University of Southampton

Faculty of Medicine

Exercise as an Immunomodulatory Mechanism in Asthma

by

DR ANNA TERESA FREEMAN MBBS BSc (Hons) MRCP

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Abstract

Faculty of Medicine
Doctor of Philosophy

FINAL THESIS

EXERCISE AS AN IMMUNOMODULATORY MECHANISM IN ASTHMA

by
Dr Anna Teresa Freeman

The prevalence of asthma remains high worldwide, and despite the development of biological treatments, many patients remain sub optimally controlled, and mortality rates have been static for decades. Exercise immunology is an expanding field, and there is growing evidence that exercise can modulate the inflammatory and immune processes in asthma. Changes in redox status have been linked with asthma severity, and modulation of redox balance via upregulation of the master antioxidant NRF2 has been proposed as the mechanism through which exercise exerts its disease modifying properties. Despite this, and national and international guidance to increase exercise, patients with asthma are less likely to engage in physical activity than non asthmatics. The aim of this thesis was to investigate the effect of an interval exercise training programme on symptom control and inflammation in patients with suboptimally controlled asthma, and the role of NRF2 driven increases in redox buffering capacity in mediating this response. A 12-week exercise intervention resulted in significant improvements in symptom and quality of life scores, in addition to improvements in asthma related inflammatory markers and lung function. In terms of mechanistic investigation, downstream markers of redox regulation increased, with demonstration of association between improvements in fitness, increases in antioxidant capacity and inflammation, through to overall improvements in lung function and asthma symptoms.

Given the early exploratory data to support my hypothesis, alongside challenging recruitment and high participant drop out rate, identification of the barriers to exercise for patients with asthma was important. Therefore, I assessed perceived barriers to exercise in the WATCH Cohort of Difficult Asthma. The perceived burden of exercise therapy in patients with difficult asthma was demonstrated to be at a level comparable to those with cardiovascular disease, and higher than in patients with cancer. A high perceived burden of exercise therapy was significantly associated with increased asthma symptoms, anxiety and depression, reduced quality of life and increased number of rescue oral steroid courses whilst more biological markers of disease such as lung function, blood eosinophil count, FeNO and hospitalisations in the previous year were not. This analysis described herein suggests exercise intervention in symptomatic asthma is tolerated and beneficial for physical fitness, and symptom control, with associated improvement in inflammatory parameters and lung function. The mechanism of these improvements may be via improved redox buffering capacity, resulting in increased tolerance to disease related stressors. Demonstration that perceived barriers to exercise are associated with higher symptom scores and psychological burden, but not biological

markers of disease severity, suggest that an exercise intervention along with psychological input may help facilitate increased uptake of a disease modifying lifestyle change. Further work is required to validate these exploratory results in a fully powered study, which will require adaptation to accommodate COVID safe protocols.

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Research Thesis: Declaration of Authorship

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Definitions and Abbreviations

AAM	Alternatively activated macrophage
ACCP	American College of Chest Physicians
ACQ	Asthma Control Questionnaire
ACSM	American College of Sports Medicine
APC	Antigen presenting cell
AQLQ	Asthma Quality of Life Questionnaire
ARE	Antioxidant response element
AT	Anaerobic threshold
ATS	American Thoracic Society
BAL	Bronchoalveolar lavage
BALF	Bronchoalveolar lavage fluid
BALT	Bronchial associated lymphoid tissue
BD	Bronchodilator
BP	Blood pressure
BR	Breathing reserve
BTS	British Thoracic Society
CI	Confidence interval
CCL-5	Chemokine ligand 5
CPET	Cardiopulmonary exercise test

CO	Cardiac output
COPD	Chronic Obstructive Pulmonary Disease
Ct	Cycle threshold
CYS	Cysteine
CysLTR1	Cysteinyl leukotriene receptor
CYSS	Cystine
CysGly	Cysteinylglycine
CysLTs	Cysteinyl leukotrienes
DTNB	Dithionitrobenzoic
DTT	Dithiothreitol
ECG	Electrocardiogram
EOA	Early onset atopic obese asthma
ERS	European Respiratory Society
FeNO	Fractional exhaled nitric oxide
FEF25-75%	Mid expiratory flow rate
FEV1	Forced expiratory volume in 1 second
FOT	Forced oscillation technique
FoxP3	Forkhead box P3
FRC	Functional residual capacity
FVC	Forced vital capacity
GINA	Global Initiative for Asthma

GLI	Global lung function initiative
GluCys	L-Glutamyl-L-Cysteine
GSH	Glutathione
GSSG	Glutathione disulphide
HADS	Hospital Anxiety and Depression Questionnaire
HCYS	Homocystine
HCYSS	Homocysteine
HDM	House Dust Mite
HIIT	High intensity interval training
HNE	Hydroxynonenal
HR	Heart rate
iNOS	Inducible Nitric Oxide Synthase
ICAM-1	Intercellular adhesion molecule 1
ICS	Inhaled corticosteroid
IGF	Insulin like growth factor
IL	Interleukin
Keap1	Kelch-like ECH-associated protein 1
LABA	Long acting bronchodilator
LAMA	Long acting antimuscarinic
LONA	Late onset non atopic obese asthma
LTRA	Leukotriene receptor antagonist

LPS	Lipopolysaccharide
MBNW	Multibreath nitrogen washout
MBP	Major basic protein
MCID	Minimal clinically important difference
MDA	Malondialdehyde
MET	Metabolic equivalent task
MICT	Moderate intensity continuous training
miR	Micro RNA
NAC	N-acetylcysteine
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NIHR	National Institute for Health Research
NK	Natural killer
NO^{2-}	Nitrite
NO^{3-}	Nitrate
NRF2	Nuclear factor erythroid 2-related factor 2
OVA	Ovalbumin
PAMP	Pathogen associated molecular pattern
PBMC	Peripheral blood mononuclear cell
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
$P_{ET}CO_2$	Partial pressure of end tidal CO2

PGD	Prostaglandin
PR	Pulmonary rehabilitation
PRR	Pathogen recognition receptor
RER	Respiratory exchange ratio
RPE	Rating of perceived exertion
RPM	Revolutions per minute
RM	Repetition maximum
RNNO	N-nitroso species
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
RSNO	S-nitroso species
RSS	Reactive sulphur species
RXNO	Total nitroso species
SGRQ	St George's Respiratory Questionnaire
SIGN	Scottish Intercollegiate Guidelines Network
SIT	Sprint interval training
SPT	Skin prick testing
SOP	Standard operating procedure
SRETP	Structured responsive exercise training programme
TGF ß	Transforming growth factor beta
TLR	Toll like receptor

TNB	2-nitro-5-thiobenzoic acid
TNF α	Tumour necrosis factor alpha
TREG	Regulatory T cell
TSLP	Thymic stromal lymphopoietin
UHS	University Hospital Southampton
UHSFT	University Hospitals Southampton NHS Foundation Trust
UoS	University of Southampton
URTI	Upper respiratory tract infection
VCAM-1	Vascular cellular adhesion molecule 1
$\dot{V}_E$	Ventilatory equivalent
VEGF	Vascular endothelial growth factor
$\dot{V}O_2$	Oxygen uptake
$\dot{V}CO_2$	Carbon dioxide output
WATCH	Wessex Asthma Cohort of Difficult Asthma

Chapter 1 Introduction

1.1 Background

The burden of respiratory disease is increasing worldwide (1). Asthma is the commonest chronic disease in children (1) and the cost of respiratory disease is high and rising (2). New approaches are needed to treat chronic respiratory conditions that are scalable at minimal cost, and thus able to offer benefit to a range of conditions and patient groups. Exercise, as pulmonary rehabilitation (PR), has an established role in chronic obstructive pulmonary disease (COPD)(3), with benefit also demonstrated in asthma patients (4). Emerging data suggest that exercise as a therapeutic intervention may modulate the immune and inflammatory basis for asthma and other respiratory diseases, and offer clinical benefit beyond improvement in daily symptom burden and quality of life (5) (6).

1.2 The lungs in health

The lungs are responsible for gas exchange, facilitating diffusion of oxgen into and removal of waste carbon dioxide from the blood. They also function as a filter to constant insults from exernal stimuli, in the form of inhaled pollutants, pathogens and allergens. As such, the lungs have developed a advanced defense mechanism involving both innate and adaptive immunity, as depicted in figure 1.1 . Dendritic cells and macrophages are the first responders to an external challenge. Dendritic cells are generated in the bone marrow and are ubiquitous in their distribution, whilst macrophages are found throughout the lung. Together, they identify and respond to acute and chronic inflammation. Macrophages are the main source of cytokines and chemokines, which in turn propagate the immune and inflammatory response to external stimuli. Macrophages have been grouped into M1 and M2 macrophages depending on their role, activation pathways, receptors and signalling pathways (7). M1, classically activated macrophages exhibit an inflammatory phenotype and function, with M2, alternatively activated macrophages demonstrating anti-inflammatory functions, thereby decreasing inflammation and encouraging tissue repair.

Any external insult will be indentified by a pathogen recognition receptor (PRR) on a antigen presenting cell (APC) or dendritic cell, by its pathogen associated molecular

pattern (PAMP). The APC then presents to naïve T cells in lymph tissue, either in lymph nodes (LN) or bronchial associated lymph tissue (BALT). T lymphocytes are subdivided into $CD4^+$, $CD8^+$ and natural killer T (NKT)cells. It is to the $CD4^+$ helper T cells that APC present their PAMPs, with $CD4^+$ T cells further dividing into Th1 and Th2 cells. Th1 and Th2 cells are responsible for responses to intracellular and extracellar pathogens respectively, and trigger differing cytokine responses(8). Cytokines are small polypeptides that regulate immunity and inflammation via autocrine, paracrine or endocrine functions (9). Th1 responses are pro-inflammatory in nature to counteract viruses and malignant cells, and are characterised by the cytokines interferon gamma (IFN), tumour necrosis factor (TNF), interleukins (IL) 1 and 12. The Th2 responses are directed at extracellular organisms, with their cytokines driving humoral immune responses to upregulate antibody production, and are characterised by expression of the cytokines IL 4, 5, 9 and 13 (8). A well regulated immune system displays balance between the two responses, and it is thought that an imbalance between the Th1 and Th2 responses drive a number of inflammatory lung conditions including asthma (10).

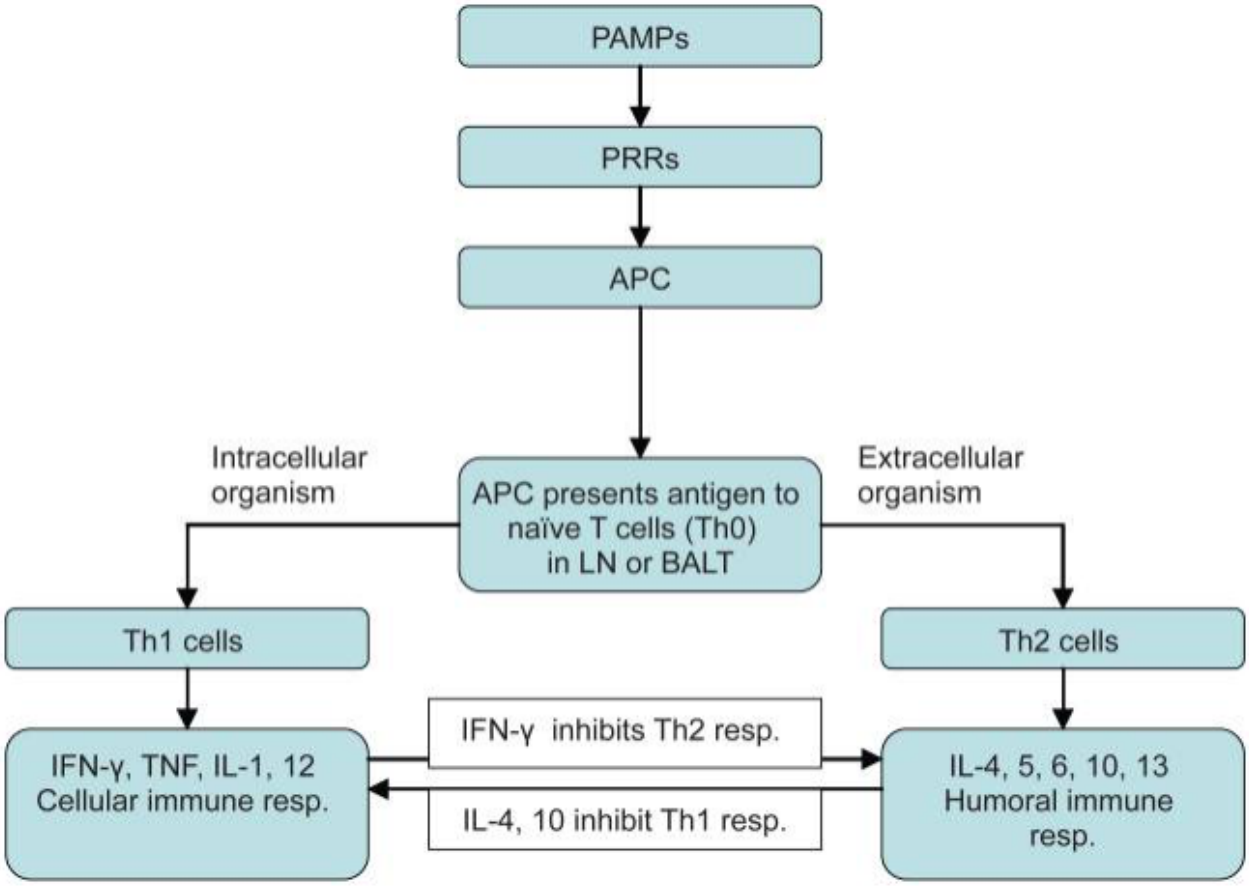


Figure 1.1 Innate and adaptive immune responses in the lung

Innate and adaptive immune responses in the lung on exposure to external inflammatory stimuli, copied from (8). Abbreviations APC; antigen presenting cell, BALT; bronchial associated lymphoid tissue, IFN; interferon, IL; interleukin, LN: lymph node, PAMPs; Pathogen association molecular patterns, PRR; pathogen recognition receptor, resp; responses TNF; tumour necrosis factor

1.3 Asthma

The Global Initiative for Asthma (GINA) defines asthma as 'a heterogenous disease', with symptoms of 'wheeze, shortness of breath, chest tightness and cough that vary over time and in intensity, together with variable expiratory flow limitation' (11), that is 'usually associated with airway hyperresponsiveness and airway inflammation'(11). This airways hyperresponsiveness is described as a diagnostic *sine qua non* for asthma (12). The heterogeneity of asthma, both in terms of its symptom severity and disease pathogenesis has led to grouping of patients into pheno and endotypes. Increasingly, description of disease processes are for these groups rather than asthma as a whole, with the relative

balance between airway hyperresponsiveness and inflammation varying between endotypes and individual patients (13, 14), and suggestion that bronchial hyperreactivity may exist in the absence of bronchial mucosal inflammation (12). The pathophysiological findings of smooth muscle hypertrophy and hyperplasia with resultant airway remodelling are evident in asthma regardless of phenotype and across severities (15). Airway remodelling has been shown to develop in the context of bronchial hyperresponsiveness without inflammation (16), and this finding may explain the bronchial hyperreactivity *sine qua non*. There is suggestion that the concept of remodelling phenotypes in addition to clinical and inflammatory phenotypes ought to be recognised in addition to clinical and inflammatory phenotypes in asthma (15). Treatments such as Calcilytics, targeting the Calcium Sensing Receptor, have been suggested to target bronchial hyperresponsiveness more specifically (12). However, pheno and endotyping have so far focussed on clinical phenotypes, which are discussed below.

1.3.1 Clinical phenotypes and endotypes in asthma

Haldar et al (14) first described asthma as a group of clinical phenotypes with mechanism driven endotypes, and identified 4 key clusters in their secondary care cohort; early onset atopic asthma, female predominant obese non-eosinophilic asthma, early onset symptom predominant asthma with minimal eosinophilic disease and male predominant later onset eosinophilic disease fewer symptoms. Most broadly, asthma has been divided into Th2 (T2) high, 'allergic' or 'eosinophilic' asthma and Th2 (T2) low asthma (see figure 1.2).

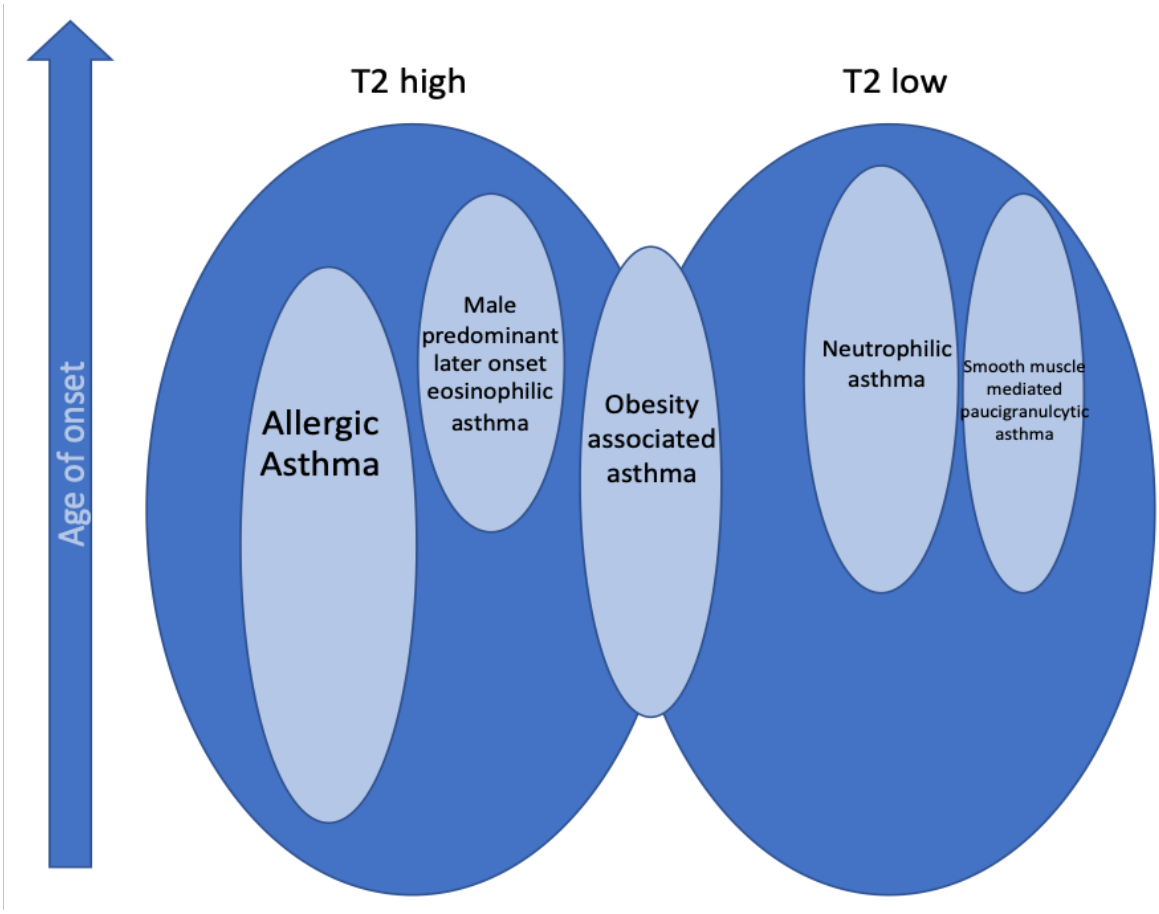


Figure 1.2 T2 and non T2 asthma

T2 and non T2 asthma, adapted from (13), demonstrating the overlap between these two classifications and the phenotypes within. Allergic asthma and eosinophilic asthma sit within T2 asthma, with obese asthma overlapping T1 and T2 disease. Neutrophilic and paucigranulocitic asthma are predominantly T1 phenotype

1.3.1.1 T2 high asthma

T2 high asthma involves chronic inflammation and airway remodelling (17). The majority of childhood onset T2 high asthma is associated with atopy (18). In patients with adult onset T2 high asthma, there is association with atopy in approximately half of patients (18), with illustration of the mechanisms of inflammation demonstrated in figure 1.3. Whilst the relationship between atopy and asthma cannot explain the pathobiological processes involved in the development of asthma, atopy can exacerbate asthma symptoms via IgE mediated inflammation. On initial exposure to specific allergens, commonly house dust mite, grass pollens and animal dander, plasma cells synthesise and release large amounts of allergen specific IgE. These specific IgE molecules attach to mast

cells, which on further exposure to the allergen, degranulate, resulting in an increase of airway and systemic inflammatory cytokines, eosinophils and mast cells, resulting in airway inflammation and bronchoconstriction (17).

As depicted in Figure 1.3, epithelial alarmins are increasingly recognised in the development of T2 high asthma. The airways are constantly exposed to external particulates, microbes and allergens, and as a result, the epithelium has to maintain continuous immune surveillance for these environmental irritants. On exposure to an external threat, the airway epithelium releases cytokines (or epithelial alarmins), including thymic stromal lymphopoietin (TSLP), IL-25 and IL-33. These epithelial alarmins are able to activate both the innate and adaptive immune system through activation of Th2 cells and innate lymphoid cells type 2 (19, 20). The alarmins act on immature dendritic cells to increase expression of OX40 Ligand (OX40L)(12). On maturation, they migrate to draining lymph nodes and activate differentiation of naïve CD4 T cells into Th2 cells (21). These Th2 cells produce type 2 cytokines including IL-4, IL-5 and IL-13, via activation of the GATA3 transcription factor. Nitric oxide (NO) may also be involved in the pathophysiology of asthma. Inducible NO synthase (iNOS) production is stimulated by pro-inflammatory cytokines and oxidants, via activation of the transcription factor NF-κB (discussed in section 1.3.1.5). There has been demonstration of increased iNOS in asthmatic airway epithelium (22), with levels of bronchial and alveolar iNOS increased in uncontrolled asthma vs controlled asthma(23). Increased NO can contribute to increased airway oedema and mucus secretion(24), and may also amplify asthmatic inflammation indirectly through inhibition of Th1 cells, with resultant increases in Th2 cells (25).

M2, alternatively activated macrophages (AAMs) are increasingly identified as having a role in Th2 immunity and are IL-25 and IL-33 responsive(26). There is demonstration of local proliferation under the control of Th2 immune processes in addition to recruitment from the blood (27), with M2 macrophages demonstrated to directly induce airway inflammation(26). It is thought that asthma demonstrates an imbalance between the two macrophage phenotypes, with M2 macrophages found in increased numbers in bronchoalveolar lavage fluid in asthma patients (28).

Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) is a transcription factor that regulates genes responsible for both the innate and adaptive immune responses. NF-κB has long been known to have a role in allergic airways disease (29), with

glucocorticoids mediating some of their effect via suppression of NF-κB induced gene transcription (30) (31). More recently, NF-κB has been indentified as one of the central genes in differentiating house dust mite (HDM) sensitised indiviuals with asthma from those with house dustmite sensitisation but not asthma, with IL-2, IL-4 and TNF α signalling as primary drivers for the development of asthma (32). In this study, peripheral blood mononuclear cells from HDM sensitised individuals with and without a co-existent diagnosis of asthma were cultured in the presence and absence of HDM extract. CD4 T cells were then isolated and gene expression patterns were investigated. Differential network analysis demonstrated a group of asthma associated genes with upstream regulator analysis suggesting that drivers for this module were IL-2, IL-4 and TNF signalling. Further reconstruction identified NF-κB as one of the hub genes involved in driving inflammation (32). NF-κB is also involved in the calcium related increase in airway smooth muscle hyperresponsiveness (33). Intracellular calcium binds calcineurin, a phosphatase which indirectly activates NF-κB (33), with administration of calcilitics shown to prevent increases in intracellular calcium in asthmatic airway smooth muscle (34). In a murine model, calcilytics have also been shown to reduce airway resistance (34), with suggestion that inhaled calcium sensing receptor antagonists may provide an effective treatment to target bronchial smooth muscle hyperresponsiveness (12).

As a result of increased IL-5, probably driven by epithelial alarmins as discussed above, patients with severe asthma are more likely to demonstrate a raised peripheral blood eosinophil count (35). IL-5 promotes eosinophil recruitment and survival, and activates the cysteinyl leukotriene receptor (CysLTR1), contributing to increased airway smooth muscle activity, increased microvascular permeability and increased airway mucus secretions(36).Those patients with elevated peripheral blood eosinophil counts experience more severe exacerbations and demonstrate poorer disease control (37). Eosinophils are terminally differentiated granulocytes that develop in the bone marrow, under the control of IL-5, which mediate inflammatory processes involved in allergy and asthma, and may also have a role in protection against viruses and parasites (13). The role of eosinophils in driving asthma symptoms and exacerbations are multifactorial. Mature eosinophils secrete a large number of mediators, including granule proteins, cytokines, chemokines and lipid mediators (38). Included in this are the granule molecules such as Major Basic Protein (MBP). MBP drives asthma symptoms through directly increasing smooth muscle reactivity (39), and also triggers degranulation of mast cells and basophils

(40). Both cells release histamine, amongst other inflammatory mediators. Histamine in turn increases intracellular calcium, which drives airway hyperresponsivness, remodelling and further increase inflammatory cytokine production (34). Eosinophils have also been shown to increase airway smooth muscle proliferation through direct contact, which triggers their release of cysteinyl leukotrienes (41). Cysteinyl leukotrienes are a lipid inflammatory mediator synthesised from arachdidonic acid, levels of which a number of studies have linked with asthma severity (42). Leukotrienes have a role in the pathophysiology of asthma, acting on airway smooth muscle as a potent bronchoconstrictor in addition to upregulation of cell cycling and proliferation of airway smooth muscle. Cysteinyl leukotrienes also act to increase vascular permeability and mucus secretion (38). Activation of the CysLT (1) receptor contributes to increased airway smooth muscle activity, increased microvascular permeability and increased airway mucus secretions(36). As a result, leukotriene receptor antagonists (LTRA) play a role in the treatment of allergic asthma (36). Eosinophils within the peripheral blood demonstrate diurnal variation, and levels up to 500/mm^3 are normal. Associations between inflammatory and physiologic indices in asthma patients have been demonstrated within sputum cell counts (43). In this study, absolute eosinophil counts showed significant correlation with airways obstruction as assessed through spirometry (specifically forced expiratory volume in 1 second (FEV1)), peak flow (PEF) variability and daily symptom scores, with absolute neutrophil counts correlating with PEF variability. Correlation between sputum eosinophil count and asthma control has been demonstrated to be a longitudinal phenomenon in clinical practice (44), with differences in sputum cell counts identified in different clusters of asthma patients (45). Within clinical management of asthma, peripheral blood eosinophil count has become more routinely embedded in clinical practice with the introduction of anti-IL-5 medications for certain endotypes of patients that remain sub optimally controlled on optimised inhaled therapy. The anti-IL-5 treatments require demonstration of a peripheral blood eosinophil count of greater than or equal to 300/mm^3 within the preceding 12 months (14), in addition to demonstration of requirement for regular maintenance or rescue courses of oral steroids.

In addition to IL-5, T2 cytokines IL-4 and IL-13 initiate and propagate inflammation in asthma, resulting in eventual airway remodelling, hyper-responsiveness and obstruction. IL-4 acts on B cells to produce antigen specific IgE (46). IL-4 and IL-13 mediate an isotype

swtich of B cells to produce IgE, with subsequent mast cell migration and degranulation (46) and resultant airway hyperresponsiveness (47). IL-13 drives smooth muscle hyperresponsiveness and remodelling, whilst stimulating the epithelium to produce mucus and increase cytokine production (20). Additionally, IL-13 induces airways hyperresponsiveness through upregulation of histamine and cysteinyl leukotriene receptors (48). IL-13 also increases inflammation via induction of chemokine expression, including CCL11/eotaxin through structural airway cells, and activation of STAT 6 in the airways. These chemokines, in turn, increases expression of acid mammalian chitinase (AMC), resulting in increased mucus secretion and airway hyperresponsiveness (46)

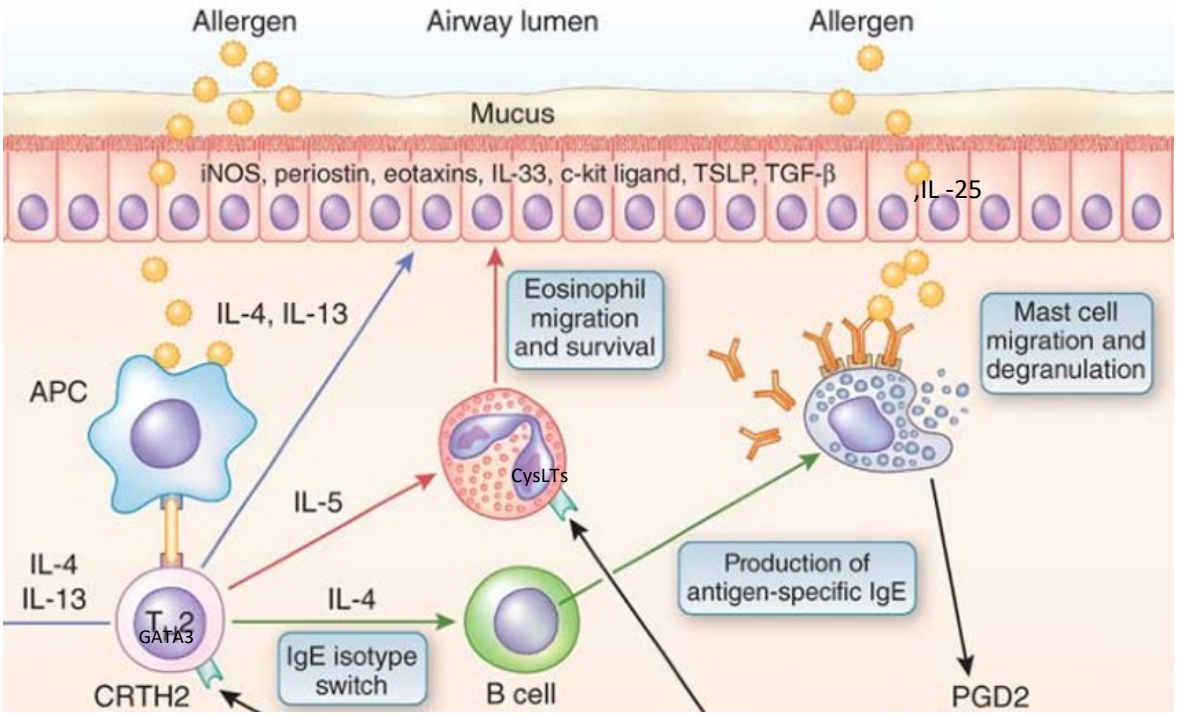


Figure 1.3 Mechanisms of T2 high asthma

Mechanisms of T2 high asthma demonstrating the role of allergic, IgE mediated inflammation, eosinophils, CysLTs and oxidative stress in driving the inflammatory response, adapted from (13). Exposure to external irritants stimulate the epithelium to secrete epithelial alarmins (IL-25, IL-33, TSLP). These epithelial alarmins then activate Th2 cells and innate lymphoid cells type 2. Th2 cells develop and secrete IL-4, IL-5 and IL-13, which in turn stimulate eosinophilic and allergic responses, in addition to epithelial and smooth muscle changes. Abbreviations: CRTH2, chemoattractant receptor-homologous molecule expressed on Th2 cells CysLTs; cysteinyl leukotrienes, GATA3; GATA3 transcription factor, IL; interleukins, iNOS; induced nitric oxide synthase, PGD2; prostaglandin D2, TSLP; thymic stromal lymphopoietin.

1.3.1.2 T2 low asthma

There is no universally agreed definition for non T2 asthma, but it is generally defined as non-eosinophilic asthma in the absence of T2 inflammatory markers (49). There are less clear pathogeneses in non-type 2 phenotypes, and these patients are therefore more limited in their treatment options (13). These non-T2 patients tend to demonstrate less airways reversibility than T2 high asthmatics, with a greater tendency to fixed airway obstruction (50). Neutrophils have been implicated in non-T2 asthma (13, 51).

Corticosteroid treatment, bacterial colonisation and smoking drive a more neutrophilic airway environment, that may contribute to this phenotype (49). Exposure to cigarette smoke, particulate matter and bacterial infection result in airway injury and release of toll-like receptors (TLRs). The activation of TLRs result in a shift towards Th1 and Th17 responses (49).IL-1 and TNF α/NF-κB pathways have been shown to correlate with neutrophilic inflammation and asthma symptoms in these patients (52), via activation of Th1 and Th17 cells. Th1 and Th17 responses result in generation of IL-8, IFN-γ, and IL-17 (see figure 1.4).

Paucicellular asthma encompasses patients without evidence of neutrophilic or eosinophilic inflammation who demonstrate bronchial hyperresponsiveness and ongoing symptoms (49). Paucicellular asthma demonstrates the uncoupling of airways obstruction from airway inflammation (53), and may reflect bronchial hyperresponsiveness driven airway remodelling, as shown by Grainge et al (16). Grainge and colleagues demonstrated bronchial hyperresponsiveness without inflammation in vivo in asthmatic airways. Dysregulation of calcium homeostasis may provide an explanatory mechanism for these findings (33). Paucicellular asthma demonstrates similar responses to neutrophilic asthma with exacerbations thought to be driven by bacterial and viral infections and environmental exposures such as smoking, see figures 1.2 and 1.4 (54). Reactive oxygen species (ROS) are thought to contribute to smooth muscle change seen in asthma, with ozone and hydrogen peroxide demonstrated to increase airways hyperresponsiveness to stimuli such as acetylcholine and bradykinin, as reviewed in (55). Increases in oxidative stress as assessed by hydrogen peroxide levels in exhaled breath have been demonstrated to correlate with increase airway hyperresponsiveness in asthma (56). A mitochondria dependent pathway for bronchial smooth muscle proliferation has been shown in asthmatic but not COPD or healthy bronchial smooth muscle cells (57). There is demonstration that the burden of oxidative stress in increased in vivo in asthmatic airway smooth muscle (58). In vitro, this airway smooth muscle resulted in increased oxidative stress and subsequent oxidatively damaged DNA, with correlation between levels of oxidative damage and degree of airway obstruction (58). Similarly, proliferation of airway smooth muscle cells was inhibited by antioxidant administration, and more specifically by blockade of NF-κB (59). Smooth muscle changes are thought to be regulated in part by genetics, with demonstration that microRNA-(miR)-221 can regulate regulate airway smooth muscle proliferation in asthmatic airways (60). Furthermore, changes in DNA

methylation are apparent in primary airway smooth muscle cells from asthma patients, which are associated with changes in gene expression for genes associated with airway smooth muscle contraction and proliferation (61).

Non T2 asthmatics are less likely to respond to corticosteroid treatment (62-64) and are often ineligible for the biological treatments, and therefore new treatments are much needed in this group. They do, however, demonstrate features such as obesity (65) and airway hyperresponsiveness that may respond to exercise training (5).

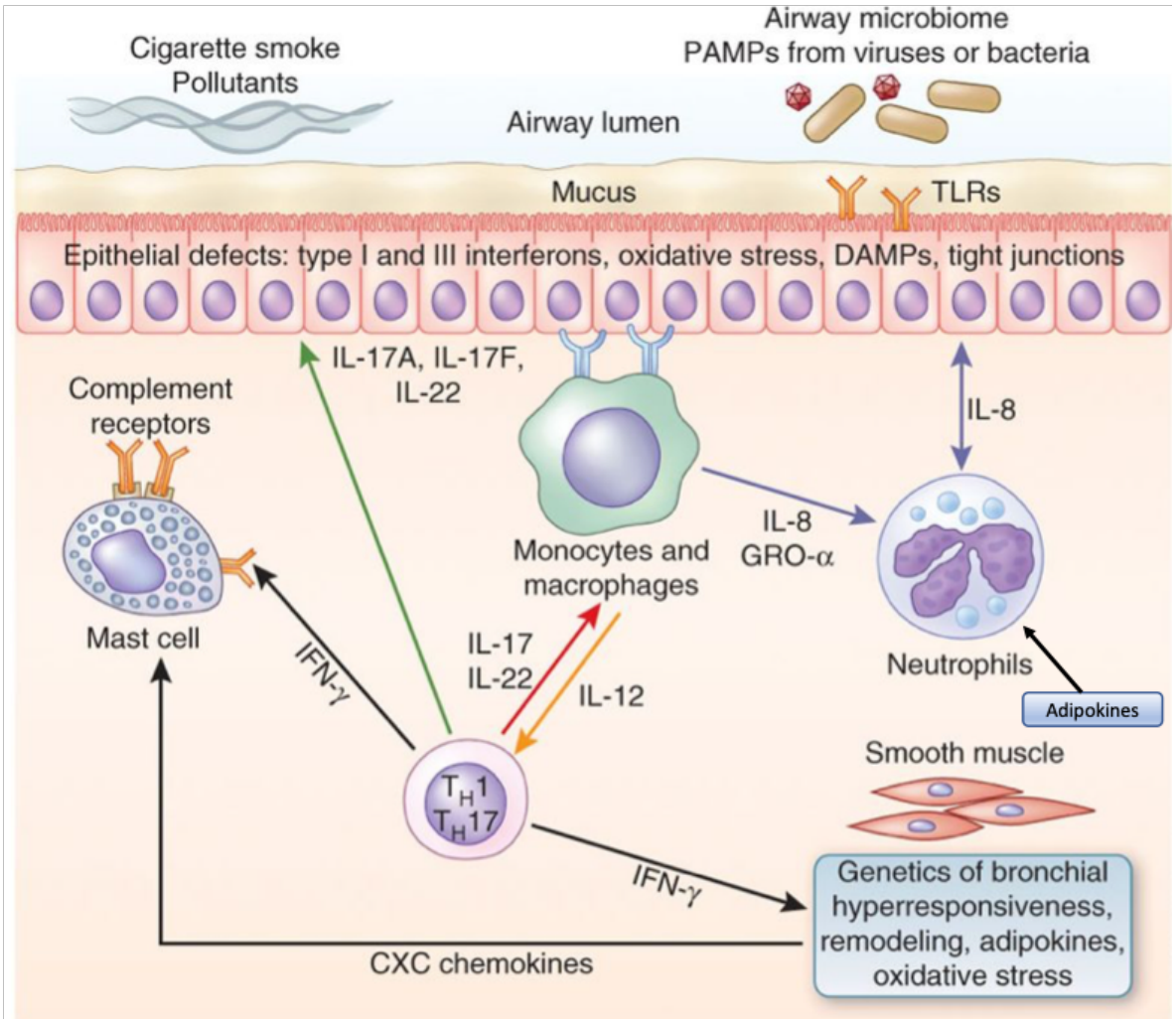


Figure 1.4 Potential factors involved in non-type 2 asthma

Potential factors involved in non-type 2 asthma including neutrophilic inflammation with involvement of IL-1/TNF α driven symptoms and the role of environmental pollutants and infections. Smooth muscle changes are driven by genetics and oxidative stress. Abbreviations: DAMP; danger-associated molecular pathway, IFN-γ, interferon-γ; GRO-α, growth-regulated oncogene-α; IL; interleukin, PAMP, pathogen-associated molecular pathway; TLR, Toll-like receptor. Adapted from (13)

1.3.1.3 Obese asthma

The obese asthma phenotype is emerging as a distinct group of patients that demonstrate poorer asthma control, reduced treatment responsiveness, and increased asthma severity and exacerbation rates (66). The mechanistic link between asthma and obesity requires further clarification, and is likely multifactorial. There is suggestion of at least two phenotypes of obese asthma (67) with early onset atopic obese asthma (EOA) and late

onset non-atopic asthma with high symptom expression (LONA)(14, 67). The pathophysiology between obese asthma phenotypes may vary, and they straddle the T2 high/ T2 low divide(67). Early onset atopic obese asthma is described as a more inflammatory driven process with its origins more typical of T2 high asthma. The inflammatory effect of excess adipose tissue is thought to compound this. EOA is thought to be the more labile, being more associated with disease exacerbation than the later onset disease (67, 68). The LONA phenotype is thought to post date the onset of obesity. The airways hyperresponsiveness is likely a result of the adipose related inflammatory milieu and obesity related reduction in functional residual lung capacity (FRC)(67), with demonstration that a fall in lung volume below normal FRC results in increased airways hyperresponsiveness (69).

Within obese asthma as a whole, there is a mechanic effect, in that adipose tissue around the upper airway results in dyspnoea due to upper airway narrowing, and limits breathing with a reduction in vital capacity and increased respiratory resistance, resulting in a restrictive lung deficit (70) (see figure 1.5). There is an inflammatory effect, in that adipose tissue is a complex and metabolically active endocrine organ. An excess of adipose tissue results in a chronic inflammatory state (71), which contributes to symptoms of asthma and airway hypersensitivity by induction or exacerbation of airways inflammation (66) (see figure 1.5). There is suggestion that obese asthma patients demonstrate reduced responsiveness to corticosteroid treatment (72), and that they continue to express high levels of type 2 cytokine dependent gene expression in sputum cells despite inhaled corticosteroid use (73). Within the later-onset, obese asthma group, neutrophilic inflammation seems to dominate the inflammatory state (74), and adipokines have been proposed as a potential biomarker that potentially link allergic and non-allergic inflammation in asthma and obesity, although the specific mechanisms remain unclarified (75). The neutrophil predominant obese group demonstrate particular treatment challenges, as, with the neutrophilic group as a whole, they often demonstrate steroid insensitivity (54), and do not meet prescribing criteria for biological treatment. A consideration with regards to an exercise intervention in asthma is whether patients with obese asthma respond to an exercise intervention differently to non-obese asthmatics. The relationship between obesity and exercise response in health has been investigated, reviewed in (76), and requires consideration when implementing an exercise intervention for patients with asthma, given the prevalence of obesity in asthma (65). At rest,

ventilation in obesity remains relatively normal due to adjustments in respiratory neural drive to counter increased external thoracic respiratory impedence (76). Obesity carries an increased metabolic demand, which is also apparent during exercise, and is a result of the increased chest wall loading during ventilation and the increased metabolic cost for any task due to increased load (77). The third component is respiratory inefficiency; there is increased lung atelectasis and reduced tidal volume that may result in increased dead space (78). However, despite this, whilst further investigation into the subtleties of ventilatory responses to exercise in obesity is required, healthy obese individuals appear able to maintain ventilatory homestasis during exercise (76).

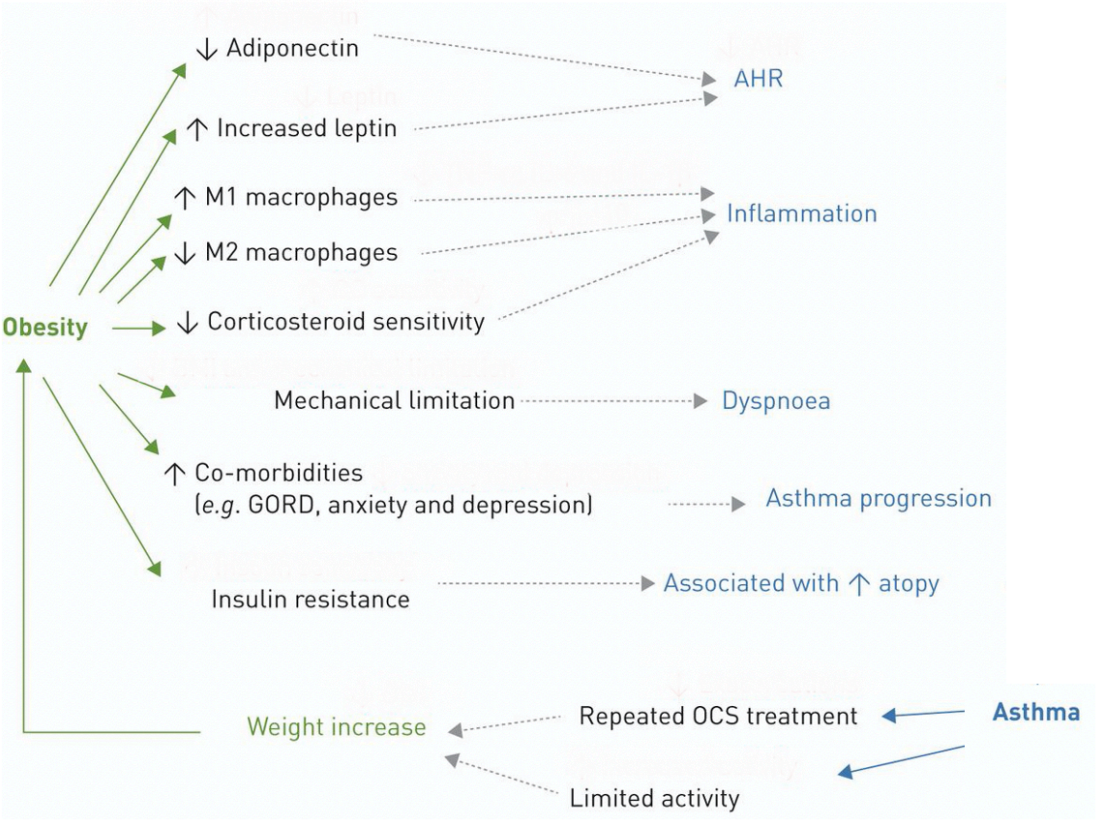


Figure 1.5 The interaction between asthma and obesity.

