increased in obesity; stimulates release of IL-6, CCL2 and vascular cell adhesion molecule 1 (VCAM1) promoting endothelial inflammation.

These cytokines may be suitable for use as biomarkers in obesity-related disease [74]. Notably, raised IL-6 and leptin are seen in people suffering with asthma with obesity suggesting a link with airway inflammation and the importance of the obesity-associated inflammatory cascade in asthma [75-77].

Over-nutrition leads to excess WAT deposition. In this state, WAT acts as a pro-inflammatory organ with increased release of these pro-inflammatory adipokines and promotion of systemic inflammatory mediators such as C-reactive protein (CRP). Furthermore, there is enhanced activated macrophage and CD8+ T cell activity seen in obesity [78]. Whilst BAT attempts to counter-act these effects through its anti-inflammatory role, BAT function is itself suppressed by obesity in a process known as "whitening" of BAT possibly caused by mitochondrial dysfunction [79, 80]. Browning of WAT also acts to attenuate the pro-inflammatory state and further research into this area may yield potential therapeutic options for obesity-associated inflammatory disease. Whilst beige adipose tissue and BAT are protective against WAT-excess-induced inflammation, the sheer surplus deposition of WAT eventually outweighs these positive effects, and a general low-level inflammatory state is seen in obesity as a result [81, 82].

1.2.4 Obesity and asthma

1.2.4.1 Lung function

Obesity affects the severity of numerous pulmonary diseases including obstructive sleep apnoea syndrome (OSAS), obesity hypoventilation syndrome (OHS), chronic obstructive pulmonary disease (COPD) and asthma [83]. This will be at least partly a consequence of dysfunctional chest wall mechanics from mass effect caused by the increased weight burden on the thorax and reflected in reduced functional residual capacity (FRC), forced vital capacity (FVC), expiratory reserve volume (ERV) and FEV_1 [84]. These effects manifest clinically as increased exertional dyspnoea, reduced exercise tolerance and can be the start of the vicious cycle of deconditioning. Beyond this, weight gain affects pulmonary function in various other ways suggesting a more complex pathobiological disruption caused by obesity. For example, increased dynamic hyperinflation has been shown in patients with asthma an obesity [85].

Hyperinflation and air-trapping in airway disease is associated with two major factors: airway obstruction and airway closure. There is evidence showing that patients with asthma and increased BMI have increased airway closure regardless of asthma control status [86]. Furthermore, this effect appears to be independent of mass effect caused by obesity as suggested by data showing raised BMI without central obesity (with waist circumference used as a surrogate marker) enhances airway closure in patients with asthma [87]. Equally important, obesity has been associated with increased bronchial hyperreactivity in people suffering with asthma, and effects on bronchial smooth muscle are more obvious in females with obesity [88, 89].

A controlled cohort study of 2959 adults aged over 35 years showed increased airflow obstruction with BMI ≥ 25 kg/m^2 [90]. Whilst this may be as a direct effect from obesity [91], there is also an effect on systemic and airway inflammation, and this will undoubtedly also impact airflow limitation.

1.2.4.2 Inflammation

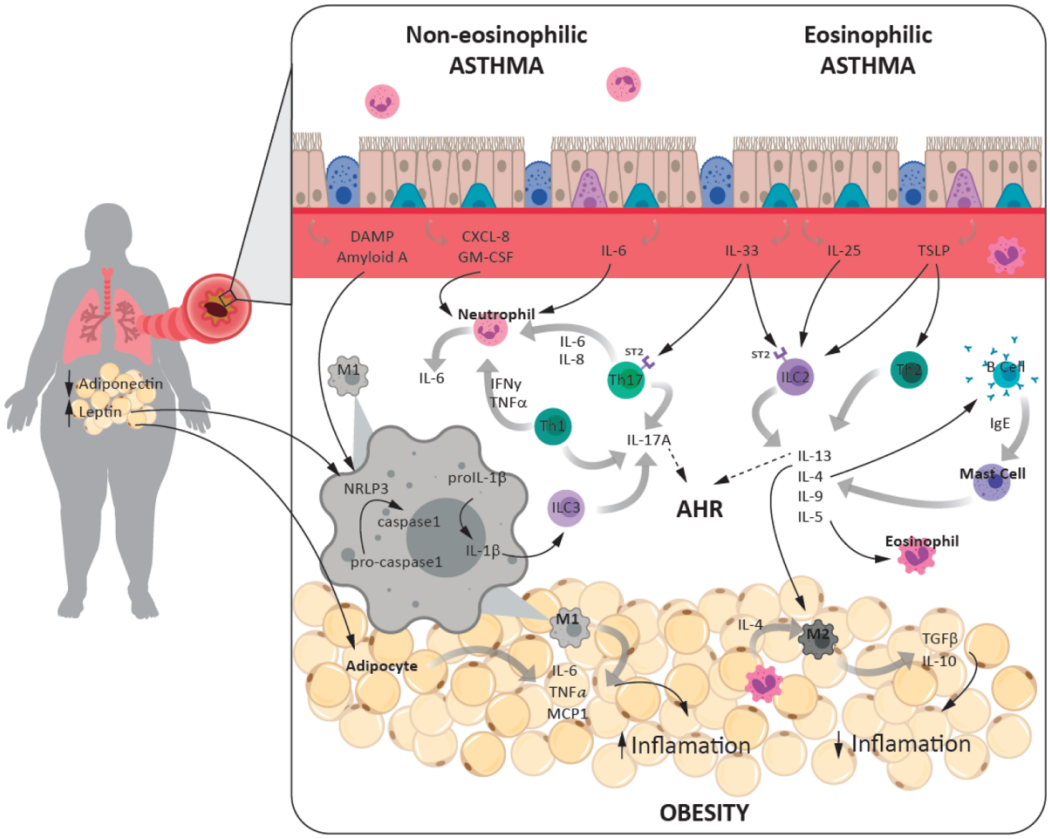
Effects of obesity on inflammatory cells in asthma also continue to be studied, with interesting findings. Murine models have shown that adipose tissue-situated eosinophils have roles in metabolism, regulate macrophage activity and, in obesity, are reduced in number [92]. This latter effect has been reversed by administration of low-calorie diet in mice [93]. A study by Desai et al [94] revealed increased submucosal eosinophilia, as well as raised IL-5, in the absence of sputum eosinophilia in individuals with asthma and obesity. This is potentially a key finding as it suggests obesity-mediated eosinophilic inflammation that may be miscategorised as non-eosinophilic owing to a lack of sputum eosinophils. It is unclear if this particular subset of patients would respond to eosinophilic-sensitive asthma treatments, something the authors also reflect upon. Sunadome et al recently reported results from a large-scale genome-wide association study of 9789 adults in Japan, 4% of whom had asthma, showing a linear correlation between BMI and peripheral neutrophil count and a rising peripheral eosinophil count, until BMI ≥ 40 kg/m^2 (when eosinophil levels

plateaued) [95]. Complicating matters further, the same study showed that healthy-BMI participants with raised eosinophils displayed an inverse relationship with increasing BMI. This suggests a more heterogeneous impact on inflammation from excess adiposity. In 2018, Farahi et al reported a study utilising single-photon emission computed tomography observing increased pulmonary uptake of radio-labelled eosinophils in participants with obesity compared to lean counterparts [96]. Furthermore, there is evidence of an additive effect on inflammatory markers in asthma and obesity together, and alteration of the microbiome negatively impacting inflammation [97].

Macrophages are the most abundant inflammatory cell in adipose tissue and obesity results in increasingly large populations of resident macrophages [62]. Comprehensive understanding of the functions of these macrophages remains unclear, and there are variable reports of the class of macrophage identified. Previously, macrophage subsets were divided into M1 (broadly pro-inflammatory) and M2 (broadly anti-inflammatory), and studies have reported that obesity in patients with asthma results in a pre-dominant M1 population in adipose tissue [98]. Other studies suggest more plasticity in macrophage behaviour, with novel subset function/dysfunction including a metabolically active subset predominant in obesity related to insulin resistance [99, 100].

Figure 1.5 (adapted from Bantulà et al [101]) summarises some of the inflammatory mechanisms in obesity-associated asthma.

Figure 1. 5 - Summary of inflammatory processes in obesity and asthma



Compounding the issue, mouse models have shown that diets high in simple carbohydrates and fats, and low in fibre, also enhance inflammation in the airways [102, 103], corroborated by a recent review of dietary impact on asthma [104]. Additionally, intake of fatty acids during a respiratory tract infection promotes bronchial pro-inflammatory mediator secretion leading to more severe exacerbations of asthma [105].

Beyond these effects, obesity results in reduced levels of an inflammatory regulator, MKP-1 (mitogen-activated protein kinase (MAPK) phosphatase-1) and increased TNF-α expression impairing the response of corticosteroids in asthma further complicating matters [106].

1.2.4.3 Adipokines

Adiponectin

Adiponectin exists in the body in several isoforms circulating as trimers, hexamers and higher molecular weight forms [107].These various isoforms likely perform different functions, but it remains unclear whether any specific isoform is responsible for pulmonary effects. Adiponectin-binding proteins, including AdipoR1 and AdipoR2, have been identified on airway epithelium and smooth muscle cells suggesting direct bronchial effects [108, 109].

Direct effects of adiponectin dysfunction have been elucidated in the context of asthma. In 2006, Shore et al reported results of a murine model showing a protective effect of adiponectin in asthma [110]. Specifically, lower levels of adiponectin, seen in obesity, resulted in increased airway hyperresponsiveness and airway inflammation. Importantly, weight loss results in increased serum adiponectin. There may also be a causal link between reduced adiponectin levels and asthma [111], and a protective effect of high levels of adiponectin against asthma [112]. Furthermore, low serum levels of adiponectin appear to be a predictor of future asthma exacerbation [113]. There are likely to be many immuno-protective effects of adiponectin in asthma, but previous evidence has shown adiponectin attenuates ACAT-1 (acyl-coenzyme A:cholesterol

acyltransferase-1) expression in human macrophages [114], diminishes TNF-α and IL-6 production, supresses NFκB effects and enhances IL-10 production [115, 116].

Leptin

Structurally similar to most cytokines, leptin is a 16-kDa protein produced in WAT with a large role in feeding regulation, in particular satiety, immunoregulation and tissue healing [117]. It acts on widespread Ob-R receptors including within the lung [118].

Excessive expression of circulating leptin seen in obesity, results in activation of the NLRP3 inflammasome in pulmonary macrophages resulting in IL-1β secretion and ILC3 stimulation down the T2-low pathway [101, 119]. Leptin appears to have a direct effect on alveolar macrophage dysfunction [120], including upregulation of leukotriene production, a key pro-inflammatory component in asthma [121]. Furthermore, Ob-R receptors have been identified on mast cells, though the clinical significance of this remains to be determined [122]. Beyond this, murine models have shown leptin-induced increased airway hyperresponsiveness [123], and *in vitro* human airway remodelling [124].

Interleukin-6

IL-6 is a pleiotropic, single-chain 21-kDa glycoprotein with roles in differentiation of B-cells, T-cells, macrophages, neutrophils, neurons, as well as haematopoiesis, and generation of hepatic acute-phase reactants [125, 126]. It acts on IL-6 receptors (IL-6R) present on hepatocytes and leukocytes, and soluble IL-6R in the serum, high levels of which have been identified in bronchioalveolar fluid in patients with asthma [127]. Precise mechanisms of obesity-mediated IL-6 effects in asthma are unclear however effects on neutrophils and T cell differentiation into Th2 cells are likely most relevant to asthma pathogenesis. Adipocytes themselves produce only a small amount of IL-6, with the majority made by non-adipocyte components of adipose tissue, such as stromal vascular cells [128]. IL-6 levels are elevated in individuals with

asthma compared to controls and higher again during asthma exacerbation [129, 130]. Whilst IL-6 can be produced by inflammatory cells, there is also evidence that raised IL-6 levels are seen in individuals with asthma when adjusted for other pro-inflammatory mediators, such as TNF-α, suggesting that presence of IL-6 is not merely due to active inflammation and may be involved with asthma pathogenesis [131]. Indeed, raised IL-6 levels have been associated with decreased FEV_1 and asthma control [132]. Raised IL-6 levels are associated with obesity and severe asthma [133].

1.2.4.4 Bidirectional link and obesity-associated asthma phenotypes

Historically, the association between asthma and obesity was thought to be one-way; people suffering with chronic asthma are often less able to perform rigorous activity and engage in a more sedentary lifestyle which, as well as being directly associated with weight gain, is associated with a process of deconditioning further reducing exercise undertaken. During this process, exercise tolerance decreases, skeletal musculature becomes less efficient at respiration and exertional dyspnoea increases often with marked worsening of symptoms following an indolent period. This often manifests itself clinically as the perception of sub-acute onset of breathlessness despite the chronicity of this vicious cycle. Weight gain is worsened by the recurrent or long-term use of OCS seen in chronic uncontrolled asthma. However, whilst this phenotype of obesity-associated asthma is recognised, the last twenty years has seen a rise in evidence that the asthma/obesity association may be bi-directional; asthma may be caused by the presence of obesity or worsened with rising BMI in a dose-dependent manner. Table 1.2 summarises key points from recent studies showing this causal link. However, this appreciation is not new, nor indeed linear. Celedòn et al [134] reported a cross-sectional study in 2001 and showed a U-shaped relationship between BMI and probability of asthma, with increased risk in both under- and over-weight individuals. Previous studies of twin cohorts have suggested genetic links between obesity and asthma, notably by Hallstrand et al in 2005 showing 8% shared genetic components for both [135]. A further study of 29183 twins reported by Thomsen et al in 2007 observed genetic correlation between obesity and asthma in the female population only

[136].These studies define asthma as self-reported, parental-reported and/or physician-reported and lack objective assessments of asthma diagnosis.

Asthma phenotypes caused by obesity tend to be adult-onset, are less-likely to be allergic or eosinophilic, and more likely to be paucigranulocytic or neutrophilic, and are associated with more difficult-to-treat disease. A well-established phenotype of obesity-associated asthma, female adult patients with asthma continues to be shown in cluster cohort studies [137-139], especially in the perimenopausal and postmenopausal age groups [140]. The difference seen between sexes and around menopause suggests a deleterious consequence from gonadal hormonal changes in women or perhaps a protective effect of male sex hormones. This is somewhat substantiated by a recent population-based cross-sectional study of 7615 adults in the USA which showed reduced OR for asthma in obese people with high oestradiol (0.43, 95% CI 0.23 - 0.78) and testosterone (0.59, 95% CI 0.37 - 0.91) levels [141], though more research in this area is needed to confirm this.

Obesity-associated asthma phenotypes are associated with declining asthma control, worse quality of life, recurrent exacerbations, higher treatment burden and increased emergency service use [142-148]. These more severe and more difficult-to-treat, less steroid responsive [106] phenotypes are also present in those with visceral adipose excess in the absence of raised BMI or central obesity. For example, a recent Japanese study of 206 adults with asthma utilising CT scanning to assess visceral adiposity showed reduced quality of life in association with visceral adipose excess independently of obesity markers [149]. The authors suggest this is related to reduced lung function, increased gastro-oesophageal reflux and associated mental health issues seen with increased abdominal fat, however, as described above, there will likely be impact from adipokine-mediated inflammation also. Asthma-related comorbidities such as GORD, anxiety, depression, and chronic rhinosinusitis worsen morbidity and mortality in asthma, however obesity-related comorbidities appear to contribute negatively also, particularly increased insulin resistance and the metabolic syndrome [150, 151]. The metabolic syndrome is characterised by three of the

following: abdominal obesity (as per WC), hypertriglyceridaemia, cholesterolopathy, hypertension and dysglycaemia.

Table 1. 2 - Links between obesity and asthma - summary of recent studies

Study	Population	Design	Key findings
Sun *et al.* **[152]**	Norwegian, ≥ 20 years of age. N = 56 105	Mendelian randomisation analysis	OR **1.36** (95% CI 1.10 - 1.68), **1.49** (95% CI 1.14 - 1.94) and **1.40** (1.02 - 1.93) per 4.1kg/m^2 BMI increase and "ever asthma", doctor-diagnosed asthma and doctor-diagnosed active asthma respectively
Abrahamsen *et al.* **[142]**	Norwegian, 16-50 year-old patients with symptomatic asthma. N = 326	Cross-sectional	OR_{adj} **2.2** (95% CI 1.2 - 4.1, p <0.05) for BMI ≥ 30 kg/m^2 association with poor asthma control
Alves *et al.* **[143]**	Brazilian, asthma patients ≥ 18 years of age. N = 473	Cross-sectional	OR_{adj} **1.46** (95% CI **0.89** - 2.39) for BMI ≥ 30 kg/m^2 in severe asthma
Souza *et al.* **[153]**	Brazilian, patients ≥ 40 years of age. N = 1026. Asthmatics, N = 116	Cross-sectional	PR_{adj} **2.3**; 95% CI 1.2-4.5 (p = 0.01) for overweight and asthma, **3.1**; 95% CI 1.6-6.0 (p = 0.001) for obesity and asthma
Park *et al.* **[154]**	South Korean, 40-79 year-old patients without asthma. N = 459 529	Cohort study, outcome was development of asthma	Multivariable HR **1.23** (95% CI 1.13 - 1.34) and **1.40** (95% CI 1.32 - 1.48) for development of asthma with BMI ≥ 30 kg/m^2 in men and women respectively
Lampalo *et al.* **[155]**	Croatian, adult patients, n = 302, divided into asthmatic and non-asthmatic groups	Cross-sectional	Increased BMI associated with asthma in women (p = 0.002)
Zhu *et al.* **[156]**	UK, 16+ years of age. N = 457 822	Cross-trait genome-wide association study	OR **1.21** SE 0.04 (p = 6.3 x 10^{-7}) for causal effect of raised BMI on later-onset asthma
Borna *et al.* **[157]**	Sweden, age 16-75 years. N = 24534	Cross-sectional	OR **2.60** (95% CI 1.63 - 4.13) for current asthma and BMI >30 kg/m^2, and OR **2.50** (95% CI 1.61 - 3.88) for physician-diagnosed asthma and BMI >30 kg/m^2
Irani *et al.* **[144]**	Lebanon, age 18+ years. N = 183	Cross-sectional	OR_{adj} **0.155** (95% CI 0.062 - 0.389, p <0.001) and **0.131** (95% CI 0.035 - 0.485, p = 0.002) for BMI 25-29.9 kg/m^2 and ≥ 30 kg/m^2 respectively (compared to normal BMI) and poor asthma control
Ohta *et al.* **[147]**	Japan, age 18+ years. N = 421	Cross-sectional	OR **1.05** (95% CI 1.02 - 1.08, p = 0.002) for BMI and asthma exacerbation
Petermann-Rocha *et al.* **[158]**	Chile, age 15+ years. N = 5499	Cross-sectional	OR **1.13** (95% CI 1.04 – 1.22. p <0.01) for BMI and asthma, OR **1.15** (95% CI 1.06 – 1.25, p <0.01) for WC
Xu *et al.* **[159]**	Multi-national, European ancestry	Mendelian randomisation analysis	OR **1.18** (95% CI 1.11 – 1.25, p = 2 x 10^{-8}) per unit increase of BMI on risk of asthma
Solet *et al.* **[160]**	Reunion Island, age 18-44 years. N = 2419	Cross-sectional	OR **1.52** (95% CI 1.02 – 2.28) for obesity and suspected asthma

Neffen *et al.* **[146]**	Multi-national, Latin American, age 12+ years. N = 594	Cross-sectional	OR_{adj} **1.71** (95% CI 1.04 – 2.84, p = 0.036) obesity and uncontrolled asthma
Vandenplas *et al.* **[161]**	Multi-national, European, adults with occupational asthma. N = 162	Cross-sectional	OR **1.98** (95% CI **0.97** – 3.97, p = **0.056**) for obesity and severe occupational asthma
Aarab *et al.* **[162]**	Netherlands, multiple ethnic groups, age 18+ years. N = 23356	Cross-sectional	OR_{adj} **1.07** (95% CI 1.06 – 1.08) for BMI and adult-onset asthma across all ethnic groups
Lurbet *et al.* **[163]**	USA, age 18+ years. N = 543 574	Cross-sectional	OR **1.75** (95% CI 1.75 – 1.76) for obesity with asthma
Klepaker *et al.* **[145]**	Norway, age 18-52 years. N = 626	Cross-sectional	OR **1.78** (95% CI 1.14 – 2.80), **1.81** (95% CI 1.03 – 3.18) for asthma with BMI ≥ 30 kg/m^2 and higher symptom score and poor asthma control respectively
Tomita *et al.* **[164]**	Japan, age 40-64 years. N = 9888	Cross-sectional	OR_{adj} **1.92** (95% CI 1.35-2.75, p <0.01), **2.24** (95% CI 1.23-4.09, p <0.01), **1.89** (95% CI 1.30-2.75, p <0.01) and **1.53** (95% CI 1.15-2.03, p <0.01) for asthma in women only and BMI 25-29.9 kg/m^2, BMI ≥ 30 kg/m^2, WC ≥ 90cm and WHt ratio ≥ 0.5 respectively
Santos *et al.* **[165]**	Brazil, age 18-45 years. N = 60202	Cross-sectional	OR_{adj} **1.49** (95% CI 1.14-1.96) for asthma and obesity
Matulonga-Diakiese *et al.* **[140]**	France, women without asthma at baseline, age 41-68 years. N = 67 872	Cohort study, outcome was development of asthma	HR_{adj} **1.91** (95% CI 1.00-3.66) and **2.08** (95%CI 1.07-4.06) for overweight/obese peri-menopausal and post-menopausal women respectively and asthma
Abbreviations: Adj (adjusted), BMI (body mass index), CI (confidence interval), HR (hazard ratio), OR (odds ratio), PR (prevalence ratio), SE (standard error), WC (waist circumference), WHt (waist-to-height)			

Adapted from Sharma and Cowan [166]

1.2.5 Biomarkers in obesity-associated asthma

In current clinical practice, T2-high biomarkers are used, where appropriate, to guide treatment decisions. These include total IgE, FeNO and blood eosinophils.

Total IgE

For over three decades, the correlation of raised IgE levels with both acute and chronic asthma has been well described [167, 168]. In health, B lymphocytes predominantly secrete IgG/IgM/IgA as part of the humoral response. In asthma and allergy, B cells are stimulated by Th2 cytokines (e.g., IL-4, IL-13) to undergo class switching to IgE-producing plasma cells and this effect has been seen in bronchial mucosa [169]. The resultant circulating IgE binds to high-affinity Fc receptors on mast cells leading to degranulation of pro-inflammatory mediators including histamine, prostaglandins, proteases, leukotrienes and chemokines [170, 171]. This inflammatory cocktail induces several airway changes such as bronchoconstriction, granulocyte recruitment, increased vascular permeability, impaired immune regulation and tissue remodelling. Mast cells in particular have been shown to affect airway hyperresponsiveness [172].

As increased IgE is seen in T2-high inflammation, it is logical that serum total IgE can be used to characterise disease. Total IgE is measured using enzyme-linked immunosorbent assay (ELISA) testing and gives a non-specific guide to the patient's allergic status. More specific radioallergosorbent tests (RAST) can be used to identify specific allergens from serum. Commonly tested and clinically relevant allergens include house dust mite, dog, cat, grass pollen and tree. In the absence of positive RAST testing but raised total IgE, skin prick testing can be performed for further allergen assessment. Raised total IgE and evidence of relevant allergen-sensitivity defines eligibility for anti-IgE MAb therapy (omalizumab).

Fractional exhaled nitric oxide

Nitric oxide (NO) has been identified as a regulator of many processes in the body including cell and tissue homeostasis, metabolism, feeding behaviour, and insulin sensitivity through vasodilation, neurotransmission, and inflammatory mediation [173]. Additionally, in the bronchi it can cause bronchodilation to help regulate airway function perhaps by acting as a neurotransmitter for efferent nerves [174, 175]. NO is produced through the actions of a family of enzymes, nitric oxide synthases (NOS), in particular inducible NOS (iNOS), stimulated by bronchial inflammation [176].

In balance, the functions of iNOS are protective against pathogens, however in excess it can be detrimental and implicated in the pathophysiology of numerous conditions including asthma [177]. Corticosteroids have been shown to reduce the over-stimulation of iNOS, reducing T2-mediated inflammation [178].

In uncontrolled asthma there is increased expression of iNOS with increased NO production [179]. The excess NO can be measured as FeNO, providing indirect evidence of active eosinophilic airway inflammation. FeNO therefore has roles in assessment of treatment adherence, disease monitoring and diagnosis. High FeNO levels are predictive of asthma exacerbation as well as declining lung function [180-183]. In practice, steroid doses are increased in response to raised FeNO levels and similarly, low FeNO may aid in titrating steroid doses down.

Eosinophils

Airway eosinophilia is a hallmark of allergic and eosinophilic asthma and has been identified in bronchial biopsy, sputum and bronchoalveolar lavage samples of patients with asthma [184-187].

Eosinophilia is known to predict exacerbations of asthma [188, 189] as well as asthma severity [190, 191]. Interestingly, eosinophilia has also been independently associated with increased cost in disease management [192].

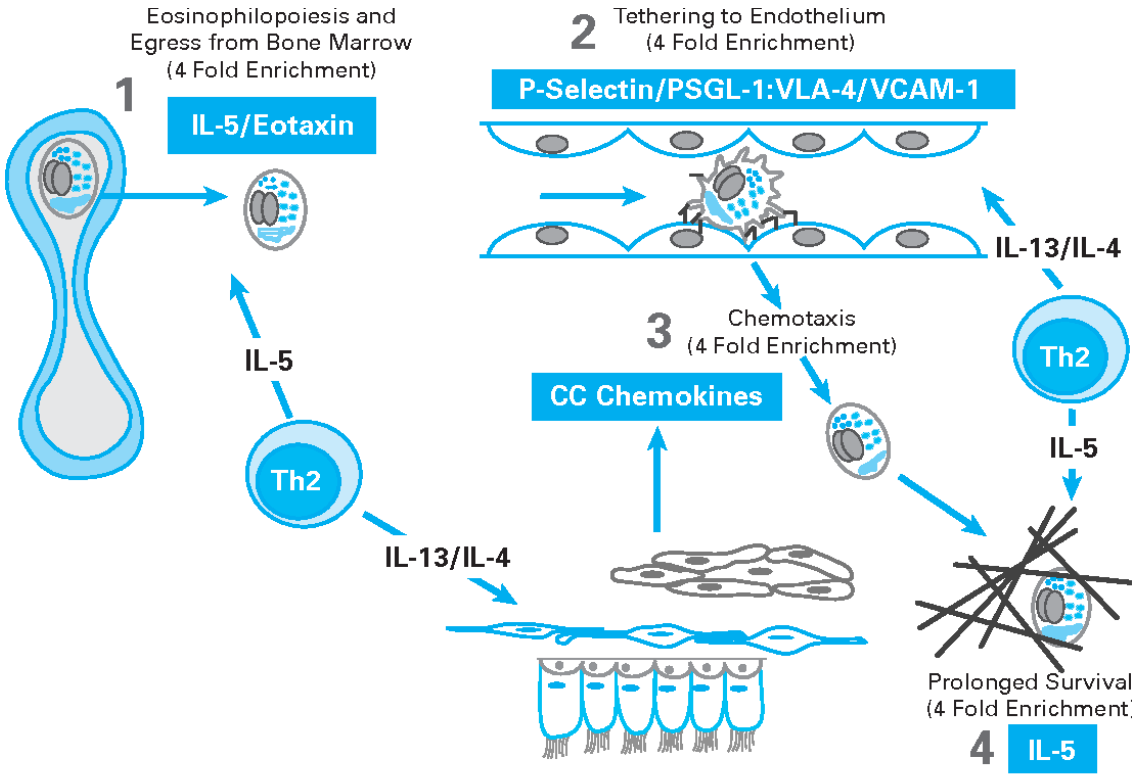
Treatment aimed at targeting eosinophilia in asthma is effective in improving asthma control [193].

Eosinophil biology is complicated and not yet fully understood. Alongside basophils and neutrophils, they are categorised as granulocytes owing to their numerous small intracellular granules filled with various proteins. Following development in bone marrow tissue, mature eosinophils briefly migrate in the bloodstream to reside in various tissues including the thymus and gut [194]. Allergen-induced Th2 and ILC-2 stimulation in the lungs causes eosinophil over-production in the bone marrow with subsequent increase in blood eosinophilia and migration to pulmonary tissues facilitated by IL-5 and eotaxins, a key group of cytokines involved in eosinophil chemotaxis [195, 196]. IL-5, along with IL-3 and GM-CSF, also has a role in prolonging eosinophil survival [197]. The alarmin, TSLP, has also been implicated in eosinophil chemoattraction and survival [198]. Increased IL-4 and IL-13 levels promote upregulation of key adhesion molecules such as P-selectin and vascular cell adhesion molecule (VCAM)-1 in pulmonary vasculature allowing eosinophil tethering and subsequent extravasation into parenchyma via chemokines to occur (see Figure 1.6) [199]. Through these mechanisms of recruitment and enhanced survival, the eosinophil is able to participate in increased airway inflammation.

Eosinophils can cause both direct and indirect inflammatory effects. Degranulation of proteins such as eosinophil peroxidase leads to direct pro-inflammatory stress [200], whilst release of mitogenic peptides promote tissue remodelling [201]. Other eosinophilic peptides have roles in plasma cell support, Th2 cell stimulation via IL-4 and ILC-2 activation [200, 202]. Prolonged exposure to toxic eosinophilic peptides can lead to tissue remodelling and fibrosis [203].

Corticosteroid use, a mainstay of asthma treatment, has been shown to impact eosinophil production, function and survival [204-206]. Administration of MAb treatments directed at IL-5 or IL-5 receptors (IL-5r) on eosinophils leads to depleted levels of blood and sputum eosinophils [207, 208].

Figure 1. 6 - Eosinophil recruitment into tissue



Adapted from AJ Wardlaw [199]. 1. haematopoiesis and bone marrow egress mediated by interleukin (IL)-5 and chemotactic signals; 2. IL-4 and IL-13 upregulation of P-selectin and vascular cell adhesion molecule (VCAM)-1 on vascular endothelium; 3. selective chemotaxis under the influence of CC chemokines generated by IL-4 and IL-13-stimulated epithelial, fibroblast and smooth muscle cells; 4. prolonged survival mediated by IL-5 (PSGL = P-selectin glycoprotein ligand; VLA = very late activation antigen).

Blood eosinophil level is a reliable predictor of sputum eosinophilia [209]. Blood eosinophil counts consistently raised >0.3x10^9/L are a marker of eosinophilic asthma, though eosinophil-directed Mab treatments also have some benefit at

>0.15x10^9/L [210]. Higher eosinophil counts are associated with poorer disease control and exacerbation risk [211].

Obesity and asthma biomarkers

No effective T2-low biomarkers have been identified. Despite this, a soon-to-be available anti-TSLP MAb, tezepelumab, has shown promise in severe asthma regardless of endotype, though greater benefit was seen in T2-high disease [44] . There are no asthma biomarkers specific to the OAA population. However, greater understanding of obesity-asthma inflammatory pathways may shed light on biomarkers that could be utilised in the future.

Previous studies have suggested that obesity may affect T2-biomarker utility, with knock-on implications for disease endotyping and subsequent determination of eligibility for MAb therapy. Indeed, the obesity-associated asthma phenotype is typically thought of as T2-low. This may not be the case if there is an effect of obesity on T2-biomarker levels as previous studies suggest. Further detail is provided in Chapter Five.

1.2.6 Obesity, asthma and sleep

The overlapping associations between obesity, sleep and asthma continue to be studied. Whilst increased nocturnal symptoms are synonymous with poor asthma control, there is evidence of poor sleep quality in asthma patients with adequate disease control [212].

Obesity is a major cause of obstructive sleep apnoea syndrome (OSAS), a condition characterised by an increased number of pathological apnoeas and hypopnoeas overnight resulting in disrupted sleep cycles, in-part because of increased soft-tissue mass around the upper airways. Gold standard for diagnosis is full polysomnography, though limited sleep studies are more pragmatic and widely available. The use of wearable technologies in this area is growing and accelerometer-derived sleep metrics are explored briefly in this thesis.

The effect of asthma severity on sleep quality is less explored. Difficult-to-treat asthma is often associated with obesity and so the three aspects (asthma, obesity and sleep) remain intimately linked. In this thesis we briefly explore the effect of mild and difficult-to-treat asthma on sleep parameters assessed using accelerometer devices (see Chapter Six).

1.2.7 Discussion

Despite being a burden on health, healthcare and the economy internationally, obesity remains relatively poorly understood. Adipose tissue is a fascinating and clinically more relevant organ than was previously thought, and it is unlikely there is a system within the human body unaffected in its excess. Beyond the significant impact on cardiovascular health, adipose excess profoundly upsets the balance of inflammation and is intimately involved with numerous inflammatory conditions including asthma. Currently available biomarkers may be of limited use in obesity-associated asthma, perhaps affecting our ability to deliver precision medicine to this population. Asthma, sleep and obesity are interwoven and their effects on each other remain poorly understood.

Based on epidemiological trends, the problem of obesity-associated asthma is likely to worsen, and new treatment options need to be explored. Whilst prevention of disease and public health policies need to be addressed, a sensible starting point is to tackle current obesity in practice. The first question to answer is whether weight loss helps disease-specific outcomes.

1.3 Weight loss in asthma

1.3.1 Early studies of weight loss in obesity

Studies of weight loss in asthma have garnered interest for around two decades. One of the earliest reports of the effect of weight loss in asthma was published in 1993 by Macgregor and Greenberg [213] who presented results following gastric banding in 40 patients with morbid obesity and asthma. They reported a significant decrease in asthma symptoms, medication use, exacerbation frequency, and disease severity, and even "remission" in 48%. They also observed worsening asthma outcomes in five patients that re-gained weight. This study paved the way for others to assess the effect of bariatric surgery in asthma.

Hakala et al [214] evaluated peak flow and spirometry before and after an eight-week low-calorie programme in a single cohort study of 14 patients with obesity and asthma. They observed an improvement in peak flow, FEV_1 and FVC, as well as decreased peak flow variability after weight loss suggesting that airflow obstruction and airway hyperresponsiveness improve with weight loss. A similar study of 24 women with obesity and asthma receiving a six-month weight loss programme showed similar effects on lung function but no significant difference in airway hyperresponsiveness as measured by methacholine challenge [215].

In 2000, Stenius-Aarniala et al reported results from an open-label randomised controlled trial [216]. They provided a 420 calorie per day weight reduction programme for eight weeks to 38 participants with asthma and BMI 30-42 kg/m^2. Whilst they reported a 14.5% reduction in total body weight following intervention, as well as improved lung function, symptoms and exacerbation frequency compared to control, the population studied was one of general asthma (I.e., not difficult-to-treat or severe) and no quality-of-life measures were assessed.

In 2007, Johnson et al [217] reported results from a proof-of-concept single cohort study in which nine adults with stable moderate asthma were prescribed

an alternate day low-calorie diet for eight weeks. The authors reported mean loss of 8.5kg (8% total body weight) with improvements also seen in peak flow (of around 50 L/min, p = 0.008), post-bronchodilator FEV_1 (10.5%, p = 0.016), asthma-related quality of life (mini-AQLQ increase of 2.1, p = 0.004) and asthma control (ACQ decrease by 1.3, p = 0.002). However, as well as the lack of longer-term outcomes, the non-randomised design and small sample size are key limitations.

Following the study by Macgregor and Greenberg, sporadic studies of bariatric surgery have also drawn interest. In 1999 Dixon et al [218] found an improvement in asthma severity in 32 patients with obesity and asthma following laparoscopic gastric banding. The authors correctly suggested that factors other than direct weight loss may be involved in the improvements observed.

Hasegawa et al [219] reported a roughly 50% decrease in emergency department or hospital attendance with asthma exacerbation in the two years following bariatric surgery in a case series retrospective study of 2261 adults with asthma and obesity (OR 0.42; 95% CI 0.35, 0.50).

In 2008, Maniscalco et al [220] reported results from a case series of laparoscopic gastric banding in 12 women against 10 control women with morbid obesity and asthma. As well as a reduction of approximately 10 kg/m^2 in BMI, the intervention group also experienced an improvement in asthma control ($p < 0.001$), dyspnoea and asthma reliever use ($p < 0.05$ for both). Five-year outcomes demonstrated persistent improvements in asthma control and quality of life [221].

Bariatric surgery studies have shown an alteration in adipose tissue make-up with increased browning of WAT and increased BAT, and therefore a reduction in pro-inflammatory mediators [59]. An improvement in airway hyperresponsiveness has also been observed [222].

It stands to reason that weight loss would be of benefit in obesity-associated asthma, not least due to the impact on overall health, but also by potentially reversing the pro-inflammatory state of obesity. Another approach could be to counteract the inflammatory effects caused by adipose imbalance directly. However, robust randomised controlled weight-loss trials are needed to further assess impact.

1.3.2 Recent trials of management in obesity-associated asthma

1.3.2.1 Surgical techniques

More recently, bariatric procedures including laparoscopic Rouz-en-Y gastric bypass, vertical sleeve gastrectomy, adjustable gastric banding and single anastomosis gastric bypass have been used as radical weight loss treatments in obesity with evidence of use in patients with asthma (Table 1.3). Open-label prospective studies of adults with asthma and obesity have shown dramatic post-operative weight loss and suggested sustained significant improvements in asthma control, quality of life, treatment burden, systemic and pulmonary inflammatory mediators [223-225], though these benefits did not extend to adults with weight loss and presence of the metabolic syndrome [224]. This further suggests the complexity of dealing with metabolic syndrome-associated asthma which may represent a separate phenotype, and that an alternate approach may be needed in this cohort. Studies have suggested surgical intervention can reduce asthma treatment burden and possibly induce asthma "remission", though these are small number, underpowered retrospective analyses with incompletely described asthma outcomes and focus more on overall health [226-228]. Further appropriately powered studies may help bring clarity to these suggestions.

1.3.2.2 Conservative treatments

Weight loss

There has been ongoing interest in non-surgical treatments of obesity-associated asthma. A controlled parallel study by Pakhale et al [229] of 22 patients with obesity and asthma showed improved asthma control, quality of life, disease severity, spirometry and airway hyperresponsiveness following a three-month weight reduction programme. Mean (SD) weight loss in the intervention arm was 16.5 ± 9.9kg.

In addition to this, a prospective Brazilian study of middle-aged women with asthma and obesity examined the effects of a three-month tailored diet and exercise program, with psychological support, primarily on dynamic hyperinflation and expiratory flow limitation, but also on asthma control, quality of life and airway inflammatory indices [230]. Both dynamic hyperinflation and expiratory flow limitation impact exercise capacity and exertional breathlessness, profoundly impacting activities of daily living, patient confidence and perception of disease. Participants who lost >5% body weight displayed improvements in all these fields compared to those who lost <5% body weight. It is unclear if these benefits were sustained in the longer term.

Dietary

A randomised uncontrolled trial in 2013 reported by Scott et al [231] studied the effects of an exercise programme, dietary restriction or both in adults with raised BMI and asthma in a three-arm design over 10 weeks. Analysis was performed on a per protocol basis and showed mean (standard deviation) percentage total body weight-loss of 1.8 (2.6)%, 8.5 (4.2)% and 8.3 (4.9)% in exercise, dietary and combined groups respectively. Improvements were observed in mean ACQ in diet (-0.6 [0.5]) and combined groups (-0.5[0.7]), and in median (IQR) AQLQ in exercise (0.49[0.03, 0.78]), diet (0.9 [0.4, 1.3]) and combined (0.5 [0.1, 1.0]) groups. The efficacy of intervention is unclear however, as there was no control group. Furthermore, the population studied was one with lower treatment burden (e.g., ICS dose 1000mcg BDP equivalent),

higher quality of life (AQLQ 5.8-6.8) and had markedly greater disease control (ACQ 1.0-1.4) compared to our cohort.

A randomised controlled trial of antioxidants in 137 adults with stable asthma has shown that a high antioxidant diet results in longer time to exacerbation of asthma and that low-antioxidant diet results in reduced lung function, increased serum CRP levels and 2.3 (95% CI 1.0, 4.9; p = 0.039) times likelihood of exacerbation over 14 weeks [232]. This effect was not observed with a tomato-based synthetic antioxidant supplement program suggesting natural antioxidant dietary intake of fruits and vegetables is needed, though no clinically relevant difference was observed in ACQ. It should be noted the population studied was one of stable asthma.

As specified earlier, iNOS is active in bronchial epithelium with likely roles in innate defence mechanisms. iNOS-induced NO release is seen in many airway conditions including asthma, and indeed this is the basis for measurement of NO (FeNO) in T2 inflammation. Adipose-mediated disruption via adipokines leads to reduced expression of iNOS [233] throughout the body, the underlying mechanism of which is unclear. A recent proof-of-concept study [234] of L-citrulline supplementation in adults with uncontrolled asthma and obesity showed improved asthma control. The study conductors postulated that there is iNOS uncoupling due to decreased l-arginine and increased asymmetric di-methyl arginine (ADMA) levels seen in obesity, resulting in reduced NO. L-citrulline (a metabolite of l-arginine) counteracts this by aiming to re-couple iNOS in bronchial epithelial cells. Despite a significant increase in FeNO after two weeks of supplementation, a reduction in ACQ was seen giving credence to the author's hypothesis. Further study in this area is warranted and could result in other conservative management options in the future.

A three-arm, parallel, randomised controlled trial of two different intensities of Mediterranean diet package with nutritionist support against control in 38 adults with symptomatic asthma showed in-group improvements with intervention in

AQLQ domains (symptoms, emotional and environmental with high intensity program; environmental with low intensity program) but no significant differences between groups and no significant weight loss [235].

It has previously been shown that obesity increases leukotriene synthesis [236] well known to be associated with inflammation in asthma, and that supplementation of polyunsaturated fatty acids can inhibit leukotriene production [237]. A recent trial of omega-3 fatty acid supplementation in 12- to 25-year-olds with uncontrolled asthma and raised BMI in the USA did not show significant difference in asthma control or exacerbation frequency [238]. This was a multicentre, double-blind, randomised, controlled study with a six-month follow-up period. The authors' self-reported limitations are valid with possible higher doses and/or longer follow-up periods needed to assess impact further. Despite this, there remains a need to further elucidate the mechanisms linking nutrition and inflammation in asthma to identify future potential therapies. Simultaneously, conservative, cost-effective weight loss options available to primary and secondary care settings are needed to tackle this growing phenotype of asthma.

Meta-analysis of these studies is not feasible due to a variety of reasons. Firstly, eight of the studies (Scott et al, Baltieri et al, Guerron et al, Forno et al, Wazir et al, Samuel et al, Grandi Silva et al and Holguin et al) lack control groups limiting the quality of evidence due to factors such as lack of causation or being able to rule out observed effects due to alternate explanations. Secondly, it is likely that several of these studies are subject to publication bias, with many lacking pre-specified outcomes and are post-hoc observations. Thirdly, sample size is an issue with two studies (Baltieri et al and Santos et al) having fewer than 20 asthma participants and those without pre-specified outcomes lacking power calculations. Finally, there is significant heterogeneity in populations studied, with many studies incorporating general asthma patient populations rather than the more relevant difficult-to-treat or severe asthma populations, and heterogeneity in interventions (though consistency in surgical methods), trial design and outcomes reported. Furthermore, two studies (Wazir et al and

Samuel et al) use “asthma remission” as a key outcome but lack definition of this, particularly relevant given asthma remission is only now appreciated globally with emerging definitions. Only Lang et al report a study with robust design (placebo-controlled, double-blind RCT) with appropriate sample size and population studied, however, this is not a weight-management trial but a nutritional supplement proof-of-concept study, and therefore not directly comparable to weight management interventions. It is evident from these studies that structured, higher quality randomised controlled trials with appropriate methodology and pre-specified outcomes are needed to assess the effects of weight loss on asthma, in particular the clinically relevant sub-populations of difficult-to-treat and severe asthma. It is these gaps that are addressed in the trial presented in this thesis.

Table 1. 3 - Summary of surgical and non-surgical intervention trials

Study	Population	Intervention	Design	Follow-up duration	Outcome(s)	Result
Scott *et al.* [231]	Australia. Adults with asthma, overweight and obese. N = 46	10 week dietary, exercise or both	Open-label, prospective, randomised, uncontrolled trial, 3-arm parallel	10-weeks	1) airway inflammation 2) ACQ, AQLQ	**Positive primary and secondary outcome** Reduced neutrophilic and eosinophilic airway inflammation with weight loss. >5% weight loss resulted in ACQ improvement in 58% and AQLQ improvement in 83%.
Baltieri *et al.* [223]	Brazil. Age 18–65-year-old women, BMI ≥ 35kg/m^2, respiratory clinician diagnosed asthma. N = 18	Bariatric surgery – RYGB	Open-label prospective cohort study, single-centre, uncontrolled	12 months after surgery	1) Systemic and sputum inflammatory markers – adiponectin, IL-6, IL-8, leptin, resistin, TNF-α, CRP 2) ACT (MCID = 3)	**Positive primary and secondary outcome** Reduced systemic IL-8, CRP, leptin, TNF- α (p value 0.002, 0.003, 0.001, 0.007 respectively). Increased systemic IL-6 (p value 0.004). Reduced pulmonary TNF- α (p value <0.001). ACT increased from 18 (range 5-23) to 25 (range 24-25), p value <0.0001. Median weight loss 45 (25-58)kg.
Santos *et al.* [225]	Portugal. Age 18+ years. Physician diagnosed obese asthmatics (n = 8), obese non-asthmatics (n = 18)	Bariatric surgery – gastric bypass or vertical gastrectomy	Open-label, prospective longitudinal study, single-centre	6-9 months after surgery	1) Pulmonary function tests 2) CARAT, ALQ 3) Asthma medication usage	**Negative primary outcome, positive secondary outcome** Improvement in lung function in both groups, with no statistically sig difference. Improved CARAT score for lower airways (4.2±4.4, p value = 0.027) and improved ALQ score (8.1±5.6, p value = 0.017) Decrease in asthma treatment step (-1.8±1.0, p value = 0.017). Mean BMI decrease in asthma group 11.3±4.7kg/m^2
Guerron *et al.* [226]	USA. Age 18+ years. Obese patients on at least one asthma medication (n = 751)	Bariatric surgery - RYGB, sleeve gastrectomy, adjustable gastric banding, duodenal switch	Retrospective analysis	3 years after surgery	Asthma medication usage	**Positive primary outcome** Adjusted rate ratios of count of asthma medications 0.73 (95% CI 0.66-0.80, p < 0.0001) and 0.54 (95% CI 0.45-0.65, p <0.0001) at 30 days post-op and 3 years post-op respectively
Forno *et al.* [224]	USA. Age 18+ years with self-reported asthma diagnosis (n= 555). Comparing those with and without metabolic syndrome	Bariatric surgery - RYGB, laparoscopic adjustable band, sleeve gastrectomy, other	Prospective observational cohort study, multi-centre	6 years after surgery	ACT (MCID = 3)	**Positive primary outcome** Proportion of metabolic syndrome negative obese asthma patients with an ACT >19 (i.e., adequate control) increased from 58% to 78% at 60 months. Outcomes for metabolic

						syndrome positive patients poorer, however many results not statistically significant. Mean weight loss 83lbs
Wazir *et al.* [228]	UK. Age 18-68. Primarily study of obese patients with T2DM. N=121 in total, n=70 with asthma	Bariatric surgery – sleeve gastrectomy, adjustable gastric band, one anastomosis gastric bypass, RYGB	Retrospective analysis	Two years after surgery	Primary outcomes related to T2DM remission. Secondary outcomes included remission of obesity-related comorbidities including asthma	**Asthma outcomes unclear – negative study** 18(25.7%) of patients with asthma had remission, however definition of remission not given, and asthma-related outcomes not specifically analysed
Samuel *et al.* [227]	UK. Adults divided into morbidly obese (BMI 40-49.9 kg/m^2), super-obese (BMI 50-59.9 kg/m^2) and super-super-obese (BMI >60 kg/m^2). N= 64 asthmatics (353 patients in total).	Bariatric surgery – laparoscopic RYGB, laparoscopic adjustable band, laparoscopic sleeve gastrectomy	Retrospective analysis	Two years after surgery	Secondary outcome included mid-term remission of obesity-related comorbidities including asthma (however criteria for asthma remission not evident)	**Asthma outcomes unclear – negative study** In the super-morbidly obese that underwent RYGB, 6 (5.9%) had remission of asthma (p value = 0.014)
Grandi Silva *et al.* [230]	Brazil. Physician diagnosed asthma in women aged 30-60 with BMI ≥35 and <40 kg/m^2. N=42. Analysis divided into two groups: those that lost >5% body weight and those that lost <5% body weight	Diet and exercise programs (3 months) with psychology support	Prospective, non-controlled study	3 months	Primary outcome - improvement of DH and EFL. Secondary outcomes include ACQ, AQLQ, airway inflammatory markers (FeNO, IL-2, IL-4, IL-5, IL-10)	**Positive primary and secondary outcomes** Improved DH during submaximal exercise and increased time to onset of DH and EFL in >5% weight group. >5% weight group had >0.5 clinically significant improvement in both ACQ and AQLQ, and statistically significant improvement in most AQLQ domains (except environmental stimuli) compared to <5% weight group. >5% weight group: • reduced FeNO (-7.94 ± 12.24 ppb, p value = 0.04) • reduced pro-inflammatory interleukins (IL-2 -25.33±72.55, and IL-4 -3.13±7.72, p values 0.02 and 0.05 respectively) • increased anti-inflammatory interleukin (IL-10 41.83±63.44, p value 0.003)
Lang *et al.* [238]	USA. Age 12-25 years. Overweight/obese patients with uncontrolled asthma. N = 98	Omega-3 fatty acid (n3 polyunsaturated fatty acid) supplementation	Randomised, double-blind, placebo-controlled, parallel design	24 weeks	Primary outcome – change in ACQ at 6 months Secondary outcomes – ACT, lung function and inflammatory biomarkers	**Negative primary and secondary outcomes** No significant difference in ACQ, ACT, lung function or biomarkers.

			study, multicentre			
Holguin *et al*. [234]	USA. Age 18-66 years. Physician-diagnosed asthma. BMI ≥ 30kg/m^2, FeNO ≤ 30 ppb. N = 41	L-citrulline (15g/day) supplementation	Open-label pilot, proof-of-concept study, multicentre, uncontrolled	2 weeks	Primary outcome – rise in FeNO Secondary outcome included ACQ	**Positive primary and secondary outcome** Increased FeNO (4.2 ppb, 95% CI 1.8 to 6.7, p value = 0.001) Decreased ACQ (-0.46, 95% CI -0.67 to -0.27, p = 0.001)
Abbreviations: ACQ (Asthma Control Questionnaire), ACT (Asthma Control Test), ALQ (Asthma Life Quality), AQLQ (Asthma Quality of Life Questionnaire), BMI (body mass index), CARAT (Control of allergic rhinitis and asthma test), CI (confidence interval), CRP (C-reactive protein), DH (dynamic hyperinflation), EFL (expiratory flow limitation), FeNO (fractional exhaled nitric oxide), IL (interleukin), RYGB (Roux-en-Y gastric bypass), T2DM (type 2 diabetes mellitus), TNF (tumour necrosis factor)						

Adapted from Sharma and Cowan [166]

1.3.3 Current management strategies in obesity-associated asthma

The notion of treatable traits in asthma continues to garner attention [239]. As highlighted above, obesity is one such treatable trait though, despite a move towards precision medicine and individualised care, clinicians in the UK are restricted to providing dietary advice and referring to local weight management teams. Access to bariatric surgery is limited due to several factors [240] and there is a significant risk further narrowing this as a viable population-wide option. Non-surgical alternatives are imperative for patients with obesity and asthma who are ineligible for radical surgery or find the risk undesirable. Pharmacological treatment of obesity-associated asthma is non-specific with use of conventional asthma therapies. There is a scarcity of options for advanced therapies in T2-low or non-T2 severe obesity-associated asthma underlining the need for precision biomarkers in this cohort. Effective weight management options may improve asthma outcomes enough to curtail the need for advanced treatments altogether.

1.3.4 Potential for treatment in obesity-associated asthma

Deeper understanding of the immunopathological pathways between over-nutrition, obesity and asthma may yield novel therapies, and this remains an area of interest. Wood *et al* [241] report two randomised controlled studies in adults with healthy-BMI or obesity and asthma examining the effects of dietary fat and carbohydrate excess on airway inflammation via a NLRP3-mediated pathway. These showed increased NLRP3 associated inflammation in lean participants after fatty acid and carbohydrate intake and in obesity-associated asthma individuals also, with increased sputum granulocyte populations and raised IL-5 and IL-1β. Research into treatments aimed at the NLRP3 axis are warranted in obesity-associated asthma.

Pharmacological treatments routinely used in diabetes have been shown to promote weight loss and therefore may provide another treatment option in obesity-associated asthma. Metformin and glucagon-like peptide 1 agonists/receptor agonists (e.g., liraglutide, semaglutide) result in substantial weight loss in non-diabetic populations [242-244]. Their effect in asthma remains

to be unequivocally proven, though both have been studied in asthma with early studies showing favourable outcomes [245, 246]. Their effects appear to be more than simply related directly to weight-loss with immunomodulatory effects suggested with both metformin and GLP-1 agonists.

1.3.5 Discussion

Weight loss appears to improve asthma outcomes in obesity-associated asthma however methodology and population selection have been variable in previous studies limiting interpretation and conclusions. This is a multi-morbid, at-risk population that is increasing in number and requires urgent attention to elucidate viable and effective treatments accessible to all. One area that needs further study and may assist with identification of new treatments, is discovery of appropriate biomarkers specific to this population. Current management strategies are not adequate alone, but there are other creative pharmacological avenues that continue to be explored that may provide an alternative. First and foremost, an effective conservative weight management option is needed for patients with uncontrolled asthma and obesity.

Chapter Two: Materials and Methods

2.1 Regulatory approval and trial conduct

Within this thesis, the presented studies were submitted for review with the Health Research Authority (HRA) and ethical approval from the West of Scotland Research Ethics Committee (REC). Studies were conducted in accordance with the ethical principles according to the UK Policy Framework for Health and Social Care Research (2017) and the World Medical Association Declaration of Helsinki Ethical Principles for Medical Research Involving Human Subjects (1964).

All participants were given a written Patient Information Sheet (PIS) and attended for discussion before consent and trial enrolment. Studies were funded by NHS Greater Glasgow and Clyde (GGC).

Full study design and protocol, including recruitment and randomisation are described in Chapter Three. Briefly, we conducted a randomised controlled open-label single centre study assessing a weight management programme (CWP) against usual care (UC). Participants were randomised 1:1 from difficult asthma clinics and ward settings across NHS GGC and attended the Glasgow Royal Infirmary Clinical Research Facility for study visits at baseline, 16-weeks and 52-weeks. Primary outcome was assessing Asthma Control Questionnaire (ACQ) change between groups at 16-weeks. Secondary outcomes included ACQ change at 52-weeks, Asthma Quality of Life Questionnaire (AQLQ) change at 16- and 52-weeks between groups, exacerbation frequency, lung function, markers of T2 inflammation and anthropomorphic measures (weight, body mass index, waist circumference). Other outcomes measured are described in this chapter and include activity levels, anxiety and depression scores and dyspnoea scores, all factors related to obesity and asthma. Asthma control and quality of life measures were chosen as primary key secondary outcomes to reflect patient-centred outcomes relevant in a real-world setting.

2.2 Assessments

2.2.1 Anthropomorphic measurements

2.2.1.1 Body mass index

BMI, initially called the Quetelet index until 1972, was first devised by Belgian mathematician and scientist Lambert Adolphe Jacques Quetelet in 1832 as an attempt to describe physical characteristics of the "normal man" [247]. Calculated as weight (kg) divided by the square of the height (m^2), BMI continues to be used to sort individuals into weight categories [248]:

- Underweight – BMI < 18.5 kg/m^2
- Healthy weight – BMI 18.5 - 24.9 kg/m^2
- Overweight – BMI 25.0 - 29.9 kg/m^2
- Obesity I – BMI 30.0 - 34.9 kg/m^2
- Obesity II – BMI 35.0 - 39.9 kg/m^2
- Obesity III – BMI ≥ 40.0 kg/m^2

However, there are limitations to BMI as an indicator of body fat status, especially considering its development in largely Caucasian populations. BMI must be interpreted with caution in particular subsets of patients including the extremes of age, non-Caucasian ethnicities, and those with differing body proportions. Even amongst the target population studies have shown that BMI has limitations. For example, when compared with body fat analysis, using bioelectrical impedance analysers, BMI ≥ 30 kg/m^2 showed good specificity (96%) but poor sensitivity (43%), while BMI ≥ 25 kg/m^2 showed poor specificity (72%) and better sensitivity (86%) [249].

In this study BMI was used alongside other markers of body fat status, discussed below.

Height was measured in centimetres using a portable stadiometer, seca 213 (seca, Hamburg, Germany, 2018), to 1mm accuracy. Participants were asked to remove footwear and stand upright and straight prior to measuring. Weight was measured in kilograms using electronic scales, Charder MS4202L (Charder Medical, Taichung City, Taiwan, 2014), to 100g accuracy. Participants were

asked to remove outer layers of clothing, footwear, and heavy items in pockets prior to measuring. These measuring devices have been calibrated and certified for use. From these each individual BMI was calculated.

2.2.1.2 Waist circumference, waist-to-hip ratio, and waist-to height ratio

Surrogate and pragmatic markers of abdominal fat include waist circumference (WC), waist-to-hip ratio (WtH) and waist-to-height ratio (WtHt). These have been used in conjunction with BMI as markers of general and abdominal obesity, and as a predictor for morbidity and mortality [250-257].

WC has been categorised by risk of morbidity as follows [248]:

	Low	High	Very high
Women	**< 80 cm**	**80 – 88 cm**	**> 88 cm**
Men	**< 94 cm**	**94 – 102 cm**	**> 102 cm**

WC was measured in centimetres using a flexible, non-stretch measuring tape to within 1mm, with the participant comfortably standing upright at the end of expiration. The measurement was taken with the tape parallel to the floor, at the halfway point between the iliac crest and the lowest rib. The tape is pulled tight to the body with skin or thin clothing underneath, but not so tight as to indent the skin or constrict the participant.

WtH was calculated by dividing the WC (cm) by hip circumference (cm). The World Health Organisation (WHO) identify increased morbidity at WtH ≥ 0.85 for women and ≥ 0.90 for men [258].

Hip circumference was measured using the same tape and participant position as above, and the measurement was taken at the widest part of the buttocks.

WtHt was calculated by dividing WC (cm) by height (cm). WtHt of ≥ 0.5 in both sexes has been implicated with increased morbidity [259, 260].

2.2.2 MRC dyspnoea scale

The Medical Research Council (MRC) dyspnoea scale has widely been used in research and clinical practice to categorise the severity of exertional breathlessness experienced by patients. Initially developed in Cardiff, Wales, and described by CM Fletcher in 1952, it was devised as a graded scale of dyspnoea in the Pneumoconiosis Research Unit [261]. It has since been revised and validated in chronic obstructive pulmonary disease (COPD) [262], and widely adopted for respiratory conditions across the board.

Breathlessness in all participants was assessed by a member of the Clinical Research Team, after discussion with each participant, and categorised as per standard MRC dyspnoea scale grades as follows (used with the permission of the Medical Research Council [263]):

MRC Dyspnoea Scale	
Grade	Description
1	Not troubled by breathlessness except on strenuous exercise
2	Breathless when hurrying on the level or walking up a slight hill
3	Walks slower than people of the same age on the level or needs to stop for breath when walking at their own pace on the level
4	Has to stop for breath after walking 100 yards or after a few minutes on the level
5	Too breathless to leave the house or breathless when dressing/undressing

Participants were graded using this system to give an MRC score.

2.2.3 Asthma control questionnaire

The ACQ was first developed and validated by Juniper *et al* [264] as a simple means of assessing asthma control objectively using a seven-point scoring system.

Shortened versions of the original ACQ were subsequently developed as pragmatic solutions in a variety of clinical scenarios, for example lack of FEV_1 availability, and have been validated for use in asthma control [265, 266]. Of these, the ACQ6 was used in this thesis which incorporates all five symptom-based questions and a sixth question enquiring about frequency of SABA use over the preceding week.

The questionnaire is quick and easy to follow, as well as being simple to interpret. All six questions are equally weighted and have scores ranging from zero to six. The final score is the mean of all six questions with a higher score associated with uncontrolled disease. A score of ≤ 0.75 suggests good control while a score of ≥ 1.5 is indicative of poor asthma control [267]. The minimal clinically important difference (MCID) is 0.5 [266].

Due to its widespread use, efficacy, simplicity and relevance as a patient-centred measure, the ACQ is the primary outcome in the studies of this thesis. Participants were given a paper copy of the ACQ to complete at baseline and each subsequent visit.

2.2.4 Asthma quality of life questionnaire

The AQLQ was first described by Juniper *et al* [268] in 1992 and was designed as a method to quantitatively assess asthma-related quality of life, a hitherto unheard-of outcome for asthma. Prior to this, outcomes for asthma were primarily clinical (e.g., number of exacerbations) or related to pulmonary function (e.g., Improvement in FEV_1, PEFR etc.) which, while important, do not consider how patients are affected by their disease. The AQLQ has been validated [269] and, like the ACQ, is widely used in clinical research.

The questionnaire comprises of thirty-two questions which are divided into separate domains, namely symptomology, limitation on activities, emotional factors, and effects from the environment. Each question is answered on a seven-point Likert scale (1 – 7) with higher numbers associated with better quality of life. The overall score is the average of all thirty-two questions with higher numbers suggesting better quality of life. Similarly average scores of the questions within each domain yields a score out of 7. The MCID is also 0.5 [270].

All participants were given paper copies of the AQLQ to complete at baseline and each subsequent visit.

2.2.5 Hospital anxiety and depression scale

Developed by AS Zigmond and RP Snaith at the Department of Psychiatry at the University of Leeds and published in 1983 [271], the Hospital Anxiety and Depression Scale (HADS) was devised as a brief method to detect two of the most common mental health disorders, depression and anxiety. It has subsequently been validated [272] and continues to be used in clinical research. This quick questionnaire has fourteen questions each with a 0 – 3 rating, and is divided into anxiety and depression sub-sections, each with seven questions, allowing a total score of 21 for each. Scores of 0-7 are within the normal range, whereas ≥ 8 are indicative of either anxiety or depression.

All participants were given paper copies of the HADS to complete at baseline and each subsequent visit.

2.2.6 Accelerometery

Participants at each visit were provided with an ActiGraph wGT3X-BT device (ActiGraph, Penascola, USA) calibrated each time to the participants height, weight, and age. These devices were initialised using the accompanying ActiLife

software (v.6.14.3; ActiGraph) to capture data at 30 Hz, before attaching to the non-dominant wrist. Devices were active for 7 days at which time they would automatically stop monitoring at the pre-designated date and time set at initialisation.

Data were then downloaded with the ActiLife software (v.6.14.3; ActiGraph) in their raw form (.gt3x files). These files were shared securely with a specialist at the University of the West of Scotland (Dr Duncan S. Buchan) who converted the files to Comma Separated Values (.csv) format before exporting into a statistical software package, R v4.1.2 (R Foundation for Statistical Computing, Vienna, Austria). From here, an evidence-based dedicated R package was used (GGIR v2.6.0) to calculate the amount of inactive time, light physical activity (LPA) and moderate-vigorous physical activity (MVPA) time. In summary, this package identified raw auto-calibrated tri-axial signals with local gravity as reference, as well as abnormally high values and non-wear time [273]. From this it was able to calculate the vector magnitude as Euclidean Norm Minus One (ENMO) (1 *g*) in milli-gravitational (m*g*) units averaged over 5s epochs [274]. Inactive time was the cumulative time spent below 30m*g* acceleration, whilst cumulative time spent between 30-99m*g* was LPA [275], and ≥100 m*g* was MVPA [276]. The data produced from this method was subsequently analysed by the author.

2.2.7 Pulse oximetry

Pulse oximetry is a non-invasive method of measuring blood oxygen saturation levels, widely used in primary and secondary care. Pulse oximeters project and resorb light passing through the chosen appendage and calculate an oxygen level based on the ratio of light resorbed through oxygenated and deoxygenated blood [277]. Measurement of oxygen saturations were taken using an Anapulse ANP100 (Surrey, UK) pulse oximeter.

2.2.8 Lung function

2.2.8.1 Spirometry and reversibility

Measurement of dynamic lung volumes, specifically FEV_1, FVC, and FEV_1/FVC, were undertaken using a calibrated Vitalograph ALPHA™ spirometer (Buckingham, UK). Calibration was performed prior to each use with participants using a 3-litre precision syringe and a paper printout of the calibration result. A log of calibration results was kept and updated after each calibration. Further annual manufacturing calibrations were undertaken, with certificates kept on record also. Assessment was performed to the standards outlined by ERS/ATS guidelines [278, 279].

Following a full inspiration, participants were asked to exhale as fast, hard and long as able into the spirometer mouthpiece with verbal encouragement as per standard practice. A minimum of three measurements were taken and a satisfactory result was accepted if there was less than 0.15L FEV_1 and FVC variation between two efforts, though more attempts were made if these conditions were not met with the initial three attempts. Confirmation of satisfactory results was undertaken by the author and included visual assessment of the printed flow-volume loop, assessment of variation as outlined above and an in-built computer-generated graded system of test quality within the spirometer.

Spirometry was performed both before and after bronchodilator (BD) use to assess reversibility. BD was delivered by participants own specific short-acting BD, e.g., Salbutamol MDI 4 Puffs, 15 minutes prior to the second set of spirometry attempts. Reversibility was defined as presence of at least 200mls and 12% increase in FEV_1 following BD use. Some participants were unable to perform spirometry, e.g., due to equipment failure or poor technique, and this was recorded into the participant case report form (CRF).

2.2.8.2 Peak expiratory flow rate

PEFR is the maximal flow rate of air, following a full inspiration, during forced exhalation. This study used the forced vital capacity manoeuvre with the spirometer. This entailed performing spirometry as above, and subsequently PEFR was obtained from the electronic print-out. PEFR is also represented graphically in the flow-volume loop; the peak of the expiratory limb represents the PEFR. Three readings are taken with the spirometry attempts and the best is recorded in litres/min, as per standard practice. Some participants were unable to perform spirometry and therefore PEFR, e.g., due to equipment failure or poor technique, and this was recorded into the participant CRF.

2.2.9 Inflammometry

2.2.9.1 Blood eosinophils

Serum eosinophils of > 0.3 x 10^9 cells/litre are considered significant in the context of asthma. Peripheral eosinophilia is used as a surrogate marker of pulmonary eosinophilia, which is suggestive of eosinophilic asthma in conjunction with the clinical signs and symptoms. Apart from this role in phenotyping, blood eosinophil count can be used as a marker of treatment response and/or as treatment adherence, as a reduction in eosinophils may suggest better disease control or treatment adherence in this phenotype. Blood eosinophils are routinely reported as part of the full blood count which was undertaken on each study visit. More detail is provided in Chapter Three.

2.2.9.2 Fractional exhaled nitric oxide

As described in Chapter One, FeNO is a marker of T2-mediated airway inflammation and has a role in asthma diagnosis, phenotyping and disease monitoring.

When first developed, FeNO measurement was initially performed using large, non-portable and expensive chemiluminescence gas analysers [280]. Since then, more operator-friendly, cost-effective and portable methods have been developed, the most widely used of which is via electrochemical analyser. These analysers contain electrochemical sensors that display electrical signals proportional to the detected gas concentrations, by using the amperometric technique in which gas oxidation/reduction (in this case NO) occurs on the surface of the sensing electrode to produce a current [280-282]. Factors such as smoking, airway calibre, nitrate-rich foods, and water, caffeine or alcohol intake can directly affect FeNO results [283-293]. In these contexts, results must be interpreted with caution.

In this study, FeNO was measured with a handheld NIOX VERO® (Aerocrine AB, Solna, Sweden) electrochemical analyser. This is a pre-calibrated system and therefore no routine calibrating is recommended. Following a deep inhalation of NO-free air through the breathing port, participants were asked to exhale at a controlled rate with a visual animated prompting aid and verbal encouragement to facilitate a constant flow rate. The test is performed with exhalation against resistance ensuring soft palate closure to prevent false readings due to upper respiratory tract NO contamination. There were no maximum/minimum number of attempts. Some participants were unable to perform FeNO, e.g., due to equipment failure, and this was recorded into the participant CRF.

Results are reported, as is standard, in parts per billion (ppb) of NO in exhaled air with results of <25 ppb considered normal, >50 ppb considered high, and 25-50 ppb a moderate area in which clinicians are advised to interpret alongside the clinical context. A change of >20% in FeNO in those with a high baseline FeNO (I.e., >50 ppb) or change >10 ppb in those with a FeNO <50 ppb at baseline over time is considered clinically significant [294].

2.2.10 Six-minute walk test

2.2.10.1 6MWT

The 6MWT is a simple, effective and reproducible measure of submaximal functional reserve and capacity for performing activities of daily living. The test involves participants walking on level, firm surfaces for 6 minutes and the total distance observed after this time is the six-minute walk distance (6MWD). A lower 6MWD in the context of a chronic respiratory condition such as asthma, is with increased morbidity and mortality; a MCID of 30m is accepted [295]. The test is validated and has been incorporated into guidelines for a variety of chronic respiratory diseases including asthma.

This test was performed in the Clinical Research Facility corridor over a marked 30m straight distance with use of a flexible measuring tape, and cones showing the start and end of the 30m. Time was recorded using a simple stopwatch. Participants were asked to walk at their usual pace for the duration of the test and could slow down or stop, if necessary, before resuming when able. The member of the CRT performing the test offered verbal encouragement as per standard practice. Baseline oxygen saturations, heart rate, blood pressure, and Borg rating (see below) were obtained prior to and after the test, as well as continuous pulse oximetry and heart rate monitoring during the test.

A second attempt of the test was made after a suitable rest time of at least 10 minutes, as per standards, due to the presence of a learning effect seen in this test [295]. The best 6MWD from the two attempts, alongside the oxygen saturations and Borg rating, were recorded for data analysis. On occasion participants were unable to perform the 6MWT due to increased fatigue or dyspnoea at baseline. A member of the CRT would offer encouragement where appropriate, however if the participant was not able, this was logged into their CRF.

Two tests, where able, were performed at each visit, though if only one test was performed, then results were taken from the singular effort.

2.2.10.2 Modified Borg Scale

The initial Borg scale was developed by GAV Borg and described in 1970 as a method to allow participants to quantitatively express their perception of level of exertion during exercise [296]. It has been through a few iterations and is now used as the modified Borg scale [297], primarily in pulmonary rehabilitation. Since then, it has been adopted and used as a marker of dyspnoea on exertion in various conditions including asthma [298]. In particular, the scale is used as part of the six-minute walk test (6MWT) protocol. The scale is as follows (adapted from [299]):

Modified Borg Scale	
Score	**Dyspnoea**
0	**None**
0.5	**Extremely mild dyspnoea**
1	**Very mild dyspnoea**
2	**Mild dyspnoea**
3	**Moderate dyspnoea**
4	**Intense dyspnoea**
5	**Rather intense dyspnoea**
6	
7	**Very intense dyspnoea**
8	
9	**Almost unbearable dyspnoea**
10	**Unbearable dyspnoea**

The scale was completed as per protocol for the 6MWT alongside a member of the Clinical Research Team.

2.3 Adverse events

Definitions

An adverse event (AE) is defined as any unintentional and unfavourable change in state, either a sign, symptom or disease, associated with treatment. All AEs were recorded in the CRF and medical notes.

The NHS Health Research Authority (HRA) defines a serious adverse event (SAE) as “an untoward occurrence that:

a) results in death

b) is life-threatening

c) requires hospitalisation or prolongation of existing hospitalisation

d) results in persistent or significant disability or incapacity; or

e) consists of a congenital abnormality or birth defect; or

f) is otherwise considered medically significant by the investigator” [300]

Reporting serious adverse events

SAEs that were either “related” (I.e., resulting from administration of the trial treatment) or “unexpected” (I.e., an event not listed in the protocol as an expected occurrence) were reported to the West of Scotland Research Ethics Committee (REC) using the appropriate “Non-CTIMP safety report to REC form” within 15 days of becoming aware of the event.

2.4 Effects of COVID-19

Due to the global SARS-CoV-2 pandemic, certain aspects of the study design and implementation were affected with significant impact on data sets and the planned outcomes. These effects will be described in each relevant chapter.

Chapter Three: A total diet replacement weight management programme for difficult-to-treat asthma and obesity: a randomised controlled trial

3.1 Introduction

Asthma affects around 360 million people worldwide [301]. Roughly 17% of patients with asthma are considered to have difficult-to-treat disease, defined as uncontrolled disease despite treatment with medium or high dose inhaled corticosteroids with a second agent. This is, in part, due to factors including inhaler technique, treatment adherence and asthma-related co-morbidities, of which obesity is a common one [5, 7].Asthma associated with obesity is a particular challenge, often being steroid resistant, and linked with persistent symptoms, frequent exacerbations, reduced quality of life, and greater mortality [106, 302].Treatment options for this phenotype are often limited. Obesity prevalence continues to rise [301] and the burden of difficult-to-treat asthma with obesity is likely only to increase too. As described in Chapter One, the effects of obesity on asthma are numerous and not entirely understood. As well as mechanical effects on chest wall dynamics, resulting in lower FRC, ERV and expiratory volume [84], obesity also results in increased bronchial hyper-reactivity [88, 89], greater airway closure [86, 87] and enhances both systemic and airway inflammation [70, 73, 77].

In the era of precision medicine, focus has shifted to asthma management strategies that target “treatable traits” [48], and co-existent obesity is one such trait, the successful management of which might lead to improved outcomes in this asthma phenotype. In 2012, a Cochrane review of four weight loss studies (n = 197) was published [303]. The studies reviewed had significant variations in both populations and methodology, and whilst the review panel concluded the quality of evidence was poor, there was some evidence that weight loss could perhaps improve asthma control. Ultimately, robust and well-designed randomised controlled studies were suggested in order to clarify the effect of weight loss on asthma outcomes. Surgical treatments appear to provide a welcome solution to the most severe cases, with recent studies of bariatric procedures showing improved asthma control, and quality of life, lower treatment burden, improved spirometry and reduced inflammatory markers, though methodology and populations vary substantially [223-226, 304]. However, surgical management is not without risk and access to these interventions is variable [240]. More readily available and safer options are needed.

The Counterweight-Plus weight management programme (CWP) has an evidence base in obesity and type 2 diabetes mellitus, resulting in mean weight loss of 10kg (approximately 1/3 participants achieving weight-loss of at least 15kg) and diabetes remission in almost half [305, 306]. It is a dietitian-led programme of total diet replacement using low-energy liquid formula over 12-weeks with subsequent stepwise food re-introduction and weight-loss maintenance phases up to one year in total. CWP is currently commercially available in the UK and is easily delivered. Effects of the CWP have not been studied in patients with asthma and obesity and we hypothesised that the programme may improve patient-centred asthma outcomes. Unlike previous trials in this area, this study assesses an evidence-based weight management programme (I.e. CWP) on a population of patients with obesity and difficult-to-treat asthma with pre-specified asthma-related outcomes using randomised controlled methods.

3.2 Hypothesis

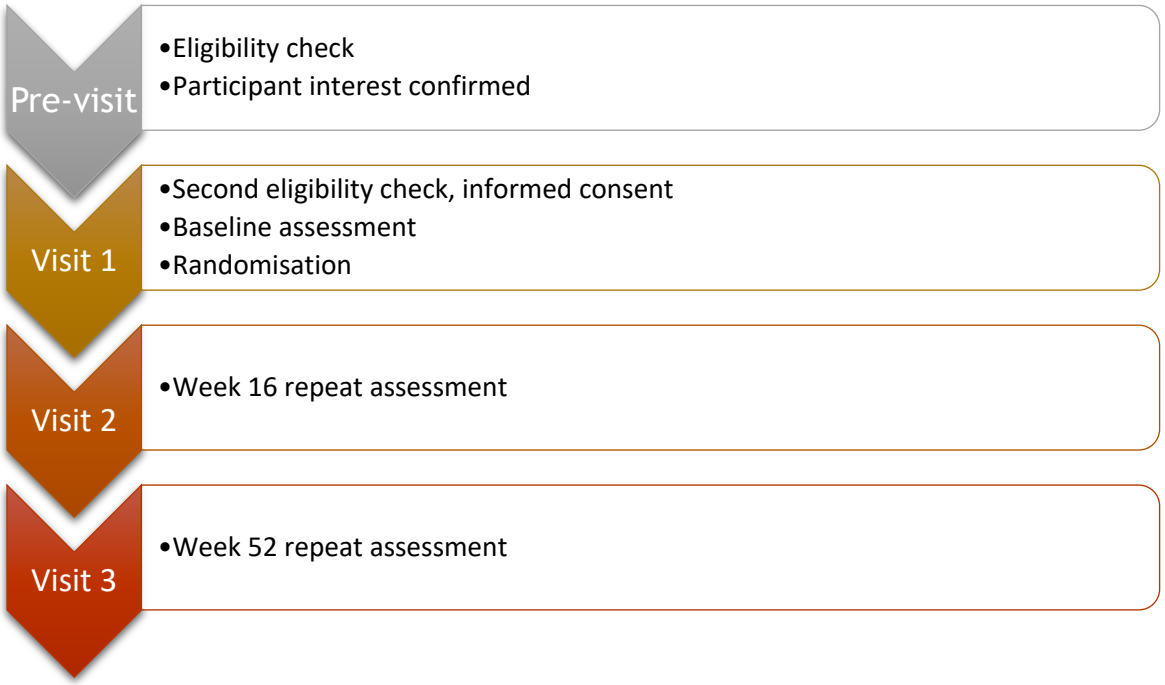
A randomised, controlled study over 16 weeks to determine the effect of the Counterweight-Plus weight management programme (CWP) compared to usual care (UC) on asthma control and quality of life in patients with difficult-to-treat asthma and obesity.