AHR: airway hyperresponsiveness; OCS: oral corticosteroid; BMI: body mass index; GORD: gastro-oesophageal reflux disease; GC: glucocorticoid; TNF: tumour necrosis factor; IL: interleukin, adapted from (79)

1.3.1.4 Exercise induced asthma

When considering an exercise intervention for asthma, an important area to understand is exercise induced asthma or exercise induced bronchoconstriction. Exercise induced

bronchoconstriction can occur with and without other symptoms of asthma (80), and is defined as a transient increase in airway resistance reflected as a 10-15% decline in FEV1 following at least 6-8 minutes of strenuous exercise (81-83). This usually occurs within 5 minutes of exercise, peaking at 10 minutes and resolving within 60 minutes (82). British Thoracic Society/ Scottish Intercollegiate Guidelines Network (BTS/SIGN) guidance suggests that most exercise induced asthma is a demonstration of poorly controlled asthma rather than a specific phenotype of disease (84), with other studies reporting that the prevalence of exercise induced asthma is between 5-20% in the general population, between 30-70% in elite athletes and up to 90% in individuals with asthma (85) . A number of pathogenic mechanisms have been identified as potential hypotheses for exercise induced bronchoconstriction (86). Increased ventilation is a physiological response to exercise, and results in both osmolar and thermal changes within the airways, as a result of increased inhalation of relatively cold and dry air. The increased water loss from the airways results in increased osmolality of the bronchial mucosal extracellular fluid. The increased osmolality causes movement of water from cells to the extracellular fluid, with a subsequent inflammatory response from the 'shrinking' cells (86). This can result in activation of mast and epithelial cells, with subsequent release of histamine, leukotrienes and chemokines (85), and the potential to cause an increase in local calcium sensing receptor signalling, which have been implicated in driving bronchial smooth hyperreactivity in asthma (12). Support for this inflammatory response as a mechanism for exercise induced bronchospasm is demonstrated in data from Hallstrand et al (87). This group demonstrated that the severity of exercise induced bronchoconstriction was associated with the percentage of columnar epithelial cells in induced sputum at baseline, and that after exercise challenge, there was an increase in these columnar epithelial cells in addition to increased histamine, tryptase, cysteinyl leukotrienes. The concentration of these epithelial cells correlated with levels of histamine and cysteinyl leukotrienes in the airways, and treatment with montelukast and loratadine were able to inhibit the release of these inflammatory mediators (87).

Thermal changes are also implicated in mediating exercise induced bronchospasm. Exercise increases ventilatory requirements, which often increases cold air inhalation. This increase in cold air inhalation results in pulmonary vasoconstriction following by reactive airway warming, and subsequent bronchial congestion and oedema (88). An increase in cold air flow also stimulates cholinergic receptors in the airways, with

resultant increased airway tone and secretions (85). Additional triggers include sport or environment specific triggers, such as chlorine exposure(89), and pollution and allergen exposure from external sports activites (90, 91). In the context of investigating the role of an exercise intervention in symptomatic asthma, it is reassuring to note that in none of the previous exercise intervention studies in asthma reported exercise induced bronchoconstriction in any of their participants (84). However, it is unclear if these patients were assessed for or excluded if at risk of exercise induced bronchoconstriction. When a maximal cardiolpulmonary exercise test was used to investigate unexplained dyspnoea in patients with difficult asthma, only 21 % of patients demonstrated evidence of exercise induced bronchospasm (92). There is suggestion in children that supervised aerobic exercise training reduced the severity of exercise induced bronchospasm, although there was no linear relationship demonstrated between improvement in fitness and reduction in exercise induced bronchospasm (93).

1.3.1.5 Redox regulation and asthma pathogenesis

Redox dysregulation and oxidative stress have been implicated in the pathogenesis of asthma (94), and may be a mechanism common to asthma across pheno and endotype. The master antioxidant transcription factor nuclear factor erythroid 2-related factor 2 (NRF2), has a key role in the homeostasis of the redox system throughout the body, and is responsible for regulation of many of the downstream processes that maintain oxidative balance via activation of the antioxidant reponse element (ARE). As depicted in figure 1.6, under normal conditions, NRF2 remains bound in the cytoplasm to its repressor protein, Kelch-like ECH associated protein 1 (Keap1). Imbalance within the redox regulation system through exposure to oxidative or reductive stressors, results in a conformational change in Keap1, which allows NRF2 to dislocate and accumulate in the nucleus of a cell. Here, it binds to the ARE in target genes such as haemoxygenase-1 (HO-1) to modulate their responses via upregulation or inhibition, including downregulation of NF-κB (95). NF-κB is discussed in greater detail earlier in this chapter in section 1.3.1.1.

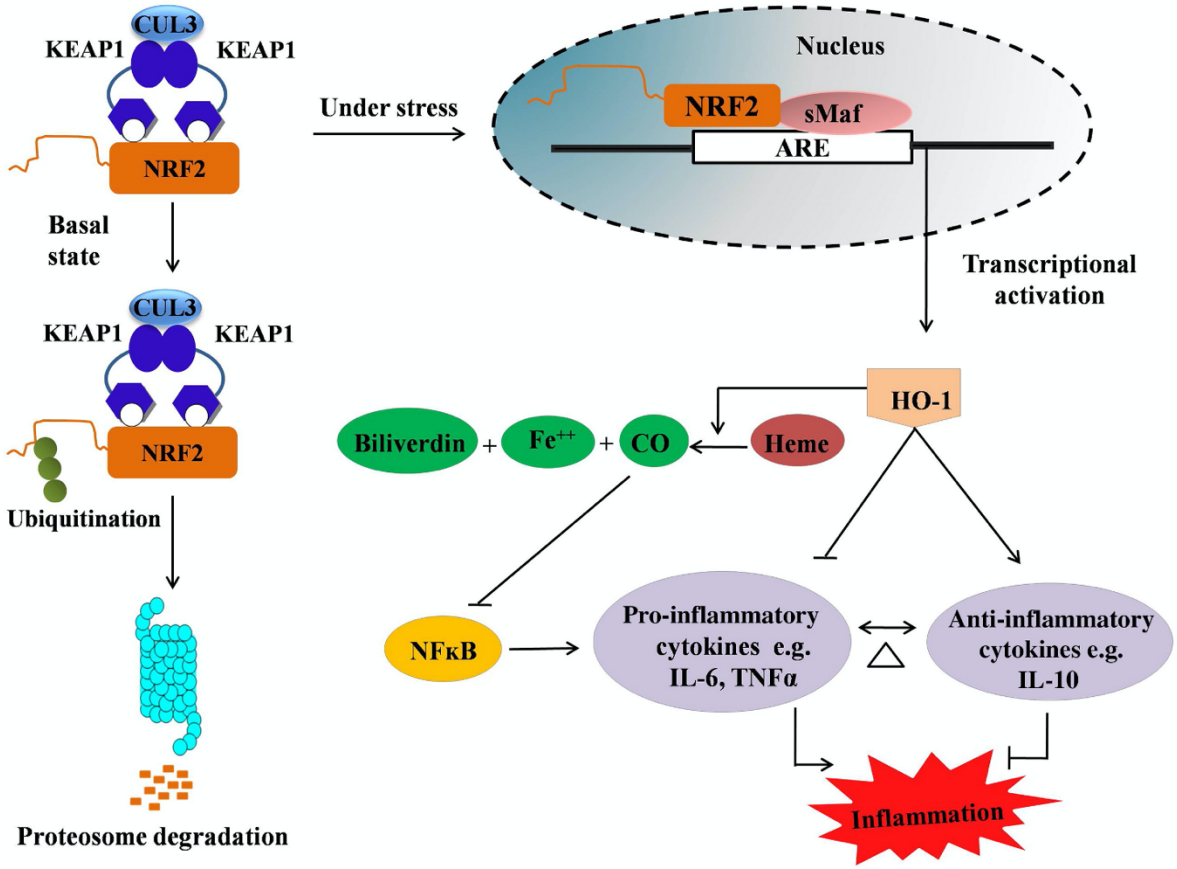


Figure 1.6 Overview of the NRF2/Keap1 pathway.

Under normal conditions, NRF2 binds to its repressor Keap1 which leads to ubiquitination followed by proteasome degradation. Imbalance within the redox regulation system through exposure to oxidative or reductive stressors, results in a conformational change in Keap1, which allows NRF2 to dislocate and accumulate in the nucleus of a cell. Here, it binds to the ARE, to target genes to modulate their responses via upregulation or inhibition, such as HO-1. HO-1 directly inhibits the pro-inflammatory cytokines as well as activating the anti-inflammatory cytokines, via downregulation of NF-κB and thus leads to balancing of the inflammatory process. Abbreviations: ARE; antioxidant response element, CO; carbon monoxide, Cul3; cullin 3, Fe; ferrous, HO; haemoxygenase, NF-κB Nuclear factor kappa-light-chain-enhancer of activated B cells, sMaf; small musculoaponeurotic fibrosarcoma proteins Copied from (96).

In animal models, NRF2 specifically has been linked with asthma severity, with disruption of NRF2 enhancing susceptibility to airway inflammation and asthma in mice, and enhancing susceptibility to diesal exhaust particles (97, 98). Thiol redox disturbances

demonstrated in children with asthma have been associated with post-translational modification of NRF2(99), with the mechanism through which allergen responses drive thiol redox disturbances depicted in figure 1.7 .

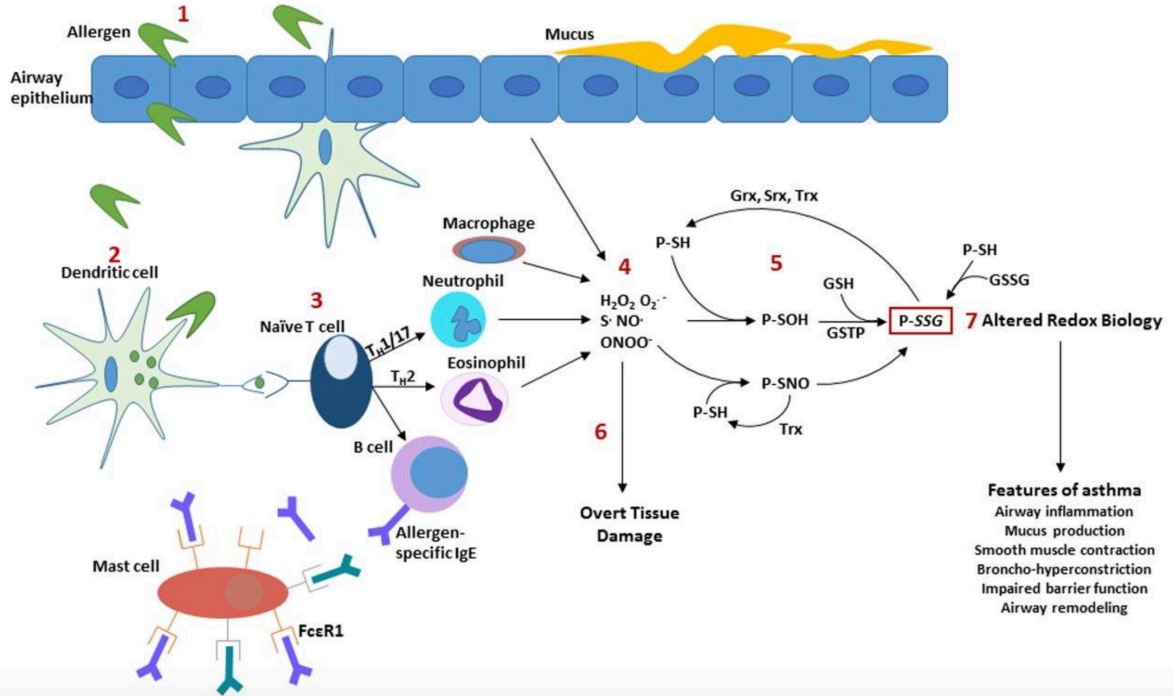


Figure 1.7 Overview of allergen-initiated innate and adaptive immune responses and associated changes in protein thiol redox status.

(1) Inhalation of allergens leads to disruption of epithelial cell homeostasis and impaired barrier function, allowing the allergen to interact with dendritic cells and other immune cells, including T cells. (2) Allergens are engulfed and processed by dendritic cells, which present antigenic epitopes to naïve T cells. (3) Naïve T cells polarize leading to recruitment of neutrophils and eosinophils (innate immune response) and activation of B cells, driving the adaptive immune response. (4) Reactive oxygen and nitrogen species are produced by inflammatory cells and structural cells including epithelial cells, resulting in oxidative modifications of reactive cysteines within proteins (P) (5). The downstream effects of oxidant overproduction have traditionally been associated with tissue damage (6). However, altered protein thiol redox biology (7) has emerged as a putative mechanism where oxidants contribute to the chronic features of asthma. H2O2, hydrogen peroxide; O2·–, superoxide; S·, thiyl; NO· nitric oxide; ONOO– peroxynitrite; P-SH, protein thiol; P-SOH, protein sulfenic acid; P-SNO, S-nitrosylation; P-SSG, S-glutathionylation; Grx, glutaredoxin; Srx, sulfiredoxin; Trx, thioredoxin; GSTP, glutathione S-transferase pi; GSSG, glutathione disulfide, copied from (100).

Redox regulation upset has been implicated in the pathogenesis of airways inflammation in obese asthma. Higher levels of oxidative stress and activation of the NF-κB pathway have been demonstrated in an asthma murine model (101), with NF-κB involved in the negative regulation of NRF2 signalling (102). A role for NRF2 in the pathogenesis of obese asthma has also been suggested through the identification of NRF2 driven mechanisms of effect for experimental treatments. Administration of a neutrophil elastase inhibitor to a rat model of obese asthma reduced symptoms and lung pathology via upregulation of the NRF2/Keap1 pathway (103). In addition, the antioxidant, reseveratrol, has been shown to upregulate NRF2 expression, and this upregulation was postulated to be responsible for the anti-asthma effects of this antioxidant in a rat model of asthma (104). In non T2 disease, NRF2 may also be of relevance, with a recent study demonstrating that neutrophil recruitment and accumulation during contact hypersensitivity in skin is regulated via NRF2 (105). In asthma mouse models, an experimental free radical scavenger has been shown to reduce oxidative stress markers and neutrophil counts in bronchoalveolar lavage fluid (BALF) accompanied by NRF2 acitvation, suggesting a role for NRF2 in neutrophilic airways disease (106). Similarly, an antioxidant herbal formulation has been demonstrated in a mouse model to suppress neutrophilic lung inflammation via activation of NRF2 and suppression of NF-κB (107). In humans, sulforaphane, a naturally occurring antioxidant phytochemical that induces NRF2, was shown to attenuate methacholine response in some asthmatic patients (108). In this study, in those patients for whom sulforaphane reduced bronchoconstriction to methacholine, there was an associated reduction in the activities of NRF2 regulated antioxidant and anti-inflammatory genes, whereas the converse was seen in non-responders. Further in vitro work demonstrated that sulforaphane-induced activation of NRF2 reduced proliferation of cultured human airway smooth muscle cells. Furthermore, in those airway smooth muscle cells isolated from severe asthma patients were compared with healthy and non severe asthmatic airway smooth muscle cells, whilst absolute levels of NRF2 expression was similar, binding of the NRF2 protein to the antioxidant reponse element was reduced in severe asthmatic airway smooth muscle cells. Downstream antioxidant mRNA and protein expression were also reduced in both severe and non severe asthmatic airway smooth muscle cells compared to healthy controls (109).

Further downstream of NRF2 and NF-κB, higher levels of cysteine oxidation of the glucocorticoid receptor have been demonstrated in children with difficult to treat asthma,

with levels correlating with disease severity and poor control (110). Similarly, children with asthma display higher levels of glutathione oxidation in BALF, with a reduction in ratio of reduced glutathione (GSH) to oxidised (GSSG) (111). This shift in the redox balance of glutathione to a more oxidised state appears to effect macrophage function, with in vitro supplementation with GSH correcting this impairment (112).

1.3.2 Asthma Diagnosis

Clinically, a diagnosis of asthma is not based on a single objective test, and reflected in recent GINA guidance, history and response to treatment, if supportive of bronchial smooth muscle hyperresponsiveness, are the intial criteria for making the diagnosis of asthma (11). Objectively, spirometry is possibly the most widely used and easily accessible test to support the diagnosis of asthma through demonstration of an obstructive deficit and/ or bronchodilator reversibility, and is recommended to confirm the clinical diagnosis of asthma (113). Spirometry assesses the amount of air that a patient can expel under force in 1 second (FEV1), the amount of air that can be expelled in total or forced vital capacity (FVC) and mid forced expiratory flow rates or forced expiratory flow 25-75% (FEF 25-75%). Asthma and other obstructive airways diseases are characterised by a reduction in FEV1 that is of a greater degree than the reduction in forced vital capacity, giving a ratio of FEV1/FVC of <0.7. Demonstration of this obstructive ratio is of diagnostic importance in COPD, as the airway obstruction is irreversible to a greater degree. In asthma, however, part of the definition includes reversibility and so a well-controlled patient may demonstrate normal spirometry. Gold standard demonstration of bronchial hyperreactivity is through a methacholine challenge, which is arduos for both patients and physiologists. A methacholine challenge requires demonstration of a drop in FEV1 of > 20% at a delivered dose (PD_{20}) of methacholine of 0.5-2.0 µmol for borderline hyperreactivity, and greater than 2.0 µmol demonstrative of airways hyperreactivity (114). This reversibility can also be demonstrated through bronchodilator reversibility testing, and significant bronchodilator reversibility reflects bronchial hyperreactivity. In this test, patients perform spirometry before and after inhalation of 400mcg of a short acting bronchodilator such as salbutamol. Variability of >200ml and 12% is diagnostic of asthma, as is variability in peak flow recordings of greater than 8%. The mean forced expiritory flow between 25 and 75% of the FVC (FEF 25-75%) is of relevance in asthma in that they are an indirect marker of small airways function, and

may show obstruction prior to deterioration in FEV1 (115). As such, spirometry has been listed by NICE as a recommended test for adults and children over 5, with bronchodilator reversibility testing recommended if an obstructive deficit is demonstrated (116).

Whilst currently used mainly in the research context, Forced Oscillation Technique (FOT) has been around since the 1970s, and is a non-invasive and objective method of measuring airways resistance, that has been demonstrated to have greater sensitivity at assessing bronchodilator reactivity than spirometry. As it is not effort dependant, it has been suggested that is has greater utility in younger children (117), with children as young as three usually able to perform FOT (118). FOT measures the impedance of the airways and lungs, in the form of reactance and resistance (see table 1.1 for definitions of indices measured in FOT and their clinical utility.

Table 1.1 Impulse Oscillometry terminology and outputs

Impulse Oscillometry terminology and outputs and their clinical rationale and relevance, copied from (118)

Impulse oscillometry terminology	
Impedence (Zrs)	*A calculation of the total force needed to propagate a pressure wave through the pulmonary system; comprised of resistance and reactance*
Resistance (Rrs)	*Energy required to propagate a pressure wave through the airways; to pass through the bronchi, bronchioles, and to distend the lung parenchyma. Resistance is determined when a pressure wave is unopposed by airway recoil and is in phase with airflow*
Reactance (Xrs)	*Energy generated by the recoil of the lungs after distension by a pressure wave out of phase with airflow*
Area of reactance (AX or XA)	*Area under the curve between the reactance values for 5Hz and the resonance frequency*
Resonanace Frequency (Fres)	*The frequency at which the lung tissue moves from passive distension to active stretch in response to the force of the pressure wave signal; graphically when reactance is zero*
Coefficient of variability (CV)	*Statistical determinany of the trial to trial variability serving as an index of reproducibility*
Frequency independent change	*When resistance values do not vary at differenct frequencies. If overall resistance is increased this may be indicative of proximal obstruction*
Frequency Dependent Change	*When resisitence varies with frequenvy more than age ependent normal values. This may be indicative of distal obstruction as shown by R5-R20*

Other clinically utilised assessments include symptom control scores such as the asthma control questionnaire (119, 120), and assessment of airway inflammation through fractional exhaled nitric oxide levels (116). Fractional exhaled nitric oxide (FeNO), is now routinely used in the assessment of airways inflammation in asthma, and its use is recommended to aid diagnosis and management in national guidelines (121). It was first postulated as a measure of inflammation in the airways over 20 years ago (25). L arginine is used to produce nitric oxide (NO), in a reaction catalysed by nitric oxide synthase, which can exist in several forms. The form of interest in asthma is inducible nitric oxide synthase (iNOS), which is inducible by inflammatory cytokines such as IL-4, IL-5 and IL-13, as previously discussed. iNOS produces a large amount of nitric oxide, and is suppressed by steroids (25). iNOS has been shown to be present in airway epithelium from asthmatic patients ex vivo(22), probabably via activation of NF-κB (122). The NO produced from this enzyme can drive and amplify the T2 inflammatory response, possibly resulting in hyperaemia, mucus secretion and increased Th2 cell proliferation (122). It is therefore is a useful non-invasive measure of eosinophilic airway inflammation (123, 124), with reasonable correlation with sputum eosinophilia (124).

1.3.3 Treatment of asthma

Treatment for asthma was revolutionised in the 1960s with the development of inhaled corticosteroids (125), which were augmented with the addition of inhaled long acting beta-2 agonists (126). Recent changes in international guidance focus on the avoidance of short acting bronchodilator therapies following indentification that these can contribute to inflammation in human airway epithelial cells (113, 127), and thus may increase asthma morbidity and mortality. Current treatment recommendations focus on anti-inflammatory therapy (detailed in figure 1.8), with an increasing understanding that disease modification as opposed to symptom management is key in improving outcomes for patients with asthma.