3.3 Method

3.3.1 Study design

This trial was a single-centre, prospective, open-label, parallel, two-arm, randomised controlled trial comparing CWP to UC in participants with obesity and difficult-to-treat asthma. Study visits were undertaken at the Clinical Research Facility in Glasgow Royal Infirmary (GRI) and occurred at baseline (Visit 1) and 16-weeks (Visit 2). All data were collected by the Clinical Research Team comprising specialist research nurses and the Clinical Research Fellow. Figure 3.1 shows the study timeline. Eligible and consented participants were allocated 1:1 to CWP or UC following randomisation using an online, password-protected, secure third-party randomisation service [307]. The trial was sponsored by NHS GGC and approval was obtained from the West of Scotland Regional Ethics Committee (18/WS/0216), before being registered at ClinicalTrials.gov (NCT03858608) [308].

Figure 3. 1 - Participant study timeline



3.3.2 Population and recruitment

Participants were recruited from GRI and New Stobhill Hospital Difficult Asthma Clinic, with the remainder from other specialist outpatient asthma services across Greater Glasgow and Clyde and hospital ward admissions. Patients at clinic had their electronic records vetted to assess potential eligibility and were then approached either at the clinic or via telephone discussion for consideration of participation. Ward patients, where applicable, were contacted after discharge to consider participation. Patients were excluded both at the time of vetting or during this initial discussion if found to be ineligible. The eligible patients were then added to a list of potential recruits and telephoned by the asthma Clinical Research Team (CRT) to confirm patient willingness to participate and eligibility once more to prevent unnecessary attendances, before scheduling an initial appointment at the Clinical Research Facility. Patients were given a patient information sheet either at clinic or via post prior to the initial appointment.

To be included, participants were required to be aged 18-75 years, and have obesity (BMI ≥ 30.0 kg/m^2) and difficult-to-treat asthma. Asthma was defined (as per GINA guidelines [4]) as presence of characteristic symptoms consistent with asthma and either airway reversibility (evidenced by an increase in FEV_1 by at least 12% and 200mls post-bronchodilator, between clinic visits or after at least four weeks treatment with OCS) or bronchial hyperreactivity (evidenced by a PD_{15} <635mg with mannitol challenge or PC_{20} <8mg/ml with histamine or methacholine challenge) in the past five years. Difficult-to-treat disease was defined (as per SIGN/BTS guidelines [6]) as high treatment burden (at least high dose ICS/LABA or moderate-dose ICS/LABA and an oral controller such as LRA or theophylline) and either frequent exacerbations requiring OCS (≥ 2 or ≥ 1 severe exacerbation requiring hospitalisation in the past 12 months) or ACQ6 score ≥ 1.5.

Participants were excluded from randomisation if there was any history of ICU admission or mechanical ventilation for asthma in the prior six months. Other exclusion criteria included:

- Asthma exacerbation requiring OCS within the preceding four weeks
- Lower respiratory tract infection requiring antibiotics within the preceding four weeks
- Pregnancy +/- breastfeeding
- Commencement of antifungal, biologic (omalizumab, mepolizumab, lebrikizumab) or Airsonett device (Ängeholm, Sweden) within the preceding six months
- Current insulin use
- Current treatment with anti-obesity medications
- Severe and/or unstable cardiac disease
- Significant respiratory or other co-morbidity likely to influence study conduct

Willing participants that met these eligibility criteria were approached with written information and allowed time to read this (at least 48 hours) before

invitation to attend the Clinical Research Facility. Here, final eligibility checks were conducted, further trial information was discussed, where appropriate, and written consent was obtained. The baseline visit (Visit 1) was then conducted alongside randomisation.

3.3.3 Assessments

At Visit 1, demographics and history (asthma, general medical and drug) were collected. Alongside this, and at all other visits, anthropomorphic measures (height, weight, BMI, waist circumference, hip circumference, waist-to-height and waist-to-hip ratios), healthcare usage over the preceding 12 months or since the previous study visit (number of high dose OCS courses, out-of-hours GP attendances, ED attendances, hospital admissions and ICU admissions), ACQ6, AQLQ, MRC dyspnoea score, HAD score, lung function (peak flow and spirometry), FeNO, 6MWT and accelerometery data were collected. These are described in detail in Chapter Two.

The healthcare usage variables described at all visits subsequent to Visit 1 were annualised using the equation:

Annualised events = (number of events x 365)/number of days since previous visit.

Venesection

Blood sampling was performed utilising a butterfly needle and vacutainer system (VACUETTE®, Monroe, North Carolina, USA). Once collected, serum samples were delivered to the relevant local laboratory for analysis.

Full blood counts were collected into 4ml Ethylenediaminetetraacetic acid (EDTA) blood tubes and processed at the Glasgow Royal Infirmary Haematology laboratory using an automated analyser, the SYSMEX XN-10 (SYSMEX, Norderstedt, Hamburg, Germany).

Several tests were analysed at the GRI Biochemistry laboratory as follows:

- Urea and electrolytes, liver function tests, bone profile, magnesium, lipid profile and CRP were collected in 5ml serum separator clot activator (SST) tubes
- Glucose was collected in 2ml sodium fluoride/potassium oxalate tubes
- HbA1c was collected in 4ml EDTA tubes
- Insulin was collected in 2ml heparin tubes - these were transported to the lab within 30 minutes after drawing blood.

All of these, except HbA1c, were processed using an automated Abbott Alinity analyser (Abbott, Abbott Park, Illinois, USA). HbA1c was processed using the Menarini HA-8180V analyser (ARKRAY, Minneapolis, USA).

3.3.4 Counterweight-Plus weight management programme

Participants assigned to the intervention arm were entered into the CWP programme which was comprised of three phases across the course of one year: the total diet replacement (TDR) phase (0-12 weeks), food re-introduction phase (13-18 weeks) and weight-loss maintenance phase (19-52 weeks). This programme was supported by a team of dietitians with experience and training in CWP. Adherence to the Counterweight Plus programme was patient reported and confirmed by the trial dieticians. Return visits were postponed by four weeks if they coincided with a lower respiratory tract infection of asthma exacerbation requiring OCS course. Any missed appointments were re-appointed within 7 days unless participants chose to withdraw and declined the option to continue attending.

Total diet replacement phase

The TDR phase consisted of low energy liquid diet replacement of all meals with a target of 825-853 kcal/day. Where needed, soluble fibre supplements were provided, and fluid intake was encouraged to avoid constipation. Medications such as antihypertensives, diuretics and hypoglycaemic agents were withdrawn at the start of the TDR phase. If diabetes or hypertension returned, the relevant

medication was reintroduced at an appropriate dose on a case-by-case basis. Aspirin prescribed for diabetes mellitus was stopped, though aspirin and/or ß-antagonist use for ischaemic cardiac disease were allowed to continue. As well as the formal study visits outlined above, the dietitian team reviewed CWP participants in the TDR phase one week after initiation and subsequently on a fortnightly basis. The TDR phase could be increased to 20 weeks in those that did not achieve >15kg weight-loss by week 12. Similarly, food re-introduction was initiated sooner than 12 weeks if any participants BMI reduced to <23.0 kg/m^2.

Food reintroduction phase

With the food reintroduction phase, participants were asked to increase their energy intake every two weeks in a stepwise manner whilst reducing use of the CWP low energy liquid products. An example of this is as follows:

Week	Total daily calorie target	Daily breakdown of intake
Week 13	1000 kcal/day	400 kcal/day with CWP low energy product, one low-fat meal (c. 360-400 kcal/day), 200mls skimmed milk, two portions of fruit and free use of vegetables
Week 15	1200 kcal/day	200 kcal/day with CWP low energy product, two low-fat meals (c. 720-800 kcal/day), 200mls skimmed milk, two portions of fruit and free use of vegetables
Week 17	1400 kcal/day	Three low-fat meals (c. 1080-1200 kcal/day), 200mls skimmed milk and free use of vegetables

Dietitians continued to review participants on a fortnightly basis. Depending on individual confidence with managing weight-loss with the supporting dietitian, flexible periods of 2-8 weeks were allowed in total for this phase.

Weight-loss maintenance phase

For the weight-loss maintenance phase (lasting until intervention end at 52 weeks), tailored calorie prescriptions were supplied by the dietitians to support stabilisation of weight and prevent weight regain. Participants were advised to eat healthy meals with a <30% fat content target, though flexibility up to 35% was allowed to aid compliance. Dietician reviews occurred on a monthly basis in this phase.

Rescue packages

In the event of weight regain, dietitian-led "rescue packages" were provided determined by the extent of weight gain. Individuals gaining >2kg were provided with further CWP low energy products to replace one main meal per day and orlistat 120mg/main meal (maximum three times a day) for four weeks. Orlistat is a licensed oral medication for obesity that reversibly inhibits gastric and pancreatic lipases reducing the digestion and absorption of exogenous fat by around 30% [309].

Participants gaining >4kg, or to within 15kg of their baseline weight, were re-started on a TDR regime (825-853 kcal/day) for four weeks with weekly dietitian review. Following this, participants were then moved to 2–4-week food reintroduction phase during which they would add one daily low-fat c. 360-400 kcal main meal per week. Orlistat was also given as above.

These packages were able to be repeated as needed until week 52.

Patient safety

The Counterweight Plus programme is a non-pharmacological treatment for weight loss and is generally well tolerated. Previously reported adverse effects

from clinical trials [305] are more common in the earlier total diet replacement (TDR) phase and less common in the food reintroduction and weight loss management phases. These adverse effects are considered mild and include constipation, sensitivity to cold, headache, dizziness, fatigue, mood disturbance, nausea, diarrhoea, indigestion, and hair loss. Serious adverse events from these trials are rare (4% of participants) but include angina pectoris, abdominal pain, abdominal strangulated herniation, gallstones, urinary tract infections, dizziness and pre-syncope. Improvement in blood pressure, lipid profile and blood glucose control have been reported with this programme, with decreased treatment burden seen. Monitoring of blood pressure and HbA1c was undertaken throughout this trial in the treatment group, as per programme protocol. Participants with type 2 diabetes mellitus and hypertension had their concurrent medications adjusted as appropriate, and re-introduction of the relative medication if indicated.

3.3.5 Usual care

Participants assigned to usual care formed the control group. They were offered opportunities at all study visits to discuss their asthma and be subject to changes in treatment in order to improve disease control (as they would at specialist clinic attendance). All previous medications continued, and participants were not discouraged from pursuing weight management elsewhere. Lifestyle advice focussing on diet and exercise, assessing inhaler technique, treatment adherence advice and asthma education was available at study visits where needed. All participants continued to be reviewed at their local asthma specialist outpatient clinics. All the above was available also to the participants of the CWP group.

3.3.6 Outcomes

3.3.6.1 Primary outcome

Difference in change in ACQ6 scores between CWP and UC over 16-weeks (from Visit 1 to Visit 2). 16-weeks was chosen for primary outcome analysis as the majority weight-loss was anticipated to occur in the initial 12-week total diet replacement phase of the intervention with weight maintenance the primary focus beyond this point, as per the DiRECT study which utilised the CWP intervention.

3.3.6.2 Secondary outcomes

Difference in change in AQLQ scores (overall and in each domain) between CWP and UC over 16-weeks (from Visit 1 to Visit 2).

Difference in proportion of participants with ≥MCID change (0.5) in ACQ6 and AQLQ between CWP and UC at Visit 2.

3.3.6.3 Other outcomes

Difference in healthcare usage (number of OCS courses, out-of-hours GP attendances, ED admissions, hospital admission and ICU admissions) between groups over 16 weeks.

Comparison of anthropomorphic measures (weight, BMI, waist-to-height and waist-to-hip ratios) between groups over 16 weeks.

Difference in MRC dyspnoea and HAD scores between groups at 16 weeks.

Difference in peak flow and spirometry (e.g., FEV_1) between groups after 16 weeks.

Difference in FeNO and peripheral eosinophil counts between groups at 16 weeks.

Comparison of 6MWD between groups over 16 weeks.

Difference in accelerometer-derived activity levels between the two groups at 16-weeks.

3.3.7 Sample size

The MCID for the primary outcome, ACQ6, is 0.5 and the standard deviation for a similar population for ACQ is also 0.5 [231]. Assuming $\alpha = 0.05$, $\beta = 0.2$ and power = 0.8, to detect a difference in the means of the Counterweight Plus group and usual care of 0.5 from baseline to Visit 2, a sample size of 30 participants (15 in each group) was needed. 40 participants were targeted, 20 in each group, to allow for a 25% dropout rate.

3.3.8 Statistical analysis

Data handling

All data were entered initially into individual CRFs and then subsequently collated onto a secure electronic spreadsheet (Microsoft Excel). Both documents were purposely created by the PI and Clinical Research Fellow and maintained by all members of the CRT. A second member of the CRT independently verified the transcription to minimise errors. Where areas of uncertainty were recognised, clarification and correction, if needed, was sought from the original paper CRF. The paper CRFs were stored in a folder within a locked facility accessible only to employees of the Clinical Research Facility. The electronic spreadsheet was password-protected and saved on the secure Clinical Research Facility server, accessible only to the CRT. Accelerometery data were processed by Dr DS Buchan at the University of the West of Scotland as described in Chapter Two.

All data is set to be archived for a minimum of 10 years through the local R&D archive system. The data will not be shared out with the CRT but may be accessed for possible ad-hoc analyses.

Statistical analysis plan

Analysis was performed on an intention-to-treat basis, with participants attending both Visits 1 and 2 included for this regardless of their adherence to intervention. Distribution was assessed using the Shapiro-Wilk test. Based on

this, continuous variables were summarised as mean (95% confidence intervals) or median (interquartile range) and compared with unpaired t-tests or Mann-Whitney U tests respectively. Where appropriate, ANCOVA (with Bonferroni correction), adjusting for baseline, was used to compare change in continuous variables over time, or change in variable was directly compared between groups using unpaired t-tests or Mann-Whitney U tests. Categorical variables were summarised as n. (%) and analysed with Pearson's chi-square, or if expected cell count was <5, with Fisher's exact test. Analysis was performed entirely by the author using IBM SPSS Statistics for Mac, version 28 (IBM Corp., Armonk, N.Y., USA) and graphs were produced using GraphPad Prism for Mac, version 9.3.1 (GraphPad Software, San Diego, CA, USA). Significance was set at $p \leq 0.05$.

3.3.9 Effects of COVID-19

The COVID-19 pandemic resulted in study disruption in various ways. Firstly, recruitment was appropriately postponed during national lockdowns in the UK and during periods of caution due to rising cases numbers in the GGC area. Specifically, recruitment was halted from March 2020 until September 2020, and again from December 2020 until April 2021. Secondly, whilst recruitment was halted, follow-up visits were switched to a remote format with telephone consultations during times of increased risk from rising cases of COVID-19, in order to optimise data collection. For example, the primary and key secondary outcomes of ACQ6 and AQLQ were obtained via telephone, and physical data such as spirometry and blood sampling became missing data. Thirdly, even when physical attendance was allowed at the Research Facility, many participants were advised to or chose to continue shielding resulting in further remote study visits. The dietitian team adapted appropriately also with a hybrid of telephone and physical consultations as appropriate. Finally, protocols were in place within the Clinical Research Facility including mask-wearing, social distancing etc, where appropriate to comply with local and government guidance and to allow study visits to continue.

3.4 Results

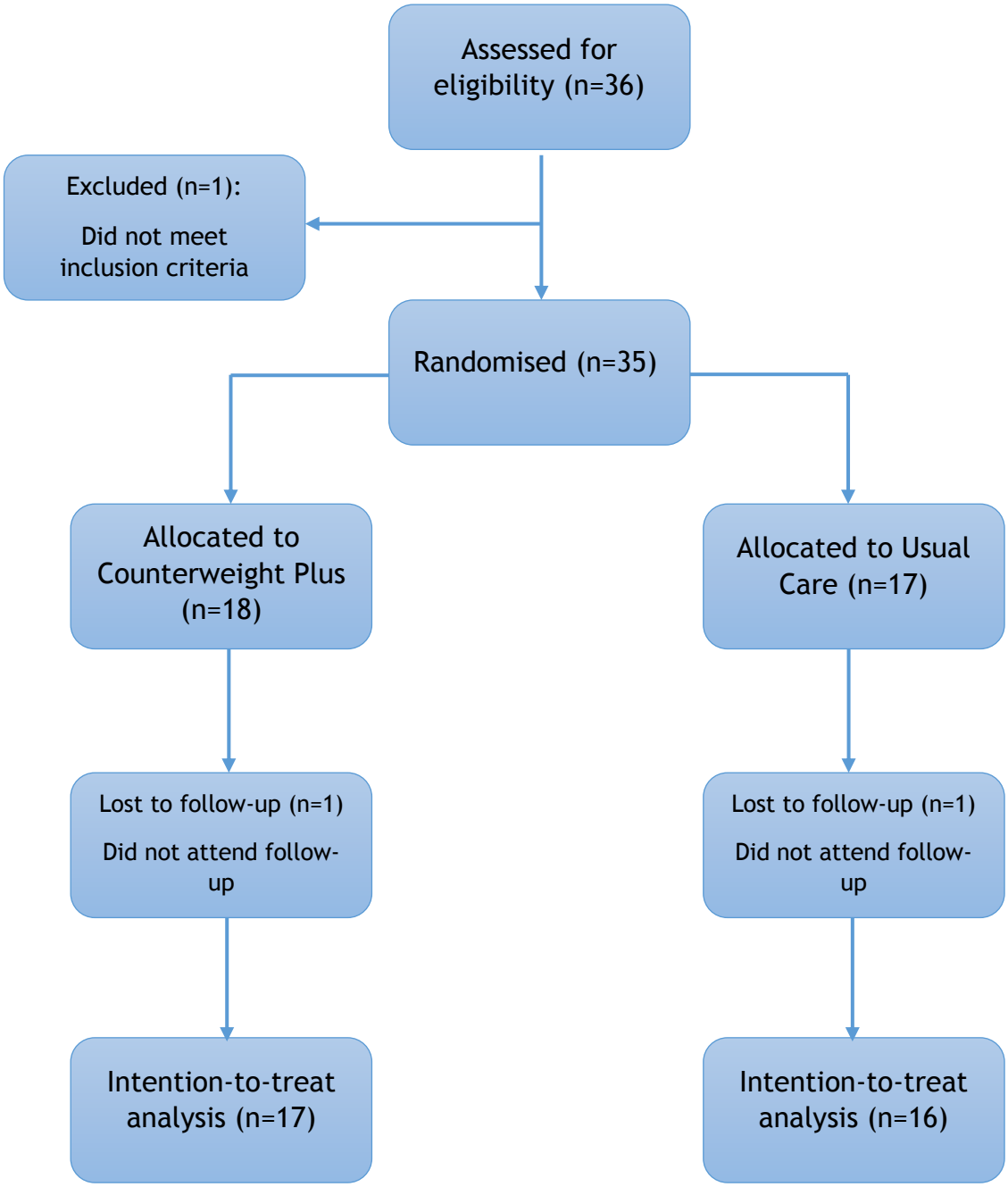
3.4.1 Recruitment

Recruitment took place between August 2019 and August 2021. The final participant attended the primary outcome visit (Visit 2 at 16-weeks) in December 2021.

Figure 3.2 (adapted from Sharma et al [310]) summarises patient recruitment. 36 individuals were screened, of which 35 proceeded to randomisation. One participant was found to be ineligible at final check having started liraglutide for obesity at a private clinic a week prior. This shows a high participation rate reflective of the interest in weight management in this population, however there was significant "pre-screening" occurring of patients attending asthma OPD. This involved "virtual" assessment of eligibility criteria before approaching each potential participant. Numbers of patients pre-screened in this manner prior to approaching were not recorded. Of the 35 participants randomised, two did not respond to contact attempts and did not attend Visit 2, leaving n = 17 for CWP and n = 16 for UC (total n = 33) for intention-to-treat primary analysis. The dropout rate was lower than anticipated and the minimum required (15 per group) was attained allowing recruitment to halt earlier than the planned target of 40.

Two participants in the CWP group discontinued intervention but attended Visit 2. One struggled to tolerate total diet replacement despite encouragement whilst the other had a change in personal circumstances and was unable to continue with intervention. These were excluded to leave a per-protocol analysis group (n = 31). All others in CWP were able to adhere to intervention with dietitian support.

Figure 3. 2 - CONSORT flow diagram



3.4.2 Baseline demographics and characteristics

Table 3.1 summarises characteristics for all patients and compares CWP and UC groups at baseline. Mean age was 53-years and almost two-thirds were female. The majority were ex-smokers (54%) or lifelong non-smokers (43%). Proportions of asthma-related co-morbidities were high including atopy (71%), allergic rhinitis (54%), GORD (86%), osteopenia/osteoporosis (43%) and mental health disorders (51%). 34% were on monoclonal antibody treatment and 17% on maintenance prednisolone suggesting a high treatment burden in this group. Median (IQR) number of OCS courses (3; 2 to 5) and mean (95%CI) ACQ6 score (2.8; 2.4, 3.1) show that this group were frequent exacerbators with poorly controlled disease. Moreover, anthropomorphic measures show this was a morbidly obese, high-risk population with median weight 102kg (91 to 119), BMI 37.5 kg/m^2 (35.0 to 42.3), mean waist-to-hip ratio 0.99 and mean waist-to-height ratio of 0.74. The study population was predominantly T2-low one with reduced median eosinophil counts and FeNO observed ($0.11x10^9/L$ and 18 ppb respectively).

Table 3. 1 - Baseline demographics and characteristics

Variable	Overall (n = 35)	CWP (n = 18)	UC (n = 17)	p value*
Age (years)	52.6 (48.3, 56.9)	56.7 (51.3, 62.1)	48.3 (41.5, 55.1)	**0.047**
Female sex	22 (62.9)	13 (72.2)	9 (52.9)	0.238
Smoking status: Current smoker Ex-smoker Lifelong non-smoker	 1 (2.9) 19 (54.3) 15 (42.9)	 0 (0.0) 12 (66.7) 6 (33.3)	 1 (5.9) 7 (41.2) 9 (52.9)	 0.236
Smoking (pack years)	15.0 (6.0 to 30.0)	15.0 (5.0 to 22.5)	5.0 (0.0 to 20.0)	0.904
Age at asthma diagnosis (years)	30.9 (23.8, 38.1)	34.3 (24.1, 44.4)	27.4 (16.6, 38.2)	0.335
Duration of asthma (years)	21.7 (16.5, 27.0)	22.5 (13.7, 31.3)	20.9 (14.3, 27.5)	0.760
Atopy	25 (71.4)	12 (66.7)	13 (76.5)	0.711
Allergic rhinitis	19 (54.3)	9 (50.0)	10 (58.8)	0.600
Perennial rhinitis	16 (45.7)	7 (38.9)	9 (52.9)	0.404
Nasal polyps	4 (11.4)	3 (16.7)	1 (5.9)	0.603
Nasal surgery	4 (11.4)	3 (16.7)	1 (5.9)	0.603
Eczema	13 (37.1)	6 (33.3)	7 (41.2)	0.631
GORD	30 (85.7)	16 (88.9)	14 (82.4)	0.658
ILO/DFB	8 (22.9)	5 (27.8)	3 (17.6)	0.691
Psychological illness	18 (51.4)	8 (44.4)	10 (58.8)	0.395
Emphysema	5 (14.3)	3 (16.7)	2 (11.8)	1.000
Bronchiectasis	1 (2.9)	1 (5.6)	0 (0.0)	1.000
SAFS/ABPA	9 (25.7)	3 (16.7)	6 (35.3)	0.264
Diabetes mellitus	4 (11.4)	4 (22.2)	0 (0.0)	0.103
Hypertension	9 (25.7)	6 (33.3)	3 (17.6)	0.443
Cardiac disease	7 (20.0)	2 (11.1)	5 (29.4)	0.214
Osteopenia/osteoporosis	15 (42.9)	6 (33.3)	9 (52.9)	0.241
BDP equivalent dose (mcg)	1600 (1600 to 2000)	1600 (1600 to 1600)	2000 (1600 to 2400)	0.077

LAMA	33 (94.3)	18 (100.0)	15 (88.2)	0.229
Maintenance prednisolone	6 (17.1)	4 (22.2)	2 (11.8)	0.658
Prednisolone dose (mg)	4.5 (1.2, 7.8)	4.5 (-1.9, 10.9)	4.5 (-1.9, 10.9)	1.000
Montelukast	27 (77.1)	14 (77.8)	13 (76.5)	1.000
Theophylline	22 (62.9)	10 (55.6)	12 (70.6)	0.358
Azithromycin	7 (20.0)	6 (33.3)	1 (5.9)	0.088
Omalizumab	4 (11.4)	1 (5.6)	3 (17.6)	0.338
Mepolizumab	8 (22.9)	4 (22.2)	4 (23.5)	1.000
Antihistamine	24 (68.6)	11 (61.1)	13 (76.5)	0.328
Nasal steroid	24 (68.6)	12 (66.7)	12 (70.6)	0.803
PPI/H2A	30 (85.7)	17 (94.4)	13 (76.5)	0.177
Previous 12 months:				
Prednisolone courses	3 (2 to 5)	4 (2 to 5)	3 (2 to 5)	0.318
Out-of-hours GP	0 (0 to 0)	0 (0 to 0)	0 (0 to 0)	0.858
ED attendance	0 (0 to 0)	0 (0 to 0)	0 (0 to 0)	0.568
Hospital admissions	0 (0 to 1)	0 (0 to 0)	0 (0 to 1)	0.684
ICU admissions	0 (0 to 0)	0 (0 to 0)	0 (0 to 0)	1.000
Weight (kg)	101.7 (91.4 to 118.7)	103.3 (96.9 to 118.3)	97.0 (86.5 to 122.0)	0.287
BMI (kg/m^2)	37.5 (35.0 to 42.3)	38.2 (35.6 to 45.3)	36.1 (32.7 to 42.5)	0.184
Waist circumference (cm)	121.0 (116.5, 125.4)	122.8 (117.3, 128.3)	119.1 (111.5, 126.7)	0.410
Hip circumference (cm)	123.0 (117.4, 128.7)	126.7 (121.4, 132.0)	119.1 (108.6, 129.6)	0.172
Waist-to-hip ratio	0.99 (0.96, 1.02)	0.97 (0.93, 1.01)	1.01 (0.97, 1.05)	0.185
Waist-to-height ratio	0.74 (0.71, 0.77)	0.76 (0.71, 0.80)	0.72 (0.68, 0.77)	0.270
MRC dyspnoea scale	3 (3 to 4)	3 (3 to 4)	3 (3 to 4)	0.807
ACQ6	2.8 (2.4, 3.1)	2.8 (2.2, 3.3)	2.8 (2.2, 3.3)	0.994
AQLQ:				
Overall	3.8 (3.4, 4.2)	3.8 (3.3, 4.4)	3.8 (3.2, 4.4)	0.957
Symptom domain	3.8 (3.4, 4.2)	3.7 (3.2, 4.3)	3.8 (3.2, 4.5)	0.829
Activity domain	3.8 (3.4, 4.2)	3.9 (3.4, 4.4)	3.7 (3.0, 4.3)	0.529
Emotional domain	3.8 (3.2, 4.3)	3.6 (2.8, 4.5)	3.9 (3.1, 4.7)	0.689
Environmental domain	4.1 (3.6, 4.6)	4.0 (3.4, 4.6)	4.2 (3.4, 5.0)	0.724
HADS: Anxiety score	8 (6 to 11)	9 (7 to 11)	7 (5 to 11)	0.463
HADS: Depression score	8 (5 to 11)	8 (5 to 11)	9 (7 to 14)	0.193

Eosinophils (x10^9/L)	0.11 (0.08 to 0.42)	0.17 (0.08 to 0.42)	0.1 (0.04 to 0.51)	0.656
FeNO (ppb)	18 (11 to 33)	15 (10 to 35)	20 (13 to 51)	0.205
PEF (L/min)	375 (334, 415)	318 (275, 360)	435 (374, 496)	**0.002**
Spirometry:				
Pre-BD FEV_1 (%)	72.1 (66.0, 78.1)	65.8 (57.1, 74.6)	78.7 (70.7, 86.7)	**0.030**
Pre-BD FEV_1/FVC (%)	70.4 (67.2, 73.5)	67.9 (62.5, 73.2)	73.0 (69.7, 76.2)	0.099
Post-BD FEV_1 change (%)	3.4 (1.3, 5.4)	5.1 (1.5, 8.7)	1.5 (-0.5, 3.6)	0.083
6MWD (m)	326 (284, 367)	315 (250, 381)	337 (282, 393)	0.596
Borg score	3 (3, 4)	3 (2, 4)	3 (3, 4)	0.278
Accelerometery (min/d):				
Inactive time	1164 (1115, 1212)	1211 (1159, 1263)	1108 (1026, 1190)	**0.025**
Time in LPA	227 (187, 266)	189 (149, 229)	271 (200, 341)	**0.041**
Time in MVPA	37.3 (22.5 to 79.7)	26.8 (12.3 to 75.3)	61.0 (36.7 to 86.1)	**0.041**

Continuous variables described as mean (95% confidence intervals) or median (first quartile to third quartile).
Categorical variables described as no. (%).
*Comparison of CWP vs UC using independent t test or Mann Whitney U test.
Abbreviations: ABPA (Allergic Bronchopulmonary Aspergillosis); ACQ6 (Asthma Control Questionnaire-6); AQLQ (Asthma Quality of Life Questionnaire); BD (Bronchodilator); BDP (Beclomethasone dipropionate); BMI (Body Mass Index); CWP (Counterweight Plus); DFB (Dysfunctional breathing); ED (Emergency Department); FeNO (Fractional exhaled Nitric Oxide); FEV_1 (Forced Expiratory Volume in 1 second); FVC (Forced Vital Capacity); GORD (Gastro-oesophageal Reflux Disease); HAD (Hospital Anxiety and Depression scale); H2A (H2-receptor antagonists); ICU (Intensive Care Unit); ILO (Inducible Laryngeal Obstruction); LAMA (Long-acting anti-muscarinic); LPA (Low Physical Activity); MRC (Medical Research Council); MVPA (Moderate to Vigorous Physical Activity); OOH (Out-of-hours); PEF (Peak Expiratory Flow); ppb (parts per billion); PPI (Proton pump inhibitor); SAFS (Severe Asthma with Fungal Sensitisation); UC (Usual Care); 6MWD (6 minute Walk Distance).

Adapted from Sharma et al [310]

Between group differences at baseline were observed in age, peak flow, FEV_1 and accelerometery data. Participants in CWP were older, had reduced peak flow and FEV_1, and evidence of more time spent inactive on accelerometery compared to UC. There were no between group differences in all other baseline variables.

3.4.3 Primary outcome

Mean change in ACQ6 was –0.4 (-1.0, 0.1) in the CWP group and 0.2 (-0.2, 0.6) in the UC group over 16 weeks. Mean difference between groups was –0.69 (-1.37, -0.01; p = 0.048). Table 3.2 and Figure 3.3 summarise ACQ6 and AQLQ changes.

Table 3. 2 - Intention-to-treat analysis of asthma control and quality of life between CWP and UC over 16 weeks

Change in variable	CWP group (n = 17)	UC group (n = 16)	Mean difference between CWP and UC	p-value*
ACQ6	-0.4 (-1.0, 0.1)	0.2 (-0.2, 0.6)	-0.69 (-1.37, -0.01)	**0.048**
AQLQ	0.8 (0.3, 1.3)	0.1 (-0.3, 0.5)	0.76 (0.18, 1.34)	**0.013**
AQLQ Symptom	1.0 (0.4, 1.5)	0.3 (-0.1, 0.6)	0.72 (0.14, 1.31)	**0.018**
AQLQ Activity	0.5 (0.0, 1.1)	-0.1 (-0.7, 0.5)	0.78 (0.08, 1.47)	**0.029**
AQLQ Emotional	1.5 (0.6, 2.3)	0.7 (0.1, 1.3)	0.72 (-0.16, 1.59)	0.104
AQLQ Environmental	0.5 (-0.3, 1.3)	-0.5 (-1.3, 0.3)	0.98 (0.01, 1.96)	**0.048**

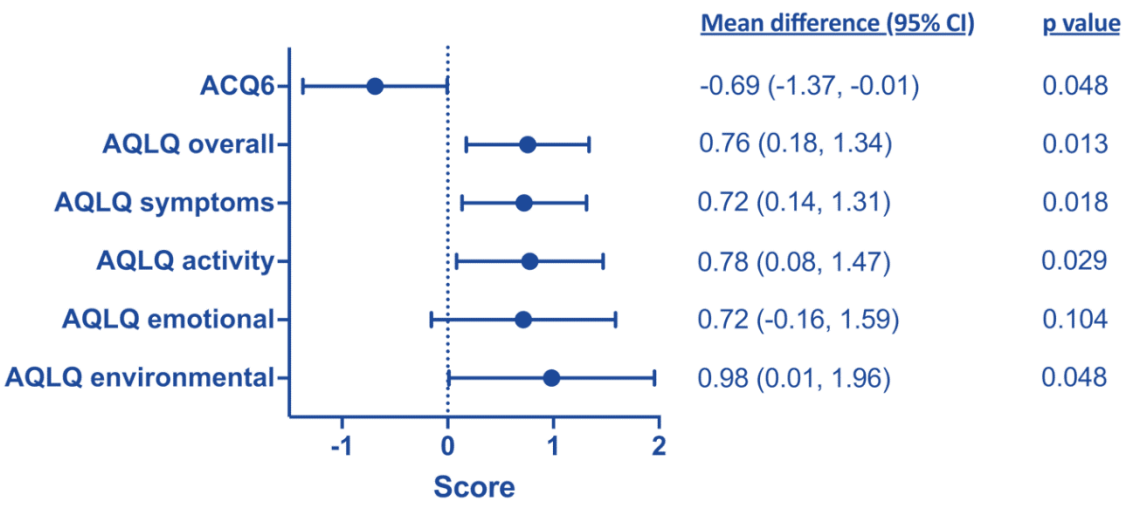
Variables described as mean (95% confidence intervals).

*Comparison of mean difference using ANCOVA adjusting for baseline.

Abbreviations: ACQ6 (Asthma Control Questionnaire); AQLQ (Asthma Quality of Life Questionnaire); CWP (Counterweight Plus); UC (Usual Care).

Adapted from Sharma et al [310]

Figure 3. 3 - Mean differences in ACQ6 and AQLQ between Counterweight-Plus and usual care over 16-weeks



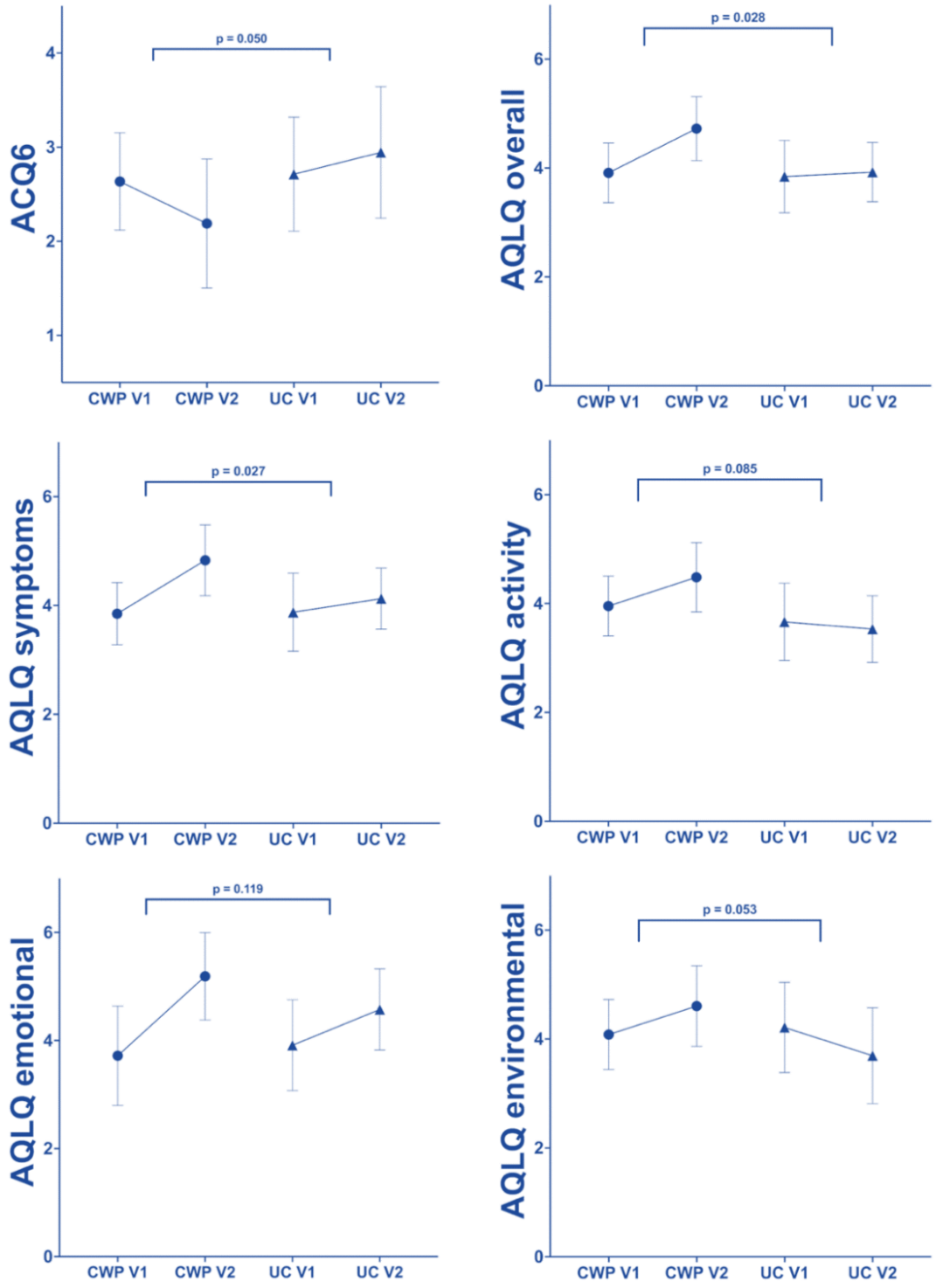
ACQ6 score left of the dashed line favours a good response. AQLQ score to the right of the dashed line favours a good response.

3.4.4 Secondary outcomes

Mean change in overall AQLQ was 0.8 (0.3, 1.3) in the CWP group and 0.1 (-0.3, 0.5) in the UC group over 16 weeks. Mean difference between groups was 0.76 (0.18, 1.34; p = 0.013). Similarly, as shown in Table 3.2 and Figure 3.3, CWP resulted in greater benefit in AQLQ symptom, activity and environmental domains compared to UC over 16 weeks. No between group difference was seen in AQLQ emotional domain.

Figure 3.4 (adapted from Sharma et al [310]) shows change in mean ACQ6 and AQLQ scores from Visit 1 to Visit 2, with p-values showing comparison by independent t-test. Table 3.3 summarises these results.

Figure 3. 4 - Change in ACQ6 and AQLQ scores between CWP and UC at baseline (V1) and 16-weeks (V2)



p value compares change in variable between CWP and UC with independent t test

Table 3. 3 - Between-visit comparison of 16-week ACQ6 and AQLQ in CWP against UC

Variable	CWP group (n = 17)	UC group (n = 16)	p-value
ACQ6:			
Visit 1	2.6 (2.1, 3.2)	2.7 (2.1, 3.3)	0.837
Visit 2	2.2 (1.5, 2.9)	2.9 (2.2, 3.6)	0.111
Change	-0.4 (-1.0, 0.1)	0.2 (-0.2, 0.6)	**0.050**
AQLQ:			
Visit1	3.9 (3.4, 4.5)	3.8 (3.2, 4.5)	0.867
Visit 2	4.7 (4.1, 5.3)	3.9 (3.4, 4.5)	**0.043**
Change	0.8 (0.3, 1.3)	0.1 (-0.3, 0.5)	**0.028**
AQLQ Symptom:			
Visit1	3.8 (3.3, 4.4)	3.9 (3.2, 4.6)	0.948
Visit2	4.8 (4.2, 5.5)	4.1 (3.6, 4.7)	0.093
Change	1.0 (0.4, 1.5)	0.3 (-0.1, 0.6)	**0.027**
AQLQ Activity:			
Visit 1	4.0 (3.4, 4.5)	3.7 (3.0, 4.4)	0.493
Visit2	4.5 (3.8, 5.1)	3.5 (2.9, 4.1)	**0.029**
Change	0.5 (0.0, 1.1)	-0.1 (-0.7, 0.5)	0.085
AQLQ Emotional:			
Visit 1	3.7 (2.8, 4.6)	3.9 (3.1, 4.8)	0.743
Visit2	5.2 (4.4, 6.0)	4.6 (3.8, 5.3)	0.248
Change	1.5 (0.6, 2.3)	0.7 (0.1, 1.3)	0.119
AQLQ Environmental:			
Visit1	4.1 (3.4, 4.7)	4.2 (3.4, 5.0)	0.792
Visit2	4.6 (3.9, 5.3)	3.7 (2.8, 4.6)	0.100
Change	0.5 (-0.3, 1.3)	-0.5 (-1.3, 0.3)	0.053

Continuous variables described as mean (95% CI).
p value compares CWP vs UC using unpaired t test.
Abbreviations: ACQ6 (Asthma Control Questionnaire); AQLQ (Asthma Quality of Life Questionnaire); CWP (Counterweight Plus); UC (Usual Care).

Adapted from Sharma et al [310]

There was a higher number of ACQ6 responders (those achieving MCID) with CWP than UC (53% vs 19% respectively, p = 0.041; NNT = 3 (95%CI 2, 27)), but no between group differences in number of AQLQ responders (overall or domains). Table 3.4 and Figure 3.5 (adapted from Sharma et al [310]) summarise this in more detail.

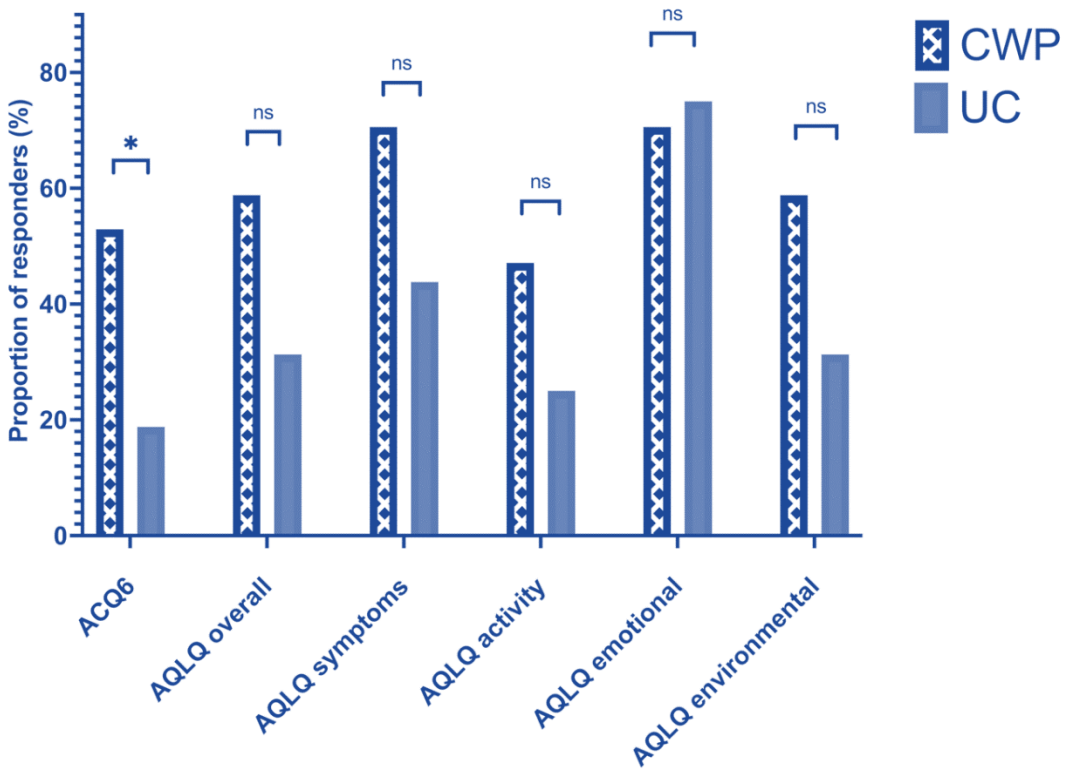
Table 3. 4 - Proportion of intention-to-treat participants achieving MCID in ACQ6 and AQLQ scores

Variable	CWP group (n = 17)	UC group (n = 16)	p-value
ACQ6 MCID change	9 (52.9)	3 (18.8)	**0.041**
AQLQ MCID change	10 (58.8)	5 (31.3)	0.112
AQLQ symptoms MCID change	12 (70.6)	7 (43.8)	0.119
AQLQ activity MCID change	8 (47.1)	4 (25.0)	0.188
AQLQ emotional MCID change	12 (70.6)	12 (75.0)	1.000
AQLQ environmental MCID change	10 (58.8)	5 (31.3)	0.112

Variables described as number (%) and compared using chi-square or Fisher's exact as appropriate.
Abbreviations: ACQ6 (Asthma Control Questionnaire); AQLQ (Asthma Quality of Life Questionnaire); CWP (Counterweight Plus); MCID (Minimal Clinically Important Difference); UC (Usual Care).

Adapted from Sharma et al [310]

Figure 3. 5 - Proportion of participants achieving MCID in ACQ6 and AQLQ with CWP and UC over 16-weeks



Compared using chi-square or Fisher's exact. * denotes significant result; ns = not significant.

Pearson's test showed a moderate-to-large positive correlation between change in weight and ACQ6 across 16 weeks (r = 0.607, p = 0.001). Univariate linear regression was significant (F[1,20] = 11.682, p = 0.003, R^2 = 36.9%) and weight change predicted ACQ6 over 16 weeks (β = 0.066 [95% CI 0.026, 0.106], p = 0.003).

Additionally, further Pearson's test showed a moderate-to-large negative correlation between change in weight and overall AQLQ across 16 weeks (r = -0.433, p = 0.022). Univariate linear regression was significant (F[1,20] = 4.613, p = 0.044, R^2 = 18.7%) and weight change predicted AQLQ over 16 weeks (β = -0.053 [95% CI –0.105, -0.002], p = 0.044).

No between group differences were observed in number of OCS courses, out-of-hours GP attendances, emergency department attendances, hospital admissions or ICU admissions over 16 weeks.

3.4.5 Other outcomes

Table 3.5 summarises other outcomes between CWP and UC. Compared to UC, weight loss was greater in the CWP group with a mean difference of –12kg (-17, -8; p < 0.001). Mean percentage body weight loss was 12% in the CWP group. Participants in CWP had a decrease of approximately 5 kg/m^2 in BMI, and a mean difference, compared with UC, of -4.6kg/m^2 (-6.3, -2.9), p<0.001. Furthermore, there was a greater improvement in mean waist-to-height ratio in CWP compared to UC of -0.06 (-0.11, -0.01), p = 0.029.

Participants in the CWP group experienced an improvement in breathlessness compared to UC with a median change in MRC dyspnoea scale of –1 (-1 to 0) and 0 (0 to 0) respectively, p = 0.004. No differences were observed between groups in HAD scale, spirometry, 6MWD, Borg score, blood eosinophils, FeNO or accelerometer outcomes.

Table 3. 5 - Intention-to-treat comparison of other outcomes between CWP and UC

Change in variable	N	CWP group	N	UC group	Mean difference between CWP and UC	p-value*
Weight (kg)	13	-13.5 (-17.5, -9.6)	9	-1.4 (-3.2, 0.4)	-12.1 (-16.9, -7.4)	**<0.001**
Total body weight (%)		-12.3 (-15.7, -8.8)		-1.2 (-3.0, 0.7)	-11.1 (-15.4, -6.9)	**<0.001**
BMI (kg/m^2)	13	-4.9 (-6.3, -3.5)	9	-0.3 (-1.1, 0.6)	-4.6 (-6.3, -2.9)	**<0.001**
WC, cm	7	-10.9 (-18.5, -3.3)	6	-1.7 (-7.4, 4.1)	-9.3 (-18.0, -0.6)	**0.039**
HC, cm		-8.2 (-16.5, 0.2)		5.3 (0.2, 10.4)	-13.5 (-22.5, -4.4)	**0.007**
Waist-to-height ratio	7	-0.07 (-0.12, -0.02)	6	-0.01 (-0.04, 0.02)	-0.06 (-0.11, -0.01)	**0.029**
Waist-to-hip ratio		-0.03 (-0.09, 0.03)		-0.06 (-0.12, 0.00)	0.03 (-0.04, 0.11)	0.318
MRC dyspnoea scale	17	-1 (-1 to 0)	16	0 (0 to 0)	n/a	**0.004**
HAD:						
Anxiety	17	1 (-1, 3)	16	1 (-1, 2)	0 (-3, 3)	0.972
Depression		-1 (-3, 2)		1 (-1, 2)	-1 (-4, 2)	0.445
Eosinophils (x10^9/L)	8	0.05 (0.00 to 0.11)	6	0.00 (-0.23 to 0.12)	n/a	0.228
FeNO (ppb)	8	1 (-3 to 21)	6	-6 (-28 to 18)	n/a	0.573
PEF (L/min)	9	38 (-16, 91)	6	7 (-36, 49)	31 (-37, 99)	0.343
Spirometry:						
Pre-BD FEV_1 (%)	8	5.5 (-3.2, 14.2)	6	3.7 (-1.4, 8.8)	1.8 (-7.5, 11.1)	0.671
Pre-BD FEV_1/FVC (%)		-1.96 (-4.23, 0.32)		1.09 (-4.25, 6.43)	-3.0 (-8.4, 2.3)	0.224
Post-BD FEV_1 (%)		3.4 (-2.8, 9.6)		4.2 (-7.3, 15.7)	-0.8 (-11.5, 9.9)	0.874
Annual healthcare use:						
Prednisolone courses		-2 (-2 to 0)		-2 (-3 to 1)		0.790
OOH GP attendances	17	0 (0 to 3)	16	0 (0 to 3)	n/a	0.737
ED attendances		0 (0 to 0)		0 (0 to 0)		0.557
Hospital admissions		0 (0 to 0)		0 (-1 to 0)		0.510
ICU admissions		0 (0 to 0)		0 (0 to 0)		1.000
6MWD (m)	8	8 (-16, 31)	5	0 (-50, 50)	8 (-34, 49)	0.698
Borg score	8	-1 (-2 to 0)	5	-1 (-3 to 0)	n/a	0.724
Accelerometery:						
Inactive time, min/d	5	-38.1 (-89.1, 12.9)	3	8.3 (-80.9, 97.6)	-46.4 (-116.9, 24.1)	0.158
Time in LPA, min/d		29.0 (-11.2, 69.2)		-9.6 (-84.3, 65.1)	38.6 (-17.9, 95.1)	0.145
Time in MVPA, min/d		9.1 (-23.0, 41.2)		1.3 (-13.4, 16.0)	7.8 (-30.4, 46.0)	0.635

Variables described as mean (95% confidence intervals) or median (first quartile to third quartile).
*Comparison using independent t test or Mann Whitney U test.
Abbreviations: BD (Bronchodilator); BMI (Body Mass Index); CWP (Counterweight Plus); ED (Emergency Department); FeNO (Fractional exhaled Nitric Oxide); FEV_1 (Forced Expiratory Volume in 1 second); FVC (Forced Vital Capacity); HAD (Hospital Anxiety and Depression scale); HC (hip circumference); ICU (Intensive Care Unit); MRC (Medical Research Council); OOH (Out-of-hours); PEF (Peak Expiratory Flow); ppb (parts per billion); UC (Usual Care); WC (waist circumference); 6MWD (6 minute Walk Distance).

Adapted from Sharma et al [310]

3.4.6 Per protocol analysis

As stated above, the per protocol analysis had a total of 31 participants, 15 in CWP and 16 in UC. CWP resulted in favourable outcomes compared to UC in weight-loss (mean difference –13kg (-17, -9); p <0.001), BMI (-5 kg/m^2 (-7, -4); p <0.001), waist-to-height ratio (-0.07 (-0.12, -0.02); p = 0.017) and MRC dyspnoea score (p = 0.002). Table 3.6 compares other outcomes from the per-protocol CWP and UC groups.

Table 3. 6 - Per protocol comparison of other outcomes between CWP and UC

Change in variable	N	CWP group	N	UC group	Mean difference between CWP and UC	p-value*
Weight (kg) Total body weight (%)	12	-14.7 (-18.0, -11.4) -13.3 (-16.2, -10.5)	9	-1.4 (-3.2, 0.4) -1.2 (-3.0, 0.7)	-13.3 (-17.2, -9.4) -12.2 (-15.6, -8.8)	**<0.001** **<0.001**
BMI (kg/m^2)	12	-5.3 (-6.4, -4.1)	9	-0.3 (-1.1, 0.6)	-5.0 (-6.5, -3.5)	**<0.001**
WC (cm) HC (cm)	6	-12.4 (-20.7, -4.1) -10.5 (-18.0, -3.1)	6	-1.7 (-7.4, 4.1) 5.3 (0.2, 10.4)	-10.8 (-19.5, -2.0) -15.8 (-23.6, -8.0)	**0.021** **0.001**
Waist-to-height ratio Waist-to-hip ratio	6	-0.08 (-0.13, -0.02) -0.02 (-0.09, 0.05)	6	-0.01 (-0.04, 0.02) -0.06 (-0.12, 0.00)	-0.07 (-0.12, -0.02) 0.04 (-0.04, 0.12)	**0.017** 0.280
MRC dyspnoea scale	15	-1 (-1 to 0)	16	0 (0 to 0)	**n/a**	**0.002**
HAD: Anxiety Depression	15	 0 (-2, 2) -2 (-4, 1)	16	 1 (-1, 2) 1 (-1, 2)	 -1 (-3, 2) -2 (-5, 1)	 0.567 0.134
Eosinophils (x10^9/L)	7	0.02 (0.00 to 0.12)	6	0.00 (-0.23 to 0.12)	n/a	0.295
FeNO (ppb)	7	0 (-3 to 26)	6	-6 (-28 to 18)	n/a	0.534
PEF (L/min)	8	39 (-23, 101)	6	7 (-36, 49)	33 (-41, 106)	0.350
Spirometry: Pre-BD FEV_1 (%) Pre-BD FEV_1/FVC (%) Post-BD FEV_1 (%)	7	 6.9 (-2.9, 16.6) -1.12 (-2.44, 0.20) 5.0 (-0.8, 10.8)	6	 3.7 (-1.4, 8.8) 1.09 (-4.25, 6.43) 4.2 (-7.3, 15.7)	 3.2 (-7.1, 13.5) -2.20 (-7.53, 3.12) 0.8 (-9.9, 11.5)	 0.510 0.346 0.867
Annual healthcare use: Prednisolone courses OOH GP attendances ED attendances Hospital admissions ICU admissions	15	 -2 (-2 to 0) 0 (0 to 3) 0 (0 to 0) 0 (0 to 0) 0 (0 to 0)	16	 -2 (-3 to 1) 0 (0 to 3) 0 (0 to 0) 0 (-1 to 0) 0 (0 to 0)	 n/a	 0.861 0.806 0.572 0.379 1.000
6MWD (m)	7	9 (-20, 37)	5	0 (-50, 50)	9 (-37, 54)	0.681
Borg score	7	-1 (-2, -1)	5	-1 (-3 to 0)	n/a	1.000
Accelerometery, min/d: Inactive time Time in LPA Time in MVPA	4	 -40.1 (-115.2, 34.9) 31.5 (-26.9, 90.1) 8.6 (-38.8, 56.0)	3	 8.3 (-80.9, 97.6) -9.6 (-84.3, 65.1) 1.3 (-13.4, 16.0)	 -48.5 (-132.9, 36.0) 41.2 (-26.1, 108.4) 7.3 (-38.6, 53.2)	 0.200 0.176 0.699

Variables described as mean (95% confidence intervals) or median (first quartile to third quartile).
*Comparison using independent t test or Mann Whitney U test.
Abbreviations: BD (Bronchodilator); BMI (Body Mass Index); CWP (Counterweight Plus); ED (Emergency Department); FeNO (Fractional exhaled Nitric Oxide); FEV_1 (Forced Expiratory Volume in 1 second); FVC (Forced Vital Capacity); HAD (Hospital Anxiety and Depression scale); HC (hip circumference); ICU (Intensive Care Unit); MRC (Medical Research Council); OOH (Out-of-hours); PEF (Peak Expiratory Flow); ppb (parts per billion); UC (Usual Care); WC (waist circumference); 6MWD (6 minute Walk Distance).

Adapted from Sharma et al [310]

Mean change in ACQ6 was –0.60 (-1.20, 0.01) in the CWP group and 0.23 (-0.17, 0.63) in the UC group over 16 weeks. Mean difference between groups was –0.86 (-1.55, -0.18; p = 0.015).

Mean change in overall AQLQ was 0.97 (0.42, 1.53) in the CWP group and 0.08 (-0.32, 0.48) in the UC group over 16 weeks. Mean difference between groups was 0.95 (0.40, 1.50; p = 0.001). Similarly, CWP resulted in greater benefit in AQLQ symptom, activity and environmental domains compared to UC over 16 weeks. No between group difference was seen in AQLQ emotional domain at 16 weeks.

Table 3.7 summarises these findings.

Table 3. 7 - Per protocol comparison of 16-week asthma outcomes between CWP and UC

Change in variable	CWP group (n = 15)	UC group (n = 16)	Mean difference between CWP and UC	p-value*
ACQ6	-0.60 (-1.20, 0.01)	0.23 (-0.17, 0.63)	-0.86 (-1.55, -0.18)	**0.015**
AQLQ	0.97 (0.42, 1.53)	0.08 (-0.32, 0.48)	0.95 (0.40, 1.50)	**0.001**
AQLQ Symptom	1.11 (0.55, 1.68)	0.25 (-0.13, 0.63)	0.89 (0.32, 1.46)	**0.003**
AQLQ Activity	0.72 (0.25, 1.19)	-0.13 (0.73, 0.46)	0.97 (0.32, 1.62)	**0.005**
AQLQ Emotional	1.45 (0.45, 2.46)	0.66 (0.07, 1.25)	0.85 (-0.62, 1.75)	0.067
AQLQ Environmental	0.66 (-0.17, 1.49)	-0.52 (-1.30, 0.26)	1.18 (0.21, 2.14)	**0.018**

Variables described as mean (95% confidence intervals).

*Comparison of mean difference using ANCOVA adjusting for baseline.

Abbreviations: ACQ6 (Asthma Control Questionnaire); AQLQ (Asthma Quality of Life Questionnaire); CWP (Counterweight Plus); UC (Usual Care).

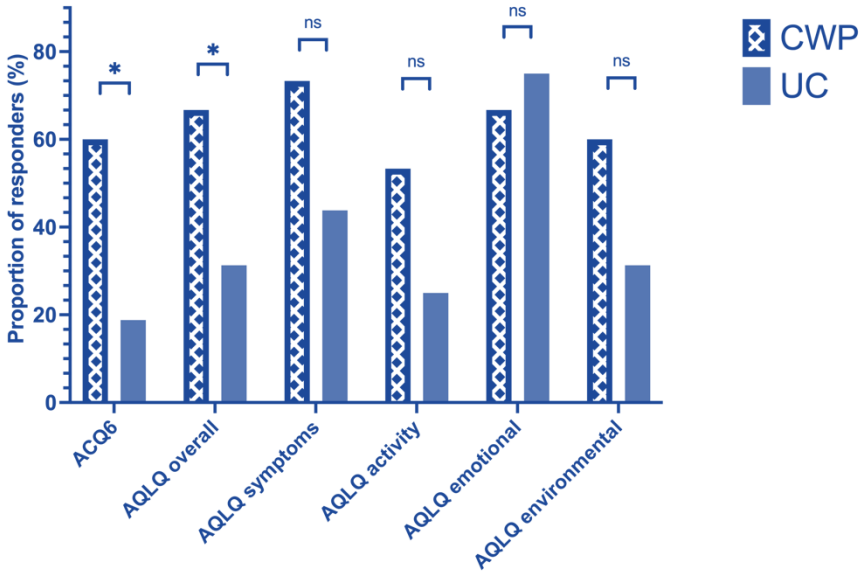
Adapted from Sharma et al [310]

There was a higher proportion of ACQ6 responders (those achieving MCID) with CWP than UC (60% vs 19% respectively, p = 0.018), and in overall AQLQ (67% vs 31% respectively, p = 0.049), though no between group differences in individual AQLQ domains. Table 3.8 and Figure 3.6 summarise these findings.

Table 3. 8 - Proportion of per protocol participants achieving MCID in asthma control and quality of life scores

Variable	CWP group (n = 15)	UC group (n = 16)	p-value
ACQ6 MCID change	9 (60.0)	3 (18.8)	**0.018**
AQLQ MCID change	10 (66.7)	5 (31.3)	**0.049**
AQLQ symptoms MCID change	11 (73.3)	7 (43.8)	0.095
AQLQ activity MCID change	8 (53.3)	4 (25.0)	0.106
AQLQ emotional MCID change	10 (66.7)	12 (75.0)	0.704
AQLQ environmental MCID change	9 (60.0)	5 (31.3)	0.108
Variables described as number (%) and compared using chi-square or Fisher's exact as appropriate. Abbreviations: ACQ6 (Asthma Control Questionnaire); AQLQ (Asthma Quality of Life Questionnaire); CWP (Counterweight Plus); MCID (Minimal Clinically Important Difference); UC (Usual Care).			

Figure 3. 6 - Proportion of per-protocol participants achieving MCID in ACQ6 and AQLQ with CWP and UC over 16-weeks



Compared using chi-square or Fisher's exact. * denotes significant result; ns = not significant.

3.4.7 *Post hoc* weight-loss stratified analysis

Table 3.9 compares ACQ6 and AQLQ scores between participants from the CWP group categorised by extent of total weight loss (<10%, 10-15% and ≥15% of total body weight). Mean change in ACQ6 was >MCID in the 10-15% and ≥15% groups only (-0.7 (0.9) and –1.2 (1.2) respectively), similar to mean change in overall AQLQ (0.6 (0.7) and 1.4 (1.4) respectively). Mean change in symptom (0.5 (0.6) in <10% group, 0.9 (0.8) in 10-15% group and 1.7 (1.4) in ≥15% group) and emotional (0.8 (1.4) in <10% group, 1.0 (1.4) in 10-15% group, 1.4 (2.0) in ≥15% group) AQLQ domains were >MCID in all three groups. Mean change in activity AQLQ domain was >MCID only in the ≥15% group (1.3 (1.2)), similar to the environmental AQLQ domain (0.9 [1.3 in the ≥15% group]). These trends suggest a benefit with >10% loss of total body weight, and greater benefit at >15%.

Table 3. 9 - Post-hoc comparison of asthma control and quality of life stratified by percentage weight loss

Change in variable	<10% group (n=3)	10-15% group (n=6)	≥15% group (n=4)	p value*
ACQ6	-0.1 (-2.0, 1.8)	-0.7 (-1.6, 0.3)	-1.2 (-3.1, 0.7)	0.390
AQLQ	0.2 (-2.1, 2.5)	0.6 (-0.1, 1.3)	1.4 (-0.8, 3.6)	0.309
AQLQ Symptom	0.5 (-1.1, 2.0)	0.9 (0.1, 1.8)	1.7 (-0.4, 3.9)	0.259
AQLQ Activity	-0.1 (-4.2, 4.0)	0.4 (-0.3, 1.0)	1.3 (-0.5, 3.1)	0.236
AQLQ Emotional	0.8 (-2.7, 4.3)	1.0 (-0.4, 2.4)	1.4 (-1.8, 4.6)	0.876
AQLQ Environmental	-0.4 (-3.8, 3.0)	0.3 (-1.7, 2.3)	0.9 (-1.2, 2.9)	0.625
Variables described as mean (95% confidence intervals). *Comparison of mean difference using ANOVA. Abbreviations: ACQ6 (Asthma Control Questionnaire); AQLQ (Asthma Quality of Life Questionnaire).				

Adapted from Sharma et al [310]

3.4.8 Safety outcomes

No intervention-related adverse events were observed during the trial period of 16 weeks. Five participants were hospitalised for unrelated or unexpected events. Three of these were in UC of which one was admitted with COVID-19 pneumonitis and two with exacerbations of asthma (one required monitoring in high dependency). Two participants were from the CWP group. One was admitted to hospital with COVID-19 gastroenteritis, and one with migraine (with a history of the same).

3.5 Discussion

3.5.1 Weight management in difficult-to-treat asthma and obesity

Results from this open label, randomised, controlled trial over 16 weeks showed improved asthma control and quality of life with the Counterweight-Plus weight management programme compared to standard care in individuals with obesity and difficult-to-treat asthma. This is evidenced by clinical improvements in ACQ6, AQLQ and AQLQ domain scores. Whilst the mean change in ACQ6 within the CWP group was below the MCID, when compared to usual care, this difference is above the MCID. Furthermore, per protocol analysis revealed greater benefit in those adherent to the CWP programme. Post-hoc weight-loss analysis suggests that weight-loss of at least 10% results in clinical benefits, and total body weight loss >15% with probable more pronounced effect. Beyond control and quality of life, CWP resulted in an improvement in exertional dyspnoea and marked reductions in weight, BMI and waist circumference, imparting benefits to overall health. The intervention is safe, can be delivered in a community setting and is effective suggesting CWP may have clinical utility as a conservative option in managing individuals with obesity and difficult-to-treat asthma. Longer-term outcomes are pending, with one-year outcomes described in Chapter Four.

There were no between-group differences observed in healthcare usage (I.e. frequency of exacerbations, OOH GP/ED attendances, hospital/ICU admissions), though 16-weeks may be too soon to expect benefits. No differences were seen either in HAD score, lung function, markers of T2 inflammation (eosinophils and FeNO) or accelerometer-derived activity levels. However, there was likely insufficient data (due to the COVID-19 pandemic) to detect any differences between groups – highlighted in the limitations below. In the last decade, there have been several weight-management studies in individuals with asthma and obesity, though with heterogeneity in method, chosen population and outcomes. [231]In 2014, Dias-Júnior et al [311] published results from a randomised (2:1) controlled trial of low-calorie intake, 10mg daily sibutramine (a serotonin-noradrenaline reuptake inhibitor with appetite suppressant effects) and 120mg daily orlistat against control in 33 participants (22 intervention, 11 control) with

severe asthma. This was a more clinically relevant population that was well-defined with criteria for severe asthma clearly stated. The authors chose to report primary results from a per protocol analysis (though this was not pre-specified), defined as weight loss >10%, which limits the real-world impact of their findings, though intention-to-treat results are available on an online supplement. These results are broadly comparable to our findings with an improvement in mean±SD ACQ from 3.02±0.19 to 2.25±0.28 in the intervention group observed with substantial weight loss. There were no asthma-related quality of life outcomes assessed. Only 12 of the 22 participants were able to adhere to the weight loss intervention provided, a much lower proportion than our study. Furthermore, the pharmacological components of the intervention are not without risk. Orlistat commonly causes unwanted gastrointestinal side-effects, and rarely hepatic injury [312], whilst sibutramine is no longer recommended for use following evidence of increased risk of myocardial infarction and stroke [313].

In 2015, Ma et al [314] reported no improvements in ACQ or asthma quality of life with a mixed lifestyle intervention (calorie-restricted diet, increased physical activity and behavioural techniques) in 330 adults with obesity and asthma. Trial pragmatism is questionable as 2022 were initially screened. Beyond this, whilst baseline weight and BMI (104kg and 37.5 kg/m^2, respectively) are similar to our population, baseline ACQ of 1.4 was considerably lower suggestive of a more controlled group. Moreover, real-world applicability is also unclear as participants taking maintenance oral corticosteroids were excluded. Overall, mean weight-loss at 6 months was markedly lower than our trial at 5kg, which may account for lack of response in asthma control and quality of life.

In 2017, Freitas et al [315] reported results from a randomised trial assessing the effects of a 3-month cardiovascular exercise programme against sham breathing and stretching exercises on ACQ and AQLQ. Again, trail pragmatism and generalisability are called into question as 51 individuals took part from 645 screened. There were no documented reasons for the ineligibility of 167 individuals. Participants requiring maintenance oral corticosteroids were

excluded. Whilst results show improvement in both asthma control and quality of life with a median weight-loss of 6kg, the population studied varied. Asthma definition and severity were not specified prior to trial initiation. Given the issues surrounding asthma heterogeneity and difficulties in confirming asthma, an unspecified definition of asthma used in this study further calls into question generalisability. Additionally, 98% of participants were female, with a substantially lower mean weight (92kg intervention group, 90kg control) and ACQ (2.0 intervention group, 2.0 control) than our population. Peripheral eosinophils (>0.3 $x10^9$/L) in this group are more suggestive of a T2-high endotype.

In 2019, Grandi Silva et al [230] reported results from a study assessing a weight-loss intervention, consisting of exercise, dietary change and psychology support, on dynamic hyperinflation in 51 adult women with moderate-severe asthma and BMI 35-40 kg/m^2. Whilst their primary outcome was related to dynamic hyperinflation, they report post-hoc results showing improved ACQ and AQLQ in the group that achieved ≥5% total body weight loss compared to those losing <5%. These results should be interpreted with caution, however, as there are several key limitations including absence of detail of the intervention given, lack of randomisation or control and unclear pre-specified definitions of primary and secondary outcomes.

A randomised controlled trial of 55 adults with asthma and obesity assessing a 10-week weight management programme with dietitian support against standard care was reported by Özbey et al [316] in 2020. Promising results of improved asthma control and quality of life observed must be weighed against limitations of the trial. The population studied was 96% female with unclear details of how asthma diagnosis was confirmed, whether asthma was still active (usually defined as requirement of medication in the previous twelve months), and what severity of disease was present. Indeed, the reported mean Asthma Control Test score of 21 indicates a population with good control. These call into question the generalisability of their findings.

Together these trials all raise suspicion as to the pragmatic, real-world applicability of their interventions. We provide clarity by studying a pre-defined difficult-to-treat population that forms the bulk of secondary and tertiary outpatient burden using an evidence-based, readily available programme.

3.5.2 Limitations

A number of potential limitations need to be acknowledged. Firstly, this was a proof-of-concept, open-label trial aiming to determine feasibility prior to a larger study and as such has low population numbers subject to discrepancies between treatment and control groups. This is evidenced by the between group differences in age, FEV_1, PEFR and accelerometery data at baseline limiting definitive conclusions. Randomisation minimises the impact of variability on outcomes. Moreover, these variables are not likely to have impacted on our primary and key secondary outcomes of asthma control and quality of life.

Secondly, the effects of restrictions from the COVID-19 pandemic (described above) resulted in substantial missing data with only 39% having complete datasets at Visit 2. Data for the primary outcome (ACQ6) and key secondary outcomes (e.g., AQLQ) were complete however variables dependent on physical attendance at the Clinical Research Facility were largely missing. This included serum sampling, lung function, 6MWT and accelerometery. A more complete dataset may have allowed conclusions to be drawn as to the effects of CWP on spirometry, peak flow, exacerbation rate and inflammation among other outcomes. The percentage of missing data in these variables was too great to justify handling methods such as multiple imputation and therefore complete case analysis alone was performed.

Thirdly, variables such as number of prednisolone course, out-of-hours GP attendances etc. were subject to recall bias.

Finally, as with all weight-loss trials, individuals were likely to be more motivated to participate which may reflect selection bias. However, this is unlikely to affect the clinical utility of the intervention.

Notwithstanding the above, important advantages of this trial include pragmatism and relevance to asthma care as described. Our population is a challenging-to-manage phenotype, one that is high-risk and often associated with limited advanced treatment options. The largely clinically equivalent groups formed by randomisation allow for greater confidence in the effect estimates described.

3.5.3 Conclusion

In patients with difficult-to-treat asthma and obesity, improvements in asthma control, quality of life, dyspnoea and anthropometry are observed over 16 weeks with the dietitian-supported Counterweight-Plus weight management programme compared to usual care. Study of longer-term outcomes is vital to ensure benefits persist, and to identify factors associated with response to treatment. Future research could also explore efficacy in the overweight (BMI 25.0-29.9 kg/m^2) population with difficult-to-treat asthma. Weight-loss of at least 10% total body weight, and ideally >15%, are suggested as targets to ensure improvement in patient-centred outcomes.

Chapter Four: One-year outcomes of the Counterweight-Plus programme for difficult-to-treat asthma and obesity

4.1 Introduction

In Chapter Three we demonstrated improvements in asthma control and quality of life from a trial comparing the Counterweight-Plus weight management programme (CWP) against usual care (UC) in individuals with difficult-to-treat asthma and obesity over 16-weeks. Substantial weight loss of around 12kg was observed with CWP compared to UC. These results emphasise the importance of weight loss in the management of difficult-to-treat asthma and obesity, an extra-pulmonary treatable trait, and the effectiveness of this structured weight management programme over 16 weeks. The current question is whether asthma-related benefits from CWP are sustained over a longer period.

One-year results from the DiRECT trial (analysing CWP in type 2 diabetes mellitus) reported by Lean et al in 2018 [305] showed mean weight loss of around 9kg ($p<0.0001$) with CWP compared to UC at 12 months, with a quarter of participants losing >15kg. Sustained weight loss and, more pertinently, improvements in asthma-related outcomes, are important to ensure before considering CWP as a viable therapy for difficult-to-treat asthma and obesity. Here we report one-year findings from a randomised controlled feasibility trial of CWP with dietitian support compared to UC in difficult-to-treat asthma and obesity. 52-weeks was chosen to assess outcomes as the CWP intervention was complete at this time-point. No visits were planned between 16-weeks and 52-weeks as no additional benefit in weight-loss was expected during the weight-maintenance phase, based on DiRECT study experience. Furthermore, additional trial-expense with additional visits in between 16 and 52-week time-points were deemed unnecessary for little gain in this feasibility study.

4.2 Aim

To assess asthma-related outcomes at one-year between CWP and UC in participants with difficult-to-treat asthma and obesity.

4.3 Method

4.3.1 Study design

This has been described previously. Briefly, this was a single centre randomised, controlled pilot study with a parallel design and 1:1 randomisation into either CWP or UC. Participants attended for one-year (+/- seven days) follow-up (Visit 3, V3) from the date of randomisation (V1) having attended at 16-weeks (V2). CWP with dietitian support continued until the one-year mark as per the protocol described in Chapter Three. Recruitment and randomisation were undertaken by the Clinical Research Fellow, and study visits were conducted by the Clinical Research Fellow and Clinical Research Nursing team at the Glasgow Royal Infirmary Clinical Research Facility.

Participants

In short, adults aged 18-75 years with a body mass index (BMI) ≥30.0 kg/m^2 and a diagnosis of difficult-to-treat asthma as per GINA/SIGN/BTS guidelines [4, 6] were recruited from specialist asthma clinics and ward admissions across NHS Greater Glasgow and Clyde from August 2019 until August 2021. All participants continued standard asthma care and were continued to be reviewed at their parent secondary/tertiary asthma care clinics. Lifestyle advice (including healthy eating and exercise in UC), inhaler technique and asthma education were performed at each study visit as required.

Measurements

Demographics, anthropomorphic measures, asthma history, medications and healthcare usage were collected at baseline and are previously described. At all visits assessments performed included questionnaires (Asthma Control Questionnaire (ACQ6); Asthma Quality of Life Questionnaire (AQLQ); MRC dyspnoea score; Hospital Anxiety Depression (HAD) scale), venesection, spirometry (Vitalograph ALPHA™ spirometer, Buckingham, UK) as per ERS/ATS standards [278], peak expiratory flow rate (PEFR), fractional exhaled nitric oxide (FeNO; NIOX VERO®, Aerocrine AB, Solna, Sweden) as per ATS guidelines [294], 6-minute walk test (6MWT) as per ERS/ATS standards [295], and accelerometery.

Both ACQ6 and AQLQ are validated tools assessing disease control and quality of life respectively in asthma [264, 270]. Minimal clinically important difference (MCID) is 0.5 for both, with an ACQ6 score of ≥1.5 in accordance with poor disease control, and a higher AQLQ score consistent with better quality of life.

Counterweight-Plus weight management programme

Full detail of the CWP protocol has previously been described, however, in summary, consisted of three dietitian-led phases across one year. Firstly, a total diet replacement phase consisting of low-energy liquid formula (around 850 kcal/day) for 12 weeks, followed by a food reintroduction phase comprising stepwise calorie-controlled meal intake with reducing formula use for six weeks. Finally, an approximate 34-week phase of weight maintenance phase with tailored calorie-controlled meals and dietitian review completed this programme. The latter two phases incorporated flexibility accounting for individual response to weight loss and stabilisation.