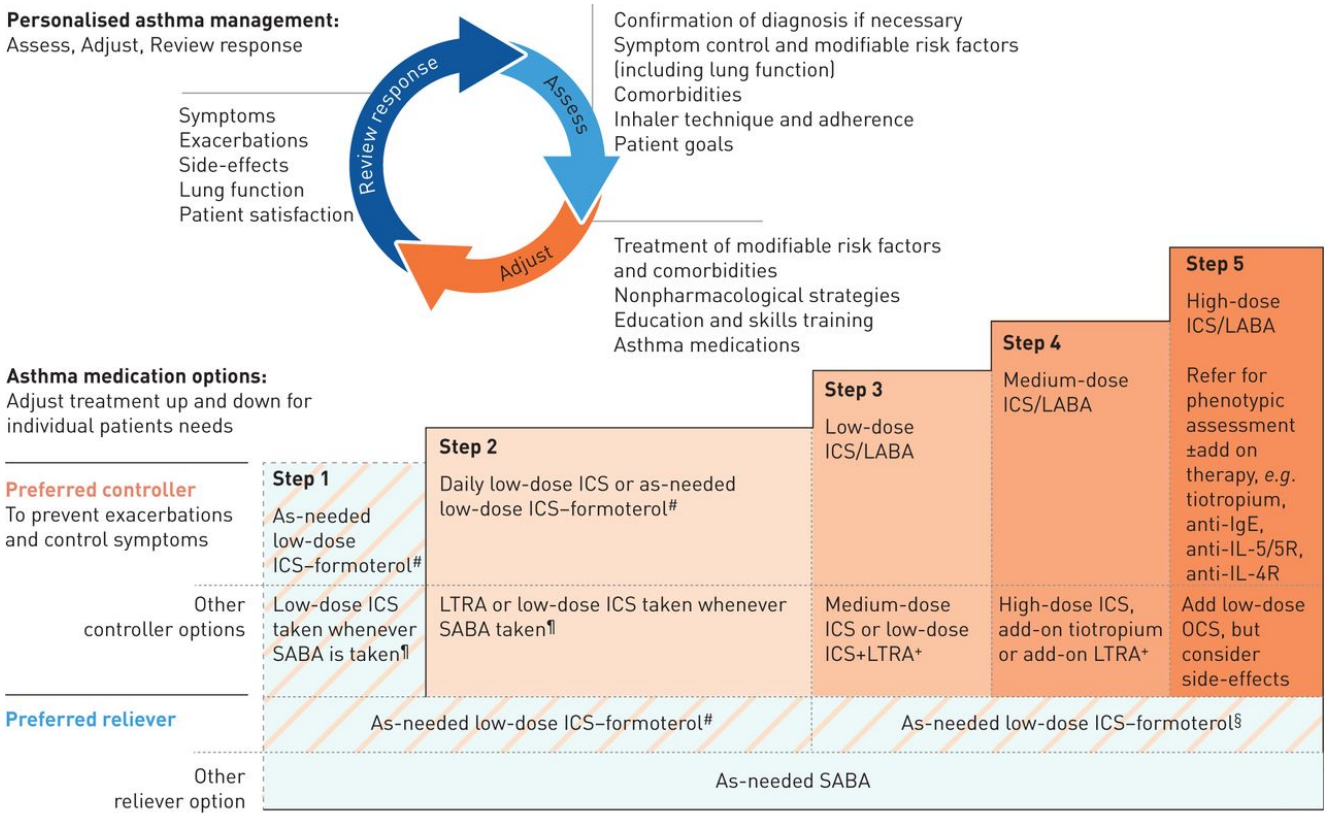


Figure 1.8 The Global Initiative for Asthma stepwise asthma treatment strategy for adults and adolescents ≥12 years of age.

Abbreviations: ICS: inhaled corticosteroid; SABA: short-acting ß2-agonist; LTRA: leukotriene receptor agonist; LABA: long-acting ß2-agonist; IL: interleukin; R: receptor; OCS: oral corticosteroid. #: off-label (data only with budesonide–formoterol); ¶: off-label (separate or combination ICS and SABA inhalers); +: consider adding house dust mite sublingual immunotherapy for sensitised patients with allergic rhinitis and forced expiratory volume in 1 s >70% predicted; §: low-dose ICS–formoterol is the reliever for patients prescribed budesonide–formoterol or beclometasone dipropionate–formoterol maintenance and reliever therapy, copied from (128).

Improved understanding of the inflammatory pathways underlying the pathogenesis of asthma has facilitated the development of an increasing number of biological treatments for those patients sub optimally controlled on inhaled treatments (129). Figure 1.9 details current biological targets and their position within the inflammatory cascade involved in asthma pathogenesis. For some patients, these biological treatments complement longer standing treatments of inhaled corticosteroids, long acting beta agonists, long acting muscarinic antagonists and leukotriene receptor antagonists (see figure 1.8).

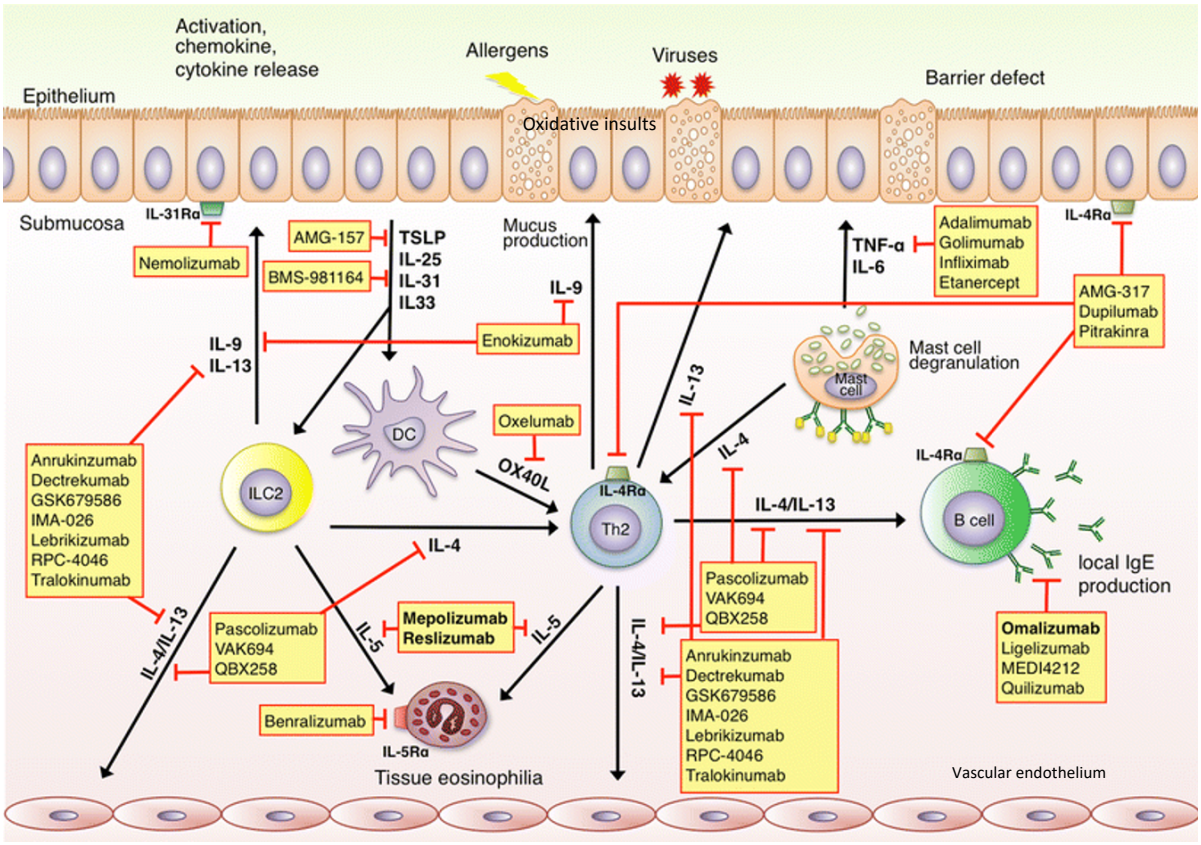


Figure 1.9 Biological treatments for asthma

copied from (130). External challenges in the form of allergens and viruses act as oxidative insults, and enter through the epithelium in lungs or skin, where they are identified and presented to T lymphocytes by dendritic cells. The dendritic cells then present to T cells, with Th1 cells responding to intracellular pathogen and Th2 responding to extracellular challenge. The alarmins (IL-25, IL-33 and TSLP) induce local OX40L expression on dendritic cells in the airways and subsequent binding by OX40 is a key molecular signal for Th2 cell differentiation. Th2 cells then secrete type 2 cytokines IL-4, IL-5 and IL-13, which exert their effects on eosinophils, mast cells, epithelial cells and airway goblet cells, resulting in type 2 inflammation. Th2 cytokines also stimulate differentiation of activated allergen specific B cells into IgE producing plama cells. The IgE specific molecules then bind to mast cells and basophils. Re-exposure to the allergen results in binding of the allergen to the specific IgE molecules and subsequent mast cell and basophil degranulation to release inflammatory mediators of histamine and prostaglandins. The various biological treatments are highlighted in yellow. DC, dendritic cell; IL, interleukin; ILC2, type 2 innate lymphoid cell.

Omalizumab is currently licensed for use in those over 6 years old with evidence of continuous or frequent treatment with oral corticosteroids and demonstration of confirmed IgE mediated asthma as an add on therapy (131). Omalizumab is a humanised monoclonal antibody that binds to serum IgE molecules, thereby preventing IgE from binding with its high-affinity (FcεRI) receptors on mast cells, antigen-presenting cells, and other inflammatory cells (132). Responsiveness to anti-IgE treatment is greater in asthma patients with an atopic phenotype (133, 134) with reduced benefit demonstrated in a non-atopic group (135). Similarly, anti-IL-5 treatments are licensed only for asthma patients who demonstrate a peripheral blood eosinophilia and frequent exacerbations, as it is only in this group of patients that a benefit has been demonstrated (136-138).

Difficult and severe asthma encompass both T2 high and T2 low asthma patients. These patients are of particular clinical and research interest, as whilst they only account for 10% of asthma in the UK, they contribute to much of the morbidity and mortality of the disease (139). Difficult and severe asthma patients are responsible for the majority of the costs associated with asthma, both in terms of direct healthcare utilization and the more hidden societal costs, through days lost to work and schooling (140, 141). Many factors are implicated in the control of difficult to treat and severe asthma, including co-morbidities and associated conditions such as obesity (67), depression and anxiety (142) , upper airway dysfunction (143), poor medication adherence (144), and genuine therapy insensitivity (145). Systematic assessment and directed treatment of these patients has been shown to improve outcomes (146, 147) but there remains a significant unmet need, with mortality from asthma remaining static in the UK since 2001(148). Whilst biological therapies are emerging as a key treatment for asthma, many patients do not demonstrate the appropriate biological marker (e.g. high blood eosinophilia or sensitivity to a perennial allergen), or remain sub optimally controlled despite biological treatment (149, 150). Thus, there is a significant unmet need for treatment for severe asthmatics. Exercise interventions may have a role in all endotypes and severities of asthma and are potentially more cost effective than biologicals (see table 1.2), assuming that the response levels are comparable, which has yet to be proven. Elucidation of the mechanisms through which exercise exerts its impact in asthma may also offer the potential to identify new targets for medication.

Table 1.2 Comparative cost of asthma treatment per month at University Hospital Southampton

Biological figures sourced from national prescribing costings and exercise intervention figures from exercise intervention studies at UHS, assuming comparable efficacy.

Treatment	**Cost per month (£)**
Exercise training thrice weekly in pairs (hospital setting)	189
Exercise training thrice weekly in pairs (community gym setting)	566
Mepolizumab	840 (151)
Omalizumab	Up to 2083 (variable dosing) (152)

1.4 Exercise as Medicine

Physical activity is defined by the World Health Organisation as 'any bodily movement produced by skeletal muscles that requires energy expenditure' (153). Physical inactivity has been implicated as a risk factor in the pathogenesis of many chronic conditions. Physical fitness is defined as a set of attributes that people have or achieve that relates to their ability to perform physical activity (154). Regular, adequate levels of physical activity are known to reduce the risk of many chronic inflammatory diseases and malignancies, in addition to improving quality of life in all age groups (155). Despite this, approximately one third of adults worldwide were insufficiently active in 2008, and physical inactivity is responsible for over 3 million deaths each year (153).

Exercise is a broad term sitting as a subheading under the more general term of 'physical activity', and covers a wide range of physical stressors of varying intensity. Comparing across all training programmes would be equivalent to comparing all inhalers for efficacy in asthma, without discriminating between drug classes. Part of the difficulty in assessing response to exercise is that variability in training programmes utilised in studies makes direct comparison very difficult.

Exercise is a subcategory of physical activity that is planned and purposeful and carried out with the intention of increasing physical fitness. It is difficult to entirely separate from physical activity, as higher levels of physical activity, regardless of where and in what context it is undertaken, have been demonstrated to negatively correlate with all cause mortality (156, 157).

In an attempt to better quantify physical activity, various definitions have been employed. A metabolic equivalent of task (MET) measures energy expenditure equivalent to the rate expended at rest or 1 MET, which is defined as 3.5ml of O_2/min/kg (158). Light intensity activities are defined as non-sedentary behaviours of less than 3 METs or 3 x the energy expenditure at rest, moderate intensity activities are defined as 3-5.9 METs and vigorous intensity METs are 6 METs or greater (159). However, these definitions can be inaccurate in participants with a lower or higher than usual fitness level, where a task that would usually require moderate intensity exercise, such as walking 3 miles/hour would, for that person, equate to a high or light intensity activity (159); something which is compounded by the original definitions of metabolic equivalents of task determined based on a single, healthy, 40 year old, 70 kg male (160). Therefore, whilst providing a guide, METs only estimate the physical cost of an activity rather than providing an objective measurement, and must be interpreted as such (158).

A cardiopulmonary exercise test (CPET) refers to an exercise test where the expired gas is collected, in order to non-invasively and objectively assess the dynamic physiological responses to exercise (161). A CPET is generally performed on either a treadmill or a cycle ergometer, with a variety of protocols reported in the literature for both (162). An advantage of cycle ergometer protocols are that they provide a controlled and accurate assessment of the workrate, and therefore allow more accurate prescription of exercise programmes. The outcome variables from a symptom limited incremental maximal exercise test (referred to for simplicity as a cardiopulmonary exercise test (CPET) for the duration of this thesis) can be used both to quantify physical fitness, and also for personalisation of exercise interventions. Amount of physical activity can also be expressed as a percentage of a person's maximal exercise capacity. This 'amount' is expressed either as a percentage of their maximum heart rate reserve or their aerobic capacity as defined by peak oxygen uptake (163). Peak oxygen uptake ($\dot{V}O_2$ peak) is a key outcome variable captured by a CPET. $\dot{V}O_2$ peak is a metabolic rate defined as the highest oxygen uptake at the end of an incremental exercise test(164). $\dot{V}O_2$ peak represents the capacity of the

pulmonary, cardiovascular and muscular systems to take up, transport and utilise oxygen Oxygen uptake can be measured by gas analysis of the oxygen content of inspired air compared to the oxygen content of expired air, with rapid development of this seen in the 1960s and 1970s, alongside technological developments allowing design and production of fast gas analysers and flow sensors (165, 166). However, exercise intensity, or the metabolic stress conferred by an exercise prescription determined by this method can be over or underestimated, as this method does not account for the anaerobic threshold (AT) variability for any given $\dot{V}O_2$ peak (167). Anaerobic threshold is also a metabolic rate, and is defined as the $\dot{V}O_2$ above which arterial lactate first begins to increase systematically during an incremental exercise test. and is dependent on a number of variables (143). The anaerobic threshold reflects increasing recruitment of type 2 muscle fibres and a subsequent increase in reliance on anaerobic glysolyis rather than aerobic respiration. To control for this variability, in order to allow equivalent metabolic stress for each individual, the calculation of intensity of exercise must include the anaerobic or lactate threshold (167). This is the way in which we have assessed and prescribed exercise training in this study, using a Structured Responsive Exercise Training Programme that takes into consideration both the anaerobic threshold and the peak oxygen uptake (168).

1.4.1 The role of cardiopulmonary exercise tests in medicine and exercise prescription

The CPET variables provide not only accurate assessment of exercise intensity but also objective, dynamic assessment of the cardiovascular, pulmonary, metabolic and haematological function of a person (164). As such, CPET has utility in diagnosis of shortness of breath where degree of symptoms do not correlate with standard cardiac and respiratory investigations (92). CPET provides a useful assessment tool for when standard, non-dynamic investigations do not provide information upon which to base a diagnosis of the cause of the unexplained shortness of breath, and is increasingly requested as part of the investigation of unexplained dyspnoea (169). CPET also has utility in mixed cardio and respiratory disease, in identifying which pathophysiological mechanism is driving the symptomatology of exercise intolerance; in mixed disease, it may not be possible to distinguish the limiting factor in the absence of a dynamic test (169). CPET has been demonstrated to be of benefit in the assessment of the cause of unexplained dyspnoea in difficult asthma patients, and is increasingly used in this context (92).

1.4.1.1 Outcome variables for CPET

Outcome variables can be broadly divided into exercise capacity variables and cardiorespiratory variables (see table 1.3 for details). For overall assessment of cardiorespiratory exercise response, it is important to interpret these combined variables in the context of symptoms and pre-test clinical assessment.

Table 1.3 Outcome variables for cardiopulmonary exercise testing

Copied from (164). Abbreviations AT; anaerobic threshold, BP; blood pressure, BR, breathing reserve, HR; heart rate, IC; inspiratory capacity, MVV;maximum voluntary ventilation, S_pO_2; oxygen saturation, $P_{ET}CO_2$; end tidal pressure of carbon dioxide, $P_{ET}O_2$; end tidal pressure of oxygen, RR; respiratory rate, $\dot{V}E$, Ventilatory equivalent, $\dot{V}E/\dot{V}CO2$; ventilatory equivalent for carbon dioxide, $\dot{V}E/\dot{V}O_2$; ventilatory equivalent for oxygen, $\dot{V}O2$; oxygen uptake, WR; workrate

Exercise capacity variables
Anaerobic threshold (AT) (ml/min and ml/kg/min)
Peak O_2 uptake ($\dot{V}O_2$peak) (ml/min and ml/kg/min)
Peak work rate (WR peak) (W)
Cardiorespiratory variables
$\dot{V}O_2$ work rate slope ($\Delta\dot{V}O_2$/ ΔWR) (ml/min/W)
Heart rate (HR) (beats/min); resting and peak exercise
Heart rate reserve (HRR) (beats/min) at peak exercise = maximum predicted heart rate-measured maximum heart rate
Oxygen pulse (ml/beat) resting and peak exercise
Arterial blood pressure (BP) (mmHg) resting and peak exercise
Arterial O_2 saturation (S_pO_2, %) resting and peak exercise
Tidal Volume (V_T; L or ml) resting and peak exercise
Respiratory rate (RR; bpm) resting and peak exercise
Ventilation ($\dot{V}E$) in litres/min resting and peak exercise
Breathing reserve (BR) in litres/min and % of $\dot{V}E$ at peak (BR=MVV-VEpeak)
Ventilatory equivalent for O_2 ($\dot{V}E/\dot{V}O_2$) at AT
Ventilatory equivalent for CO_2 ($VE/\dot{V}CO_2$) at AT
$VE/\dot{V}CO_2$ slope
End tidal pressure of O_2 ($P_{ET}O_2$ mmHg) resting and peak exercise
End tidal pressure of CO_2 ($P_{ET}CO_2$ mmHg) resting and peak exercise
Spirometry variables
FEV_1 (L)
FVC (L)
Maximum voluntary ventilation (MVV) either directly measured or as an estimate (FEV_1 x 40) (litres/min)
Inspiratory Capacity (IC) (L)

1.4.1.2 Exercise capacity outcome variables: Anaerobic Threshold and Peak Oxygen Uptake

The primary outcome variables used for the prescription of exercise interventions are Anaerobic Threshold (AT) and $\dot{V}O_2$ peak (oxygen uptake at peak exercise). AT can be viewed as more objective than $\dot{V}O_2$ peak in the context of it not being effort dependent, or volitionally influenced. $\dot{V}O_2$ peak is identifiable by a non-linear increase in ventilation, as respiration increases in response to the increasing CO_2 production that results from anaerobic metabolism. Determination of the anaerobic threshold involves indentification of three key criteria; 1) excess $\dot{V}CO_2$ in comparison to $\dot{V}O_2$, as a result of increasing metabolic acidosis from increasing anaerobic glycolysis, 2) hyperventilation relative to oxygen; $\dot{V}E$ becomes driven by the excess CO_2 production from increasing anaerobic glycolysis rather than oxygen demand and 3) exclusion of non-specific hyperventilation as the cause of rising $\dot{V}CO_2$, through a combination of the absence of a fall in $P_{ET}CO_2$ and maintained or falling $VE/\dot{V}CO_2$ as $VE/\dot{V}O_2$ begins to rise (164). In healthy untrained people, AT occurs at 45-65% of $\dot{V}O_2$ peak, but is relatively lower in heart disease and higher following training (170) (171). It should be reproducible independent of the ramp gradient used in the test, and is calculated as an average over 20-30 seconds at peak workrate. $\dot{V}O_2$ peak could be described as less objective than AT as it is volitionally dependent. $\dot{V}O_2$ peak is often used, incorrectly, interchangeably with $\dot{V}O_2$ max, which is a physiological endpoint that cannot be volitionally influenced. $\dot{V}O_2$ max refers to the maximum oxygen uptake achievable by an individual, beyond which no increased effort can increase. Athletes or very fit individuals can achieve their $\dot{V}O_2$ max on an incremental exercise test, and this requires demonstration of a plateau in $\dot{V}O_2$ in the context of increasing workrates, to demonstrate that maximal uptake has been achieved (164, 172). For confirmation that $\dot{V}O_2$ max is a true maximal oxygen uptake as opposed to a peak oxygen uptake, verification or repeated testing can be employed (172). As demonstration of a true $\dot{V}O_2$ max in an incremental exercise test is often not possible other than in fit, healthy individuals, for the purpose of this thesis, $\dot{V}O_2$ peak will be used. The normal ranges for both AT and $\dot{V}O_2$ peak vary with age, sex and other anthropometric varibales and results from a number of key studies are summarised in figure 1.10. There are wide distribution of data, and this may reflect the differences in the populations selected; most of the widely cited studies used volunteers, which is likely to result in selection bias of participants with an interest in physical fitness (173). The SHIP study aimed to address this by using a population study as the platform for participant selection (174). The

ATS/ACCP recommend the use of the two most widely used sets of reference values (Jones et al(175) and Hansen et al(176)) in their statement on cardiopulmonary exercise testing, and therefore I have used Hansen et al calculations in this thesis (177). The effort dependent nature of $\dot{V}O_2$ peak is also of consideration with regards to reference values, although reproducibility appears relatively reliable (178). Within patient variability in the context of pre-operative exercise testing has been investigated in patients undertaking CPET as part of pre operative preparation for aortic aneurysm repair. Here, patients underwent 4 cycle ergometer based CPETs over a 6 week period, with instructions to keep their exercise levels at a constant. The same blinded investigator reported all 4 tests. Results demonstrated a typical within-patient error (% coefficient of variation) in AT across the four repeat tests was 10% (95% confidence interval, CI, 8–13%). Expressed in raw units the typical error in AT was approximately 1.3 ml O_2/kg/min (95% CI, 1.1–1.6 ml/kg/min) (179). The intraclass correlation coefficient for the four tests was 0.74 (95% CI, 0.55–0.89). These data were used in the design of the EMPOWER study, which based their sample calculation on detection of a difference in peak oxygen uptake of 2ml/kg/min(168). There are also differences in oxygen uptake observed depending on the mode of exercise, with demonstration of differing $\dot{V}O_2$ peak values for cycling and running protocols (180, 181).