4.3.2 Outcomes

4.3.2.1 Asthma-related outcomes

The primary outcome of the trial was difference in change in ACQ6 scores from baseline (V1) to 16-weeks (V2) between CWP and UC reported in Chapter Three. Further asthma-related outcomes included difference in change in ACQ6 and AQLQ from baseline to one year between groups, and in each AQLQ domain (symptoms, activity, emotional and environmental); change in ACQ6 and AQLQ across V1, V2 and V3; and difference in proportion of participants with ≥MCID change (0.5) in ACQ6 and AQLQ between CWP and UC at V3.

4.3.2.2 Other outcomes

Difference in healthcare usage (number of prednisolone courses, out-of-hours GP attendances, Emergency Department (ED) admissions, hospital and Intensive Care Unit (ICU) admissions between groups over one year.
Comparison of anthropomorphic measures (weight, BMI, waist-to-height and waist-to-hip ratios) between groups over one year.

Difference in MRC dyspnoea and Hospital Anxiety and Depression (HAD) scores between groups at one year.
Difference in peak flow and spirometry between groups after one year.
Comparison of 6MWD between groups over one year.

4.3.3 Statistical analysis

Patients attending both V1 and V3 were included for intention-to-treat analysis. Continuous data were described as mean (95% CI) or median (IQR) depending on distribution and compared using unpaired t-tests or Mann Whitney U respectively. Comparison across the three time points (V1, V2, V3) between UC and CWP was performed using repeated measures analysis of variance (ANOVA) or the Friedman test depending on data distribution, with further mixed model two-way ANOVA comparing the two groups where statistical assumptions have been met. Categorical variables were described as number (percentage) and compared using chi-square or Fisher's exact test as appropriate.

Predictors of response were identified by comparing baseline characteristics of the responders to non-responders in the CWP group and assessed using logistic regression to identify factors associated with a positive response (defined as achieving MCID in ACQ6 or AQLQ as specified) between V1 and V3.

Missing data were analysed, and key variables missing at random (MAR) were subjected to multiple imputations [317] described in further detail in the results. Any data missing completely at random (MCAR) was assessed by complete case analysis.

All analyses were performed by the author with IBM SPSS Statistics for Mac, version 28 (IBM Corp., Armonk, N.Y., USA) and graphs were produced using GraphPad Prism for Mac, version 9.5.1 (GraphPad Software, San Diego, CA, USA). Significance was set at $p \leq 0.05$. Post-hoc tests including per protocol analysis are described in each relevant section below. Data analysis was performed by the author on anonymised data.

4.3.4 Effects of COVID-19

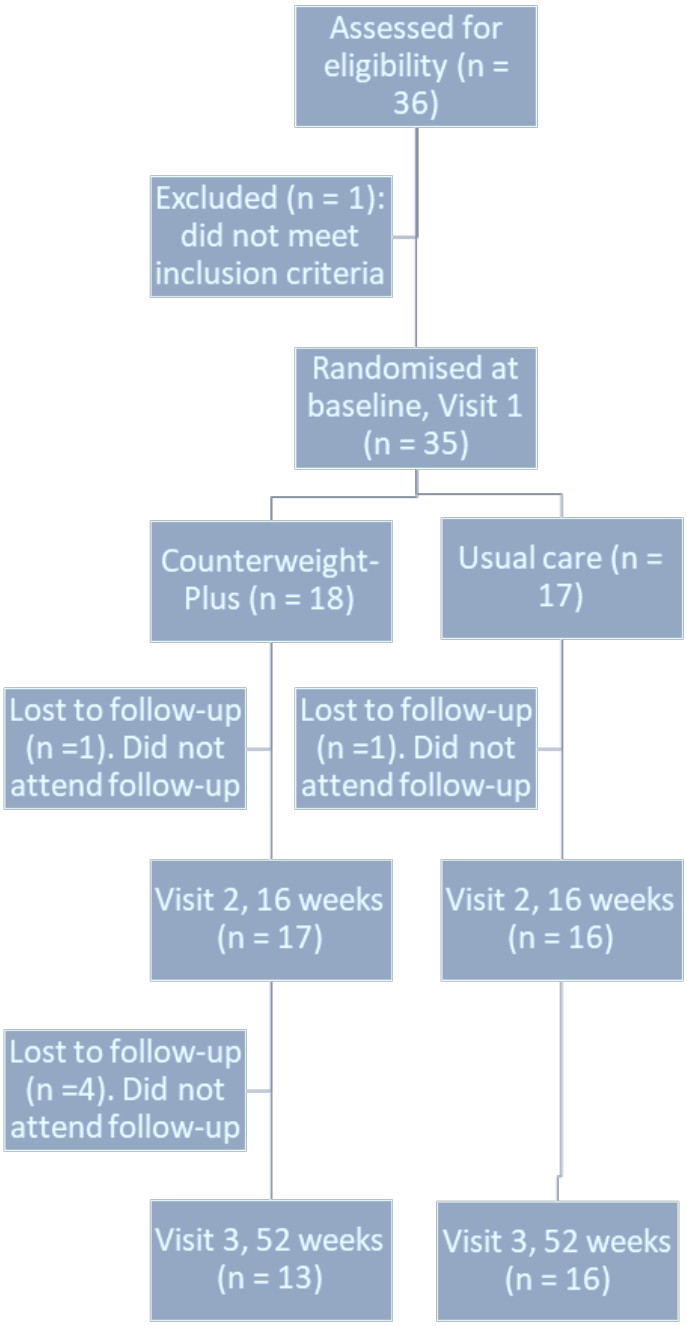
As described previously, the effects of the COVID-19 pandemic continued to result in a mixed virtual/in-person methods to maximise data collection. The result was ongoing loss of physical data variables limiting conclusions specifically for these. These variables were deemed to be MCAR (subject to complete case analysis) due to the nature of the lockdown effects.

4.4 Results

4.4.1 General characteristics and missing data

Baseline characteristics between CWP and UC, described previously, show a poorly controlled population with reduced quality of life and frequent exacerbations. We recruited 36 participants (one excluded at baseline due to ineligibility), 33 attended V2 (primary outcome), of which 29 participants attended at V3 (one year) and were included for intention-to-treat analysis; 13 CWP and 16 UC (Figure 4.1).

Figure 4. 1 - CONSORT flow chart



In total six participants did not attend at one-year: four lost to follow-up and did not respond to attempts to contact, one due to mental health issues predating the trial and one who felt unable to commit to the intervention.

Missing data for the key asthma-related outcomes ACQ6, AQLQ and annualised healthcare use were MAR, and a multiple imputation model was employed with Rubin's rules to generate pooled estimates. Five imputations were performed applying an automated method using SPSS with either an iterative Markov chain Monte Carlo or monotone method depending on the pattern of missing values. For completeness, complete case analysis is also reported in Table 4.1 for these variables. Other variables with missing data were deemed MCAR due to the effect of virtual follow-up visits and complete case analysis was appropriate here.

Table 4. 1 - Complete case intention-to-treat analysis of asthma control, quality of life and healthcare use variables across one year comparing CWP and UC

	Group	N	V1	V2	V3	Repeated measures ANOVA/Friedman test		
						F statistic/chi squared	p value	Effect size
ACQ6	CWP	13	2.5 (1.9, 3.1)	1.9 (1.1, 2.7)	2.2 (1.2, 3.2)	1.911(1.4,16.6)^	0.185	0.137
	UC	16	2.7 (2.2, 3.3)	2.9 (2.3, 3.6)	2.6 (2.0, 3.3)	0.786(2,30)	0.465	0.050
AQLQ	CWP	13	4.0 (3.3, 4.7)	4.9 (4.2, 5.6)	4.5 (3.7, 5.3)	3.681(2,24)	**0.040**	0.235
	UC	16	3.8 (3.2, 4.5)	3.9 (3.4, 4.5)	3.9 (3.3, 4.6)	0.091(2,30)	0.914	0.006
AQLQ symptom	CWP	13	4.0 (3.3, 4.7)	5.1 (4.2, 5.9)	4.5 (3.6, 5.4)	3.700(2,24)	**0.040**	0.236
	UC	16	3.9 (3.2, 4.6)	4.1 (3.6, 4.7)	4.2 (3.5, 4.9)	1.202(2,30)	0.315	0.074
AQLQ activity	CWP	13	4.0 (3.3, 4.7)	4.5 (3.7, 5.4)	4.3 (3.4, 5.2)	1.442(2,24)	0.256	0.107
	UC	16	3.7 (3.0, 4.3)	3.5 (2.9, 4.2)	3.6 (2.9, 4.3)	0.088(2,30)	0.916	0.006
AQLQ emotional	CWP	13	3.8 (2.7, 5.0)	5.6 (4.7, 6.4)	4.5 (3.6, 5.4)	7.731(2,24)	**0.003**	0.392
	UC	16	3.9 (3.0, 4.8)	4.6 (3.9, 5.2)	4.3 (3.5, 5.0)	3.622(2,30)	**0.039**	0.195
AQLQ environmental	CWP	13	4.0 (3.1, 4.8)	4.7 (3.8, 5.7)	4.8 (3.7, 5.8)	2.042(2,24)	0.152	0.145
	UC	16	4.2 (3.4, 5.0)	3.7 (2.9, 4.5)	3.7 (2.8, 4.6)	0.864(2,30)	0.432	0.054
Annualised Prednisolone boosts*	CWP	13	4 (2 to 6)	0 (0 to 7)	0 (0 to 2)	10.7 (2)	**0.005**	0.412
	UC	16	3 (2 to 5)	3 (0 to 6)	2 (1 to 4)	0.4 (2)	0.824	0.012
Annualised OOH GP attendances*	CWP	13	0 (0 to 0)	0 (0 to 3)	0 (0 to 0)	2.8(2)	0.247	0.108
	UC	16	0 (0 to 0)	0 (0 to 3)	1 (0 to 2)	2.5 (2)	0.285	0.079
Annualised ED attendances*	CWP	13	0 (0 to 0)	0 (0 to 0)	0 (0 to 0)	2.6 (2)	0.1	0.273
	UC	16	0 (0 to 0)	0 (0 to 0)	0 (0 to 0)	2.0 (2)	0.368	0.063
Annualised Hospital admissions*	CWP	13	0 (0 to 1)	0 (0 to 0)	0 (0 to 0)	1.6 (2)	0.449	0.062
	UC	16	0 (0 to 1)	0 (0 to 0)	0 (0 to 0)	5.2 (2)	0.074	0.163
Annualised ICU admissions*	CWP	13	0 (0 to 0)	0 (0 to 0)	0 (0 to 0)	n/a		
	UC	16	0 (0 to 0)	0 (0 to 0)	0 (0 to 0)			

Variables described as mean (95%CI) and compared with repeated measures ANOVA (F-statistic and effect size η_p^2 [partial eta squared]). Unless non-parametric (denoted by *): these variables described as median (IQR) and compared with Friedman chi-squared (effect size Kendall's W).
^Greenhouse-Geisser correction as Mauchly's test of sphericity violated.
Annualised health-care use variables compare change from baseline data (No. of events in prior 12 months) to V2/V3 ([No. of events × 365] / No. of d between visits).
Abbreviations: ACQ6 (Asthma Control Questionnaire 6), AQLQ (Asthma Quality of Life Questionnaire), ED (Emergency Department), ICU (Intensive Care Unit), OOH (Out-Of-Hours), V1/V2/V3 (Visit 1/2/3).

4.4.2 Asthma outcomes

Mean ACQ6 reduced by 0.5 (95%CI -0.2, 1.1) from V1 to V3 with CWP, and by 0.1 (-0.6, 0.7) with UC, with a mean difference in change in ACQ6 between groups of 0.4 (-0.5, 1.2); p = 0.409 (Tables 4.2 and 4.3). Repeated measures ANOVA showed no significant change in ACQ6 within the CWP group ($F[2, 32] = 1.9$; $p = 0.168$) or UC group ($F[2, 30] = 0.8$; $p = 0.465$) across the three visits, and mixed model two-way ANOVA showed no difference between groups across the three visits ($F[2, 30] = 2.1$; $p = 0.136$).

Table 4. 2 - Intention-to-treat analysis comparing asthma control and quality of life over one year between CWP and UC

	Group	N	Mean (95% CI)			Repeated measures ANOVA		
			V1	V2	V3	F statistic	p value	Effect size (η_P^2)
ACQ6	CWP	17	2.6 (2.1, 3.2)	2.2 (1.5, 2.8)	2.2 (1.6, 2.8)	1.887(2,32)	0.168	0.105
	UC	16	2.7 (2.2, 3.3)	2.9 (2.3, 3.6)	2.6 (2.0, 3.3)	0.786(2,30)	0.465	0.050
AQLQ	CWP	17	3.9 (3.3, 4.5)	4.7 (4.2, 5.3)	4.5 (3.9, 5.1)	4.700(2,32)	**0.016**	0.227
	UC	16	3.8 (3.2, 4.5)	3.9 (3.4, 4.5)	3.9 (3.3, 4.6)	0.091(2,30)	0.914	0.006
AQLQ symptom	CWP	17	3.8 (3.2, 4.5)	4.8 (4.3, 5.4)	4.5 (3.8, 5.1)	5.296(2.32)	**0.010**	0.249
	UC	16	3.9 (3.2, 4.6)	4.1 (3.6, 4.7)	4.2 (3.5, 4.9)	1.202(2,30)	0.315	0.074
AQLQ activity	CWP	17	4.0 (3.4, 4.5)	4.5 (3.9, 5.1)	4.3 (3.7, 5.0)	2.018(2,32)	0.149	0.112
	UC	16	3.7 (3.0, 4.3)	3.5 (2.9, 4.2)	3.6 (2.9, 4.3)	0.088(2,30)	0.916	0.006
AQLQ emotional	CWP	17	3.7 (2.9, 4.6)	5.2 (4.4, 5.9)	4.5 (3.8, 5.2)	6.517(2,32)	**0.004**	0.289
	UC	16	3.9 (3.0, 4.8)	4.6 (3.9, 5.2)	4.3 (3.5, 5.0)	3.622(2,30)	**0.039**	0.195
AQLQ environmental	CWP	17	4.1 (3.4, 4.8)	4.6 (3.8, 5.4)	4.8 (3.9, 5.6)	1.963(2,32)	0.157	0.109
	UC	16	4.2 (3.4, 5.0)	3.7 (2.9, 4.5)	3.7 (2.8, 4.6)	0.864(2,30)	0.432	0.054

Imputed dataset used

Abbreviations: ACQ6 (Asthma Control Questionnaire 6), ANOVA (analysis of variance), AQLQ (Asthma Quality of Life Questionnaire), CWP (Counterweight-Plus weight management programme), UC (Usual Care), V1/V2/V3 (Visit 1/2/3), η_P^2 (partial eta squared).

Table 4. 3 - Intention-to-treat analysis comparing change in asthma control and quality of life measures over one-year between CWP and UC

	Group	N	Change V1-V3	Mean difference	p value
ACQ6	CWP	17	-0.5 (-1.1, 0.2)	-0.4 (-1.2, 0.5)	0.409
	UC	16	-0.1 (-0.7, 0.6)		
AQLQ	CWP	17	0.6 (-0.1, 1.2)	0.5 (-0.4, 1.3)	0.254
	UC	16	0.1 (-0.5, 0.7)		
AQLQ symptom	CWP	17	0.6 (-0.1, 1.4)	0.2 (-0.7, 1.2)	0.595
	UC	16	0.4 (-0.3, 1.0)		
AQLQ activity	CWP	17	0.4 (-0.3, 1.0)	0.4 (-0.5, 1.3)	0.370
	UC	16	-0.1 (-0.7, 0.6)		
AQLQ emotional	CWP	17	0.8 (-0.1, 1.7)	0.5 (-0.5, 1.4)	0.357
	UC	16	0.3 (-0.2, 0.8)		
AQLQ environmental	CWP	17	0.7 (-0.1, 1.4)	1.2 (0.0, 2.4)	**0.047**
	UC	16	-0.5 (-1.5, 0.5)		

Imputed dataset used
P value compares CWP vs UC using independent t test.
Abbreviations: ACQ6 (Asthma Control Questionnaire 6), AQLQ (Asthma Quality of Life Questionnaire), CWP (Counterweight-Plus weight management programme), UC (Usual Care), V1/V3 (Visit 1/3)

2The proportion of participants achieving MCID in ACQ6 was greater at V2 with CWP than UC (53% vs 19% respectively; p = 0.041), but not at V3 (53% vs 25%; p = 0.101; Table 4.4). The 53% of participants achieving MCID at V2 with CWP had sustained this improvement at V3 (Figure 4.2).

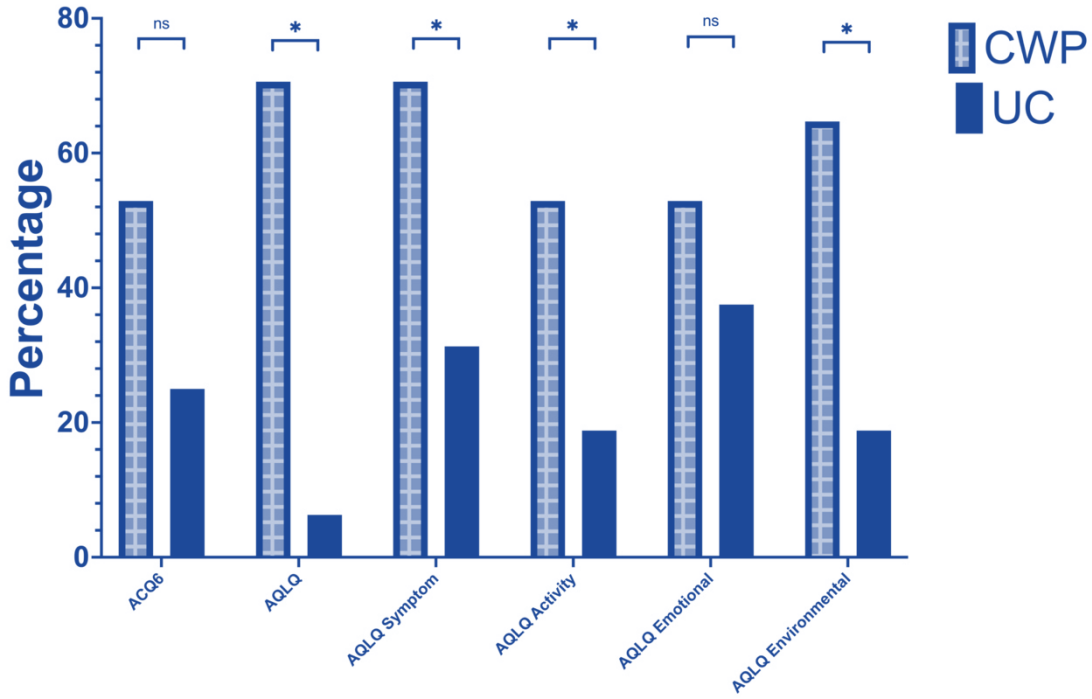
Pearson's test showed a moderate-to-large positive correlation between change in weight and ACQ6 across one year (r = 0.511, p = 0.021). Univariate linear regression was significant (F[1,18] = 6.348, p = 0.021, R^2 = 26.1%) and weight change predicted ACQ6 over on-year (β = 0.056 [95% CI 0.009, 0.102], p = 0.021).

Table 4. 4 - Proportion of intention-to-treat participants achieving MCID in asthma control and quality of life scores at 16-weeks and 52-weeks

16 weeks (V2)	**CWP (n=17)**	**UC (n=16)**	**p value**
ACQ6	9 (52.9)	3 (18.8)	**0.041**
AQLQ	10 (58.8)	5 (31.3)	0.112
AQLQ Symptoms	12 (70.6)	7 (43.8)	0.119
AQLQ Activity	8 (47.1)	4 (25.0)	0.188
AQLQ Emotional	12 (70.6)	12 (75.0)	1.000
AQLQ Environmental	10 (58.8)	5 (31.3)	0.112
52 weeks (V3)	**CWP (n=17)**	**UC (n=16)**	**p value**
ACQ6	9 (52.9)	4 (25.0)	0.101
AQLQ	12 (70.6)	1 (6.3)	**<0.001**
AQLQ Symptoms	12 (70.6)	5 (31.3)	**0.024**
AQLQ Activity	9 (52.9)	3 (18.8)	**0.041**
AQLQ Emotional	9 (52.9)	6 (37.5)	0.373
AQLQ Environmental	11 (64.7)	3 (18.8)	**0.008**

Imputed dataset used
P value compares CWP vs UC using either chi-squared or Fisher's exact test.
Abbreviations: ACQ6 (Asthma Control Questionnaire 6), AQLQ (Asthma Quality of Life Questionnaire), CWP (Counterweight-Plus weight management programme), UC (Usual Care)

Figure 4. 2 - Proportion of participants achieving minimal clinically important difference in ACQ6 and AQLQ scores with CWP and UC over one year



Compared using chi-square or Fisher's exact (* = $p<0.05$, ns = not significant)

AQLQ improved across the three visits in the CWP group from 3.9 (3.4, 4.5) at baseline to 4.5 (3.8, 5.1) at one year (F [2, 32] = 4.7; p = 0.016), with no difference in the UC group (F[2, 30] = 0.1; p = 0.914; Table 4.2). Mixed model ANOVA comparing the two groups across one year suggested a trend towards improvement with CWP compared to UC (F[2, 30] = 2.6; p = 0.092) with post-hoc pairwise comparison with Bonferroni correction showing improvement in AQLQ in CWP between V1 and V2 (0.8 [0.1, 1.5]; p = 0.016) and no difference between V2 and V3 (-0.2 [-0.9, 0.4]; p = 1.000).

Pearson's test demonstrates a large positive correlation between change in weight and log-transformed AQLQ across one year (r = -0.665, p = 0.009). Univariate linear regression was significant (F[1,12] = 9.514, p = 0.009, R^2 = 44.2%) and weight change predicted log-transformed AQLQ over on-year (β = -0.031 [95% CI -0.052, -0.009], p = 0.009).

3A similar result was observed in the AQLQ symptom domain with improvement in the CWP group from 3.8 (3.3, 4.4) at baseline to 4.5 (3.8, 5.1) at one year (F[2, 32] = 5.3; p = 0.010) with no difference in the UC group (F[2, 30] = 1.2; p = 0.315). Mixed model ANOVA was also similar (F[2, 30] = 3.1; p = 0.059) and pairwise comparison with Bonferroni correction showing improvement in AQLQ symptom domain with CWP between V1 and V2 (1.0 [0.3, 1.7]; p = 0.004) and no difference between V2 and V3 (-0.4 [-1.2, 0.4]; p = 0.674).

4There was no significant change in AQLQ activity domain, nor difference between groups across the three visits.

5AQLQ emotional domain improvement was observed in the CWP group from 3.7 (2.8, 4.6) at baseline to 4.5 (3.8, 5.2) at one year (F[2, 32] = 6.5; p = 0.004) and in the UC group from 3.9 (3.1, 4.8) at baseline to 4.3 (3.5, 5.0) at one year (F[2, 30] = 3.6; p = 0.039). Mixed model ANOVA showed no difference between groups across the three visits.

6No changes were observed in AQLQ environmental domain in the CWP group (F[2, 32] = 2.0; p = 0.157) nor UC group (F[2, 30] = 0.9; p = 0.432) across the three visits. Mixed model ANOVA suggested a trend towards improvement with CWP compared to UC across the three visits (F[2, 30] = 3.0; p = 0.065) with pairwise comparison with Bonferroni correction showing a difference of 0.7 (-0.1, 1.4; p = 0.091) between V1 and V2 with CWP and no significant change from V2 to V3 (-0.3 [-0.9, 0.3]; p = 0.484).

7At one-year, a greater proportion of participants in the CWP group compared to the UC group achieved MCID in AQLQ (71% vs 6%; p < 0.001), AQLQ symptom domain (71% vs 31%; p = 0.024), AQLQ activity domain (53% vs 19%; p = 0.041) and AQLQ environmental domain (65% vs 19%; p = 0.008; Table 4.4; Figure 4.2). There was no between-group difference in the proportion achieving MCID in AQLQ emotional domain at one year (CWP 53%, UC 38%; p= 0.373).

Median annualised exacerbation frequency (I.e., high dose OCS courses) reduced with CWP from 4 (2 to 5) at baseline to 0 (0 to 2) at one year (Friedman chi-squared (2) = 14.8; p<0.001), with no change observed in the UC group (Friedman chi-squared (2) = 0.4; p = 0.824; Table 4.5; Figure 4.3). No changes were demonstrated across the three visits in either group in out-of-ours GP attendances, emergency department attendances, hospital admissions of intensive care admissions.

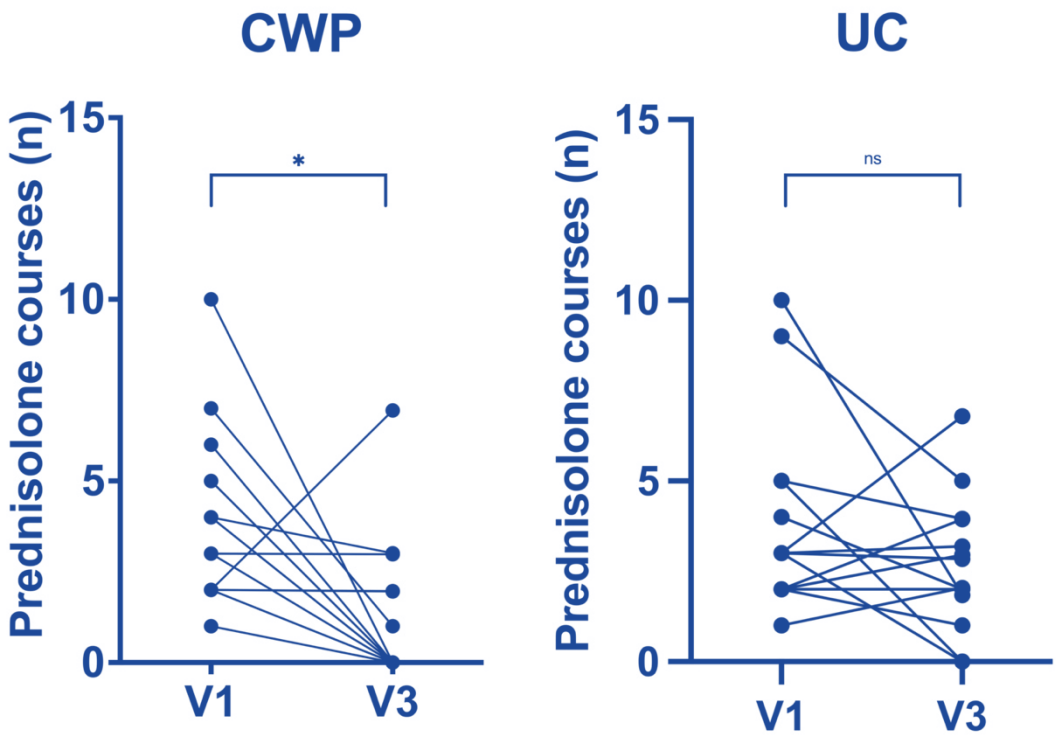
Pearson correlation test showed a moderate positive correlation between weight loss and reduction in exacerbation frequency (r = 0.496, p = 0.026). Univariate linear regression was significant (F[1,18] = 5.9, p = 0.026, R^2 = 25%) and weight change predicted exacerbation frequency over one-year (β = 0.148 [95% CI 0.020, 0.275], p = 0.026).

Table 4. 5 - Intention-to-treat analysis of other outcomes across one year between CWP and UC

	Group	N	V1	V2	V3	Repeated measures ANOVA/Friedman test		
						F statistic/chi squared	p value	Effect size
Weight, kg*	CWP	9	101.7 (95.5 to 112.0)	88.8 (82.0 to 90.7)	87.1 (85.9 to 93.3)	14.0 (2)	**<0.001**	0.778
	UC	8	106.0 (80.9 to 128.0)	105.6 (80.9 to 124.9)	108.6 (87.1 to 145.5)	1.8 (2)	0.417	0.109
BMI, kg/m2*	CWP	9	37.5 (35.6 to 41.8)	32.6 (30.1 to 35.1)	33.1 (31.4 to 37.6)	11.0 (2)	**0.004**	0.613
	UC	8	37.1 (31.5 to 47.8)	37.2 (31.0 to 47.0)	37.5 (32.6 to 54.4)	1.8 (2)	0.417	0.109
Annualised prednisolone courses*	CWP	17	4 (2 to 5)	0 (0 to 5)	0 (0 to 2)	14.8 (2)	**<0.001**	0.435
	UC	16	3 (2 to 5)	3 (0 to 6)	2 (1 to 4)	0.4 (2)	0.824	0.012
MRC dyspnoea*	CWP	13	3 (3 to 4)	2 (2 to 3)	3 (2 to 4)	5.9 (2)	0.052	0.227
	UC	15	3 (3 to 4)	3 (3 to 4)	3 (3 to 4)	0.4 (2)	0.839	0.012
HADS: Anxiety	CWP	13	8 (6, 10)	8 (5, 12)	8 (5, 11)	0.1 (2,24)	0.866	0.012
	UC	16	8 (6, 10)	9 (6, 12)	8 (6,11)	0.6 (2,30)	0.572	0.037
HADS: Depression	CWP	13	7 (5, 9)	6 (3, 9)	7 (4, 10)	0.7 (2,24)	0.516	0.054
	UC	16	10 (7, 12)	10 (8, 13)	10 (8, 12)	0.2 (2,30)	0.833	0.012

Imputed dataset used
Variables described as mean (95%CI) and compared with repeated measures ANOVA (F-statistic and effect size η_p^2 [partial eta squared]). Unless non-parametric (denoted by *): these variables described as median (IQR) and compared with Friedman chi-squared (effect size Kendall's W).
Annualised prednisolone courses compare change from baseline data (No. of events in prior 12 months) to 52 weeks ([No. of events × 365] / No. of d between visits)
Abbreviations: BMI (Body Mass Index); CWP (Counterweight Plus); HAD (Hospital Anxiety and Depression scale); MRC (Medical Research Council); UC (Usual Care); V1/2/3 (Visit 1/2/3)

Figure 4. 3 - Frequency of annualised prednisolone courses from baseline (V1) to one-year (V3) with CWP and UC



P values compare V1 to V3 with Wilcoxon-signed rank test within each group. * = p<0.05, ns = not significant

4.4.3 Other outcomes

Significant improvement was observed in median weight loss in the CWP group from 101.7kg (95.5kg to 112.0kg) at baseline to 87.1kg (85.9kg to 93.3kg) at one year (Friedman chi-squared (2) = 14.0; p <0.001), with no change in the UC group (Friedman chi-squared (2) = 1.8; p = 0.417; Table 4.5). Median weight change was –14kg (-14.8kg to –9.2kg) across one year with CWP and 1.9kg (-7.3kg to 7.9kg) with UC (p = 0.015; Table 4.6). Similar anthropometric improvements in BMI, waist and hip circumference, and waist-to-height ratio are summarised in Tables 4.5 and 4.6.

Table 4. 6 - Intention-to-treat change in other outcomes at one-year between CWP and UC

	Group	N	Change V1-V3	p value
Weight, kg*	CWP	10	-14.0 (-14.8 to -9.2)	**0.015**
	UC	10	1.9 (-7.3 to 7.9)	
BMI, kg/m2	CWP	10	-4.2 (-6.4, -2.0)	**0.036**
	UC	10	-0.1 (-3.6, 3.4)	
Waist circumference, cm*	CWP	7	-17.0 (-21.0 to 12.0)	**0.002**
	UC	6	0.8 (-4.0 to 7.0)	
Hip circumference, cm*	CWP	7	-10.0 (-17.0 to -5.2)	**0.022**
	UC	6	4.0 (-3.5 to 11.3)	
Waist-to-height ratio*	CWP	7	-0.1 (-0.1 to -0.1)	**0.005**
	UC	5	0.0 (0.0 to 0.0)	
Waist-to-hip ratio	CWP	7	0.0 (-0.1, 0.0)	0.876
	UC	6	0.0 (-0.1, 0.0)	
Annualised healthcare use:				
Prednisolone courses	CWP	17	-3 (-5, -1)	0.109
	UC	16	-1 (-3, 1)	
OOH GP attendances	CWP	17	0 (-1, 1)	0.193
	UC	16	1 (0, 2)	
ED attendances*	CWP	17	0 (0 to 0)	0.402
	UC	16	0 (0 to 0)	
Hospital admissions*	CWP	17	0 (0 to 0)	0.510
	UC	16	0 (-1 to 1)	
ICU admissions*	CWP	17	0 (0 to 0)	1.000
	UC	16	0 (0 to 0)	
MRC dyspnoea*	CWP	13	-1 (-2 to 1)	0.268
	UC	16	0 (-1 to 1)	
HADS: Anxiety	CWP	13	0 (-2, 2)	0.804
	UC	16	0 (-1, 1)	
HADS: Depression	CWP	13	0 (-3, 3)	0.946
	UC	16	0 (-2, 2)	
Eosinophils, x10^9/L*	CWP	5	0.00 (-0.03 to 0.19)	0.662
	UC	6	0.01 (-0.29 to 0.07)	
FeNO, ppb*	CWP	3	1 (-2 to 6)	0.876
	UC	7	8 (-4 to 21)	
PEFR, L/min	CWP	5	41 (-99, 182)	0.203
	UC	7	-28 (-88, 32)	
Pre-BD FEV1, %	CWP	5	12.8 (1.0, 24.8)	**0.020**
	UC	6	-4.0 (-14.6, 6.6)	
Pre-BD FEV1/FVC, %	CWP	5	-5.1 (-9.0, -1.2)	0.565
	UC	6	-3.6 (-8.8, -1.7)	
Post-BD FEV1, %	CWP	5	12.2 (2.1, 22.3)	**0.020**
	UC	6	-2.8 (-12.5, 6.8)	
6MWD, m	CWP	4	30 (-28, 88)	0.482
	UC	4	12 (-37, 61)	

Imputed dataset
Variables described as mean (95% confidence intervals) or median (first quartile to third quartile), latter denoted by *.
P value shows comparison using independent t test or Mann Whitney U test (latter with variables denoted by*).
Abbreviations: BD (Bronchodilator); BMI (Body Mass Index); CWP (Counterweight Plus); ED (Emergency Department); FeNO (Fractional exhaled Nitric Oxide); FEV_1 (Forced Expiratory Volume in 1 second); FVC (Forced Vital Capacity); HAD (Hospital Anxiety and Depression scale); ICU (Intensive Care Unit); MRC (Medical Research Council); OOH (Out-of-hours); PEF (Peak Expiratory Flow); ppb (parts per billion); UC (Usual Care); V1/3 (Visit 1/3); 6MWD (6 minute Walk Distance).

No between-group differences were detected across the three visits in MRC dyspnoea scale, Hospital Anxiety and Depression scores, eosinophils, FeNO, or peak flow. Change in post-bronchodilator FEV_1 from baseline to one-year was greater with CWP (12.2%, 95% CI 2.1%, 22.3%) than UC (-2.8%, 95% CI –12.5%, 6.8%; p = 0.020; Table 4.6).

4.4.4 Post hoc analysis by type 2 biomarkers and weight loss

4.4.4.1 Type 2 biomarkers

Post-hoc analysis comparing outcomes by T2-biomarkers over one year (using paired t-test or Wilcoxon signed-rank test for parametric and non-parametric data respectively) in the CWP group are shown in Table 4.7. For the purposes of this analysis, T2-high disease was defined as having either FeNO ≥ 25ppb and/or blood eosinophil count ≥ 0.15 $x10^9$/L [318], and the converse as T2-low, and a p-value <0.1 was accepted to suggest a trend.

Table 4.7 - Comparison of CWP participants at one-year by type 2 inflammatory status

	Group	N	V1	V3	Change V1-V3	p value
ACQ6	T2 High	11	2.9 (2.2, 3.6)	2.1 (1.2, 3.1)	-0.7 (-1.5, 0.1)	**0.067**
	T2 Low	6	2.2 (1.3, 3.1)	2.3 (0.6, 3.9)	0.1 (-1.3, 1.5)	0.907
AQLQ	T2 High	11	3.7 (3.0, 4.4)	4.4 (3.5, 5.3)	0.7 (0.1, 1.3)	**0.036**
	T2 Low	6	4.3 (3.1, 5.5)	4.6 (3.3, 5.9)	0.3 (-1.6, 2.3)	0.680
AQLQ symptom	T2 High	11	3.8 (3.0, 4.5)	4.6 (3.7, 5.4)	0.8 (0.2, 1.4)	**0.012**
	T2 Low	6	4.0 (2.7, 5.3)	4.2 (2.8, 5.7)	0.2 (-2.2, 2.6)	0.833
AQLQ activity	T2 High	11	3.7 (3.0, 4.4)	4.1 (3.1, 5.1)	0.4 (-0.4, 1.2)	0.303
	T2 Low	6	4.4 (3.4, 5.5)	4.7 (3.5, 5.9)	0.3 (-1.4, 2.0)	0.714
AQLQ emotional	T2 High	11	3.4 (2.3, 2.5)	4.5 (3.5, 5.5)	1.1 (0.3, 1.9)	**0.010**
	T2 Low	6	4.3 (2.3, 6.4)	4.5 (3.6, 5.5)	0.2 (-2.4, 2.7)	0.862
AQLQ environmental	T2 High	11	3.7 (2.9, 4.6)	4.6 (3.5, 5.8)	0.9 (-0.1, 1.9)	**0.081**
	T2 Low	6	4.7 (3.7, 5.7)	5.0 (3.8, 6.2)	0.3 (-1.2, 1.7)	0.656
Annualised healthcare use*:						
Prednisolone courses	T2 High	11	4 (2 to 6)	0 (0 to 2)	-2 (-6 to 0)	**0.003**
	T2 Low	6	4 (2 to 4)	0 (0 to 2)	-4 (-4 to 0)	0.340
OOH GP attendances	T2 High	11	0 (0 to 1)	0 (0 to 0)	0 (-1 to 0)	0.684
	T2 Low	6	0 (0 to 1)	0 (0 to 1)	0 (-1 to 1)	0.655
ED attendances	T2 High	11	0 (0 to 0)	0 (0 to 0)	0 (0 to 0)	0.317
	T2 Low	6	0 (0 to 0)	0 (0 to 1)	0 (0 to 1)	0.317
Hospital admissions	T2 High	11	0 (0 to 1)	0 (0 to 0)	0 (-1 to 0)	**0.083**
	T2 Low	6	0 (0 to 0)	0 (0 to 0)	0 (0 to 0)	0.317
ICU admissions	T2 High	11	0 (0 to 0)	0 (0 to 0)	0 (0 to 0)	1.000
	T2 Low	6	0 (0 to 0)	0 (0 to 0)	0 (0 to 0)	1.000
MRC dyspnoea	T2 High	9	3 (3, 4)	3 (2, 4)	0 (-1, 1)	0.438
	T2 Low	4	3 (2, 4)	3 (0, 5)	-1 (-4, 3)	0.703
HADS:						
Anxiety	T2 High	9	9 (6, 11)	8 (4, 12)	0 (-3, 3)	0.876
	T2 Low	4	9 (6, 12)	7 (5, 10)	0 (-2, 2)	1.000
Depression	T2 High	9	7 (5, 10)	8 (4, 12)	0 (-3, 4)	0.779
	T2 Low	4	8 (3, 13)	6 (-2, 14)	0 (-11, 10)	0.945

Continuous variables described as mean (95% confidence intervals) or median (first quartile to third quartile), latter denoted by*.
P value compares V1 vs V3 using paired t test or if non-parametric (denoted by*) Wilcoxon signed-rank test.
Annualised health-care use variables compare change from baseline data (No. of events in prior 12 months) to 52 weeks ([No. of events × 365] / No. of d between visits)
Abbreviations: ACQ6 (Asthma Control Questionnaire 6), AQLQ (Asthma Quality of Life Questionnaire), ED (Emergency Department), HADS (Hospital Anxiety and Depression Score), ICU (Intensive Care Unit), MRC (Medical Research Council), OOH (Out-Of-Hours), T2 (Type 2), V1/V3 (Visit 1/3).

Over a year, a trend suggesting improvement was observed in the T2-high group in ACQ6 (mean difference –0.7, 95% CI –1.5, 0.1; p = 0.067), AQLQ (mean difference 0.7, 95% CI 0.1, 1.3; p = 0.036), AQLQ symptom domain (mean difference 0.8, 95% CI 0.2, 1.4; p = 0.012), AQLQ emotional domain (mean difference 1.1, 95% CI 0.3, 1.9; p = 0.010), AQLQ environmental domain (mean difference 0.9, 95% CI –0.1, 1.9; p = 0.081), number of prednisolone courses (median difference –2, IQR –6 to 0; p = 0.003) and number of hospital admissions (median difference 0, IQR –1 to 0; p = 0.083) whilst no differences were observed in the T2-low group.

4.4.4.2 Weight-loss extent

Table 4.8 compares outcomes by weight loss (those that lost >10% weight vs those that lost <10% weight) over one year in the CWP group, again using either paired t-test or Wilcoxon signed-rank as appropriate. 70% of participants in the CWP group lost >10% total body weight. Weight loss of 10% was selected following previously reported suggestion that this was the level required to observe improvement in asthma control and quality of life measures (Chapter Three).

Table 4.8 - Comparison of CWP participants at one-year of those that lost >10% total body weight against those that lost <10% body weight

	Group	N	V1	V3	Change V1-V3	p value
ACQ6	<10% weight	3	2.7 (-1.0, 6.3)	3.1 (-0.7, 6.9)	0.5 (-4.6, 5.6)	0.732
	≥10% weight	7	2.1 (1.3, 3.0)	1.1 (0.2, 1.9)	-1.1 (-1.9, -0.3)	**0.018**
AQLQ	<10% weight	3	3.8 (-1.4, 9.1)	3.4 (1.9, 4.9)	-0.4 (-6.7, 5.9)	0.809
	≥10% weight	7	4.3 (3.4, 5.2)	5.5 (4.7, 6.3)	1.2 (0.4, 2.1)	**0.011**
AQLQ symptom	<10% weight	3	4.5 (-0.3, 9.3)	3.2 (-0.7, 7.1)	-1.3 (-7.7, 5.1)	0.483
	≥10% weight	7	4.1 (3.1, 5.1)	5.4 (4.4, 6.4)	1.3 (0.4, 2.2)	**0.010**
AQLQ activity	<10% weight	3	3.2 (-1.7, 8.2)	3.2 (2.5, 3.9)	0.0 (-5.5, 5.5)	0.982
	≥10% weight	7	4.4 (3.6, 5.2)	5.4 (4.4, 6.5)	1.0 (0.0, 2.0)	0.052
AQLQ emotional	<10% weight	3	4.3 (-1.9, 10.5)	3.7 (2.4, 4.9)	-0.7 (-7.4, 6.1)	0.713
	≥10% weight	7	4.3 (2.6, 6.0)	5.6 (4.6, 6.5)	1.3 (-0.2, 2.8)	0.074
AQLQ environmental	<10% weight	3	3.4 (-3.4, 10.1)	4.4 (2.0, 6.9)	1.1 (-4.8, 7.0)	0.519
	≥10% weight	7	4.5 (3.7, 5.3)	5.8 (4.8, 6.9)	1.3 (0.4, 2.2)	0**.011**
Annualised healthcare use*:						
Prednisolone courses	<10% weight	3	2 (2 to 3)	3 (2 to 5)	1 (-9, 10)	1.000
	≥10% weight	7	3 (3 to 5)	0 (0 to 0)	-4 (-7, -1)	**0.018**
OOH GP attendances	<10% weight	3	0 (0 to 1)	3 (2to 3)	3 (1 to 3)	0.285
	≥10% weight	7	0 (0 to 0)	0 (0 to 0)	0 (0 to 0)	0.317
ED attendances	<10% weight	3	0 (0 to 0)	0 (0 to 0)	0 (0 to 0)	1.000
	≥10% weight	7	0 (0 to 0)	0 (0 to 1)	0 (0 to 1)	0.180
Hospital admissions	<10% weight	3	0 (0 to 1)	0 (0 to 0)	0 (-1 to 0)	0.317
	≥10% weight	7	0 (0 to 0)	0 (0 to 0)	0 (0 to 0)	0.655
ICU admissions	<10% weight	3	0 (0 to 0)	0 (0 to 0)	0 (0 to 0)	1.000
	≥10% weight	7	0 (0 to 0)	0 (0 to 0)	0 (0 to 0)	1.000
MRC dyspnoea	<10% weight	3	3 (3 to 4)	3 (3 to 4)	1 (-5, 6)	0.655
	≥10% weight	7	3 (3 to 3)	2 (1 to 2)	-1 (-2, -1)	**0.024**
HADS:						
Anxiety	<10% weight	3	8 (-1, 16)	8 (0, 15)	0 (-1, 2)	0.423
	≥10% weight	7	7 (4, 10)	6 (2, 11)	-1 (-4, 2)	0.496
Depression	<10% weight	3	7 (-3, 17)	11 (8, 14)	4 (-8, 17)	0.274
	≥10% weight	7	7 (4, 10)	5 (0, 10)	-2 (-6, 2)	0.208

Continuous variables described as mean (95% confidence intervals) or median (first quartile to third quartile), latter denoted by*.
P value compares V1 vs V3 using paired t test or if non-parametric (denoted by*) Wilcoxon signed-rank test.
Annualised health-care use variables compare change from baseline data (No. of events in prior 12 months) to 52 weeks ([No. of events × 365] / No. of d between visits)
Abbreviations: ACQ6 (Asthma Control Questionnaire 6), ANOVA (analysis of variance), AQLQ (Asthma Quality of Life Questionnaire), ED (Emergency Department), HADS (Hospital Anxiety and Depression Score), ICU (Intensive Care Unit), MRC (Medical Research Council), OOH (Out-Of-Hours), V1/V3 (Visit 1/3).

Over one year, improvements were observed in the ≥10% weight loss group in ACQ6 (mean difference –1.1, 95% CI –1.9, -0.3; p = 0.018), AQLQ (mean difference 1.2, 95% CI 0.4, 2.1; p = 0.011), AQLQ symptom domain (mean difference 1.3, 95% CI 0.4, 2.2; p = 0.010), AQLQ activity domain (mean difference 1.0, 95% CI 0.0, 2.0; p = 0.052), AQLQ emotional domain (mean difference 1.3, 95% CI –0.2, 2.8; p = 0.074), AQLQ environmental domain (mean difference 1.3, 95% CI 0.4, 2.2; p = 0.011), number of prednisolone courses (mean difference –4, 95% CI –7, -1; p = 0.018), and MRC dyspnoea score (mean difference –1, 95% CI –2, -1; p = 0.024) whilst no differences were observed in the <10% weight loss group.

4.4.5 Per protocol analysis

4.4.5.1 Asthma-related outcomes

Ten participants from the CWP group completed the one-year programme and attended V3 and were included for per protocol analysis compared to the 16 in UC. Mean difference in ACQ6 between groups was not significant over one year (CWP vs UC: -0.5, 95%CI –1.6, 0.6; p = 0.328; Table 4.9), though repeated measures ANOVA suggested a trend towards improvement with CWP, F(2,18) = 3.023, p = 0.074, partial eta squared = 0.251 (Table 4.10) Post-hoc tests with Bonferroni correction in the CWP group showed a mean difference from V1-V2 of –0.81 (95%CI –1.68, 0.06; p = 0.069) and no change between V2-V3 (0.20, 95%CI –0.55, 0.95; p = 1.000). 6 (60%) of participants in CWP achieved MCID in ACQ6 compared to UC (4 [25%]; p = 0.109).

Table 4. 9- Per protocol analysis comparing change in asthma control and quality of life measures over one-year between CWP and UC

	Group	N	Change V1-V3	Mean difference	p value
ACQ6	CWP	10	-0.6 (-1.6, 0.4)	-0.5 (-1.6, 0.6)	0.328
	UC	16	-0.1 (-0.7, 0.6)		
AQLQ*	CWP	10	0.8 (0.5 to 1.8)	n/a	**0.003**
	UC	16	0.1 (-0.2 to 0.2)		
AQLQ symptom*	CWP	10	0.8 (0.3 to 1.3)	n/a	0.201
	UC	16	0.2 (-0.3 to 1.0)		
AQLQ activity	CWP	10	0.7 (-0.4, 1.8)	0.8 (-0.4, 1.9)	0.174
	UC	16	-0.1 (-0.7, 0.6)		
AQLQ emotional	CWP	10	0.7 (-0.8, 2.2)	0.4 (-0.8, 1.6)	0.594
	UC	16	0.3 (-0.2, 0.8)		
AQLQ environmental	CWP	10	1.2 (0.3, 2.2)	1.8 (0.4, 3.2)	**0.017**
	UC	16	-0.5 (-1.5, 0.5)		

Variables described as mean (95%CI) unless denoted by * (median [IQR])
P value compares CWP vs UC using independent t test or Mann Whitney U if denoted by*.
Abbreviations: ACQ6 (Asthma Control Questionnaire 6), AQLQ (Asthma Quality of Life Questionnaire), CWP (Counterweight-Plus weight management programme), UC (Usual Care), V1/V3 (Visit 1/3)

Table 4.10 - Per protocol analysis comparing asthma control and quality of life over one year between CWP and UC

	Group	N	Mean (95% CI)			Repeated measures ANOVA		
			V1	V2	V3	F statistic	p value	Effect size (η_p^2)
ACQ6	CWP	10	2.3 (1.6, 3.0)	1.5 (0.7, 2.3)	1.7 (0.9, 2.5)	3.023(2,18)	0.074	0.251
	UC	16	2.7 (2.2, 3.3)	2.9 (2.3, 3.6)	2.6 (2.0, 3.3)	0.786(2,30)	0.465	0.050
AQLQ	CWP	10	4.2 (3.3, 5.0)	5.3 (4.6, 5.9)	4.9 (4.1, 5.7)	4.099(2,18)	0.034	0.313
	UC	16	3.8 (3.2, 4.5)	3.9 (3.4, 4.5)	3.9 (3.3, 4.6)	0.091(2,30)	0.914	0.006
AQLQ symptom	CWP	10	4.2 (3.3, 5.1)	5.4 (4.7, 6.2)	4.8 (3.9, 5.6)	3.413(2,18)	0.055	0.275
	UC	16	3.9 (3.2, 4.6)	4.1 (3.6, 4.7)	4.2 (3.5, 4.9)	1.202(2,30)	0.315	0.074
AQLQ activity	CWP	10	4.1 (3.2, 4.9)	4.8 (4.0, 5.6)	4.8 (3.9, 5.6)	2.747(2,18)	0.091	0.234
	UC	16	3.7 (3.0, 4.3)	3.5 (2.9, 4.2)	3.6 (2.9, 4.3)	0.088(2,30)	0.916	0.006
AQLQ emotional	CWP	10	4.3 (3.2, 5.4)	6.0 (5.2, 6.9)	5.0 (4.1, 5.9)	4.814(2,18)	0.021	0.348
	UC	16	3.9 (3.0, 4.8)	4.6 (3.9, 5.2)	4.3 (3.5, 5.0)	3.622(2,30)	0.039	0.195
AQLQ environmental	CWP	10	4.2 (3.2, 5.2)	5.1 (4.1, 6.1)	5.4 (4.2, 6.6)	*5.190(1.2,10.4)	0.041	0.366
	UC	16	4.2 (3.4, 5.0)	3.7 (2.9, 4.5)	3.7 (2.8, 4.6)	0.864(2,30)	0.432	0.054

Abbreviations: ACQ6 (Asthma Control Questionnaire 6), ANOVA (analysis of variance), AQLQ (Asthma Quality of Life Questionnaire), CWP (Counterweight-Plus weight management programme), UC (Usual Care), V1/V2/V3 (Visit 1/2/3), η_p^2 (partial eta squared).
*Greenhouse-Geisser correction (Mauchly's test of sphericity violated)

A greater proportion of participants achieved MCID improvement with CWP compared to UC at one year (Table 4.11) n overall AQLQ score (80% vs 6% respectively, p<0.001), AQLQ symptom domain (80% vs 31% respectively, p = 0.016), AQLQ activity domain (70% vs 19% respectively, p = 0.015) and AQLQ environmental domain (80% vs 19%, p = 0.004). No between-group difference was seen in AQLQ emotional domain.

Table 4.11 - Proportion of per protocol participants achieving MCID in asthma control and quality of life scores at 16-weeks and 52-weeks

16 weeks (V2)	**CWP (n=10)**	**UC (n=16)**	**p value**
ACQ6	7 (70.0)	3 (18.8)	**0.015**
AQLQ	7 (70.0)	5 (31.3)	0.105
AQLQ Symptoms	8 (80.0)	7 (43.8)	0.109
AQLQ Activity	6 (60.0)	4 (25.0)	0.109
AQLQ Emotional	7 (70.0)	12 (75.0)	1.000
AQLQ Environmental	6 (60.0)	5 (31.3)	0.228
52 weeks (V3)	**CWP (n=10)**	**UC (n=16)**	**p value**
ACQ6	6 (60.0)	4 (25.0)	0.109
AQLQ	8 (80.0)	1 (6.3)	**<0.001**
AQLQ Symptoms	8 (80.0)	5 (31.3)	**0.016**
AQLQ Activity	7 (70.0)	3 (18.8)	**0.015**
AQLQ Emotional	4 (40.0)	6 (37.5)	1.000
AQLQ Environmental	8 (80.0)	3 (18.8)	**0.004**

P value compares CWP vs UC using either chi-squared or Fisher's exact test.

Abbreviations: ACQ6 (Asthma Control Questionnaire 6), AQLQ (Asthma Quality of Life Questionnaire), CWP (Counterweight-Plus weight management programme), UC (Usual Care)

Improvements in overall AQLQ score over one year with CWP (median change 0.8, IQR 0.5 to 1.8) were observed compared to UC (0.1, IQR –0.2 to 0.2; p = 0.003; Table 4.9, and repeated measures ANOVA within the CWP group was significant, F(2,18) = 4.099, p = 0.034, partial eta squared = 0.313 (Table 4.10). Post-hoc tests with Bonferroni correction in the CWP group showed a mean difference from V1-V2 of 1.12 (95% CI 0.11, 2.13; p = 0.030) and no change between V2-V3 (-0.38, 95% CI –1.30, 0.54; p = 0.776). A similar result was observed in the AQLQ environmental domain with a mean between-group difference of 1.8 (95%CI 0.4, 3.2; p = 0.017) favouring CWP at one year. Repeated measures ANOVA confirmed a significant difference in the CWP group over one year, F(1.2,10.4) = 5.190 with Greenhouse-Geisser adjustment, p = 0.041, partial eta squared = 0.366.

There was no between group difference in AQLQ symptom, activity or emotional domain scores, however, trends towards improvement with CWP in AQLQ symptom and activity domains were observed with repeated measures ANOVA (F[2,18] = 3.413, p = 0.055, partial eta squared = 0.275; F[2,18] = 2.747, p = 0.091, partial eta squared = 0.234 respectively). Post-hoc tests with Bonferroni correction for AQLQ symptom domain showed improvement with CWP from V1-V2 (mean change 1.23; 95%CI 0.24, 2.22; p = 0.016) with no difference between V2-V3 (-0.68; 95%CI –1.96, 0.60; p = 0.457). Post-hoc tests with Bonferroni correction for AQLQ activity domain showed a trend toward improvement with CWP from V1-V2 (0.78; 95%CI –0.08, 1.64; p = 0.080) with no difference from V2-V3 (-0.08; 95%CI –1.00, 0.84; p = 1.000). Repeated measures showed improvement for both CWP and UC in AQLQ emotional domain (F[2,18] = 4.814, p = 0.021, partial eta squared = 0.348; F[2,30] = 3.622, p = 0.039, partial eta squared = 0.195 respectively).

Median annualised exacerbation frequency reduced in the CWP group from 3 (IQR 2 to 5) at V1 to 0 (0 to 3) at V3 (Friedman chi-squared (2) = 7.9, p = 0.019) with no change observed in UC from 3 (2 to 5) at V1 to 2 (0 to 3) at V3 (Friedman chi-squared (2) = 0.4, p = 0.824; Table 4.12). No differences were seen in either CWP or UC over one year in out-of-hours GP attendances, emergency department attendances, hospital or ICU admissions.

Table 4.12 - Per protocol analysis of other outcomes across one year between CWP and UC

	Group	N	Mean (95% CI)/Median (IQR)*			Repeated measures ANOVA/Friedman test		
			V1	V2	V3	F statistic/chi squared	p value	Effect size
Weight, kg*	CWP	9	101.7 (95.5 to 112.0)	88.8 (82.0 to 90.7)	87.1 (85.9 to 93.3)	14.0 (2)	<0.001	0.778
	UC	8	106.0 (80.9 to 128.0)	105.6 (80.9 to 124.9)	108.6 (87.1 to 145.5)	1.8 (2)	0.417	0.109
BMI, kg/m^2*	CWP	9	37.5 (35.6 to 41.8)	32.6 (30.1 to 35.1)	33.1 (31.4 to 37.6)	11.0 (2)	0.004	0.613
	UC	8	37.1 (31.5 to 47.8)	37.2 (31.0 to 47.0)	37.5 (32.6 to 54.4)	1.8 (2)	0.417	0.109
Annualised prednisolone courses*	CWP	10	3 (2 to 5)	2 (0 to 8)	0 (0 to 3)	7.9 (2)	0.019	0.397
	UC	16	3 (2 to 5)	3 (0 to 6)	2 (1 to 4)	0.4 (2)	0.824	0.012
MRC dyspnoea*	CWP	10	3 (3 to 3)	2 (2 to 3)	2 (2 to 3)	7.5 (2)	0.024	0.374
	UC	15	3 (3 to 4)	3 (3 to 4)	3 (3 to 4)	0.4 (2)	0.839	0.012
HADS: Anxiety	CWP	10	7 (5, 10)	7 (3, 11)	7 (4, 10)	0.2 (2,18)	0.831	0.020
	UC	16	8 (6, 10)	9 (6, 12)	8 (6,11)	0.6 (2,30)	0.572	0.037
HADS: Depression	CWP	10	7 (5, 9)	5 (1, 8)	7 (3, 11)	1.5 (2,18)	0.246	0.144
	UC	16	10 (7, 12)	10 (8, 13)	10 (8, 12)	0.2 (2,30)	0.833	0.012

Variables described as mean (95%CI) and compared with repeated measures ANOVA (F-statistic and effect size η_p^2 [partial eta squared]). Unless non-parametric (denoted by *): these variables described as median (IQR) and compared with Friedman chi-squared (effect size Kendall's W).
Annualised health-care use variables compare change from baseline data (No. of events in prior 12 months) to V2/V3 ([No. of events × 365] / No. of d between visits).
Abbreviations: BMI (Body Mass Index); CWP (Counterweight Plus); ED (Emergency Department); HAD (Hospital Anxiety and Depression scale); ICU (Intensive Care Unit); MRC (Medical Research Council); OOH (Out-of-hours); UC (Usual Care); V1/2/3 (Visit 1/2/3)

4.4.5.2 Other per protocol outcomes

Median MRC dyspnoea scores improved in CWP from 3 (3 to 3) at V1 to 2 (2 to 3) at V3 (Friedman chi-squared (2) = 7.5, p = 0.024) with unchanged results observed in UC from 3 (3 to 4) at V1 to 3 (3 to 4) (Friedman chi-squared (2) = 0.4, p = 0.824; Table 4.12).

Anthropomorphic measures were identical to those reported in the intention-to-treat analysis. No between-group differences were observed over one year in anxiety and depression scores, eosinophils, FeNO, peak flow or 6-minute walk test.

Table 4.13 - Per protocol change in other outcomes at one-year between CWP and UC

	Group	N	Change V1-V3	p value
Weight, kg*	CWP	10	-14.0 (-14.8 to -9.2)	**0.015**
	UC	10	1.9 (-7.3 to 7.9)	
BMI, kg/m^2	CWP	10	-4.2 (-6.4, -2.0)	**0.036**
	UC	10	-0.1 (-3.6, 3.4)	
Annualised healthcare use:				
Prednisolone courses	CWP	10	-2 (-5, 0)	0.314
	UC	16	-1 (-3, 1)	
OOH GP attendances	CWP	10	0 (-1, 2)	0.536
	UC	16	1 (0, 2)	
ED attendances*	CWP	10	0 (0 to 0)	0.310
	UC	16	0 (0 to 0)	
Hospital admissions*	CWP	10	0 (0 to 0)	0.551
	UC	16	0 (-1 to 1)	
ICU admissions*	CWP	10	0 (0 to 0)	1.000
	UC	16	0 (0 to 0)	
MRC dyspnoea*	CWP	10	-1 (-2 to 0)	0.077
	UC	16	0 (-1 to 1)	
HADS:				
Anxiety	CWP	10	-1 (-2, 1)	0.554
	UC	16	0 (-1, 1)	
Depression	CWP	10	0 (-4, 3)	0.844
	UC	16	0 (-2, 2)	

Variables described as mean (95% confidence intervals) or median (first quartile to third quartile), latter denoted by *.
P value shows comparison using independent t test or Mann Whitney U test (latter with variables denoted by*).
Annualised health-care use variables compare change from baseline data (No. of events in prior 12 months) to 52 weeks ([No. of events × 365] / No. of d between visits)
Abbreviations: BMI (Body Mass Index); CWP (Counterweight Plus); ED (Emergency Department); HAD (Hospital Anxiety and Depression scale); ICU (Intensive Care Unit); MRC (Medical Research Council); OOH (Out-of-hours); UC (Usual Care); V1/3 (Visit 1/3)

4.5 Discussion

4.5.1 One-year outcomes of weight management

We report results from a dedicated randomised controlled trial assessing asthma-related outcomes at one-year of a weight management programme compared to usual care. Participants in the CWP group experienced sustained weight loss over one-year, as well as reduction in other anthropometric parameters such as BMI and waist-to-height ratio, with no difference observed with UC. This is the first study observing sustained weight loss from a conservative weight management programme at one-year in people with difficult-to-treat asthma and obesity. Moreover, the extent of the weight loss is important (median loss of 14kg in CWP vs weight gain of 2kg in UC, p = 0.015), likely to be significant to overall patient wellbeing. Importantly, CWP resulted in sustained improvement in asthma-related quality of life over one year which was not observed for usual care, with 71% of individuals receiving CWP achieving clinically significant (≥0.5 MCID) improvement in AQLQ. Likewise, CWP was associated with substantially greater proportion achieving clinically significant improvements in AQLQ symptom, activity and environmental domains.

Despite an improvement in ACQ6 using CWP compared to UC over 16-weeks, we did not observe any difference between groups at one year, likely due to being underpowered at the one-year time point. However, 53% of the CWP group achieved MCID in ACQ6 at 16-weeks and these improvements were sustained at one year. Moreover, 70% of the CWP group at one-year lost >10% weight and showed marked improvement in ACQ6 compared to baseline (mean difference –1.1, 95% CI –1.9, -0.3; p = 0.018). This was a feasibility study aimed at assessing asthma control with CWP at 16-weeks and as such, resulted in substantial missing data at the one-year point. This missing data and the resultant use of multiple imputations may have introduced bias. However, the complete case analysis showed broadly similar results supporting our interpretation and the authors are confident that a larger study would quantify differences in ACQ6 at one-year. Unsurprisingly, participants who failed to provide 12-month data were those who showed no improvements in weight, ACQ6 or AQLQ over the first 16-

weeks suggesting dissatisfaction with lack of weight loss and/or quality of life as potential factors in attending one-year follow-up.

Encouragingly, over one-year, CWP was associated with a reduction in frequency of prednisolone boosts whilst there was no change for UC, implying that weight loss might reduce frequency of asthma exacerbations. This measure was reliant on both participant recall and electronic case record where able but was subject to recall bias. There was a suggestion of improvement in MRC dyspnoea score with CWP over one year in the intention-to-treat analysis (Friedman chi-squared (2) = 5.9, p = 0.052), further highlighted by the per protocol analysis in the supplemental file (Friedman chi-squared (2) = 7.5, p = 0.024) and in accordance with our previously reported improvement at 16 weeks.

Post-hoc analysis suggests that benefits in asthma control, quality of life and exacerbation frequency are greater in those that lose more weight, specifically >10% of total body weight compared to <10%. Interestingly, participants with T2-high profiles also appeared to respond more than those with T2-low disease for ACQ6, AQLQ and number of prednisolone courses. The underlying mechanisms by which those with T2-high disease might derive greater benefit from weight loss are unclear. To our knowledge this is the first report comparing effects of weight loss on asthma outcomes in T2-high and T2-low asthma. Previously, a report by Baltieri et al [223] showed weight-loss-induced increase in anti-inflammatory and decrease in pro-inflammatory mediators in people with obesity and asthma, however T2-inflammatory markers were not studied. A recent study by Pinkerton et al [319] shed some light in this area by identifying a link between T2-high inflammation and obesity. They observed increased IL-5, IL-13 and CC chemokine receptor type 3 (CCR3) with obesity, the latter involved with eosinophil chemotaxis. Notably, they also report increased eosinophilic airway tissue inflammation but a paucity of eosinophils in the airway lumen itself. These finding parallels that of Farahi et al [96] who showed increased parenchymal eosinophil uptake using SPECT/CT imaging and suggested a disparity between airway tissue and sputum eosinophils counts. Links between adipokine imbalance, particularly leptin, and eosinophil biology dysfunction have

been described previously [94, 320, 321]. Potential mechanisms of obesity on airway nitric oxide synthase uncoupling with downstream effects on FeNO have been described [322]. Despite this, a comprehensive understanding of the effects of obesity on airway inflammation, and conversely of weight-loss, in people with asthma is lacking. It is feasible that obesity-associated asthma sub-endotypes exist beyond our understanding of T2-high and T2-low disease. Caution must be taken interpreting these results as this was not pre-defined outcome and the sample size is low.

Most studies of weight loss in asthma to date have evaluated short-term outcomes with few studies documenting long-term benefits. Scott et al [231] observed improvements in asthma control and quality of life after ten weeks of either dietary or combined dietary and exercise interventions, but no longer-term outcomes were assessed. Özbey et al [316] reported improved asthma-related control and quality of life indices with a 10-week weight loss programme however, as well as studying a well-controlled population, no longer-term outcomes were assessed. Freitas et al [315] had reported improvements in weight loss, as well as ACQ and AQLQ after three months using an exercise regime compared to a sham group. At twelve months weight gain was observed in the intervention group, however values were not reported, and neither was the effect on ACQ or AQLQ. Johnson et al [323] performed a single-arm study of an online weight loss intervention in participants with obesity and uncontrolled asthma (n = 43) and observed improved asthma control and quality of life in those that lost >5% body weight. However, as well as lacking a control group, patients were followed up for six months in total. Only Ma et al [314] assessed ACQ at one year using a behavioural and lifestyle interventional protocol including calorie-restriction compared to usual care and found no improvement. However, this population had a lower baseline mean ACQ (1.4) suggesting a better controlled population than our study and the weight loss observed was considerably lower (5kg) than with CWP, likely inadequate to result in improvement in asthma control or quality of life.

4.5.2 Limitations

Potential limitations and risk of bias have been acknowledged in Chapter Three. Most significantly, these include missing data both for laboratory-measured variables such as lung function, blood tests, FeNO, six-minute walk test and accelerometery due to the effects of the pandemic, and of one-year follow-up patient datasets due to loss of follow-up. This open-label trial was conducted in a real-world setting vulnerable to bias, however clinical benefits remain relevant for both the patient and healthcare.

The use of multiple imputation methods for missing data in small sample sizes must be acknowledged. Complex multiple imputation models in small sample sizes are subject to overfitting and this is a potential limitation here. However, similar signals were observed in the complete case analysis from our dataset suggesting this is not the case here. Nonetheless, caution in interpreting exact effect sizes is advised. Utilising other methods such as last observation carried forward or solely relying on complete case analysis are less appropriate and robust methods and more likely to introduce bias. Ultimately, all methods have advantages and disadvantages, and in this study, we report both imputed and complete case analysis to provide a more comprehensive overview of the effects of the intervention on the relevant variables.

This was a feasibility trial with 16-week analysis as the primary outcome. A further larger study is now required to more comprehensively assess effectiveness of CWP in asthma on spirometry and markers of inflammation, in particular T2 inflammation. Conclusions from our T2 analysis must be interpreted with caution as this was a post-hoc analysis and a dedicated study is required to confirm and build upon the signal found.

4.5.3 Conclusion

Ours is the first study of weight management in participants with obesity and asthma to observe sustained weight loss at one-year resulting in benefits in both anthropometric and asthma-related outcomes, specifically asthma-related quality of life and frequency of exacerbations. Encouragingly, whilst no between-group differences were observed at one-year in asthma control, those that experienced improvement in asthma control at four months displayed

sustained improvement longer-term. Moreover, the majority in the intervention arm experienced weight-loss greater than 10% total body weight resulting in significant asthma control improvement, further highlighting the benefit of CWP. We also offer insight into a potential effect of weight-loss in T2-high asthma necessitating further attention to obesity-mediated airway inflammation. Two-year outcomes continue to be assessed which may provide deeper insight into the merits of weight management in people with obesity and difficult-to-treat asthma.

Chapter Five: The impact of obesity on biomarkers of type 2 inflammation in difficult-to-treat asthma

5.1 Introduction

In the last two decades there has been a greater appreciation of the links between obesity and asthma, with a causal link now proposed between raised body mass index (BMI) and diagnosis of asthma [152, 154-157, 324-326]. Patients with asthma associated with obesity are more likely to have steroid-resistant uncontrolled disease and severe exacerbations, in addition to reduced quality of life [106, 145, 302, 327-329]. As specified in Chapter One, OAA is often linked to T2-low endotypes. Presently in the UK, MAb treatments for severe asthma target the T2-high pathway: omalizumab (anti-IgE); mepolizumab and reslizumab (anti-IL5); benralizumab (anti-IL5R); dupilumab (anti-IL4R). This reflects the currently available range of biomarkers utilised in asthma management; unfortunately, there are no biomarkers available to direct advanced treatments in T2-low asthma. Recent studies over the last 15 years have proposed a negative association between adipose excess and the clinically relevant T2-biomarkers, total IgE, blood eosinophils and FeNO [330-333]. The implication from this on MAb eligibility could be profound though these previous studies have not adjusted for key covariates, limiting our interpretation of their findings. In particular, none have accounted for both inhaled (ICS) and oral corticosteroid (OCS) dose, the use of which have previously been shown to decrease both FeNO and peripheral eosinophil counts [334, 335]. Further investigation is therefore needed to characterise the impact of raised BMI on T2-biomarkers allowing for corticosteroid use. Accurate classification is needed, not only to determine eligibility for advanced therapies, but also to allow dedicated research into subgroups with T2-low asthma. The impact of obesity on T2-biomarkers could therefore affect attempts at characterising T2-high and T2-low disease, limiting conclusions in this population.

A recent analysis of the UK Severe Asthma Registry (UKSAR) categorised patients with severe asthma by T2 endotype using a biomarker approach based on FeNO and blood eosinophil count [318]. Interestingly, they report that a significant portion of the T2-low asthma group had a historically raised blood eosinophil count and were therefore previously T2-high. This observation may, in part, be due to corticosteroid dosing and its known suppressing effects on FeNO and peripheral eosinophils. Yet, obesity may also be a relevant factor in the

determination of T2-high/low profiling. Indeed, a significantly higher BMI was seen in the T2-low asthma group than the T2-high group in the UKSAR study (32.1 ± 7.8 kg/m^2 vs 30.2 ± 6.7 kg/m^2 respectively, $p < 0.001$). Whether higher BMI might be masking the T2-high endotype in patients that are still inherently responsive to T2-high treatments or resulting in a true T2-low endotype that is unresponsive to these therapies is unclear. There are no studies assessing the efficacy of monoclonal antibody treatment in severe asthma and obesity with lower T2-biomarkers.

The pathobiology of adipose tissue is complex (see Chapter One) and continues to be studied in asthma, however it is becoming more evident that weight excess causes an imbalance in adipokine production with increased systemic and airway pro-inflammatory mediators (e.g., leptin, interleukin-6, tumour necrosis factor α, resistin, chemerin) as well as reduced protective anti-inflammatory mediators (adiponectin, interleukin-10) [70, 73]. It is possible that there is an adipokine-mediated disturbance to T2-biomarkers.

Certain biomarkers are already known to be altered by factors affecting their utility in clinical practice. There is evidence, for instance, that FeNO levels are altered by age, height, allergic or perennial rhinitis, diet, active chest infection and cigarette smoking [294, 336-338].

5.2 Hypothesis

Increased BMI reduces T2-biomarker levels (total IgE, FeNO and peripheral eosinophils) in patients with asthma when correcting for appropriate covariates including oral and inhaled steroid dose. We undertook a retrospective analysis of datasets from two local trials (NCT03630432, NCT03858608); trial protocols are described elsewhere [308, 339].

5.3 Method

5.3.1 Population

Data were gathered from the above trials for adult patients (aged 18-75 years) with difficult-to-treat asthma and raised BMI (overweight, BMI 25.0 - 29.9 kg/m^2; obese BMI ≥ 30 kg/m^2), and mild asthma with either healthy (< 25 kg/m^2) or raised BMI (overweight and obese). Both trials were single-centre, open-label, randomised, controlled trials undertaken at the CRF at GRI. One trial evaluated the effects of pulmonary rehabilitation in patients with raised BMI and difficult-to-treat asthma [339], with a subsequent sub-study evaluating activity levels in this cohort compared to mild asthma patients of all weight categories. The other studied the effect of a weight loss programme on difficult-to-treat asthma in patients with obesity, described in Chapters Three and Four [308]. Patients consenting to these trials agreed to their data being used for future analyses such as this.

5.3.2 Measurements

All data from the initial trials was collected by the Clinical Research Team including the specialist research nurses and clinical research fellow. Participants were identified from the GRI and New Stobhill Hospital Difficult Asthma Clinic, with the remainder from other specialist outpatient asthma services across Greater Glasgow and Clyde and hospital ward admissions. Data were taken from the baseline visits from each trial. Amongst the data collected were patient demographics, past medical and asthma-related history, drug history, smoking status, height, weight, BMI, peripheral eosinophil counts and FeNO. The processes for these are identical to the ones described in Chapters Two and Three. Historical total IgE was obtained, where available, for this analysis from each patient's electronic record. The most recent total IgE was used in cases where multiple results existed.

5.3.3 Eligibility criteria

Inclusion criteria for the difficult-to-treat patients (see Chapter Three) include:

- Asthma – characteristic symptoms and either airflow obstruction with FEV_1 variability or bronchial hyperreactivity in the last five years (as per GINA guidelines [4])
- Difficult-to-treat disease – treatment with either daily OCS or high dose ICS and either high ACQ score (≥1.5) or ≥2 exacerbations requiring OCS or ≥1 hospitalisation with acute exacerbation of asthma in the preceding twelve months (as per SIGN/BTS guidelines [6])

Mild asthma patients with active disease (defined as recorded asthma diagnosis and asthma medication use in the preceding twelve months) were identified from primary care. These patients were required to have maximum treatment of moderate dose ICS/LABA combination, an ACQ score <1.5, <2 course of OCS and no acute exacerbations of asthma requiring hospitalisation in the preceding twelve months.

Patients with a recent exacerbation (within four weeks) or lower respiratory tract infection, severe or unstable co-morbidities (e.g., cardiac), initiation of biologic therapy (within six months) or ICU admission in the preceding six months were excluded.

5.3.4 Statistical analysis

Results were processed for all participants overall and compared by the following sub-groups:

- asthma severity (mild and difficult-to-treat),
- T2-status (T2-high and T2-low),
- BMI tertile.

As per the recent UKSAR analysis [318], T2-status was described with a biomarker approach, with serum eosinophil counts ≥ $0.15x10^9$/L or FeNO ≥ 25 parts per billion (ppb) labelled as T2-high disease. Participants with levels lower

than these were classed as T2-low. For BMI tertile stratification, participants were listed in order of increasing BMI and divided into three equal groups. This was carried out for the overall group of participants and the difficult-to-treat asthma group.

Categorical variables were summarised as absolute number (percentage) and compared with the chi-square test or Fisher's exact test (if any expected cell count <5). For continuous variables, distribution was assessed using histograms, checking skewness and kurtosis, and normality tests (Kolmogorov-Smirnov). Following this, continuous variables were summarised as either mean (standard deviation) or median (interquartile range) and compared with the unpaired t-test or Mann-Whitney U test (for two groups), or analysis of variance (ANOVA) or Kruskal-Wallis test (three or more groups) where appropriate.

Correlation between each individual biomarker (FeNO, peripheral eosinophil count, total IgE) and BMI was assessed with scatter plots and Spearman's rank coefficient testing. Relationships between any correlating biomarker and BMI were further described with a stepwise multiple linear regression model (with Bonferroni correction) provided linear assumptions were validated by residual analysis, or data were transformed where appropriate. Clinically relevant covariates, such as ICS and OCS dose, were locked into any models for the purpose of this study.

Data analysis was performed entirely by the author using IBM SPSS Statistics (version 27.0.1.0). A p-value of ≤ 0.05 was set for statistical significance.

5.4 Results

5.4.1 Demographics and anthropomorphics

A total of 153 participants were included for this analysis (see Figure 5.1, adapted from Sharma et al [340]), of which 102 had difficult-to-treat disease (25 in the "overweight" BMI range, 77 in the "obesity" range) and 51 with mild disease (25 healthy-BMI, 15 overweight and 11 with obesity).