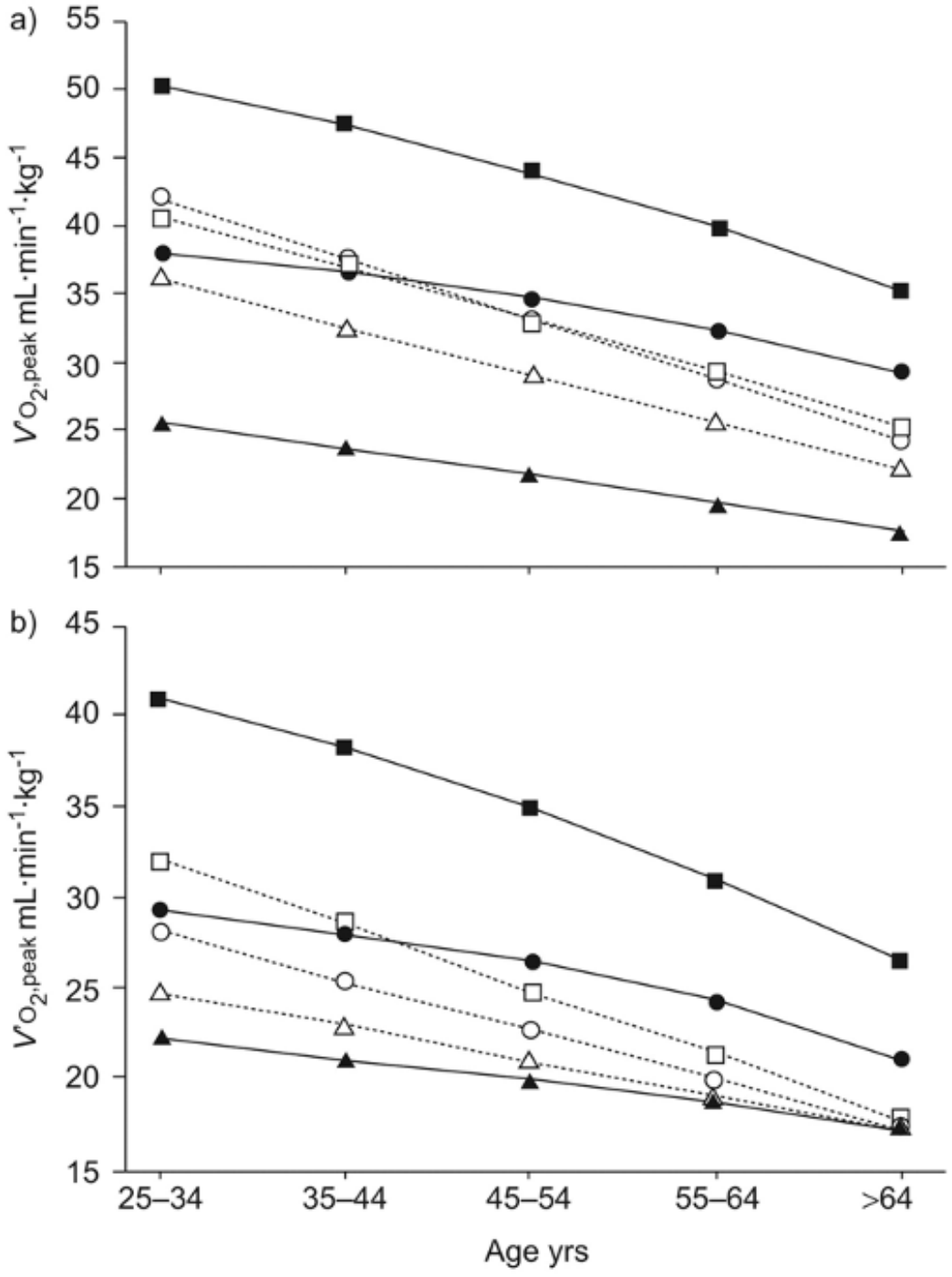


Figure 1.10 Peak oxygen uptake ($\dot{V}O_2$ peak) across 10-yr age categories

Peak oxygen uptake ($\dot{V}O_2$ peak) across 10-yr age categories for a) males and b) females for patients in the SHIP study(174), with comparison with Jones(175), Wasserman(170) and Neder(173) data performed for an exemplary individual (height 170 cm, weight 70 kg). ——: SHIP study data; ·····. ▪: 95th percentile; •: median; ▲: 5th percentile for SHIP study dataset(174) ; □: data from Jones et al (175).; ○: data from Wasserman et al. (170); ◿: data from Neder et al. 23 (173); adapted from (174).

1.4.1.3 O_2 Pulse

O_2 pulse is derived from $\dot{V}O_2$ / heart rate (HR), and reflects the amount of oxygen consumed per heartbeat (182). O_2 pulse is used as an estimate of stroke volume (SV), based on the assumption that values of arterial oxygen content at peak exercise. Given cardiac output (CO) is equal to SV x HR, O_2 pulse therefore provides an indirect assessment of cardiac output. In the initial stages of exercise, the increased cardiac output is as a result of increases in stroke volume rather than heart rate, with heart rate contributing to increases in cardiac output towards peak exercise. A flattening or downward deflection of the oxygen pulse kinetics during an incremental exercise test therefore most commonly reflects cardiogenic performance limitations (182).

1.4.1.4 Respiratory variables

In addition to AT and $\dot{V}O_2$ peak, the variables of breathing reserve (BR), in both L/min and % reserve, the relationship between minute ventilation ($\dot{V}E$) and CO_2 output ($\dot{V}E/\dot{V}CO_2$ slope) and Partial Pressure of End Tidal CO_2 ($P_{ET}CO_2$) are also of relevance in determining respiratory limitation to exercise.

Maximal Voluntary Ventilation (MVV) is the largest amount of air a person can inhale and exhale during a 12-15 second interval. In CPET, MVV acts as a surrogate measure of volunteers' absolute ventilatory capacity and is used to indirectly calculate ventilatory reserve in a maximal exercise test. MVV can either be measured directly or calculated by multiplying the FEV1 by 40 (183). However, it is noteworthy that when concordance between the actual MVV and estimated MVV were compared, whilst good correlation was seen between actual and estimated MVV, poor concordance was demonstrated with the actual MVV value (184). Conversely, patients with chronic respiratory diseases may change the accuracy of the measured MVV (184). The calculation of MVV as a 40 x multiple of FEV1 is preferentially used by the majority of the ERS taskforce for standardisation of cardiopulmonary exercise testing in chronic lung diseases and is therefore the formula adopted in this thesis (162). Breathing reserve (BR) is a measure of ventilatory flow limitation and is affected by factors limiting airflow and functional lung volumes (185). Breathing reserve is calculated as a percentage of a participants maximal voluntary ventilation that remains at peak exercise, and in healthy individuals should be ≥20% (170, 171). It is a useful variable to determine between patients who are dyspnoeic

due to cardiac disease, in which case BR should be normal. In those with respiratory limitations, who often demonstrate reduced or absent BR at peak exercise and are therefore limited in their maximal exercise tolerance by an inability to increase their ventilation to accommodate greater oxygen utilisation.

$\dot{V}E/\dot{V}CO_2$ is a measure of pulmonary dead space and VQ mismatch, and is calculated from the relationship between minute ventilation and anaerobic production of CO_2. It therefore provides a guide as to ventilatory efficiency, and the slope of these two varibles should be ≤ 32, with anything greater than this considered abnormal (170). A high $\dot{V}E/\dot{V}CO_2$ slope can occur in cardiac and metabolic diseases in addition to pulmonary compromise(171), typically with demonstration of a concomittently low (rather than normal or high) $P_{ET}CO_2$in these non respiratory causes (185) .

Another variable of relevance in CPET with regards to respiratory disease is Partial pressure of end tidal carbon dioxide ($P_{ET}CO_2$), which has been demonstrated to have great prognostic utility in both cardiac and pulmonary disease. In health, $P_{ET}CO_2$ at rest runs between 36 and 42 mmHg, (182) with an increase in $P_{ET}CO_2$ between rest and AT of between 5-8mmHg is expected, with a slight decrease thereafter. A persistently low $P_{ET}CO_2$ can be reflective of ventilation perfusion mismatch as a result of respiratory pathology or hyperventilatory breathing patterns, or poor pump transport of CO_2 from tissues to lungs as a result of cardiac disease (182).

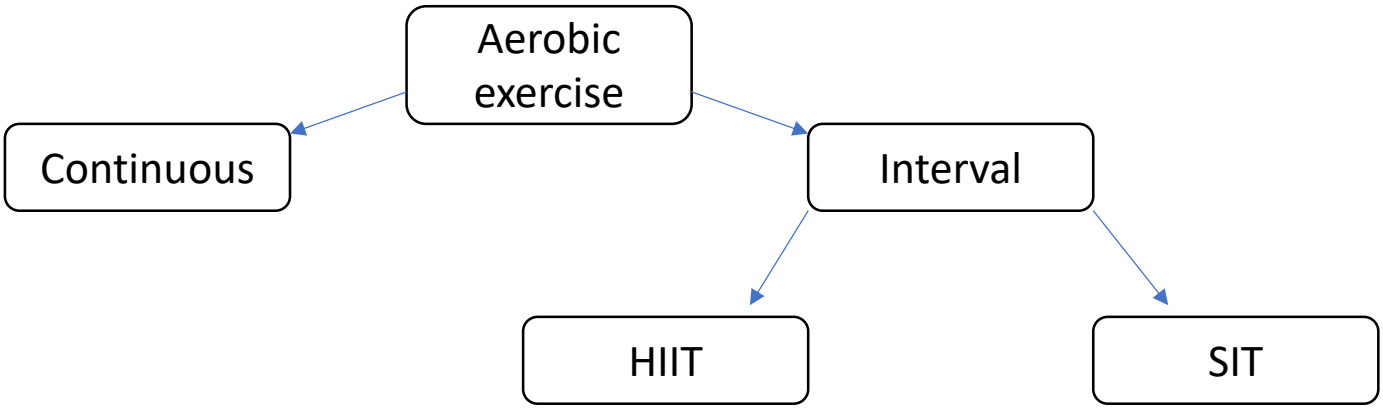
1.4.2 Exercise prescription

The goal of exercise prescription is to individually tailor exercise intervention in order to improve physical fitness and health (186). The FITT-VP (Frequency (how often), Intensity (how hard),Time (duration or how long), Type (mode or what kind), total Volume (amount),and Progression (advancement)) principle is used as a structure for exercise prescription, and is consistent with evidence based exercise recommendations from professional scientific statements (187).

1.4.2.1 Aerobic exercise or cardiorespiratory exercise

Aerobic exercise is defined by the American College of Sports Medicine (ACSM) as any activity using large muscle groups that is rhythmic and continuous (187), with these muscle groups relying on aerobic metabolism for their energy supply (188). Aerobic exercise has been demonstrated to have impact on health across the lifespan (189, 190).

The benefits are indisputable and in most cases outweigh any associated risks (186). Aerobic (or cardiorespiratory) exercise can be grouped into continuous/steady state exercise and interval training. Continuous training is defined as 30-60 minutes of aerobic exercise at a fixed intensity (186), with definitions of training intensities described in table 1.4. Interval training involves intense bouts of exercise, with subsequent recovery times, and can be classified into high intensity interval training (HIIT) and sprint interval training (191).HIIT can then be further subdivided by volume, with high volume HIIT including repeated episodes of near maximal effort separated by recovery time at a moderate intensity(191), whereas low volume HIIT has fewer or shorter intervals (191). Sprint interval training (SIT) is defined as supramaximal exercise bouts separated by active



recovery periods (191).

Figure 1.11 Types of aerobic exercise

Abbreviations; HIIT: high intensity interval training, SIT: Sprint interval training

The FITT-VP principles for prescription of the two modalties of exercise training are the same, and discussed below. A large amount of work has focussed on comparing interval and continuous intensity training modalties and their impact on different disease populations, and this is discussed in the context of asthma in section 1.6 of this thesis. In healthy individuals, a recent meta-analysis compared low volume HIIT with moderate intenstity continuous training (MICT) and a non-exercising control in terms of impact on cardiorespiratory fitness and body composition and concluded that low volume HIIT was a time efficient treatment for improving cardiorespiratory fitness (192). Similarly, meta-analyses have confirmed that HIIT is comparable or superior to MICT for improving fitness(193), vascular function (194) and cardiometabolic risk factors(195).

1.4.2.1.1 Frequency of exercise intervention for aerobic exercise prescription

The frequency of exercise intervention advised by the American College of Sports Medicine (ACSM) depends on the intensity of the exercise prescribed, but are based on demonstration that improvements in cardiorespiratory fitness are reduced with training less than 3 times/per week, with additional gain plateauing at greater than 5 sessions per week(187). They conclude that moderate intensity aerobic exercise done at least 5 days/week, or vigorous intensity aerobic exercise done at least 3 days/week, or a weekly combination of 3–5 days/week of moderate and vigorous intensity exercise is recommended for most adults to achieve and maintain health/fitness benefits'(186).

1.4.2.1.2 Intensity of exercise intervention for aerobic exercise prescription

With regards to intensity of exercise intervention, ACSM recommendations are based on the understanding described by the overload principle of training; that below a certain threshold, an exercise intervention may not challenge the body sufficiently to increase V̇O2 max, and that this may vary with baseline levels of fitness (187). The ACSM definitions for intensity of aerobic exercise are detailed in table 1.

Table 1.4 ACSM classification of aerobic exercise intensity

Copied from (187). Abbreviations HR; heart rate, HRR; heart rate reserve, MET; metabolic equivalent of task, RPE; borg rating of perceived exertion, $\dot{V}O_2$; oxygen uptake

	Relative intensity				Absolute intensity	Absolute intensity (METs) by age		
Intensity	%HRR	%HR max	% $\dot{V}O_2$ max	Perceived exertion	METs	Young (20-39 years)	Middle-aged (40-64)	Older > 65
Very light	<30	<57	<37	< very light	<2	<2.4	<2	<1.6
Light	30-39	57-63	37-45	Very-fairly light	2-2.9	2.4-4.7	2-3.9	1.6-3.1
Moderate	40-59	64-76	46-63	Fairly light to somewhat hard	3-5.9	4.-7.1	4-5.9	3.2-4.7
Vigorous	60-89	77-95	64-90	Somewhat to very hard	6-8.7	7.2-10.1	6-8.4	4.8-6.7
Near maximal	>89	>95	>90	>Very hard	>8.7	>10.1	>8.4	>6.7

As discussed in the introduction to section 1.4, a number of ways of measuring exercise intensity have been employed, including those based on % of $\dot{V}O_2$ peak or HRR, but neither of these control adequately for variability in anaerobic threshold and therefore the level of metabolic stress applied. In addition to baseline fitness as a variable to control for with exercise prescription, there is also variation in response or dose needed depending on the type of exercise intervention, with interval exercise interventions offering greater variability in training programme with similar or improved outcomes in cardiorespiratory fitness when compared to steady state(186). The ACSM conclude that the intensity recommended is 'moderate (e.g., 40%–59% heart rate reserve (HRR) or $\dot{V}O_2$) to vigorous (e.g., 60%–89% HRR or $\dot{V}O_2$) intensity aerobic exercise for most adults', and that 'light (e.g., 30%–39% HRR or $\dot{V}O_2$) to moderate intensity aerobic exercise can be beneficial in individuals who are deconditioned'. (186). The ACSM additionally suggest that interval training 'may be an effective way to increase the total volume and/or average exercise intensity performed during an exercise session and may be beneficial for adults'

1.4.2.1.3 Time or duration of exercise intevention for aerobic exercise prescription

The American (196), UK (197) and World Health Organisation Guidelines (198) on physical activity recommend either 150 min of moderate-intensity activity or 75 min of vigorous-intensity activity per week or a combination of both intensities. These recommendations are based upon data from large population studies (199) and have been supported in subsequent publications (200). The implication from these recommendations are that moderate and vigorous intensity exercise are comparable in terms of health benefits in a 1:2 ratio, with a recent (small) meta-analysis concluding that vigorous and moderate intensity activities in comparable volumes reduced all cause mortality to the same degree (201). However, the authors concluded there were a sparcity of studies investigating the comparative benefit of continuous exercise of differing intensities and that further work was needed

1.4.2.1.4 Type of exercise intervention for aerobic exercise prescription

Type of exercise recommended to improve cardiorespiratory fitness is broadly described as rhythmic aerobic exercise involving large muscle groups, and is divided by the ACSM into 4 categories (see table 1.5).

Table 1.5 Modes of aerobic exercise to improve cardiorespiratory fitness

Adapted from (186)

Exercise type	Recommended for	Examples
Endurance activities requiring minimal skill or physical fitness	All adults	Walking, leisurely cycling, aqua aerobics, slow dancing
Vigorous intensity endurance exercise activities requiring minimal skill	Adults who are habitually physically active or of average physical fitness	Jogging, running, rowing, aerobics, spinning, elliptical exercise, stepping exercise, fast dancing
Endurance activities requiring skill to perform	Adults with acquired skill and average fitness	Swimming, cross country skiing, skating
Recreational sports	Adults with a regular exercise programme and average fitness	Racquet sports, basketball, soccer, downhill skiing, hiking

1.4.2.1.5 Volume of exercise prescription

Volume of exercise prescription is measured as a combination of the FITT-VP principles described above. For research purposes, MET-minutes per week or kilocalories per minute per week tend to be used to estimate gross energy expenditure of an individual and are based on published tables listing laboratory or field measured oxygen costs of specific activities (160, 202).

1.4.2.1.6 Progression of exercise prescription

Tolerance to an exercise intervention can develop over time, with the increase in fitness resulting in a reduction in the 'dose' of exercise reducing as anaerobic threshold and oxygen uptake increase with training; the same level of intervention delivers a lower level of metabolic stress as a result of improved fitness. Therefore, an exercise training intervention needs to increase to compensate for this in order to maintain equivalent levels of metabolic stress on an individual. The Structured Responsive Exercise Training Programme (SRETP) developed and used at University Hospital Southampton adjusts for this with interim reassessment of fitness through CPET testing (168). The rate of progression depends on a number of variables including baseline fitness, training responses and exercise programme goals (186). The exercise training intervention can be progressed by modification of any of the FITT-VP parameters, and the recommendation from the ACSM is that a gradual and individualised approach is needed (186).

1.4.2.2 Resistance exercise training

This thesis has so far focussed on aerobic exercise intervention and the subtypes therein. Less is understood about the effects of resistance training on health, but it is known that sedentary adults lose between 3-8% of their muscle mass per decade, and that resistance exercise training may help counteract this (203). Resistance exercise is defined as exercises to improve muscular fitness, specifically in relation to their strength, endurance and power (187) . Resistance training has been demonstrated to improve lipoprotein profiles, improve insulin sensitivity and reduce blood pressure, although the mechanisms of effect remain unclear (203). The FITT-VP principles can be applied to resistance training as described below.

1.4.2.2.1 Frequency of resistance exercise training

For those who are not regularly engaged in resistance training, the recommendations are to train each large muscle group for 2-3 days per week, ensuring that each training session for a specific muscle group is split by > 48 hours to allow adequate recovery (186, 204).

1.4.2.2.2 Intensity and Time (duration) of resistance exercise training

Intensity is based upon an individual's 1 repetition maximum (1RM) defined as the maximum load an individual can lift in one repetition(204), and the ACSM definition of differing intensities are detailed below in table 1.6.

Table 1.6 ACSM Classification of resistance exercise intensity

Adapted from (187).Abbreviations: RM; repetition maximum

Resistance Exercise	
Intensity	Relative intensity as % 1RM
Very light	<30
Light	30-49
Moderate	50-69
Vigorous	70-84
Near-maximal/maximal	≥85

To improve muscular strength and mass, a resistance exercise that allows an indidual to complete 8-12 repetitions, usually at a resistance of 60-80% of an individual's 1RM is required (186). For an increase in endurance, it is recommended that the number of repetitions be increased, and therefore the resistance as a percentage of 1RM may need to be decreased.

1.4.2.2.3 Type of resistance exercise

The description of resistance exercise is complex, with many types of resistance training equipment and types of exercises available. In terms of equipment, there are free weights, machines with stacked or pneumatic resistance, and resistance bands, in addition to resisitance exercise which rely on an individual's own body weight to provide the resistance. From a research perspective, machines with stacked or pneumatic weights are more controllable and less technique dependent than the other types of resistance exercise, and also less likely to cause injury. In terms of the exercises themselves, they can be broadly divided into 3 groups and are summarised in table 1.7 below.

Table 1.7 Types of resistance exercise

Type of resistance exercise	Examples
Multijoint/compound	chest press, shoulder press, pull-down, rows, push-ups, leg press, squats, deadlifts
Single joint	biceps curls, triceps extensions, quadriceps extensions, leg curls, calf raises
Core muscle exercise	Planks and bridges

The ACSM summarise their recommendation for type of resistance exercise as follows; 'Both multijoint and single-joint exercises targeting agonist and antagonist muscle groups are recommended for all adults as part of a comprehensive resistance training program'(186). Technique is also key in safe and effective resistance exercise training and the ACSM recommend that instruction from a qualified health professional be employed in individuals without previous experience of resistance training (186).

1.4.2.2.4 Volume and Progression of resistance training

Volume of resistance training comprises a combination of repetitions and load of resistance training intervention and is therefore described above. Progression is recommended to maintain adaptive metabolic stress as the effect of training is seen, by ' gradually increasing resistance, number of sets, or frequency of training (186).

1.5 Exercise in health: immunomodulatory effects

Exercise has immuno-modulatory effects in healthy humans (205). There is increasing understanding of the impact of moderate intensity exercise but very little mechanistic work exists as to how and why this is achieved. Extreme and high intensity exercise are best described as they have been explored in the context of athletic training.