Figure 5. 1 - Study population by asthma severity and weight category

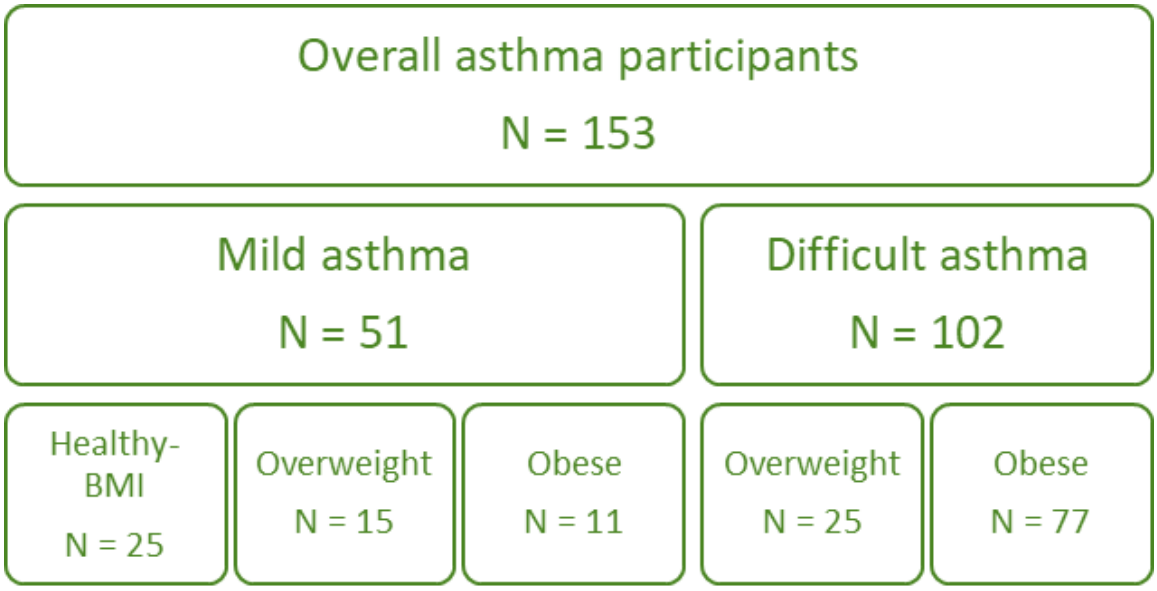


Table 5.1 shows baseline demographics and clinical characteristics both overall and compared by asthma severity (mild and difficult-to-treat).

Table 5. 1 - Baseline demographic and clinical characteristics overall and by asthma severity

Variable	Overall n = 153	Mild asthma n = 51	Difficult asthma n = 102	p value*
Age, years	54 (44 - 64)	56 (32 - 64)	54 (46 - 63)	0.713
Female sex	91(59.5)	29(56.9)	62(60.8)	0.641
BMI, kg/m^2	31.1 (26.6 - 36.9)	25.3 (23.2 - 29.0)	33.9 (30.0 - 39.2)	**<0.001**
Smoking status: Lifelong non-smoker Current smoker Ex-smoker	 87 (57.2) 9 (5.9) 56 (36.8)	 37 (72.6) 2 (3.9) 12 (23.5)	 50 (49.0) 7 (6.9) 45 (44.1)	**0.014**
Atopy	73 (47.7)	7 (13.7)	66 (64.7)	**<0.001**
Allergic rhinitis	104 (68.0)	33 (64.7)	71 (69.6)	0.540
Perennial rhinitis	66 (43.1)	16 (31.4)	50 (49.0)	**0.038**
Equivalent BDP ICS dose, mcg	1600 (800 - 2000)	400 (200 - 800)	1900 (1600 - 2000)	**<0.001**
OCS dose, mg	5 (5 - 10)	n/a	5 (5 - 10)	n/a
FeNO, ppb	22 (14 - 43)	22 (17 - 27)	23 (13 - 50)	0.308
Eosinophils, x10^9/L	0.2 (0.1 - 0.4)	0.1 (0.1 - 0.3)	0.3 (0.1 - 0.4)	**0.020**
Total IgE, kU/L	148 (32 - 372)	156 (132 - 560)	141 (31 - 386)	0.394

Reported as median (IQR) or no. (%) unless stated otherwise.
Abbreviations – BDP (beclomethasone diproprionate), BMI (body mass index), FeNO (fractional exhaled nitric oxide), ICS (inhaled corticosteroid), IgE (immunoglobulin E), IQR (interquartile range), OCS (oral corticosteroid), ppb (parts per billion).
*Mild vs Difficult asthma. Continuous variables compared using Mann-Whitney; categorical variables compared using chi-squared or Fisher's exact.

Adapted from Sharma et al [340]

Table 5.2 compares all participants (n = 153) by BMI tertile with the first tertile consisting of participants with the lowest BMI's and the third tertile the highest. Table 5.3 displays an identical comparison but of the difficult-to-treat group (n = 102).

Table 5. 2 - Asthma patients stratified by BMI tertile

Variable	First Tertile n = 51	Second Tertile n = 51	Third Tertile n = 51	p value *	p value **
Age, years	56 (32 - 64)	58 (45 - 65)	54 (47 - 60)	0.701	0.791
Female sex	27 (52.9)	27 (52.9)	37 (72.5)	0.066	**0.041**
BMI kg/m^2	25.0 (23.2 - 26.7)	31.1 (29.5 - 32.9)	39.7 (36.8 - 43.9)	**<0.001**	**<0.001**
Smoking status: Lifelong non-smoker Current smoker Ex-smoker	 38 (76.0) 2 (4.0) 10 (20.0)	 25 (49.0) 4 (7.8) 22 (43.1)	 24 (47.1) 3 (5.9) 24 (47.1)	**0.026**	**0.010**
Atopy	15 (29.4)	28 (54.9)	30 (58.8)	**0.005**	**0.003**
Allergic rhinitis	36 (70.6)	32 (62.7)	36 (70.6)	0.619	1.000
Perennial rhinitis	21 (41.2)	22 (43.1)	23 (45.1)	0.923	0.689
Equivalent BDP ICS dose, mcg	800 (400 - 1600)	1600 (1600 - 2000)	1600 (1600 - 2000)	**<0.001**	**<0.001**
OCS dose, mg	6.0 (5.0 - 11.0)	5.0 (5.0 - 10.0)	5.0 (5.0 - 10.0)	0.891	0.733
FeNO, ppb	25 (18 - 41)	23 (16 - 70)	18 (12 - 30)	**0.024**	**0.014**
Eosinophils, x10^9/L	0.2 (0.1 - 0.3)	0.3 (0.1 - 0.4)	0.2 (0.1 - 0.4)	0.643	0.799
Total IgE, kU/L	117 (32 - 243)	223 (68 - 720)	96 (20 - 334)	0.086	0.984
T2-high status	37 (72.5)	40 (81.6)	33 (64.7)	0.163	0.393

Reported as median (IQR) or no. (%) unless stated otherwise.
T2-high status defined as either: FeNO ≥ 25 ppb or eosinophils ≥ 0.15 x10^9/L.
Abbreviations – BDP (beclomethasone diproprionate), BMI (body mass index), FeNO (fractional exhaled nitric oxide), ICS (inhaled corticosteroid), IgE (immunoglobulin E), IQR (interquartile range), OCS (oral corticosteroid), ppb (parts per billion).
*All tertiles. Continuous variables compared using Kruskal-Wallis; categorical variables compared using chi-squared.
**First vs Third tertile. Continuous variables compared using Mann-Whitney; categorical variables compared using chi-squared or Fisher's exact.

Adapted from Sharma et al [340]

Table 5. 3 - Patients with difficult-to-treat asthma stratified by BMI tertile

Variable	First Tertile n = 34	Second Tertile n = 34	Third Tertile n = 34	p value *	p value **
Age, years	58 (47 - 65)	58 (38 - 65)	52 (44 - 59)	0.122	**0.046**
Female sex	24 (70.6)	13 (38.2)	25 (73.5)	**0.004**	0.787
BMI, kg/m^2	28.7 (26.8 - 30.1)	33.9 (32.8 - 35.7)	41.9 (38.9 - 46.9)	**<0.001**	**<0.001**
Smoking status: Lifelong non-smoker Current smoker Ex-smoker	 21 (61.8) 1 (2.9) 12 (35.3)	 9 (26.5) 3 (8.8) 22 (64.7)	 20 (58.5) 3 (8.8) 11 (32.4)	**0.022**	0.586
Atopy	21 (61.8)	22 (64.7)	23 (67.6)	0.879	0.612
Allergic rhinitis	22 (64.7)	21 (61.8)	28 (82.4)	0.136	0.099
Perennial rhinitis	17 (50.0)	15 (44.1)	18 (52.9)	0.760	0.808
Equivalent BDP ICS dose, mcg	2000 (1600 - 2400)	1700 (1600 - 2000)	1800 (1600 - 2000)	0.696	0.415
OCS dose, mg	5.0 (5.0 - 10.0)	8.75 (5.0 - 11.5)	5.0 (2.0 - 10.0)	0.264	0.299
FeNO, ppb	42 (20 - 66)	20 (12 - 55)	17 (12 - 38)	**0.027**	**0.008**
Eosinophils, x10^9/L	0.3 (0.2 - 0.5)	0.3 (0.1 - 0.5)	0.2 (0.1 - 0.3)	**0.048**	**0.022**
Total IgE, kU/L	162 (35 - 305)	147 (53 - 591)	89 (21 - 395)	0.601	0.371
T2-high status	32 (94.1)	28 (84.8)	21 (61.8)	**0.003**	**0.001**

Reported as median (IQR) or no. (%) unless stated otherwise.
T2-high status defined as either: FeNO ≥ 25 ppb or eosinophils ≥ 0.15 x10^9/L.
Abbreviations – BDP (beclomethasone diproprionate), BMI (body mass index), FeNO (fractional exhaled nitric oxide), ICS (inhaled corticosteroid), IgE (immunoglobulin E), IQR (interquartile range), OCS (oral corticosteroid), ppb (parts per billion).
*All tertiles. Continuous variables compared using Kruskal-Wallis; categorical variables compared using chi-squared.
**First vs Third tertile. Continuous variables compared using Mann-Whitney; categorical variables compared using chi-squared or Fisher's exact.

Adapted from Sharma et al [340]

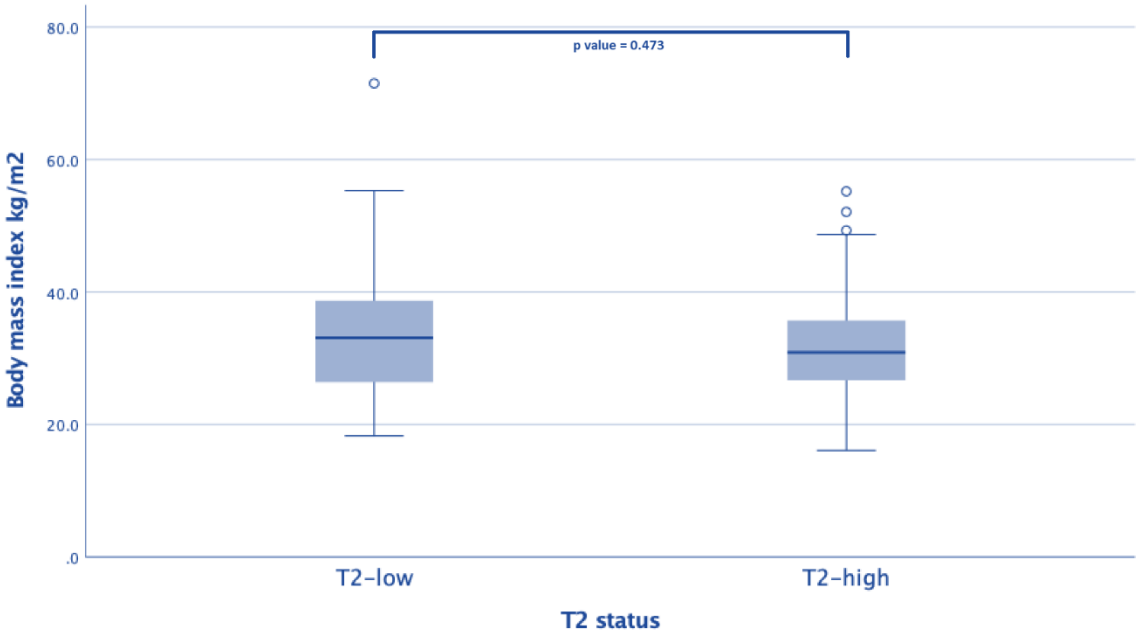
Altogether, the majority of participants were female (60%) with a median age of 54 years and either non- or ex-smokers (57% and 37% respectively). Median BMI was 31 kg/m^2, and when stratified by disease severity, a higher BMI was shown in the difficult-to-treat asthma group compared to the mild asthma group (34 kg/m^2 and 25 kg/m^2 respectively, $p < 0.001$). Comparison by T2-status showed no difference between groups in the overall dataset (Table 5.4); median BMI in the T2-high group was 31 (27, 36) kg/m^2 and 33 (26, 39) kg/m^2 in the T2-low group, $p = 0.473$ (see Figure 5.2, adapted from Sharma et al [340]).

Table 5. 4 - Comparison of all participants by T2-status

Variable	T2-high n = 110	T2-low n = 41	p value*
Age, years	57 (44 - 65)	54 (39 - 61)	0.212
Female sex	63(57.3)	27(65.9)	0.339
BMI, kg/m^2	30.9 (26.6 - 35.7)	33.1 (26.0 - 38.9)	0.473
Smoking status: Lifelong non-smoker Current smoker Ex-smoker	 61 (56.0) 6 (5.5) 42 (38.5)	 25 (61.0) 3 (7.3) 13 (31.7)	 0.715
Atopy	55 (50.0)	18 (43.9)	0.505
Allergic rhinitis	77 (70.0)	26 (63.4)	0.440
Perennial rhinitis	46 (41.8)	19 (46.3)	0.618
Equivalent BDP ICS dose, mcg	1600 (850 - 2000)	1000 (450 - 1600)	**0.006**
OCS dose, mg	7.5 (5 - 10)	5.0 (5.0 - 10.0)	0.405
FeNO, ppb	28 (17 - 51)	17 (12 - 20)	**<0.001**
Eosinophils, x10^9/L	0.3 (0.2 - 0.4)	0.1 (0.1 - 0.1)	**<0.001**
Total IgE, kU/L	152 (32 - 444)	111 (47 - 281)	0.541

Reported as median (IQR) or no. (%) unless stated otherwise.
T2-high status defined as either: FeNO ≥ 25 ppb or eosinophils ≥ 0.15 x10^9/L.
Abbreviations – BDP (beclomethasone diproprionate), BMI (body mass index), FeNO (fractional exhaled nitric oxide), ICS (inhaled corticosteroid), IgE (immunoglobulin E), IQR (interquartile range), OCS (oral corticosteroid), ppb (parts per billion).
*T2-high vs T2-low asthma. Continuous variables compared using Mann-Whitney; categorical variables compared using chi-squared or Fisher's exact.

Figure 5. 2 - Comparison of BMI of all participants by T2-status



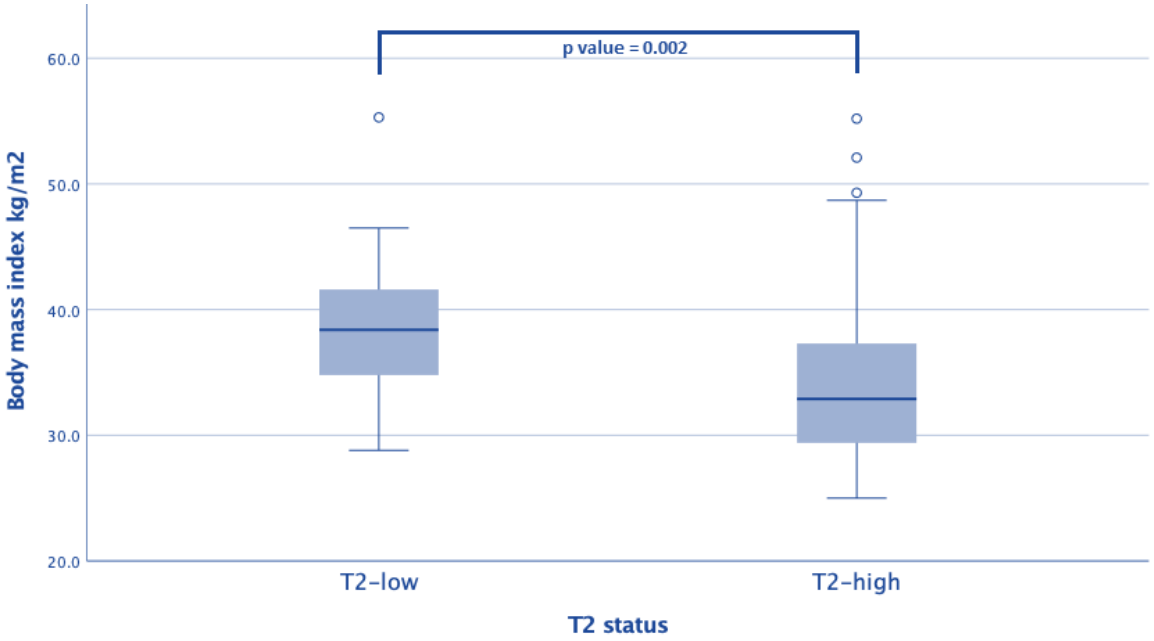
Comparison of BMI by T2-status within the difficult-to-treat asthma group (Table 5.5) showed that the T2-low group had a significantly higher median BMI of 38 (34, 42) kg/m^2 than the T2-high group (33 (29, 38) kg/m^2; p = 0.002; Figure 5.3 adapted from Sharma et al [340]). Note there were no observed differences between groups in ICS or OCS dose.

Table 5. 5 - Comparison of participants with difficult-to-treat asthma by T2-status

Variable	Difficult-to-treat T2-high n = 81	Difficult-to-treat T2-low n = 20	p value*
Age, years	57 (46 - 65)	52 (40 - 56)	**0.031**
Female sex	46 (56.8)	15 (75.0)	0.136
BMI, kg/m^2	32.9 (29.3 - 37.7)	38.4 (34.4 - 42.0)	**0.002**
Smoking status: Lifelong non-smoker Current smoker Ex-smoker	 40 (49.4) 4 (4.9) 37 (45.7)	 9 (45.0) 3 (15.0) 8 (40.0)	 0.283
Atopy	50 (61.7)	16 (80.0)	0.124
Allergic rhinitis	55 (67.9)	16 (80.0)	0.289
Perennial rhinitis	38 (46.9)	12 (60.0)	0.295
Equivalent BDP ICS dose, mcg	2000 (1600 - 2000)	1600 (1600 - 2000)	0.230
OCS dose, mg	7.5 (5.0 - 10.0)	5.0 (5.0 - 10.0)	0.405
FeNO, ppb	36 (15 - 62)	16 (11 - 19)	**<0.001**
Eosinophils, x10^9/L	0.3 (0.2 - 0.4)	0.1 (0.1 - 0.1)	**<0.001**
Total IgE, kU/L	151 (32 - 466)	104 (38 - 260)	0.485

Reported as median (IQR) or no. (%) unless stated otherwise.
T2-high status defined as either: FeNO ≥ 25 ppb or eosinophils ≥ 0.15 x10^9/L.
Abbreviations – BDP (beclomethasone diproprionate), BMI (body mass index), FeNO (fractional exhaled nitric oxide), ICS (inhaled corticosteroid), IgE (immunoglobulin E), IQR (interquartile range), OCS (oral corticosteroid), ppb (parts per billion).
*T2-high vs T2-low asthma. Continuous variables compared using Mann-Whitney; categorical variables compared using chi-squared or Fisher's exact.

Figure 5. 3 - Comparison of BMI within the difficult-treat asthma group by T2-status



Corresponding to this, within the difficult-to-treat group, there was a significantly lower proportion of T2-high participants in the third BMI tertile (the group with the largest BMI) than the first BMI tertile (62% and 94% respectively; p = 0.001).

Notably, compared by disease severity, there were fewer lifelong non-smokers and more ex-smokers in the difficult-to-treat group (49% and 44% respectively) compared to the mild asthma group (73% and 24% respectively; p = 0.014).

5.4.2 Atopic status and rhinitis

Overall, 48% had positive atopic status, with a larger proportion of atopic individuals in the difficult-to-treat asthma group than in the mild asthma group (65% and 14% respectively, p < 0.001). Atopy was more prevalent with rising BMI tertile across the whole dataset but not within the difficult-to-treat asthma group. 68% had a diagnosis of allergic rhinitis. 43% suffered from perennial rhinitis overall, with a larger proportion in the difficult-to-treat asthma group than in the mild asthma group (49% vs 31% respectively, p = 0.038), but no differences between BMI tertiles.

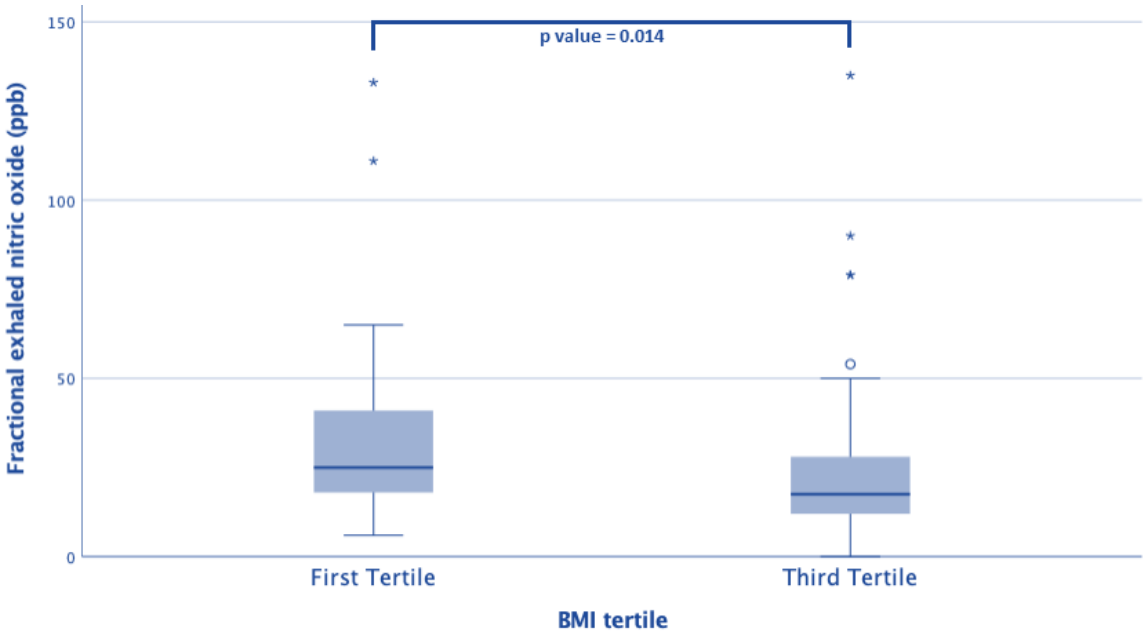
5.4.3 Corticosteroid comparison

Overall, median inhaled beclomethasone dipropionate (BDP) dose was 1600 (800, 2000) mcg. Compared by disease severity, we observed an expected higher median dose in the difficult-to-treat group (1900 (1600, 2000) mcg) than in the mild group (400 (200, 800) mcg; p < 0.001). No differences were observed in BDP dose within the difficult-to-treat asthma group when compared by BMI tertile or T2-status. In the difficult-to-treat asthma group, median oral prednisolone dose was 5 (5, 10) mg, and, of note, no difference was observed when categorised by either BMI tertile or T2-status.

5.4.4 Type 2 biomarkers of inflammation

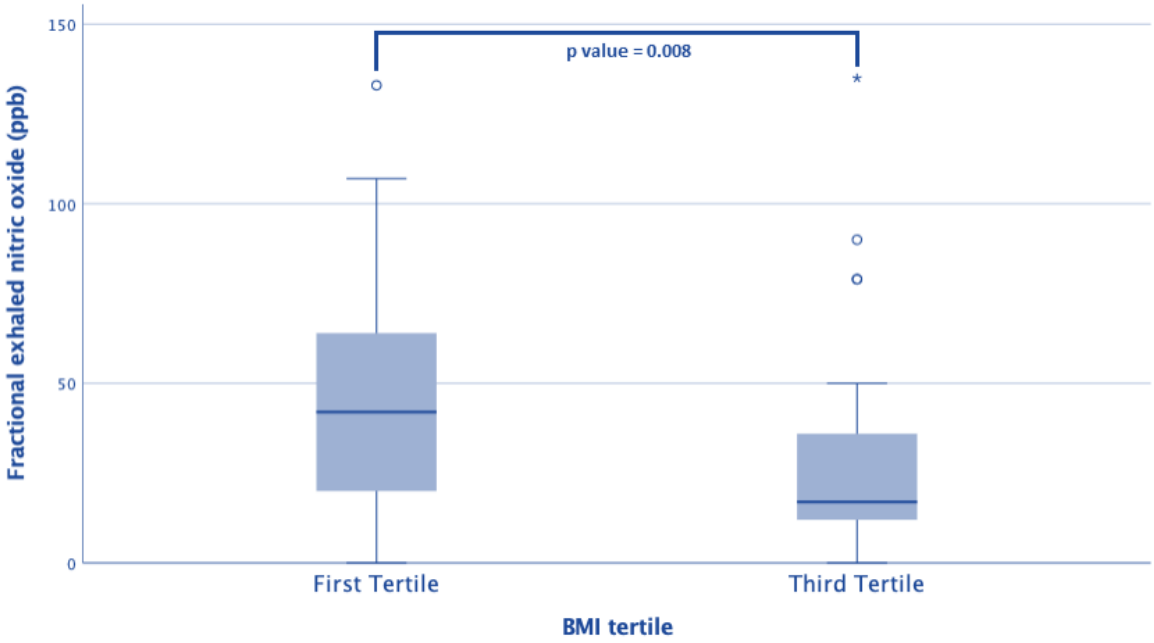
In the overall dataset (n = 153), median FeNO was 22 (14, 43) ppb. There were no observed differences comparing mild and difficult-to-treat disease. Median FeNO was highest in the first BMI tertile (the lowest BMI category) at 25 (18, 41) ppb, compared to the second (23 (16, 70) ppb) and third tertiles (18 (12, 30) ppb; p = 0.024 comparing all tertiles; p = 0.014 comparing first and third tertiles; see Figure 5.4 adapted from Sharma et al [340]).

Figure 5. 4 - Comparison of fractional exhaled nitric oxide (FeNO) levels of all participants by BMI tertile



Within the difficult-to-treat asthma group, when stratified into BMI tertiles, median FeNO was again higher in the first tertile (42 (20, 66) ppb) than the second (20 (12, 55) ppb) and third (17 (12, 38) ppb; p = 0.027 comparing all tertiles; p = 0.008 comparing first and third tertiles; see Figure 5.5 adapted from Sharma et al [340]).

Figure 5. 5 - Comparison of fractional exhaled nitric oxide (FeNO) within the difficult-to-treat asthma group by BMI tertile



Participants with difficult-to-treat asthma had higher peripheral eosinophils compared to those with mild disease (0.3 x10^9/L and 0.1 x10^9/L respectively; p = 0.020). When stratified into BMI tertiles, no difference was observed between groups in the overall dataset (n = 153), as shown in Figure 5.6 (adapted from Sharma et al [340]). However, within the difficult-to-treat group (n = 102), higher eosinophil counts were seen in the first BMI tertile (0.3 x 10^9/L) compared to the third BMI tertile (0.2 x 10^9/L; p = 0.022; see Figure 5.7 adapted from Sharma et al [340]).

Figure 5. 6 - Comparison of eosinophil levels in all participants by BMI tertile

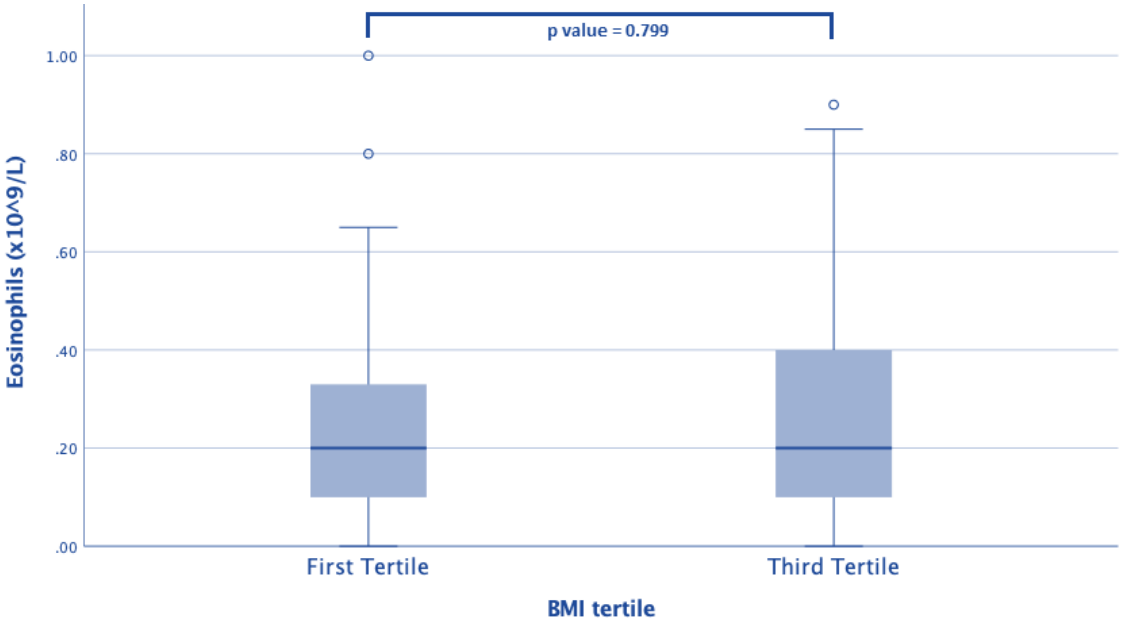
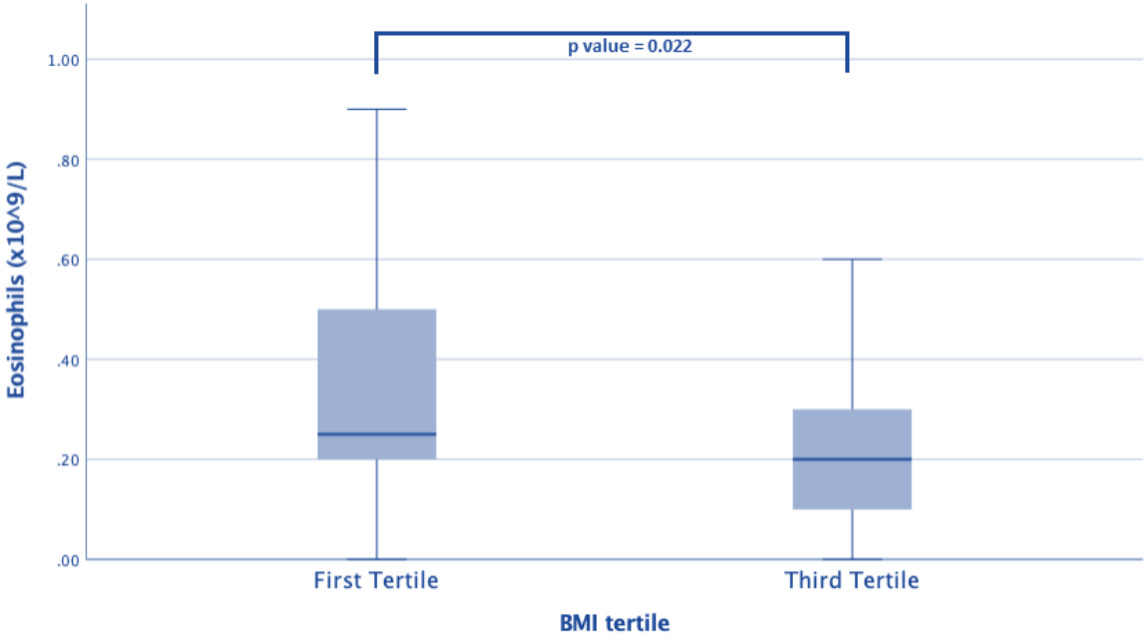


Figure 5. 7 - Comparison of eosinophil levels within the difficult-to-treat asthma group by BMI tertile

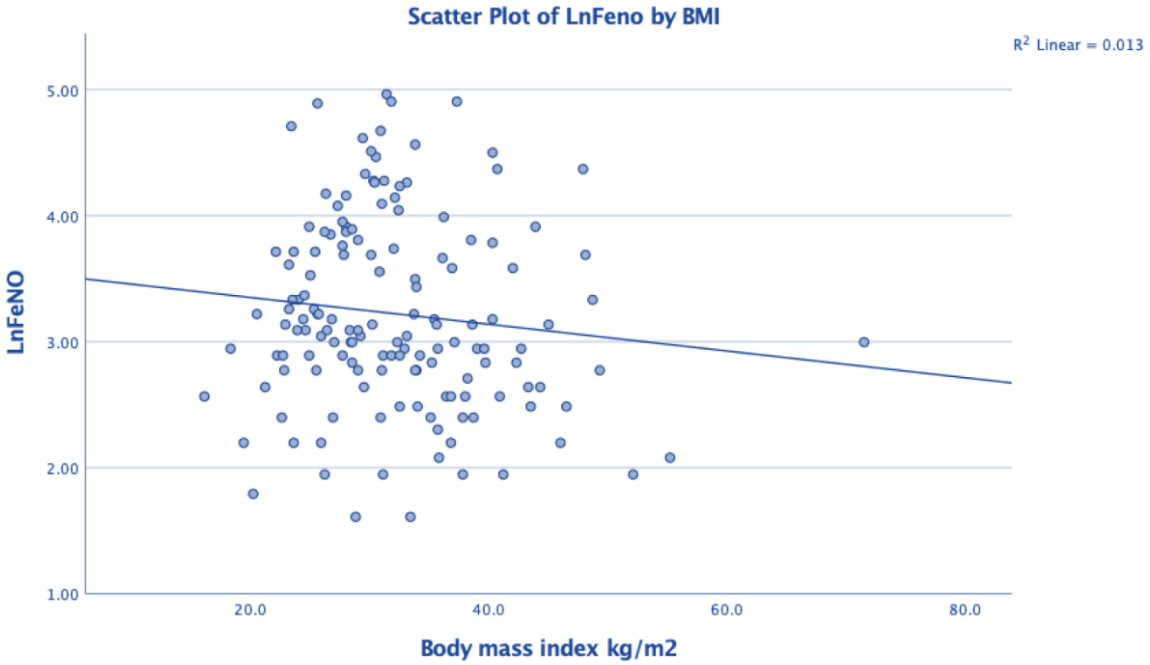


No differences were observed in total IgE between asthma severity groups, T2-status groups or BMI tertiles.

5.4.5. Spearman's rank

No correlations were identified between any of the T2-biomarkers (FeNO, peripheral eosinophils and total IgE) and BMI using Spearman's rank analysis in the overall dataset (n = 153). Within the difficult-to-treat asthma group (n = 102), a negative correlation was observed between FeNO and BMI, ρ = -0.309 (two-tailed p value = 0.002; see Figure 5.8 adapted from Sharma et al [340]).

Figure 5. 8 - Correlation between log-transformed FeNO and BMI in the difficult-to-treat asthma group



No correlations were observed between either peripheral eosinophils or total IgE and BMI in the difficult-to-treat asthma group.

5.4.6 Linear regression

Following correlation analysis, linear regression was performed to further categorise the relationship between BMI and FeNO. Although multiple regression was the aim from the statistical plan, to adjust for several variables including corticosteroid use, initial univariate analysis was undertaken of the overall dataset (n = 153) with FeNO as the dependent variable. The overall model was not significant and did not show BMI as a significant predictor:

$F(1,149) = 1.21$; $p = 0.273$; $R^2 = 0.8\%$ ($R = 9\%$).

Subsequent multiple linear regression was undertaken to investigate whether BMI could predict FeNO when adjusted for clinically relevant covariates, namely, age, sex, atopic status, smoking status, allergic rhinitis, perennial rhinitis, inhaled and oral corticosteroid dose. Employing a stepwise approach for the overall dataset (n = 153), it was observed that BMI was a significant predictor of FeNO when adjusting for these variables ($\beta = -2.848$, $p = 0.019$). Overall, the model was a good fit, a significant predictor, and had no evidence of multicollinearity:

$F(9,18) = 3.20$; $p = 0.017$; $R^2 = 62\%$ ($R = 79\%$); VIF 1.3 - 2.3.

Linear assumptions were confirmed at the time of testing, for example normality (assessment of skewness, kurtosis, histogram and P-P plots) and homoscedasticity (residual scatterplot assessment).

From this model, in this dataset, it can be inferred that for every increase in BMI of 1 kg/m^2, a decrease in FeNO of 3 ppb is observed.

No relationships between peripheral eosinophils or total IgE and BMI were identified.

5.5 Discussion

5.5.1 Obesity and T2-biomarkers

This study was conducted to assess for an association between BMI and T2-biomarkers of asthma after adjusting for confounding variables, in particular corticosteroid dose. We observed a negative relationship between BMI and FeNO levels. Comparison between BMI tertiles in the overall dataset of FeNO showed lower FeNO levels in higher BMI tertiles. This observation was reproduced in the difficult-to-treat asthma group, in which there was no difference in ICS or OCS use between tertiles. This is an interesting finding as it suggests that the lower FeNO levels observed were not secondary to corticosteroid dose. Furthermore, within the difficult-to-treat asthma group, we reported a higher BMI in the T2-low group than the T2-high group (38 kg/m^2 and 33 kg/m2 respectively) with no differences seen in corticosteroid use between these groups, again suggesting the lower T2-biomarkers were not related to corticosteroid dose. Moreover, we identified a higher proportion of T2-high asthma in lower BMI tertiles in the difficult-to-treat group. Spearman's rank in the difficult-to-treat asthma group confirmed a negative correlation between FeNO and BMI and subsequent multiple regression analysis in the overall dataset described a significant relationship whilst correcting for age, sex, atopy, smoking, allergic and perennial rhinitis, ICS and OCS. These variables are all known to affect FeNO levels impacting interpretation in practice. The initial univariate regression analysis was not significant, and this is often the case when the model is too simple and at risk of omitted variable bias. The described effect of BMI on FeNO, independently of corticosteroid use, is vital to outline due to knock-on effects on eligibility of effective treatments in T2-high disease. For example, asthma patients with higher FeNO levels benefit from increased corticosteroid dosing. Furthermore, severe asthma with raised FeNO responds to IL-4R monoclonal antibody therapy (dupilumab).

Previous studies have suggested a negative association between FeNO and BMI. In 2006, Barros et al [330] were the first to assess this however they did not account for oral corticosteroid use, amongst others, in regression modelling. Komakula et al [331] then studied patients with moderate-severe asthma against

healthy controls, finding a negative relationship correcting for age, sex, atopic status, asthma control, gastro-oesophageal reflux, use of LABA and LRA therapy, though not inhaled or oral corticosteroid use. The authors suggested that adipose excess leads to iNOS uncoupling by affecting L-arginine/ADMA causing reduced bronchial NO despite increased oxidative stress [322, 333]. In 2018, Lugogo et al [332] reported an effect of BMI on sputum eosinophilia and FeNO, as well as serum eosinophils and total IgE, however correcting for sex, age and race only. Alongside FeNO, we demonstrated reduced peripheral eosinophils in higher BMI tertiles within the difficult-treat asthma group. Correlation and regression analysis were not significant in our study, however the study from Lugogo et al [332] identified a negative association.

Peripheral eosinophil counts were lower in the higher BMI tertiles within the difficult-to-treat asthma cohort. Whilst the absolute difference in the median does not appear sizeable at first glance, it is noteworthy that minimum blood eosinophil count eligibility criteria in the UK for biologics such as mepolizumab is 0.3 $x10^9$/L. Spearman's rank analysis did not meet significance, however previous studies [332] have shown negative correlation between rising BMI and eosinophil count. Previous murine models have shown that obesity increases bone marrow production of mature eosinophils and enhances transit into the lungs, though with reduced movement into the bronchial lumen itself [341]. Whether the reduced serum eosinophil counts seen in obesity reflect rapid transit from the bone marrow to the lungs remains unclear but is possible.

Obesity, therefore, may affect eligibility for advanced therapies that target T2-high disease. It may be that this is due to a masking effect and the underlying disease process remains responsive to T2-high treatments, in which case biomarker thresholds may need to be adjusted in this group, or there may be inherent obesity-mediated inflammatory changes that result in a "true" T2-low endotype.

The obesity-associated asthma phenotype is felt to reflect more T2-low disease. The prevalence of T2-low asthma worldwide remains to be elucidated and continues to be studied [318]. We demonstrated that obesity affects FeNO interpretation putting accurate endotyping in jeopardy for this population. This may explain, in part, the difficulty in accurately characterising T2-low disease.

5.5.2 Limitations

There are a number of possible limitations to acknowledge. Firstly, this was a single centre *post hoc* observational analysis not powered to assess correlation or associations between each T2-biomarker and BMI. Therefore, a dedicated prospective study is needed to confirm our findings. Assuming an alpha of 0.05 and beta 0.2, a power calculation based on our FeNO data indicates that a definitive study would need a sample size of 246 to compare FeNO between groups.

Secondly, sub-groups were not weighted equally, with half the participants having difficult-to-treat asthma and obesity (77/153). The analysis also lacked suitable comparators with no non-asthma participants or healthy-BMI participants with difficult-to-treat asthma.

Thirdly, total IgE was not available from the two trials from which data were extracted and as a result was retrospectively obtained where available from each participant's electronic patient record. There are two key issues with this, the first being lack of data as most participants with mild asthma did not have total IgE measured at any time. Secondly, participants may have had a markedly different BMI at the time at which total IgE was measured, calling into question the validity for this analysis. These factors affected our ability to comment on any possible link between BMI and total IgE.

Fourthly, T2-status was allocated based on a combined biomarker assessment of FeNO and peripheral eosinophils, however, total IgE was not used for

pragmatism despite being a key distal component of the T2-high pathway. It is noteworthy that the median total IgE was high even in the T2-low group (111 kU/L).

Next, the result from the multivariate regression model with FeNO must be interpreted with caution. The univariate analysis was not significant and there is a risk of potential collider bias with a falsely significant result from the multivariate regression. A dedicated prospective study would be needed considering appropriate variables a priori.

Finally, although BMI is a useful marker of obesity, as outlined in Chapter Two, it does not assess other relevant factors such as central adipose accumulation.

Taking these limitations into account, no definitive clinical conclusions can be derived, and these results should be considered exploratory and interpreted with caution. Nonetheless, a notable strength was the multiple linear regression modelling correcting for clinically relevant covariates, in particular, inhaled and oral steroid use.

5.5.3 Conclusion

Results from this analysis demonstrate an inverse relationship between higher BMI and FeNO after adjusting for confounding variables including corticosteroid use, though must be interpreted with caution. It remains unclear if obesity masks underlying T2-high disease or modifies airway inflammation into a T2-low endotype. Further study is needed to confirm our observations and elucidate mechanisms between obesity and T2-inflammation in asthma, especially considering the implications for accurate disease endotyping and tailoring T2-high therapies (including MAb) in patients with obesity-associated asthma. It may be necessary to consider altering T2-biomarker thresholds for eligibility criteria to monoclonal antibody therapy in patients with severe obesity-associated

asthma. Further research assessing monoclonal antibody effectiveness in T2-low asthma with obesity may be justified based on this.

Chapter Six: Comparison of sleep parameters in mild and difficult-to-treat asthma using accelerometery

6.1 Introduction

In the healthy state, sleep needs to be of adequate quality and duration, needs to be timed correctly and result in refreshment for daytime activities [342]. Poor sleep health includes short or excessive sleep duration and fragmentation and leads to daytime somnolence, lethargy, impaired concentration, reduced productivity, and mood disorders [343-345]. Beyond this, there are systemic sequelae of poor sleep health including cardiovascular disease, type 2 diabetes mellitus, hypertension, and increased risk of death [346, 347]. Normal sleep duration for adults (age 26-64 years) is recommended to be 7-9 hours [348-350].

Obesity in asthma patients is associated with both shorter and excessive sleep duration, sleep onset and wake time variability, as well as increased asthma severity in both adult and paediatric populations [351-356]. Likewise, short or excessive sleep duration and poor sleep quality are risk factors not only for obesity [357] but also for onset of asthma [358, 359] and increased asthma exacerbations, asthma-related healthcare burden, poorer quality of life and mortality [360-363]. As specified in Chapter One, over half of patients with severe asthma have obesity. It is unclear if sleep-related disorders in difficult-to-treat or severe asthma are related to obesity, and what the underlying mechanisms are.

Complicating matters, treatments such as corticosteroids and theophylline are known to affect sleep quality. Moreover, nocturnal asthma symptoms are a hallmark of poor asthma control, and this is thought to be due to impaired lung dynamics and circadian disruption of airway inflammatory cells [364].

Obesity and obstructive sleep apnoea syndrome (OSAS) are closely associated and the link between OSAS and poor asthma control and quality of life has been well established [365-367]. In addition [368], other significant obesity-associated comorbidities known to worsen asthma outcomes include gastro-oesophageal reflux disease (GORD) and the metabolic syndrome [369] however, the impact of sleep quality on asthma appears to be independent of GORD and OSAS [361].

As stated in Chapter One, obesity is linked with increased airway inflammation and often a neutrophilic cell profile in the airways of asthma patients, however, there is evidence to suggest this may be due to OSAS [30, 370, 371]. Indeed, patients with OSAS have displayed increased systemic and airway inflammatory markers including exhaled nitric oxide [372, 373].

Actigraphy (including that using wGT3X-BT actigraphy devices) has been validated against polysomnography to measure sleep variables to predict health-related outcome measures in both the general and asthma populations [374-377]. These tri-axial devices measure acceleration in gravitational acceleration (*g*), allowing objective measurements of physical activity, sedentary time and sleep. Sleep measures can be obtained using a validated algorithm specifically designed for wrist-worn accelerometers [378]. This method is validated for use without the need for patient sleep logs [379]. In short, when the device is used on the wrist, arm angle can be calculated every 5 seconds from the *z* axis using the three acceleration sensors (in units of *g*) relative to the horizontal plane. Angle changes can then be compared over subsequent 5 second time periods. Frequency of angle changes below a certain threshold (determined by the algorithm but can be user-defined) indicate sleep; specifically, at initiation devices detect the absence of angle change >5 degrees for 5 minutes as sustained inactivity from which sleep parameters can be derived [378]. Raw accelerometer data is converted via a widely available and validated statistical package, GGIR [273] using R (R Foundation for Statistical Computing, Vienna, Austria) into meaningful variables allowing direct data comparison between accelerometer devices.

The gold standard investigation for sleep breathing disorders is polysomnography however, resources for this are limited and data collection is labour intensive. Accelerometers may offer a more cost-effective alternative. Obesity, sleep and asthma are intimately linked and we took the opportunity to explore the role of accelerometery in asthma patients further by assessing differences in sleep

metrics between mild and difficult-to-treat asthma, with a view to assessing factors affecting difficult-to-treat asthma patients relative to weight

6.2 Hypothesis

Little is known about accelerometer-derived sleep measures in patients with asthma grouped by severity. We hypothesised that sleep parameters vary significantly between mild and difficult-to-treat asthma populations. We further hypothesised that any differences observed may be due to presence of obesity in the difficult-to-treat asthma population.

6.3 Method

6.3.1 Study design and outcomes

We performed an observational, cross-sectional, post-hoc, proof-of-concept analysis of two recent local trials to compare sleep parameters in mild and difficult-to-treat asthma participants utilising accelerometer technology. Specifically, we compared each sleep parameter (sleep time, sleep window, sleep efficiency, sleep onset time and wake time) at baseline between these two groups. Patients with raised BMI were present in both groups, however there were also participants with healthy BMI in the mild asthma group.

6.3.2 Population and recruitment

Baseline data were obtained from 133 participants from two recent trials that were performed between 2017 and 2021 in our research unit. The first was a trial of pulmonary rehabilitation in difficult-to-treat asthma associated with raised BMI (including a sub-study comparing physical activity levels between difficult-to-treat asthma patients with raised BMI and mild asthma patients with both raised and healthy-BMI). The second was the trial of a weight management programme in difficult-to-treat asthma described in detail in Chapter Three of this thesis (trial identifiers: NCT03630432, NCT03858608). Full protocol for the pulmonary rehabilitation trial is described elsewhere [339].

In brief, difficult-to-treat asthma was defined as presence of characteristic symptoms with bronchodilator reversibility on spirometry assessment (12% and 200mls increase in FEV_1 after bronchodilator use, or between visits or after steroid use) or bronchial hyperreactivity identified on bronchial challenge testing; asthma treatment with high-dose ICS or maintenance OCS; and either poor asthma control (Asthma Control Questionnaire (ACQ) score >1.5) or ≥ 2 asthma exacerbations requiring OCS or ≥ 1 asthma exacerbation requiring hospitalisation in the 12 months prior to assessment. Mild asthma patients were recruited from primary care with a diagnosis of asthma and asthma treatment prescribed in the preceding 12 months. Mild disease was defined by maximum preventer treatment of moderate dose ICS/LABA combination, ACQ ≤ 1.5, <2

exacerbations requiring OCS treatment and no hospital admissions with asthma in the preceding 12 months.

6.3.3 Assessments

As described elsewhere, data obtained for all participants included demographics, atopic status, anthropomorphic measures (height, weight, BMI), drug history including maintenance oral corticosteroid (OCS) and biologic use, number of exacerbations requiring OCS, ACQ6, Asthma Quality of Life Score (AQLQ), fractional exhaled nitric oxide (FeNO) and serum eosinophil levels (see Chapter Two for more detail).

During the initial trials undertaken above, participants were asked to wear an ActiGraph wGT3X-BT device (ActiGraph, Pensacola, Florida, USA) on their non-dominant wrist 24 hours a day for 7 days (excluding prolonged water exposure events such as bathing, swimming etc.). The data used in this analysis are baseline data, i.e. not affected by interventions from the original trials. Each device was initialised prior to use with basic patient data including study number, date of birth, height, weight and non-dominant wrist side. Devices were returned to the Clinical Research Facility and data were subsequently downloaded via the ActiLife software (ActiGraph, Pensacola, USA; version 6.14.3) onto a secure device. As specified in Chapter Two, raw data (.gt3x format) was securely transferred to an expert in the use of accelerometers (Dr Duncan S Buchan) at the University of the West of Scotland. Here, data were converted to .csv format and processed using the aforementioned GGIR package (version 2.6.0) in R (version 4.1.2) before secure return to the author for statistical analysis.

Variables generated for this analysis using this method were the number of nights devices were used, mean sleep window time, mean sleep time, sleep efficiency, sleep onset time and wake time. Definitions of these are as follows:

- Sleep window – difference in time from falling asleep to waking up; includes time spent awake overnight
- Sleep time – accumulated sustained inactivity sojourns/bouts overnight
- Sleep efficiency – ratio of sleep time to sleep window

Both sleep onset and wake times were described in hours as decimals after midnight the day before, and then converted to hours and minutes; for example, 25.5 hours equates to 01:30 (24-hour clock time) as 24 hours after midnight is 1am and 0.5 hours converted into minutes is 30 mins.

6.3.4 Statistical analysis

The full dataset was analysed before stratifying by asthma severity for further analysis. Normality was assessed for each variable using visual inspection of histograms, Q-Q plots and Shapiro-Wilk testing. All variables were continuous and described as mean (95%CI) or median (IQR) and compared using independent t-tests or Mann Whitney U tests based on their distribution. All data were analysed using IBM SPSS Statistics (version 28.0) and significance was set at 0.05. All times were displayed as 24-hour clock time or hours and minutes as appropriate.

6.4 Results

133-patient data sets were identified from the initial trials. Nine participants were excluded from this analysis as outliers due to reduced adherence to accelerometer monitoring (defined as fewer than 3 nights use) leaving 124 participants in total; 44 with mild asthma and 80 with difficult-to-treat asthma. Of the difficult-to-treat asthma group (n = 80), 63 (78.8%) were obese and 17 (21.3%) overweight (BMI 25.0 - 29.9 kg/m^2). In the mild asthma group (n = 44), 20 (45.5%) had healthy-range BMI (18.0 - 24.9 kg/m^2), 14 (31.8%) were overweight, and 10 (22.7%) obese.

6.4.1 Baseline characteristics

Table 6.1 displays baseline characteristics overall and by asthma severity. Median age was 57 (47, 64) with no difference between asthma severity groups. 56% were female and 94% were non-smokers (56% lifelong non-smokers, 38% ex-smokers), with no between group differences observed. There were significant differences between mild and difficult-to-treat asthma groups in proportion of atopy (14% vs 61% respectively; p <0.001), weight (75kg vs 92 kg respectively; p<0.001), BMI (26 kg/m^2 vs 34 kg/m^2 respectively; p<0.001), number of prednisolone boosts in twelve months (0 vs 4 respectively; p<0.001), ACQ6 (0.4 vs 2.7 respectively; p<0.001), and AQLQ (6.4 vs 4.0 respectively; p<0.001), some of these differences relating to the criteria defining each group. Both FeNO and eosinophil levels were higher in the difficult-to-treat group (33ppb vs 21ppb, p = 0.023 and 0.3x10^9/L vs 0.1x10^9/L, p = 0.017 respectively) than the mild group.

Table 6. 1 - Baseline characteristics

Variable	Overall n = 124	Mild asthma n = 44	Difficult-to-treat n = 80	p-value
Age, years	57 (47, 64)	60 (48, 72)	56 (48, 65)	0.843
Female sex	69 (55.6)	25 (56.8)	44 (55.0)	0.845
Smoking status: Never smoker Ex-smoker Current smoker	 69 (55.6) 47 (37.9) 8 (6.5)	 30 (68.2) 12 (27.3) 2 (4.5)	 39 (48.8) 35 (43.8) 6 (7.5)	 0.114
Atopy	55 (44.4)	6 (13.6)	49 (61.3)	**<0.001**
Weight, kg	84.6 (73.0, 99.5)	75.3 (65.7, 84.9)	92.3 (76.8, 107.8)	**<0.001**
BMI, kg/m^2	31.0 (26.5, 36.4)	25.7 (21.9, 29.6)	33.6 (28.8, 38.5)	**<0.001**
Maintenance prednisolone	28 (22.6)	n/a	28 (35.0)	n/a
Biologic	13 (10.5)	n/a	13 (16.3)	n/a
Prednisolone boosts	2 (0, 4)	0 (0, 0)	4 (3, 6)	**<0.001**
ACQ6	1.7 (0.5, 3.0)	0.4 (0.0, 0.8)	2.7 (1.9, 3.6)	**<0.001**
AQLQ overall	4.6 (3.8, 6.2)	6.4 (5.9, 6.9)	4.0 (3.3, 4.8)	**<0.001**
FeNO, ppb	23 (16, 45)	21 (16, 26)	33 (12, 54)	**0.023**
Eosinophils, $x10^9/L$	0.2 (0.1, 0.4)	0.1 (0.0, 0.2)	0.3 (0.2, 0.5)	**0.017**

Continuous variables described as median (interquartile range).
Categorical variables described as n (%).
p-value compares mild vs difficult-to-treat groups with Mann Whitney U for continuous and chi square or Fisher's exact for categorical variables.
Abbreviations: ACQ6 (Asthma Control Questionnaire), AQLQ (Asthma Quality of Life Questionnaire), FeNO (fractional exhaled nitric oxide), ppb (parts per billion).

6.4.2 Sleep parameters

Table 6.2 summarises sleep metrics overall and in mild and difficult-to-treat asthma groups. The median number of nights accelerometery was recorded was 6 (6, 6). In the overall dataset, the median sleep onset time was 00:08 (23:02, 01:23) and the median wake time was 07:54 (06:48, 09:22), whilst the median sleep window time was 7hrs 49mins (6hrs 29mins, 8hrs 56 mins), median sleep time was 6hrs 35mins (5hrs 2mins, 7hrs 45mins) and the median sleep efficiency was 85% (81, 90).

Table 6. 2 - Accelerometer-derived sleep parameters

Variable	Overall n=124	Mild asthma n=44	Difficult-to-treat asthma n=80	p value*
No. of nights used	6 (6, 6)	6 (5, 6)	6 (6, 6)	0.333
Sleep time	6:35 (5:02, 7:45)	6:50 (6:05, 7:45)	6:26 (4:56, 7:44)	0.353
Sleep window	7:49 (6:29, 8:56)	8:03 (7:02, 8:50)	7:38 (6:08, 8:59)	0.339
Sleep efficiency (%)	85.4 (81.0, 90.2)	86.3 (82.1, 90.5)	85.4 (80.2, 90.0)	0.471
Sleep onset	00:08 (23:02, 01:23)	23:41 (22:52, 00:45)	00:24 (23:16, 02:02)	**0.019**
Wake time	07:54 (06:48, 09:22)	07:41 (06:43, 08:13)	08:03 (06:48, 10:01)	0.097

Variables described as median (IQR).
Sleep time, sleep window, sleep onset and wake time displayed as hours:mins.
*Mann Whitney U test comparing mild vs difficult-to-control asthma groups

Comparing mild to difficult-to-treat asthma, no differences were observed in sleep time, sleep window, sleep efficiency or wake time. Sleep onset time was significantly later in the difficult-to-treat asthma group compared to mild asthma (00:24 vs 23:41 respectively, $p = 0.019$).

In the overall dataset (I.e., mild and difficult-to-treat groups together), Spearman's rank showed no correlation between sleep-onset time and ACQ (marker of asthma control); $rho = 0.049$, $p = 0.589$. Additionally, both unadjusted and adjusted (correcting for weight) linear regression using sleep-onset time as the dependent variable and ACQ as the independent variable showed no relationship between asthma control and sleep-onset time: unadjusted $F(1,122)=0.28$, $p = 0.866$; adjusted for weight $F(2,121)=0.160$, $p = 0.852$.

6.5 Discussion

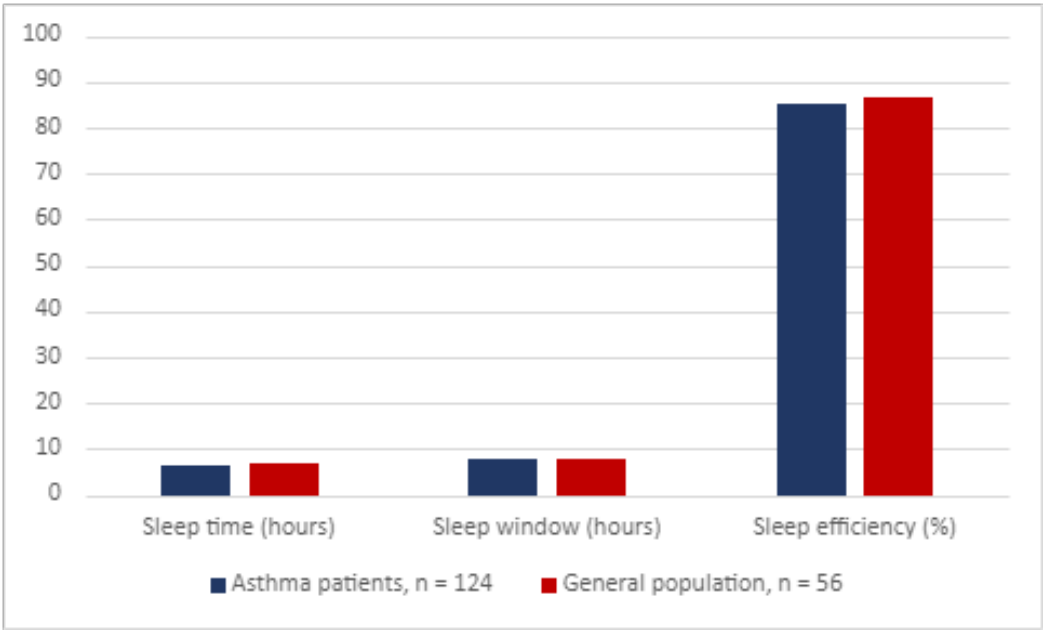
6.5.1 Accelerometer-derived sleep metrics and asthma

We observed no differences in sleep duration or sleep efficiency between mild and difficult-to-treat asthma groups, but whilst there was no difference in wake time, there was a later time of sleep-onset in the difficult-to-treat group. It is plausible that this finding is as a result of difficulty in initiating sleep in the difficult-to-treat asthma group. This is perhaps not surprising given the diurnal variation of asthma with increased symptoms nocturnally possibly resulting in poor sleep initiation. However, we identified no correlation between asthma control (ACQ) and sleep onset time suggesting this delay is not related to uncontrolled symptoms. Interestingly correlation and regression analysis suggest this difference is not related to asthma control even when adjusted for weight, a key factor in sleep health. Moreover, sleep duration was no different between mild and difficult-to-treat groups, again suggesting no impact on sleep with uncontrolled symptoms. Patients from our cohort had a lower sleep duration than the recommended amount (6.59hrs; 5.04, 7.75) suggesting poorer sleep health despite good sleep efficiency. Factors associated with delayed sleep initiation and reduced sleep duration in difficult-to-treat asthma remain to be elucidated and require further study. No further analysis assessing possible factors in difficult-to-treat asthma patients relative to weight were undertaken as a signal was not found between mild and difficult-to-treat groups.

We observed an excellent adherence rate (93%) to accelerometer use overnight suggesting this is a tolerable method of sleep metric data collection. Given the ease to set-up, the lack of manpower needed or requirement for polysomnography services, accelerometery may be a viable option to assess sleep variables though further study is needed to confirm these findings.

When compared to accelerometer-derived outcomes from previous studies, our data is similar to both general adult and asthma populations. A study in 56 young healthy adults (mean age 24.5 ± 4.5 years) using similar accelerometer devices showed similar results with mean (SD) sleep time (6hrs 56mins ± 49mins), sleep window (7hrs 59mins ± 51mins) and sleep efficiency (87% ± 4), sleep-onset (00:05 ± 90mins) and wake times (08:20 ± 84 mins), as shown in Figure 6.1 [380].

Figure 6. 1 – Comparison of accelerometer-derived sleep metrics in our cohort of patients with asthma and a previous study of a general population cohort



In contrast, a study reported by Castner et al [381] revealed conflicting results when comparing sleep metrics using fitness trackers against accelerometers in 47 women with uncontrolled asthma. They observed a mean sleep time of 7hrs 52mins ± 106mins, substantially longer in our study, although sleep efficiency was similar (88%). Sleep window time was not reported, though simple calculation from the given sleep time and efficiency would suggest a higher sleep window of around 8hrs 58mins compared to our observed 7hrs 49mins. Conversely, a small study using actigraphy in 10 patients with mild-to-moderate asthma [375] reported a shorter mean sleep time of 5hrs 54mins ± 74mins but observed a similar mean sleep window time of 7hrs 34mins ± 40mins. Again, whilst sleep efficiency wasn't reported, the calculated sleep efficiency was 78%

which appears markedly lower than our data. However, this study was limited by the small sample size.

6.5.2 Limitations

Our retrospective analysis has potential limitations. Firstly, groups were not equally weighted with more patients with difficult-to-treat asthma than mild asthma. Secondly, the initial trials data did not include objective assessments of daytime or nocturnal sleep (e.g., Epworth sleep score, Pittsburgh sleep quality index), nor any sleep logs. Thirdly, this analysis was not powered to assess sleep outcomes. Finally, this analysis did not account for factors such as sleep-disordered breathing that may influence outcomes, which should be addressed in future studies. Despite this, key strengths of our study are the sample size, higher than in previous studies, and observed excellent tolerance of accelerometer use (93%). To our knowledge this is the first comparison of mild and difficult-to-treat asthma sleep outcomes using accelerometery and we highlight a difference in sleep initiation between groups unrelated to asthma control and weight. Further study is warranted to explore the relationship between asthma severity and sleep-metrics and whether interventions targeting sleep health can improve asthma outcomes. Assuming an alpha of 0.05, beta 0.2 and power 0.8, and based on our data of sleep onset time, a sample size of 166 (n = 83 in each group) is needed for a definitive study.

6.5.3 Conclusion

Overall, our patient cohort with asthma had comparable results to previous studies of the general population for accelerometer-derived sleep outcomes. Generally, there were no differences in sleep parameters between participants with mild and difficult-to-treat asthma, though there appeared to be a later onset of sleep in difficult-to-treat asthma, the clinical consequence of which is unclear. This difference may be independent of asthma control, suggesting other factors are involved. Accelerometery appears to be a cost-effective pragmatic alternative to polysomnography. Future study is needed in the field of accelerometer-derived sleep parameters and asthma, in particular to assess the impact of improving sleep quality on asthma control and quality of life.

Chapter Seven: Discussion

7.1 Principal findings

This series of studies highlights the complexity of the combination of obesity and difficult-to-treat asthma, whilst providing a potential management option in this cohort. The outcome from this body of work is insufficient to impact clinical practice alone but provides a stepping stone upon which future work can potentially progress to real-world changes.

We have demonstrated efficacy of a dietitian-supported total diet replacement weight management programme in participants living with this combination, particularly on asthma control and quality of life, as well as breathlessness, over four months. Beyond this, we observed sustained weight loss and possible benefits in asthma-related quality of life and reduction in the number of exacerbations requiring oral corticosteroids with the same intervention after one year compared to standard care. Moreover, whilst the benefits reported after four months in asthma control were not replicated at the one-year mark, the participants that experienced improvement in asthma control at four months sustained this at one-year. Additionally, seventy percent of participants in the intervention arm lost greater than ten percent of their total body weight, and these participants experienced significant improvement in asthma control compared to standard care. This is the first randomised controlled study of weight management, to our knowledge, to assess asthma-related control and quality of life at one-year and provides vital information regarding feasibility as well as strong signals supporting its use in the longer term. Previous studies of weight management in this cohort have reported short term outcomes only, and none have reported weight-loss to this extent. Post-hoc analysis comparing outcomes relative to type-2 inflammatory status suggested improved asthma control, quality of life and reduction in asthma exacerbations in the T2-high group with no changes observed in the T2-low group. This is a potentially novel finding and suggests interplay between adipokine-mediated and T2-inflammation, however caution must be used with interpretation due to low sample size. Additionally, we reported the effects of obesity on type 2 inflammatory biomarkers in patients with difficult-to-treat asthma to provide further insight in this area. In this, participants were grouped into tertiles based on their BMI. FeNO levels and the proportion of participants with a T2-high

status were lower in the highest BMI tertile compared to the lowest even in the difficult-to-treat asthma cohort, in which there was no difference between tertiles in corticosteroid dose. Linear modelling showed that FeNO levels decreased with increasing BMI when adjusted for age, sex, atopy, smoking status, rhinitis, inhaled and oral corticosteroid dose. These findings suggest that obesity decreases T2-biomarkers, in particular FeNO, and that these effects may not be entirely related to steroid use, as previously thought. However, the possibility of collider bias and the retrospective nature of this study limit any clinically important conclusions. The effect of weight loss on markers of T2-biomarkers could not be elucidated due to missing data, but this should be a focus to address in future research.

Laterally, we assessed accelerometer-derived sleep metrics in participants with asthma, nominally to ascertain differences between participants with obesity and varying severity of asthma. However, despite a significant difference in weight and asthma control between the mild and difficult-to-treat asthma groups, sleep outcomes were similar for the most part. Sleep-onset time was later in the difficult-to-treat asthma group compared to the mild asthma group though there was only a forty-minute difference. The significance of this clinically is therefore uncertain. Overall, these results were surprising given the known effects of both obesity and poorly controlled asthma on sleep. Indeed, the results appear comparable to previous studies of the general population. We observed excellent adherence to the accelerometers, and this may be important given the pragmatic and logistic benefits of accelerometery over polysomnography. It is feasible that accelerometery may be useful as a screening tool to reduce burden on sleep services. Once again, the effects of weight loss on accelerometer-derived sleep parameters in people with difficult-to-treat asthma and obesity were not assessed due to missing data and, moreover, as there were broadly no clinically relevant differences at baseline between mild and difficult-to-treat groups, may not be justified going forwards.

Overall, this series of studies provides further evidence for weight management in difficult-to-treat asthma and obesity, highlights possible effects of obesity on

exhaled nitric oxide interpretation and describes similar sleep metrics in patients with obesity and asthma groups of varying severity.

7.2 Strengths and limitations

7.2.1 Strengths

7.2.1.1 Weight management trial

Studies in asthma are often fraught with heterogeneity in aims, methods and sub-populations resulting in conclusions that can be inappropriate in a real-life setting. This is largely due to the complexity of asthma as a whole, with multiple phenotypes and endotypes, overlap with other airways disease and differing pathological changes to the airways depending on disease severity. Most of the economic burden of asthma is attributed to the difficult-to-treat and severe asthma populations and, by and large, this should often be the target population. A common error is to study an intervention in a general asthma population often neglecting a key sub-population and deriving unclear conclusions. A prime example of this is early studies of the anti-IL5 monoclonal antibody, mepolizumab, now considered a bastion of biologic efficacy, which initially showed insignificant outcomes [382] due to inappropriate population selection. A key strength of the total diet replacement weight management programme trial reported in this thesis, is the selection of a relevant population, one of individuals with difficult-to-treat asthma associated with obesity. This is evidenced clearly by the poor baseline ACQ6 and AQLQ scores, and number of exacerbations in the preceding year. This population has been highlighted, time and again, as struggling with poor asthma-related quality of life, uncontrolled disease, impairment in activity and workplace productivity and significant health and economic burden. Focussing our intervention on this population is therefore justified. As highlighted above, studies on longer-term outcomes of weight management in asthma are sparse and our one-year outcomes provide welcome evidence in this vacant area.

The primary outcome for many asthma studies is FEV_1, a key predictor of asthma control and risk of exacerbation. However, patient-centred outcomes such as asthma control and quality of life are arguably of greater relevance to both patients and healthcare providers, and therefore another key strength is our choice in primary outcome and key secondary outcomes. Mechanistically, weight reduction is likely to improve dynamic lung volumes, but the clinical relevance

of this, in the context of airway disease, would be unclear. Directing efforts to optimising asthma control and quality of life, whilst still assessing secondary outcomes such as spirometry, is also therefore justified.

A further, though not pre-specified, benefit of this intervention is the potential for impact on overall health. Since the obesity epidemic was popularised a quarter of a century ago, the effects on overall health and wellbeing have been well described, prominently for cardiovascular health, diabetes and endocrine disease. The pulmonary world is playing catch-up, with effects beyond thoracic wall dynamics only recently being highlighted. Whilst we have not described effects of CWP on non-respiratory systems beyond anthropometric measures, weight loss would certainly be beneficial to overall health and thus likely to be even more appealing as a treatment option, if financially justified. A key strength from this work is the sustained weight loss observed after one year. This is not insubstantial and is encouraging, highlighting the potential impact CWP may have in the real-world.

This is not the first study to use CWP, as specified in this thesis, with success previously reported in the field of diabetes mellitus. A strength highlighted from these other publications, which remains relevant, is of the ease of delivery of the intervention and its favourable safety profile allowing potential for delivery in a primary care setting. This is not an intervention requiring tertiary specialist support, nor even physician or GP support. It can be delivered by trained personnel, in this case dietitians, potentially increasing intervention availability to the general population. Furthermore, as a non-pharmacological non-surgical intervention, it is more appealing to both patients and healthcare professionals.

7.2.1.2 Other studies

Our retrospective analysis assessing the effects of obesity on T2-biomarkers was timely. A recent analysis [318] of 2225 patients attending severe asthma clinics across the UK helped to advance our understanding of T2-high/T2-low population profiles. However, we noted a disparity in BMI between the two groups resulting

in stimulating correspondence to the authors highlighting the potential effects of obesity on T2-biomarker levels. The authors suggested the differences observed were likely due to increased possibly inappropriate corticosteroid doses in the T2-low group resulting in weight gain. This valid argument provided the impetus for our study with the aim of assessing the relationship between BMI and T2-biomarkers (FeNO, eosinophil count and total IgE), adjusting for both inhaled and oral corticosteroid dose. By doing so we were able to provide a further signal that obesity may affect T2-biomarkers independently of corticosteroids. The clinical implications of this may be hugely significant, impacting our ability to deliver precision medicine to this cohort

The analysis of sleep parameters using accelerometery in asthma (Chapter Six) is the largest of its kind. Beyond sample size, another key strength is the high adherence seen using these devices highlighting their potential for further research. Finally, this was the first study to compare sleep metrics using accelerometers in both mild and difficult-to-treat asthma.

7.2.2 Limitations

7.2.2.1 Weight management trial

Conducting research over the course of the COVID-19 pandemic posed a number of challenges. The main limitation for our randomised controlled study of CWP was data loss. Recruitment for this trial began in August 2019. The planned two-year recruitment period was met, however due to the nature of national lockdowns and strict necessary restrictions subjected to patients with lung disease, flexibility and initiative was necessary to ensure the trial could continue in a way that maximised data collection. We elected to replace face-to-face study visits with virtual visits, thus allowing us to collect data for our primary and key secondary outcomes. As these were questionnaires, no compromises were needed to obtain this data for the primary outcome. Despite this change, data requiring physical attendance were not able to be obtained during times of stricter lockdowns. These included spirometry, FeNO, blood sampling and 6-minute walk tests among others. As a result, we cannot confidently derive any conclusions from our study as to the effect of weight loss on spirometry,

exercise tolerance or airway inflammation. Ethically, this was the correct decision. The risks of COVID-19 in the days before vaccination were unclear in patients with asthma and a cautious approach to research was appropriate to ensure patient safety as the priority. Even after lockdowns were eased, a great deal of uncertainty remained in this cohort of patients and avoiding hospital or research facility attendance was still advisable. The missing data, therefore, continued for further follow-up visits on several occasions. Active participant recruitment was halted altogether during lockdowns, so no baseline visits took place. Fortunately, we maintained our target recruitment over the two-year period by keeping a list of potential participants and recruiting enthusiastically once restrictions eased.

In Chapter Four, we describe within group improvements in asthma outcomes with CWP but not between groups. At one year this study was underpowered limiting our ability to conclusively compare CWP with UC in this regard. Beyond this, we observed a significant improvement in frequency of exacerbations as evidenced by the reduction in number of courses of prednisolone in the CWP group from baseline to one year and compared to the UC group. This is a potentially clinically important finding, but caution must be taken as, firstly, number of prednisolone courses required was based on participant recall (in part, supplemented by the electronic patient record where available) and secondly, follow-up numbers were not absolute values, but annualised values calculated to allow comparison to baseline data. Nonetheless, a signal is there, and a larger dedicated trial is warranted to confirm this finding.

Finally, the one-year results reported signify the end of the CWP for each participant in the intervention arm. The bulk of the weight loss was undoubtedly observed at four months, however dietitian support continued with rescue packages if weight gain was noted. Whether benefits of weight loss (and asthma-related outcomes) persist beyond dietitian-input over a participant-managed period, remains to be seen. A two-year analysis is planned which may provide further insight.

7.2.2.2 Other studies

The analyses described in Chapters Five and Six are retrospective studies not powered to detect changes in their respective dependent variables. The groups compared in both (mild and difficult-to-treat asthma) are not equally weighted, and no control group data were available. For these reasons, conclusions drawn from both must be interpreted with caution and prospective dedicated trials are needed to confirm findings. Additionally, the analysis of sleep metrics does not incorporate comparison of accelerometer-derived measures against polysomnography, the gold standard in sleep medicine investigation. Whilst previous studies have found accelerometer-derived measures comparable to polysomnography, this is the first assessment comparing mild to difficult-to-treat asthma and it is feasible that clinically relevant differences may be found in this cohort.

7.3 Conclusions and future directions

Obesity-associated asthma is complex and remains poorly understood. Obesity is an often-ignored extra-pulmonary treatable trait. More recently efforts have been made to elucidate potential mechanisms between the two. Previous trials of weight loss have yielded mixed results. Yet, we demonstrate an effective weight management programme with clinically important improvements in asthma-related outcomes both in the short and longer term. This is a safe, non-pharmacological option that may reduce treatment burden and improve global health. This trial should be regarded as exploratory, and caution should be taken with interpretation of post-hoc outcomes. A larger sample trial is warranted, one that has the potential to increase our understanding of the effects of weight loss in difficult-to-treat asthma and obesity and that can assess cost-effectiveness also. Areas of focus should include assessing effects on lung function, airway inflammation and exercise tolerance as was initially planned in this trial. With a larger sample size, it may be possible to comment on which specific aspects of the ACQ6 and AQLQ improve further pinpointing the advantage. It would also be useful to further compare the response in T2-high versus T2-low endotypes. Our trial suggests greater improvement in the former, but a larger sample trial may be able to expand on this and comment on possible mechanisms, as well as provide more information as to the effects of obesity on T2-biomarkers. Feasibility studies in the overweight population (I.e., BMI 25-30 kg/m^2), populations of differing ethnicity and comparing sexes and hormonal differences should be considered. The latter has been touched upon in Chapter One, with a defined cohort of post-menopausal women with difficult-to-treat or severe asthma identified. People from non-Caucasian ethnicities are likely to respond differently to CWP due to factors such as different visceral adipose fat deposition (e.g., South Asian populations with central obesity). Finally, an assessment of adipokine levels, alongside markers of airway inflammation in participants undertaking weight management, may provide possible biomarkers and predictors of response. Consideration should also be given to pharmacological treatments associated with weight loss in future proof-of-concept trials with both metformin and GLP-1 agonists showing potential.

In summary, in people living with difficult-to-treat asthma and obesity, use of a total diet replacement weight management programme, Counterweight-Plus, is feasible to improve asthma-related control and quality of life over one-year compared to usual care. Further studies are now justified to continue our exploration into the best management of this under-served phenotype.

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