1.5.1 Acute Immune and Inflammatory responses to high-intensity exercise:

Exercise induces the hypothalamic-pituitary-adrenal (HPA) axis and sympathetic nervous system, with release of adrenocorticotrophic hormone, and subsequent cortisol, adrenaline and noradrenaline (206, 207). Responses vary with intensity, duration and type of exercise (208). There are immune consequences of the HPA response with an acute blood leukocytosis (neutrophils and lymphocytes) likely related to elevated catecholamine levels, and a potentially associated reduction in granulocyte and macrophage oxidative burst capacity. Subsequent and sustained neutrophilia is presumed secondary to increased circulating cortisol (209). Endogenous glucocorticoids demonstrated to promote the maturation of neutrophils in the bone marrow and encourage mobilisation of those cells into the circulation (210), in addition to preventing apoptosis in neutrophils (211). Further effects are seen with NK cell activity transiently decreased, due to cortisol-induced redistribution of blood NK cells to other tissues (212), with effects normalising after a few hours (213). Cortisol elevation also causes suppressed major histocompatibility complex (MHC) II expression and antigen presentation in murine macrophages, suggesting impairment of the antigen presentation process to T lymphocytes and their subsequent response to antigens (214, 215). When adrenaline is infused to simulate levels released in sepsis, TNFα production by monocytes is attenuated in response to lipopolysaccharide (LPS) with adrenaline stimulating IL-10 release (216), implying catecholamines may also be potentially involved in exercise-induced alteration in cytokine levels.

The local release of inflammatory mediators directly from muscle after exercise modulates immune responses (217). Infiltration of muscle tissue by monocytes and neutrophils, whose role is to phagocytose injured tissue, result in signalling and increased numbers of peripheral blood granulocytes and monocytes due to the acute muscle injury (218). In inflammation associated with infection, TNFα and IL-1β subsequently stimulate production of IL-6, resulting in a systemic inflammatory response. After acute exercise in humans, IL-6 is produced locally as an initial response, with IL-6 mRNA upregulation in contracting skeletal muscle (219). This local IL-6 production stimulates circulating blood mononuclear cells to produce anti-inflammatory IL-1ra (220), along with increased anti-inflammatory IL-10, and inhibition of TNFα, creating an anti-inflammatory milieu. Support for local IL-6 production is demonstrated in a study where recombinant human IL-6 or

placebo were infused into volunteers for 3 hours (221). In those who received the IL-6 infusion, there were enhanced plasma levels of IL-1ra and IL-10. There were also increases in cortisol and neutrophils with a reduction in lymphocytes. Plasma adrenaline, and body temperature, heart rate and mean arterial pressure did not change, which the authors concluded indicated that an important function of IL-6 is to limit the potentially negative effects of sustained inflammation (221). The cortisol increase was implicated as likely to be responsible for the changes in neutrophils and lymphocytes. It has therefore been suggested that muscle derived IL-6 has both a role in the anti-inflammatory effect of exercise and in exercise induced leukocyte trafficking (221).

1.5.2 Longer term and resting effects of high intensity exercise in health

The acute neuroendocrine response to exercise is attenuated with training, with a reduced impact from the same absolute workload over time (222). The response to other forms of stressors in trained subjects appears less marked than in untrained individuals, with a lower cortisol response to a standardized psychosocial stressor in more physically active women (223). Also, resting levels of inflammatory markers in endurance athletes are lower when compared to overweight and unfit adults (224) (225, 226). Higher physical activity levels have been associated with a long term reduction in white cell count, CRP, IL-6 and TNFα despite the acute elevation post exercise (227).

1.5.3 Moderate intensity exercise

Moderate intensity exercise improves immune function in the elderly and in young, healthy males, with significant increases in leukocyte, lymphocyte and neutrophil count (228), In some chronic inflammatory conditions, moderate intensity training has demonstrated improved immune function (227), with reduced sickness days, increased salivary IgA (229) and serum immunoglobulins (213). In obese males, moderate intensity continuous training appeared to improve the Th1 mediated immune response pattern, with increased IL-6, IFNγ and leptin (230). In physically active adults, moderate intensity exercise at 70% of $\dot{V}O_2$ peak promoted an anti-inflammatory status with increased IL-10/TNFα ratio(231). Vaccine responses in mouse models (232) and humans (233-235) were improved by moderate intensity training, suggesting potentially beneficial effects. These finding may have relevance in the context of airways hyperresponsiveness and inflammation in asthma; IL-10 is induced by inhaled corticosteroids (236), which have

been demonstrated to reduce airways hyperresponsiveness, inflammation and remodeling in asthma (237). Similarly, a reduction in infection driven exacerbations, well established as triggers and promotors of disease progression in asthma (238), has potential to impact on airways hyperresponsiveness, inflammation and remodelling in asthma.

1.5.4 Interval exercise training

Interest has increased in interval exercise training, as a more rapid way to achieve exercise training gains in a shorter period of time. With regards to immune responses, high intensity interval training comprising a 5 k run at 100% $\dot{V}O_2$peak:rest in a 1:1 ratio demonstrated a higher energy expenditure than the same distance run at a constant rate, with an increase in IL-6 and IL-10 levels and improved IL-10/TNFα ratio in comparison to non-exercised controls (231). It is unclear from this paper whether these data are from serum or plasma samples. Another study of recreational male runners demonstrated that, contrary to evidence from studies with continuous intensity exercise in athletes (229, 239, 240), HIIT increased salivary IgA secretion over a period of 9 training sessions (241). These findings suggest that interval training may be a more appropriate mode of training for upper respiratory tract infection (URTI) prone patients, such as those with airways disease, given salivary IgA's role in mucosal immunity .

1.5.5 Exercise and infections

Upper respiratory tract infection (URTI) is the most frequently occurring infectious disease in man worldwide with an associated large impact on health economics [28], and therefore the impact of any intervention on the occurrence of URTI is important. As a result of this, there has been longstanding interest in the impact of exercise on respiratory infections. The relationship between exercise and URTI is complex, and represents a J shaped curve, with a reduced incidence of URTI with moderate and regular exercise, and increased susceptibility to URTI in both non-exercising and extreme exercise groups (242).

A Cochrane review of the association between exercise and URTI (243) found that the number of days with URTI symptoms significantly reduced with exercise. A prospective study of 1002 adults monitored rate of upper respiratory tract symptoms and self-

reported physical activity and physical fitness. The number of days and severity of URTI symptoms were reduced in those with both high and low levels of self-reported aerobic activity, in comparison to sedentary controls,(244) inferring that any level of exercise is beneficial to some extent.

The only consistent link between exercise modulated immune function and URTI is altered mucosal immunity; with an association between reduced salivary IgA concentrations – a key facet of immune protection and rates of URTI (245). Moderate exercise training is associated with both increased levels of salivary IgA and reduced incidence of URTI symptoms (229). In America's Cup elite sailors, the decline in relative salivary IgA in the three weeks preceding an URTI contributed to URTI risk, with the size of the decrease being of more relevance than actual values (239). The relevance of relative value of IgA rather than absolute may be explained by the variability in salivary IgA between individuals, due to influence of oral health on salivary IgA secretion (246). In elite female tennis players, a higher incidence of URTI was associated with increased training duration and load, and competition level, and a reduction in salivary IgA secretion rate after 1 hour of tennis play (247). Pre-exercise salivary IgA concentration and secretion rate and decline in salivary IgA after 1 hour of intense tennis play were related to the duration and load of training undertaken during the previous day and week (247). URTI rates in extreme athletes are highest post-intensive training or competition (205) rather than following the usual seasonal variation, suggesting the transient, post-acute exercise immunosuppression provides opportunity for pathogens to infect.

It may be that the anti-inflammatory benefit conferred by moderate exercise increases to pathological immunosuppression in extreme exercise, that then results in the increased risk of URTI. This hypothesis is supported by results from a study of young adults involved in regular endurance sports training over winter months (246). This study found that illness prone subjects had ~2.5 times higher IL-4 and IL-10 production, as well as higher training loads, in addition to reduced salivary IgA levels. It is noteworthy that salivary IgA concentrations can be highly variable, with salivary flow rate and seasonal change also impacting on measured levels(246). Despite these issues, the data around infection rate and exercise remain interesting, and saliva provides an easily accessible biological fluid for further investigation in this context (248).

However, most studies are subjective, using self-reported symptoms for diagnosis of URTI. Relevant to this, Cox et al(249) characterised the aetiology of upper respiratory tract symptoms in elite athletes, and found that whilst 89% of the cases were thought by a physician to be viral or bacterial in origin, only 57% were associated with an identified pathogen. Another study found pathogens for upper respiratory tract symptoms in only 30% of the cases (250). Even allowing for inconsistencies in symptom interpretation, exercise appears to influence rates of upper respiratory tract infections through alteration of immune functions and this transient immunosuppression, or 'open window effect', may explain the link between respiratory infections and exercise. The transient post exercise immunosuppression may be of bearing in relation to the effect of exercise in patients with asthma.

The immune response to bacteria is modulated by exercise in a complex pattern. Peripheral blood phagocytosis of bacteria increases with extreme exercise (251), but is conversely reduced in nasal lavage samples (252). Oxidative burst activity (stimulated by bacteria) in marathon runners falls after heavy exertion whilst nasal mucociliary clearance reduces post marathon, and this effect persists for several days (253).

Whilst there is induction of acute phase proteins such as C-reactive protein (CRP), stimulating cytokine release, as described above in section 1.5.1 (254), serum immunoglobulin concentrations are relatively unaffected (255).

1.5.6 Redox adaptations to exercise: a mechanistic explanation?

Increasingly, a redox regulation modification is proposed as a mechanism through which exercise exerts its systemic effects. The capacity of a cell or organism to buffer changes in reductive or oxidative load is intrinsic to overall health, and failure to do so adequately is thought to be contributory at a mitochondrial level to aging and chronic disease processes (256). The 'reactive species interactome' expands on oxidative stress to include reactive nitrogen and sulphur species in maintaining homeostasis within the redox regulatory system (256). In health, the redox buffering capacity of an organism is able to absorb changes in mitochondrial redox status following an external oxidative or reductive load, such as, in the case of asthma, allergenic or irritant stimuli, without any cellular perturbations that result in downstream inflammation. If this capacity is reduced in the context of chronic oxidative stress or chronic inflammation, then the system struggles to

absorb these without cellular upset and a subsequent inflammatory response. This response then further compromises the system with resultant impairment of the redox regulatory system and a cascade of cellular upset and inflammation that drives the chronic inflammatory state central to many diseases (see figure 1.12). In support of this hypothesis, it has been demonstrated that oxidative stress impairs antiviral responses in a model using primary bronchial epithelial cells (PBECs) from asthma patients. Here, treatment of the PBECs with a viral stimulus and an oxidative stressor impaired antiviral signalling in response to viral exposure (257).

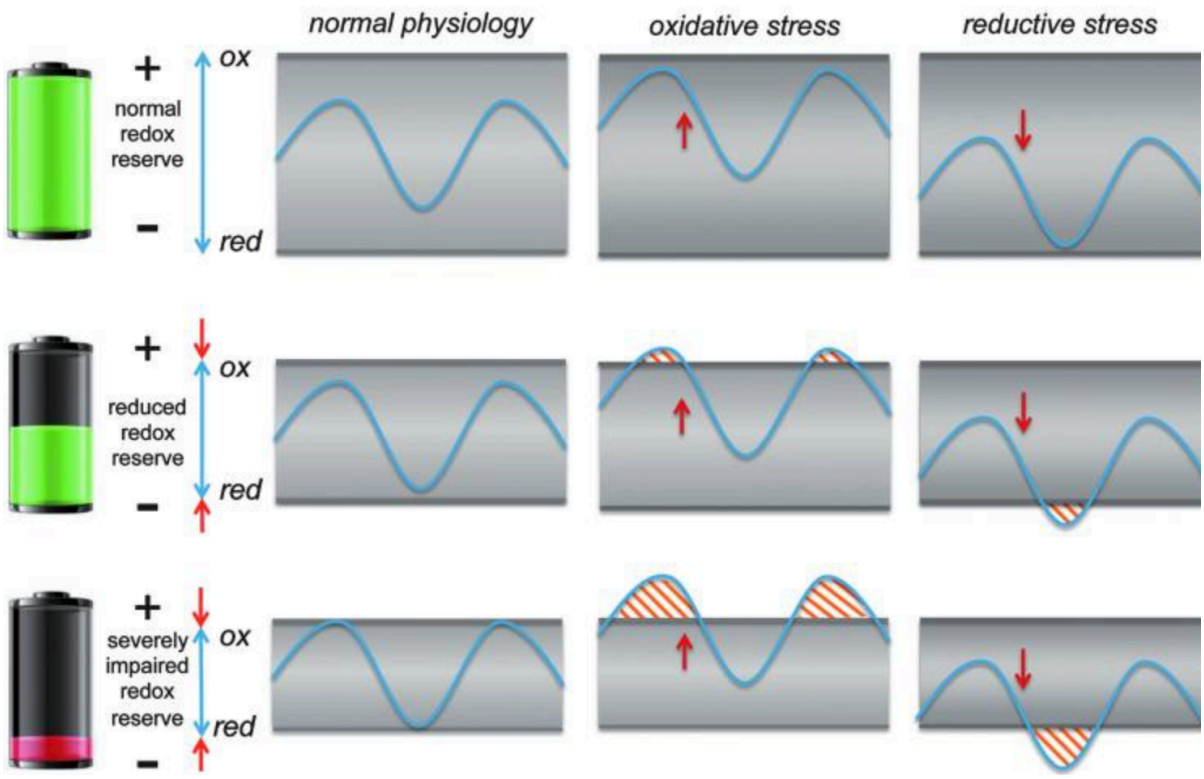


Figure 1.12 Simplified scheme to depict redox buffering,

Simplified scheme to depict redox buffering, which is the capacity of a cell or organism to buffer changes in reductive or oxidative load without impacting on mitochondrial function. In health (upper diagram), adequate reserve (or a full battery) allows redox stressors to be accommodated without cellular pertubations. Increased pressure on the redox buffering system, such as in repeated exposure to allergenic or irritant stimuli in the context of a chronic inflammatory condition, overstretches the capacity of this buffer and results in mitochondrial compromise (middle diagram). This compromise then results in further imparment of the redox buffering system (lower diagram), with the reserve of the cell or oganism depleted beyond its capacity to cope. The intermittent exposure to repeated bouts of controlled oxidative stress in the form of regular exercise is thought to increase the caopacity of the redox buffering system or 'recharge the battery'. Copied from (256).

Exercise is thought to modify this buffering capacity through an increased level of redox reserve, mediated through upregulation of the transcription factor NRF2. Regular exercise has been shown to upregulate NRF2 protein abundance and subsequent levels of NRF2 dependent antioxidant enzymes, which are thought to provide greater capacity to absorb

additional reductive or oxidative stressors. This concept is initially counterintuitive, as acute exercise results in ROS which historically were thought to be damaging. However, there is increasing evidence to suggest that the role of ROS in cellular and immune homeostasis is more complex than it was originally thought to be. ROS may also have a role in protecting cells from ageing and disease related damage (258). This protective role, re-stabilising balance within the redox regulatory system, is through upregulation of NRF2 (259). It is increasingly accepted that upregulation of NRF2 via the repeated oxidative challenge of exercise is the mechanism through which exercise exerts its systemic effects. Repeated 'doses' of oxidative stress from regular bouts of exercise result in upregulation of antioxidant defences via increased NRF2 activity, allowing improved tolerance of and capacity to buffer changes in oxidative load from the broader environment (260). The NFE2L2 gene encodes for the transcription factor NRF2, which is in turn responsible for activating over 500 genes, that are mostly cytoprotective in their function. NRF2 exerts its effect through intra nuclear binding to antioxidant response elements (AREs), which are located in the promotor regions of genes (see figure 1.13).

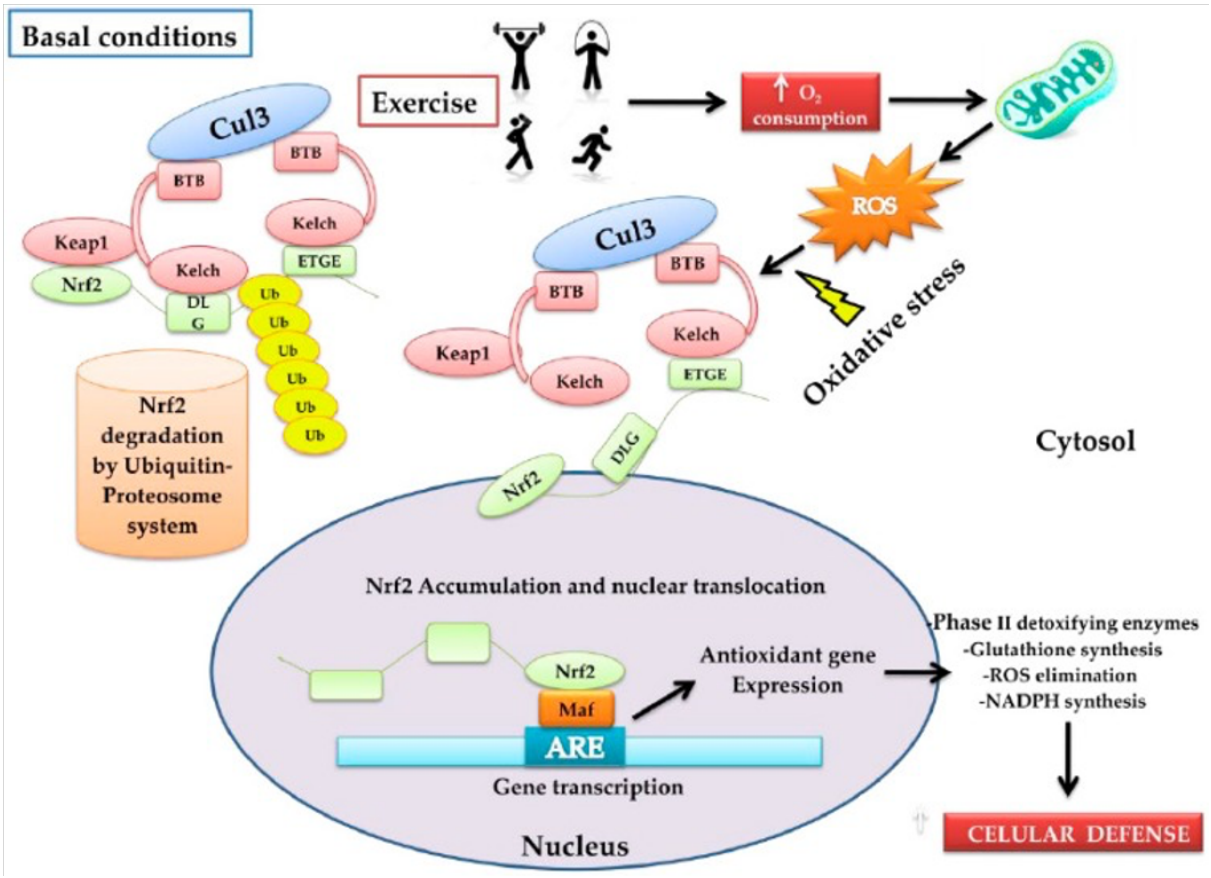


Figure 1.13 Exercise related induction of NRF2

Exercise acts as a form of oxidative stressor which stimulates a conformational change in Keap1, which allows NRF2 to dislocate and accumulate in the nucleus of a cell. Here, it binds to the ARE, to target genes to modulate their responses via upregulation or inhibition of phase 2 detoxifying enzymes, such as HO-1. HO-1 directly inhibits the proinflammatory cytokines as well as activating the anti-inflammatory cytokines, via downregulation of NF-κB and thus leads to balancing of the inflammatory proces; adapted from (260). Abbreviations ARE; antioxidant response element, HO; haemoxygenase, Keap1; Kelch-like ECH-associated protein 1, Maf; musculoaponeurotic fibrosarcoma proteins, NADPH; Nicotinamide-adenine dinucleotide phosphate NRF2; Nuclear factor kappa-light-chain-enhancer of activated B cells, ROS; reactive oxygen species.

There is support for the activation of NRF2 by acute exercise mainly in animal models, with increases in NRF2 gene and protein expression in skeletal muscle (261) and myocardium (262) after a single bout of exercise in mice. In humans, there were

increased NRF2 protein activation in PBMCs after an acute exercise challenge, in both young and older males, although there was impairment in the nuclear import with ageing. In animals, these NRF2 related changes vary with intensity and duration of exercise training, with greater increases demonstrated with interval training. The relevance of these findings to the phenomenon of bronchial smooth muscle hyperreactivity and mucosal inflammation have been demonstrated in a murine model investigating whether NRF2 disruption impacted on susceptibility to airway inflammation (97). Here, NRF2 deficient mice demonstrated increased airway hyperresponsiveness, increase airway inflammation, increased Th2 cytokines and goblet cell hyperplasia following ovalbumin sensitsation and challenge when compared to their wild-type littermates. This increase in Th2 inflammation was accompanied by reduced expression of downstream NRF2 dependent antioxidant genes (97). There does not appear to be any comparison between the anti-inflammatory effect of upregulation of NRF2 expression by exercise versus more traditional, corticosteroid based therapies in animal models. Human data are not sufficient to conclude if the effect of exercise training is similarly dose dependent (260).

1.5.6.1 Assessment of redox status and reserve

An assessment of overall redox reserve is necessary to determine the effect of exercise on redox regulation in asthma. This emerging research combines a number of experiments to form a broader picture of redox status.

1.5.6.1.1 Nitrite, nitrate and other nitrosative species

Nitrite (NO^{2-}) and nitrate (NO^{3-}) are end products of endogenous nitric oxide metabolism, and were initially thought to be inert (263). Nitrite is the reduced form of nitrate and is a non-specific marker of antioxidant activity. Gas-phase chemiluminescence technique allows detection of nitroso species in tissues and biological fluids. The amount of total nitroso species (RXNO) is quantified after sample pre-treatment with acidic sulphanilamide.

The amount of N-nitroso species (RNNO) is quantified after sample incubation with 0.2% $HgCL^2$ and acidic sulphanilamide. S-nitroso species (RSNOs) present in a sample are quantified by calculation of the difference between concentration of total nitroso (RXNO) and N-nitroso (RNNO).

The concentration of RNNOs and RXNOs are determined after reductive cleavage by an iodide/triiodide containing reaction mixture and subsequent determination of the NO released into the gas phase by its chemiluminescent reaction with ozone (O3). NO reacts with O^3 to form nitrogen dioxide (NO^2). A proportion on NO^2 arises in an electronically excited state (NO^{2*}), which, on decay to its ground state, emits light in the near-infrared region and is quantified by a photomultiplier. Provided O^3 is present in excess and reaction conditions are kept constant, the intensity of light emitted is directly proportional to NO concentration.

1.5.6.1.2 Lipid Oxidation

Lipid oxidation is prevalent in food systems, and a major factor in food deterioration resulting in development of toxic compounds. Accumulation of products of lipid oxidation are also important in the pathogenesis of age related and oxidative related diseases (264), and blood lipid profiles have been shown to be associated with childhood asthma (265). Lipid peroxides are unstable indicators of oxidative stress in cells that decompose to form more complex and reactive compounds such as Malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE). Oxidative modification of lipids can be induced in vitro by a wide array of pro-oxidant agents and occurs in vivo during aging and in certain disease conditions. Measuring the end products of lipid peroxidation is one of the most widely accepted assays for oxidative damage. These aldehydic secondary products of lipid peroxidation are generally accepted markers of oxidative stress.

Assessment of thiobarbituric acid reactive substances (TBARS) is widely used to measure this and provide an assessment of lipid peroxidation as a contributor to redox reserve (266), with the Thiobarbituric Acid Reactive Substances (TBARS) assay used for for screening and monitoring lipid peroxidation. MDA forms a 1:2 adduct with thiobarbituric acid.

1.5.6.1.3 The Thiol redox metabolome

A thiol is a compound carrying a sulfhydryl group. They are classified into low molecular weight thiols (e.g. cysteine, homocysteine, and glutathione) and high molecular weight thiols such as protein thiols. Free thiols are those that are in their reduced state, and bound thiols exist in their oxidised state. Dependent on their relative concentrations, thiols define the redox status and reserve of a cell (266). Total thiol status of plasma,

especially thiol groups, are the major determinant of the plasma antioxidant status of the body. Serum protein thiols have been found to be decreased in various disease conditions. Free thiol levels in plasma and urine are determined by spectrophotometric method using dithionitrobenzoic acid (DTNB). DTNB produces a measurable yellow-coloured product when it reacts with sulfhydryl groups. DTNB reacts with a free sulfhydryl group to yield a mixed disulphide and 2-nitro-5-thiobenzoic acid (TNB) and the level of TNB compound is generally measured by absorbance at 412nm using a spectrophotometric detector.

1.5.6.1.4 Ferric Reducing Antioxidant Capacity of Plasma

The ferric reducing antioxidant capacity of plasma (FRAP) assay was first described in the 1990s as a method for assessing overall antioxidant capacity of a biological fluid(267). The principle underlying this assay is based on understanding that the reduction of ferric (Fe^{3+}) to ferrous (Fe^{2+}) ions at low pH results in the formation of an intense, blue-coloured ferrous-tripyridyltriazine complex with a maximum absorption at 593 nm. A standard curve of known concentration of ferrous ions is used to compare with absorbance of the unknown reacted sample at 593 nm.

1.6 Exercise in Asthma

As an introduction to this thesis, I have so far described the role of the lungs in health, and the pathobiology behind the differing phenotypes in asthma. I have described exercise capacity assessment and exercise prescription. I have described the immunobiological responses to exercise in health, and the proposed mechanism of effect via increased resilience of the redox buffering capacity through intermittent exposure to the oxidative stress of exercise. I now move on to link asthma to exercise, through description of the effects of exercise on the inflammatory processes driving asthma, and propose that increased resilience of the redox regulation system may underlie the disease modulating effects of exercise within this disease.

1.6.1 Animal Models

The effect of moderate exercise on inflammation and the immune system has been investigated, using Ovalbumin (OVA) sensitised mice as a model for asthma (see table 1.8 for a summary of the main exercise in asthma OVA model studies and significant

findings). These studies are described with the caveat that, as with any animal model of human disease, the OVA mouse model does not entirely replicate human asthma (268). Benefits of using OVA models include the relatively low cost of the OVA model, and that this model is the most used allergen for animal modelling of asthma. However, OVA does not induce airway inflammation in humans and therefore its utility as an asthma model has been questioned. The pattern of induction of inflammation in asthma animal models differ to the development of human asthma, again raising questions with regards to model suitability (269). Another question is the comparability of exercise responses in animals and humans. Musculoskeletal differences may result in differing tissue responses to a given exercise intervention, and the additional stress of involuntary exercise in animals may confound understanding of the response to voluntary exercise. Voluntary exercise with the use of food reward is often limited to low internsity exercise responses (270). The inclusion of sedentary and exercised non-sensitised groups, in addition to sedentary and exercised sensitised groups in many of these animal studies provide reassurance that the changes demonstrated are not a result of changes in the immune response as a result of ovalbumin sensitisation. Despite these caveats, animal models remain the easiest way to understand the pathophysiology of allergic asthma (269).These OVA mouse models have demonstrated decreases in leukocyte infiltration, cytokine production (IL-4, IL-5, IL-13), adhesion molecule expression, and structural remodelling within the lungs following a four-week period of moderate intensity exercise (271), (272),(17). In addition, Vieira at al (272) and Silva et al(17) demonstrated a reduction in activation of NF-κB with aerobic training. Exercise reduced airway remodelling, mucus synthesis, airway smooth muscle thickness and tissue resistance and elastance, and increased IL-10 and IL-1ra. Silva et al(17) found this change occurred independently of T regulatory cell activity (FoxP3) whereas Lowder et al (273) found that exercise increased levels of FoxP3+ cells in lungs and mediastinal lymph nodes of OVA sensitized mice. Exercise training in this OVA mouse model also demonstrated effects on macrophages, which are known to play a role in modulation of the body's inflammatory response.

Fernandes et al (274) trained OVA sensitised mice at 50% of their $\dot{V}O_2$ max and demonstrated a reduction of eosinophilic infiltration and levels of IL-4 and IL-6 into the lungs with exercise training, with an increase in M1 and M2 macrophages in the lungs, with reduced presence of M2 macrophages only in the lymph nodes. Levels of activated Tregs and were higher and dendritic cells expressed an anti-inflammatory profile in the

sensitized and exercise trained mice, which were postulated as the main source for the increase in IL-10 and TGFβ demonstrated in this group.

A single bout of moderate intensity exercise in OVA sensitised mice causes similar decreases in leukocyte infiltration, including eosinophils, within the lungs (275). There was decreased phosphorylation of the NF-κB p65 subunit and reduced IL-5, IL-13 and prostaglandin E2. There were attenuation of inflammatory chemokines. However, a single bout of exercise had no effect on airway hyper-responsiveness, epithelial cell hypertrophy, mucus production, or airway wall thickening.

Alterations in airway epithelium are important in inflammation and airway remodelling in asthma. Vieira et al (276) investigated the effects of aerobic exercise on airway epithelial cells in OVA sensitized mice, and showed that low intensity aerobic exercise reduced oxidative and nitrosative stress. There was reduced epithelial expression of NF-κB and P2X7R (a plasma membrane receptor involved in control of pro inflammatory cytokine expression) and increased expression of epithelial IL-10. Aerobic exercise decreased the OVA-induced expression of insulin like growth factor (IGF)-1M, vascular endothelial growth factor (VEGF), epidermal growth factor (EGFr) and transforming growth factor (TGF)ß, all growth factors important in airway remodelling in asthma.

When aerobic conditioning was performed before and during sensitisation of OVA mice, rather than after, a similar reduction in inflammation was observed with all timepoints of sensitisation. Exercise prior to and during sensitisation reduced the OVA induced migration of eosinophils and lymphocytes into the airways, and reduced expression of T2 cytokines, as well as expression on ICAM-1, VCAM-1, CCL5, TGFß and VEGF. Airway remodelling and production of allergen specific IgE and IgG-1 were reduced (277), suggesting exercise may be protective to subsequent inflammatory insults.

Table 1.8 Summary of findings from animal studies

Abbreviations: OVA: ovalbumin; BAL: bronchoalveolar lavage; $\dot{V}O_2$peak: peak oxygen uptake; IL: interleukin; Ig: immunoglobulin; NF-κB: nuclear factor-κB; VCAM: vascular cell adhesion molecule; PGE2: prostaglandin E2; AT: anaerobic threshold; Treg: regulatory T-cell; Th: T-helper; cysLT: cysteinyl leukotriene; LTB4: leukotriene B4; LTC: leukotriene synthase; LTA4H: leukotriene A4 hydrolase; AHR: airway hyperresponsiveness; TGF-β: transforming growth factor β; ICOSL: inducible T-cell co-stimulator ligand.

Study	Mouse model	Intervention	Significant Outcomes
Pastva A, Estell K, Schoeb TR, Atkinson TP, Schwiebert LM J Immunol. 2004;172(7):4520-6	N=6-9 female BALB/cJ mice; sedentary and nonsensitized (S), sedentary and OVA sensitized (SO), exercised and non-sensitized (E), and exercised and OVA sensitized (EO)	Thrice weekly 45-minute sessions for 4 weeks of aerobic exercise at 50% $\dot{V}O_2$ peak	Reduced BAL IL-4,5 and OVA specific IgE Reduced chemokine production Reduced lung cellular infiltrate, mucus production, and epithelial hypertrophy decreased both the phosphorylation of IκBα and translocation of NF-κB subunit p65
Pastva A, Estell K, Schoeb TR, Schwiebert LM Brain Behav Immun. 2005;19(5):413-22.	Female BALB/cJ mice split into 6 groups; sedentary and nonsensitised (S), sedentary and OVA-sensitized with placebo (SO/placebo), sedentary and OVA-sensitized with RU486 treatment exercised and nonsensitised (E), exercised and OVA-sensitized with placebo (EO/placebo), and exercised and OVA-sensitized with RU486	Thrice weekly 45-minute sessions for 4 weeks of aerobic exercise at 50% $\dot{V}O_2$ peak	RU486 blocked the exercise-induced reductions in cellular infiltration of the airways KC and soluble VCAM-1 protein levels in the bronchoalveolar lavage fluid and NF-κB translocation and DNA binding within the lung to levels similar to those observed in sedentary OVA-sensitized mice
Vieira, R. P. Claudino, R. C. Duarte, A. C. Santos, A. B. Perini, A. Faria Neto, H. C. Mauad, T. Martins, M. A. Dolhnikoff, M. Carvalho, C. R. Am J Respir Crit Care Med Vol 176. pp 871–877, 2007	N= 8 per group ovalbumin sensitised male BALB/c mice vs sensitised and non-sensitised controls	Low intensity training at 50% maximal speed and moderate intensity at 75% maximal speed for 60 minutes 5 x week for 28 days	Reduced airway wall and BAL eosinophils in both exercised groups Decreased peribronchial density of cells positive for IL-4,5, and increased IL-10 Reduced airway wall collagen and elastin fibres in airways Normalisation of bronchoconstriction index
Hewitt M, Creel A, Estell K, Davis IC, Schwiebert LM American journal of respiratory cell and molecular biology. 2009;40(1):83-9.	Female BALB/cJ mice	Single 45-minute bout of aerobic exercise at 50-75% $\dot{V}O_2$ peak	decreased leukocyte infiltration, including eosinophils decreased phosphorylation of the NF-κB p65 subunit decreased IL-5, IL3 and PGE2
Silva RA, Vieira RP, Duarte AC, Lopes FD, Perini A, Mauad T, et al. The European respiratory journal. 2010;35(5):994-1002.	N=7 male BALB/c mice in 4 groups; control; AT; ovalbumin (OVA); and OVA+AT	5x 60-minute sessions for 4 weeks at 50% $\dot{V}O_2$ peak	increase of IgE and IgG reduction of eosinophils, CD3+, CD4+, IL-4, IL-5, IL-13, NF-κB, airway remodeling, mucus synthesis, smooth muscle thickness and tissue resistance and elastance increase in IL-10 and IL-1ra independently of Foxp3

Study	Mouse model	Intervention	Significant Outcomes
Lowder, T. Dugger, K. Deshane, J.Estell, K. Schwiebert, L. M Brain Behav Immun. 2010 Jan;24(1):153-9.	female C.Cg-Foxp3tm2Tch/J reporter (Foxp3+ reporter) mice bred on a Balb/cj background	45 mins 3 x week for 4 weeks at 50-75% maximum O_2 consumption	Reduced BAL macrophages, eosinophils and lymphocytes Enhanced the suppression function of CD4+CD25+Foxp3+ Treg cells
Vieira, R. P. Toledo, A. C. Ferreira, S. C. Santos, A. B. Medeiros, M. C. Hage, M. Mauad, T. Martins Mde, A. Dolhnikoff, M. Carvalho, C. R. Respir Physiol Neurobiol. 2011 Mar 15;175(3):383-9	N= 8 per group ovalbumin sensitised male BALB/c mice vs sensitised and non-sensitised controls	Low intensity training 60 minutes 5 x week for 28 days	Reduced total cells and eosinophils in BALF Reduced % of goblet cells in airway walls Reduced epithelial expression of IL-4,5, IL-13, and increased IL-10 Reduced inducible nitric oxide synthase, and other markers of oxidative and nitrosative stress Reduced NF-kB and P2X7 receptor
Silva AC, Vieira RP, Nisiyama M, Santos AB, Perini A, Mauad T, et al. International journal of sports medicine. 2012;33(5):402-9.	N=8 male BALB/c mice in 4 groups; Control (non-trained and non-sensitized); AC (aerobic conditioning and non-sensitized); OVA (non-trained and OVA-sensitized); and OVA + AC (aerobic conditioning and OVA-sensitized)	60 minutes 5 days per week for 8 weeks at 50% $\dot{V}O_2$ peak prior to and during OVA sensitisation	Inhibition of OVA-induced migration of eosinophils and lymphocytes to the airways, reduced IgE and IgG1 titres inhibition of Th2 cytokines
Dugger KJ, Chrisman T, Jones B, Chastain P, Watson K, Estell K, et al. Brain Behav Immun. 2013;34:67-78.	female wild type mice on a BALB/c split into 4 groups; *Sedentary* OVA-sensitized (SO); *Exercised* OVA-sensitized (EO); *Sedentary* non-sensitized (S); and *Exercised* non-sensitized (E).	45 minutes thrice weekly for 4 weeks at 13.5 m min^{-1}	Surface expression levels of lung-homing chemokine receptors were comparable across groups. Lung-derived Th cells from exercised OVA-sensitized mice exhibited decreased migratory function versus controls; Th cells from exercised mice are less responsive to lung-homing chemokines.
Bruggemann TR, Avila LC, Fortkamp B, Greiffo FR, Bobinski F, Mazzardo-Martins L, et al.	N=8 male Swiss mice per group; control swimming OVA-sensitized and OVA-sensitized + swimming	30 minutes high intensity swimming for 3 weeks	decreased OVA-increased total IgE, IL-1, IL-4, IL-5 and IL-6 levels, total cells, lymphocytes and eosinophils in bronchoalveolar lavage fluid, increased IL-10 and glutathione levels Increased glutathione peroxidase and catalase in the swimming only group
Alberca-Custodio RW, Greiffo FR, MacKenzie B, Oliveira-Junior MC, Andrade-Sousa AS, Graudenz GS, et al. Frontiers in immunology. 2016;7:237.	N=16 per group; Control, Exercise (Exe), OVA, and OVA + Exe groups	60 minutes 5x week for 4 weeks	decreased eosinophils neutrophils lymphocytes and macrophages in BAL Decreased eosinophils lymphocytes and macrophages in airway walls. Reduced collagen elastic fibers, mucus production, and smooth muscle thickness Reduced IL-5, IL-13 , CysLT , LTB4 in BAL. Reduced 5-LO, LTA4H, $CysLT_1$ receptor, $CysLT_2$ receptor, LTC4 synthase, and BLT2 expression by peribronchial leukocytes and airway epithelium. Reduced AHR to Methacholine
Fernandes P, de Mendonca Oliveira L, Bruggemann TR, Sato MN, Olivo CR, Arantes-Costa FM.	N=10 BALB/c mice in 4 groups; Control, Exercise (EX), OVA, and OEX group	5 weeks exercise for 60 minutes 5 x week at 50% $\dot{V}O_2$ peak	Increased IL-10 and TGF-β Increased recruitment of M2 in the lungs, influx and activation of

Study	Mouse model	Intervention	Significant Outcomes
Frontiers in immunology. 2019;10:854			regulatory T cells (Tregs) and CD4 and CD8 lymphocytes Decreased pro-inflammatory common dendritic cells' expression of co-stimulatory molecules Increased anti-inflammatory ICOSL in plasmacytoid dendritic cells

1.6.2 Human Studies

There are fewer human studies in this field, which tend to focus on improvements in clinical outcomes rather than mechanistic understanding behind these. This reliance on symptom scores without mechanistic data to support increases the risk of subjective bias, and confounding from the more generalised effects of exercise as opposed to asthma specific effects. In atopic, asthmatic, school aged children, a 12-week moderate intensity exercise programme reduced mite specific IgE, with no change in FeNO, blood eosinophil levels or serum CRP (278). A further study examined moderate to severe asthmatics who completed a twice-weekly exercise programme of 3 months' duration, with a control group of matched patients undergoing a breathing training programme. Here, induced sputum eosinophil counts fell in the training group after exercise, as did FeNO, with greatest reductions seen with highest baseline levels (279). Asthma symptoms reduced in the training group (279). Others have shown that aerobic training at moderate intensity reduces bronchial hyperreactivity, with reduced IL-6 and monocyte chemoattractant protein 1 (MCP-1). Quality of life, as measured by the Asthma Quality of Life Questionnaire (AQLQ) results and asthma exacerbation rates also improved (6). The effects of exercise and weight loss were explored in a randomised control trial of obese asthmatic adults; the combined programme demonstrated an improvement in clinical symptoms and aerobic capacity, accompanied by weight loss. There were also reductions in FeNO, CCL2, IL-4, TNFα and leptin, with increased levels of vitamin 25(OH)D, IL-10 and adiponectin (5). However, it is impossible in this study to tease out whether the improvement in inflammatory parameters were a result of the reduction in inflammation from adipose tissue or as a result of disease modulation of asthma. High intensity intermittent exercise training without strength training has been looked at in asthmatic patients (280), and shown to be beneficial in terms of symptom scores as assessed by Asthma Control Questionnaire (ACQ) and AQLQ but did not demonstrate significant changes in anti-inflammatory parameters. In another context, Del Giacco et al (281)

monitored a professional football team across a season, and found that blood NK absolute count and percentage increased in both atopic and non-atopic athletes, and IL-2 and IL-4 production reduced. The most marked reduction in IL-4 was seen in atopic individuals suggesting that the immunological mechanisms observed in the short term in murine models and humans are translatable to real life, longer-term situations, although mechanistic data in asthmatic humans are lacking.

Table 1.9 Adult human exercise intervention studies

Adult human exercise intervention studies, type of exercise intervention and their significant outcomes, adapted from (282); FEV1: forced expiratory volume in 1 s; FVC: forced vital capacity; PEF: peak expiratory flow; FEF25–75%: forced expiratory flow at 25–75% of FVC; MVV: maximum voluntary ventilation; BMI: body mass index; V'O2max: maximal oxygen uptake; QoL: quality of life; FeNO: exhaled nitric oxide fraction; 6MWT: 6-min walk test; RPE: rate perceived exertion; AQLQ: Asthma Quality of Life Questionnaire; ICS: inhaled corticosteroid; IL: interleukin; MCP-1: monocyte chemoattractant protein 1; WR: work rate; $\dot{V}O_2$ peak: peak oxygen uptake; RM: repetition maximum; OSA: obstructive sleep apnoea; ACT: Asthma Control Test; ACQ: Asthma Control Questionnaire; TNF: tumour necrosis factor; HDM: house dust mite; HR: heart rate.

Study	**Patient demographics**	**Intervention**	**Significant Outcomes**
Farid, R. Azad, F. J. Atri, A.E. Rahimi, M. B. Khaledan, A. Talaei-Khoei,M. Ghafari, J.Ghasemi, R. Iranian Journal of asthma and immunology Vol. 4, No. 3, September 2005	N= 18 vs 18 control Mean age 29 Exercise induced asthma Atopic	3 x weekly 20 minutes aerobic exercise for 8 weeks	Improved FEV1, FVC, PEF, FEF 25-75% and MVV
Mendes FA, Almeida FM, Cukier A, Stelmach R, Jacob-Filho W, Martins MA, et al. 2011;43(2):197-203	N=34 vs 34 control Median age 34 Median BMI 25.8 Moderate or severe persistent asthma	2 x 30-minute training session on indoor treadmill for 3/12 60-80% $\dot{V}O_2$ max	Reduced Feno Reduced sputum total and eosinophil counts Increased no of symptom free days Improved $\dot{V}$O2 max
Mendes, FA., Gonçalves, RC., Nunes, MPT., Saraiva-Romanholo, BM., Cukier, A., Stelmach, R., Jacob-Filho, W., Martins, MA, Carvalho, CRF. CHEST 2010; 138(2):331–337	N=44 VS 45 control Median age 39 Median BMI 25.2 Moderate or severe persistent asthma	2 x 30-minute training session on indoor treadmill for 3/12 60-80% $\dot{V}O_2$ max	Improved $\dot{V}$O2 max Improved asthma related QoL Reduced Beck Depression Inventory Reduced State-Trait Anxiety Inventory Scores
Turner, S.., Eastwood, P. Cook, A. Jenkins, S. Respiration 2011;81:302–310	N=19 VS 15 control Mean age 71 Mean BMI 26.8 Moderate to severe asthma with fixed airflow obstruction	1 x weekly 20 minutes walking at 80% of average walking speed on 6MWT Circuit training based on Borg RPE scale	Improved AQLQ activity and symptom domains
Scott HA, Gibson PG, Garg ML, Pretto JJ, Morgan PJ, Callister RClin Exp Allergy. 2013;43(1):36-49.	N=10 exercise vs 15 dietary intervention vs 13 diet and exercise Mean BMI 33.7	Personal training 60 minutes per week and visit gym 3 x week for 12 weeks	Improved symptoms with diet and combined Improved QoL with all 3 interventions Reduced sputum eosinophils with exercise Reduced BMI

Study	Patient demographics	Intervention	Significant Outcomes
Franca-Pinto A, Mendes FA, de Carvalho-Pinto RM, Agondi RC, Cukier A, Stelmach R, et al. Thorax. 2015;70(8):732-9	N =22 vs 21 control Mean age 40 Mean BMI 26.5 91% atopy Mean maintenance ICS 909 Moderate-severe asthma	2 X weekly 35 minutes (25-minute training, 5 mins warm up/cool down) 'vigorous' aerobic training programme on an indoor treadmill for 3 months	Improved bronchial hyperreactivity by 1 doubling dose Reduced serum IL-6 and MCP-1
Freitas PD, Ferreira PG, Silva AG, Stelmach R, Carvalho-Pinto RM, Fernandes FL, et al. 2017;195(1):32-42.	N=28 vs 27 control Mean age 45.9 Mean BMI 38.1 Obese participants	2 x weekly constant WR aerobic training 50-75% $\dot{V}O_2$ peak and resistance training 60% 1 RM	Reduced FeNO Improved FEV1/FVC Improved ACQ score Decreased serum CCL2, IL-4, IL-6, TNF α, leptin Increased Vit D, adiponectin, IL-10
Turk Y, van Huisstede A, Hiemstra PS, Taube C, Braunstahl GJObes Surg. 2017;27(11):3055-60.	N=44 vs 30 controls Obese mean BMI 44.75	Thrice weekly 40-60 minutes high intensity interval training for 12 weeks	Improved asthma control at surgery Improved asthma related quality of life
Candemir I, Ergun P, Kaymaz D. Wien Klin Wochenschr. 2017;129(19-20):655-64.	N=35 Mean age 45	30 minutes sessions thrice weekly for 8 weeks at 85% $\dot{V}O_2$ peak plus quadriceps resistance training	Improved ACT Reduced BMI in obese participants Reduced emergency admissions and hospitalisations
Toennesen LL, Meteran H, Hostrup M, Wium Geiker NR, Jensen CB, Porsbjerg C, et al. The journal of allergy and clinical immunology In practice. 2017	N=29 exercise; 29 diet and exercise, 33 diet, 34 control Mean age 38.2-43.7 Mean BMI 24.9-26.1 72-85% atopy 59-76% ICS	2-5 repeats of 5-minute interval training consisting of 1. 30 secs @30 $\dot{V}O_2$ max 2. 20 secs @ 60% $\dot{V}O_2$ max 3. 10 secs @ 90% $\dot{V}O_2$ max	Improved ACQ
Freitas PD, Silva AG, Ferreira PG, A DAS, Salge JM, Carvalho-Pinto RM, et al Med Sci Sports Exerc. 2018 Jul;50(7):1367-1376	N=28 vs 27 control Mean age 45.9 Mean BMI 38.1 Obese participants	2 x weekly constant WR aerobic training 50-75% $\dot{V}O_2$ peak and resistance training 60% 1 RM	Greater proportion of patients with improved depression symptoms Lower risk of developing OSA Improved sleep quality
Prossegger J, Huber D, Grafetstätter C, Pichler C, Braunschmid H, Weisböck-Erdheim R, Hartl A Int J Environ Res Public Health. 2019 Jun 8;16(11)	N=18 exercise, n=24 control Mean age 40.6 HDM sensitisation	Four 3–5 h hiking/snow-shoe tours with an average altitude difference of 411 m and 11 km in distance per day and four all day skiing sessions with an average of 42 km in ski slopes in three different ski regions (2000–2500 m)	Improved FeNO Reduced nasal eosinophil count Improvement in allergic symptoms

<table>
<tr><th>Study</th><th>Patient demographics</th><th>Intervention</th><th>Significant Outcomes</th></tr>
<tr><td rowspan="4">Saxer, S. Schneider, S. R.Appenzeller, P. Bader, P. R. Lichtblau, M. Furian, M.
Sheraliev, U. Estebesova,B.
Emilov, B. Sooronbaev, T.Bloch, K. E. Ulrich, S. BMC Pulmonary Medicine (2019) 19:134</td><td colspan="3">N= 24 vs 24 control</td><td rowspan="4">5 x weekly 30-45-minute guided walks, endurance training, strength training and education for maximum 5 hours per day for 3 weeks at either 760m above sea level or 3100m above sea level</td><td rowspan="4">ACQ in both groups
PEF variability in both groups</td></tr>
<tr><td></td><td>Low altitude</td><td>High altitude</td></tr>
<tr><td>Mean age</td><td>47</td><td>43</td></tr>
<tr><td>Mean BMI</td><td>24</td><td>26</td></tr>
<tr><td>Jaakkola, J. J. K. Aalto, S. A. M. Hernberg, S.Kiihamaki, S. P. Jaakkola, M. S. Scientific RepoRtS | (2019) 9:12088</td><td colspan="3">N= 44 vs n= 45 control
Mean age 39.7
Mean BMI 24.97</td><td>3 x weekly 30 minutes aerobic exercise at 70-80% maximal HR for 24 weeks</td><td>Improved ACT score</td></tr>
<tr><td>Evaristo et al., JACI in practice 2020, Vol. 8, Iss. 9, (Oct 2020): 2989-2996.e4.6</td><td colspan="3">N=29 vs n=25 breathing group</td><td>12 week intervention of twice weekly aerobic training at 60% of heart rate recovery for 30 minutes vs 12 weeks of breathing exercises based on the Pranayama yoga breathing technique</td><td>Improved ACQ and AQLQ in both groups
Aerobic group 2.6 times more likely to experience improved clinical control at 3 months and more likely to experience days without need for rescue inhalers</td></tr>
</table>

Table 1.10 Exercise interventions in children with asthma

Exercise interventions in children with asthma copied from (282); AT: anaerobic threshold; $\dot{V}O_2$peak: peak oxygen uptake; OCS: oral corticosteroids; pAQLQ: paediatric Asthma Quality of Life Questionnaire; Ig: immunoglobulin; BMI: body mass index; FVC: forced vital capacity; FEV1: forced expiratory volume in 1 s; 6MWT: 6-min walk test

Study	**Patient demographics**	**Intervention**	**Significant Outcomes**
Counil, F. P. Varray, A. Matecki, S. Beurey, A. Marchal, P. Voisin, M.Prefaut, C. Journal of Pediatrics, Elsevier, 2003, 142 (2), pp.179-184. 10.1067	N= 9 vs 7 control Mean age 13 Atopic with bronchodilator reversibility	3 x weekly 45 minutes 1-minute sprint at work rate at AT followed by 4 minutes recovery cycling	Improved $\dot{V}O_2$ peak and AT
Moreira A, Delgado L, Haahtela T, Fonseca J, Moreira P, Lopes C, et al. Eur Respir J. 2008;32(6):1570-5	N = 17 vs 17 control Children aged 12.7 (+/- 3.5 years) Atopic On maintenance OCS	50 minute twice weekly training session for 3 months 30-35 mins submaximal training	Improved pAQLQ Reduced total IgE and mite specific IgE
Onur, E. Kabaroglu, C. Gunay, O. Var, A. Yilmaz, O. Dundar, P. Tikiz, C. Guvenc, Y. Yuksel, H. Allergol Immunopathol (Madr). 2011 Mar-Apr;39(2):90-5	N = 15 with 15 asthmatic controls and 15 healthy controls	8-week exercise training intervention	Decreased oxidant stress markers Increased antioxidant enzyme activity Improved lung function
Willeboordse, M. van de Kant, K. D. G. Tan, F. E. Mulkens, S. Schellings, J. Crijns, Y. Ploeg, Lv van Schayck, C. P. Dompeling, E. PLoS One. 2016; 11(6): e0157158.	N=43 with 44 control Median age 12.3 Median BMI 25; 53% of group obese	60 minutes 2 x week at 60-75% maximal heart rate for 6 months	Improved asthma control and quality of life, reduced weight and improved FVC
Lu, K. D. Cooper, D. Haddad, F. Zaldivar, F. Kraft, M. Radom-Aizik, S. Pediatr Res. 2017 Aug;82(2):261-27	N=12 vs 14 healthy adolescents Mean BMI 22	Acute exercise challenge followed by 60 minutes thrice weekly for 8 weeks	Reduced glucocorticoid receptor expression in several lymphocyte subsets after an acute challenge and 8 weeks training

Study	Patient demographics	Intervention	Significant Outcomes
Abdelbasset, W. K. Alsubaie, S. F. Tantawy, S. A. Abo Elyazed, T. I. Kamel, D. M. Patient Prefer Adherence. 2018 Jun 15;12:1015-1023	n=19 with 19 control Children aged 8-12 years with moderate persistent asthma	40 minute thrice weekly training sessions at 50-70% maximum heart rate for 10 weeks	Improved FEV1 and FVC Improved $\dot{V}O_2$ peak Improved 6MWT
Lucas, J. A. Moonie, S. Hogan, M. B. Evans, W. N. Public Health. 2018 Jun;159:123-128	86 asthma 142 non asthmatic obese mean BMI percentile 98.14 Mean age 11.26	40 minutes physiologist designed exercise 1 x weekly	Reduced weight and improved $\dot{V}O_2$ peak

Consistent with these early findings, a systematic review of the effect of physical training on airway inflammation in asthma concluded that whilst some studies suggest physical training may reduce airway inflammation in asthmatics, more information was necessary (283). However, the majority of these studies address symptomatology as a primary outcome with less attention to objective measures such as changes in inflammation or bronchial hyperresponsiveness. In addition, by definition of the intervention or treatment, these studies are not single or double blinded. There have been attempts at a control group in terms of a sham intervention (284), but this cannot provide the same comparability as a fully blinded study. This problem has been discussed in the literature, and an extension to the CONsolidated Standards Of Reporting Trials (CONSORT) Statement, the Consensus on Exercise Reporting Template (CERT) has been recently developed (285). This may provide a useful additional structure to increase comparability across exercise intervention research. A recent review of the design and conduct of exercise training interventions concluded that explorative or uncontrolled trials were acceptable only in pilot or feasibility studies, or to determine baseline variability of the primary endpoint (286). A crossover trial with an adequate wash out period may be a method through which to evaluate exercise interventions, but determination of an adequate washout period for a specific training intervention may not be available, particularly with regard to immunological modification (286).

1.6.3 In search of a mechanistic link

The mechanisms of how exercise exerts anti-inflammatory effects in asthma remain unclear. Similarly to the immunomodulatory effects of exercise in health, reduced NF-κB activation (271), increases in glucocorticoid receptor expression(287), increases in the regulatory T cell forkhead box P3 (FoxP3), (273) (which is specifically expressed on $CD4^{+}CD25^{+}$ and plays a role in the suppression of autoimmunity (288)), T cell trafficking in response to an allergen challenge(289) and increased IL-10 and IL-1ra have been proposed. These theories are mainly based on animal work (see figure 1.14) (272) (17).

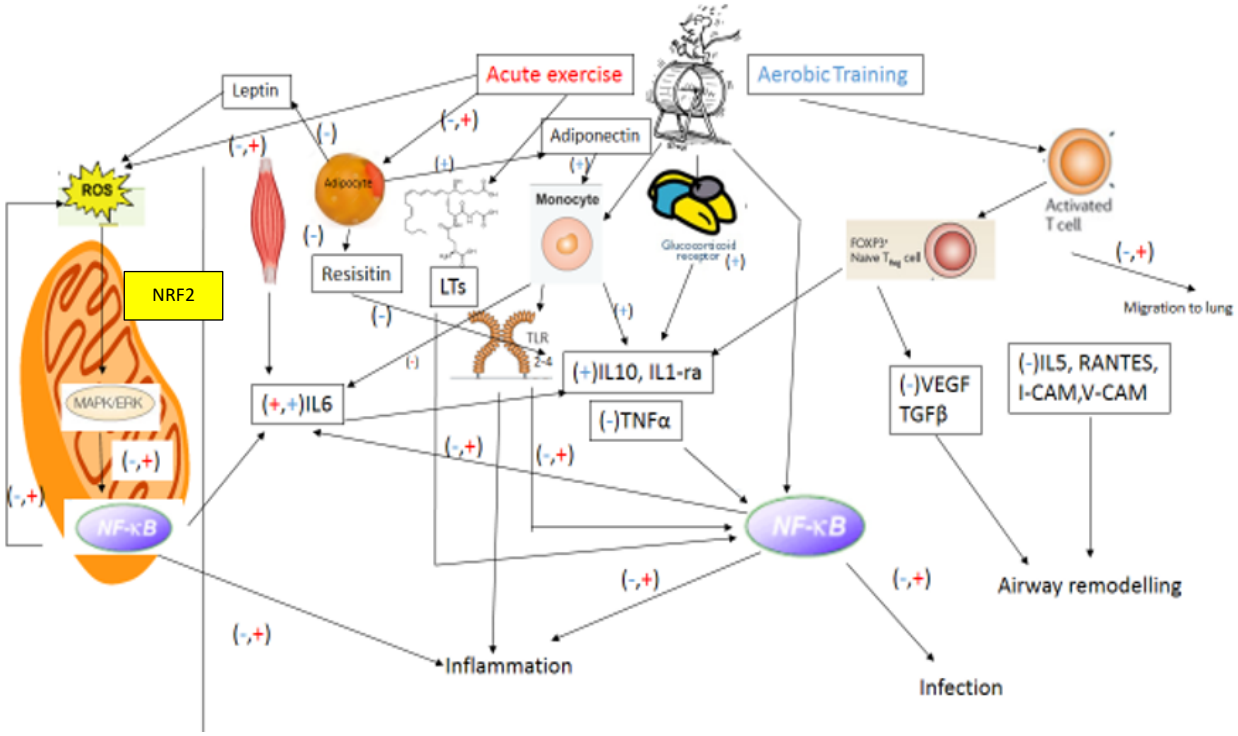


Figure 1.14 Proposed mechanisms of anti-inflammatory effect of exercise in asthma

Proposed mechanisms of anti-inflammatory effect of exercise in asthma demonstrating interaction between anti and pro-inflammatory cytokines released as a result of exercise and the role of adipokines and reactive oxygen species in this interaction. Abbreviations FoxP3; forkhead box P3, I-CAM; intercellular adhesion molecule, IL; interleukin, NF-κB, NRF2; RANTES; ROS; reactive oxygen species TGFß; Transforming growth factor beta VCAM;vascular cellular adhesion molecule VEGF; vascular endothelial growth factor

Alberca Custodio et al., (290) looked specifically at whether moderate intensity exercise modulates the leukotriene pathway in OVA mice. They found that moderate aerobic

exercise reduced eosinophilic inflammation, with reduced eosinophils in BAL and airway walls, and reduced IL-5 and IL-13 in BAL, with reduced total numbers of cysLTs in the BAL and airway epithelial cells of exercised OVA mice, as well as reduced airway hyperresponsiveness. As NF-κB is activated by cysLTs, this provides support for the role of NF-κB and cysLTs as a mechanism for reduced inflammation in exercise.

Pastva et al (287) treated exercised OVA mice with the glucocorticoid receptor antagonist RU486. In this study, exercise-induced reduction in airway infiltration, and bronchoalveolar lavage levels of KC and VCAM-1 were reduced in the presence of RU486 to levels of sedentary mice, as were NF-κB translocation and DNA binding within the lung, and exercise induced increases in Glucocorticoid Receptor nuclear translocation. Silva et al(291) found that the OVA induced reduction in glucocorticoid receptor expression was attenuated at day 3 of exercise training. Exercise training reversed the OVA induced increase in the expression of NF-κB at 7 days, with increased expression of IL-10 and IL-1ra, and no change in the expression of FoxP3. Eosinophil migration into the airways, and expression of intercellular adhesion molecule (ICAM)-1 and vascular cellular adhesion molecute (VCAM)-1 in the exercised OVA group reduced at this time point, as did IL-4, IL-5, exotoxin, CCL5, vascular VEGF and TGFß. These data suggest that glucocorticoid receptor changes may initiate the anti-inflammatory changes induced by exercise.

Human studies investigating glucocorticoid responsiveness in children with difficult to treat asthma demonstrated greater oxidation of the glucocorticoid receptor that promotes post translational modification and may impair receptor function(110). This role for redox regulation in the pathogenesis of asthma and the potential mechanism through which exercise training exerts its anti-inflammatory effect correlates with results from murine asthma models of exercise training as described above, which also implicate altered glucocorticoid receptor function in the mechanism of improvement in inflammation with exercise (287, 291).

Colleagues in Southampton have previously described and reviewed the concept of the ‘reactive species interactome’ as a more complete way of conceptualising the redox regulatory system (256). The ‘reactive species interactome’ describes the interactions between reactive oxygen species with reactive nitrogen and sulphur species that facilitate sensing and adaptation to environmental stimuli and stressors at a cellular level (256). It may be that regular exposure to oxidative stress in the form of exercise training increases

resilience to inflammatory stimuli through adaptive increased tolerance of oxidative stress. An exploratory study has demonstrated changes in components of the reactive species interactome with exercise as a stressor at sea level and altitude (292), but data in humans with asthma are otherwise lacking.

These anti-inflammatory changes may all be downstream results of upregulation of NRF2, with its associated increase in redox buffering driving a reduction in cellular upset and inflammation as a result of external stressors in asthma. There is suggestion that regular exercise exposure upregulates NRF2 activity in middle aged women with asthma, and that in those who did not regularly exercise, an acute exercise upregulated both NRF2 and reduced FeNO four hours post exercise. The authors propose that the reduction in FeNO was attenuated acutely in those who exercise regularly as they were benefitting from the sustained anti-inflammatory response to regular exercise (293).

Further clarification of the mechanisms behind changes in inflammatory parameters and symptom improvements in patients with asthma are needed, to allow improved targeting of exercise programmes to best clinical effect.

1.7 Barriers to Exercise in Asthma

This thesis has discussed the potential benefits of exercise in asthma, with reviews concluding that exercise appears to have beneficial effects on symptom control, inflammation and lung function in patients with sub optimally controlled asthma (79). In addition, exercise is recommended in national and international guidelines for asthma management (11, 84). Despite this, physical activity levels in patients with severe asthma remain impaired (294). Patients with difficult and severe asthma comprise a small proportion of all patients with asthma. However, they are disproportionately more likely to demonstrate poorly controlled symptoms and inflammation on optimised treatment regimens. This poor control drives a significant proportion of healthcare costs (295). In related disease areas, such as COPD, exercise interventions are being offered at scale using novel technologies that could be harnessed for prevalent diseases such as difficult asthma (296). Understanding of the barriers to exercise is crucial in increasing activity in patients with difficult asthma, and in implementing a successful exercise training programme to improve their health outcomes (297).

In the general population, reasons for physical inactivity are due to a combination of insufficient leisure time for physical activity and increased mechanisation of occupational and domestic activities (153). In patients with asthma there may be additional disease related barriers to exercise. Understanding these may facilitate design of an exercise intervention.

Alongside patients with severe asthma, patients with relatively mild disease have also been shown to avoid physical activity because they are concerned about triggering symptoms (298). However, asthma severity, as assessed by FEV1 and methacholine challenge, were not predictive of $\dot{V}O_2$ (maximal oxygen uptake) peak as a marker of aerobic fitness. These findings suggest that disease severity does not determine fitness in asthma patients who manage to overcome perceived barriers to exercise and undertake regular physical activity (298, 299). Relatively few studies have investigated the barriers and facilitators to exercise and physical activity in asthma. However, those which have focus predominantly on adolescents. This is partly because asthma tends to affect younger populations at a time when they should be establishing healthy lifestyles. This is therefore a critical point for intervention to encourage long-term adoption of physical activity (300). Whilst qualitative studies suggest healthy participants and asthma patients think exercise is beneficial (301), a study of elementary school teachers demonstrated few were aware that students with asthma need not avoid exercise (302). Other barriers have also been identified that prevent this group of patients in engaging with physical activity: lack of time is more likely to be reported as a barrier in younger patients (301) and fear of exacerbating symptoms is a common theme amongst adolescents (303) and adults (301), with patients with more severe disease more likely to view exercise as detrimental. Intensity of physical activity undertaken by asthma patients has been shown to be positively correlated with peak expiratory flow (304). Although causation could not be determined in this cross-sectional study, it raises the question as to whether those with less severe disease are able to undertake more activity or whether those who undertake more activity are able to modulate their disease burden, as supported by findings in a recent review (305). Obesity and musculoskeletal problems, conditions that are common in asthma and exacerbated by oral steroid therapy, were also listed as a reason for not exercising, as were extreme weather conditions (301). Facilitators included the desire to be healthy and encouragement from a motivated companion or physician. Lifestyle activities have been shown to be more acceptable to patients as a way to

increase their physical activity levels (301). In terms of intrinsic characteristics, patients with less asthma knowledge, lower self-efficacy and more negative attitudes towards asthma were more likely to view exercise negatively (301). Similar themes were noted in a group of middle aged African American women with poorly controlled asthma, who participated in focus groups to determine barriers to walking. Domains identified in this group included limited physical capability, lack of knowledge, lack of self-monitoring skills, lack of areas to walk, lack of social support and beliefs about consequences and capability (306). Given the emerging evidence that exercise can reduce disease burden in asthma in addition to the broader health benefits, understanding the disease specific barriers in this group is the first step to implementation of exercise interventions.

1.8 Summary

In health, there is an emerging picture of beneficial training effects on inflammation. However, excessive exertion or training can lead to adverse effects and so these considerations must be held in balance when exploring the ideal level of training to effect clinical benefit in asthma.

Exercise in the context of asthma seems to have a beneficial effect, both in improvement of symptom burden and inflammation, serving to address both of the primary aims of asthma management cited by NICE in its most recent guidelines (116). NRF2, as a master regulator of antioxidant processes, has been implicated in the response in health to exercise and in the pathogenesis of asthma across a range of phenotypes (307). An intervention that can modulate the pathobiology of asthma across the board would be disease changing, and yet the mechanisms of effect of exercise training have not been investigated within asthma. Furthermore, exercise training is not employed in the routine clinical management of asthma. Incorporation of exercise into routine clinical care of asthma patients requires further clarification of an optimum training programme and understanding of the mechanisms of effect. This thesis describes the development and implementation of an exercise training programme for patients with symptomatic asthma (as defined by an enrolment ACQ score of greater than or equal to 1.5), with sampling that enabled laboratory work to better understand the mechanism of effect of exercise in asthma. I also attempted to better understand barriers to exercise in difficult asthma, an

appreciation of which will be essential to the wider implementation of exercise training programmes for asthma patients.

1.9 Hypothesis

I hypothesised that a personalised interval exercise training programme (Structured Responsive Exercise Training Programme or SRETP) delivered to symptomatic asthma patients would result in clinical improvement as assessed by the asthma control questionnaire (ACQ) and asthma quality of life questionnaire (AQLQ) Further to this, I hypothesised that the clinical improvement demonstrated would be a result of modulation of inflammation and immunity in the airways, via NRF2 driven increases in redox buffering capacity, resulting in increased tolerance to disease related stressors. These improvements would be driven by increased tolerance to reactive species stressors as a result of exposure to regular environmental stress through exercise training, and the change in expression of NRF2 would predate the improvement in clinical markers of asthma. I aimed to demonstrate this through analysis of the samples taken from our exercise-trained patients and via methods detailed in this thesis. Additionally, I hypothesised that there are both asthma specific and generalised barriers to exercise within difficult asthma patients, that can be identified to help design and target exercise training interventions to this group. I aimed to demonstrate the specific barriers to exercise in this group of patients through analysis of questionnaire data that I collected from the Wessex Asthma Cohort of Difficult Asthma (WATCH) cohort. I then investigated associations between specific barriers and disease endotypes within this cohort through data already collected.

Specifically, I hypothesised that:

1. SRETP administered to sub optimally controlled asthma patients with evidence of airways hyperreactivity will
    a. Be safe, feasible and tolerable in this group of patients
    b. Improve asthma related symptom scores and quality of life
    c. Reduce systemic inflammation and airways hyperreactivity
    d. Increase antioxidant capacity through upregulation of NRF2 activity that occurs prior to improvements in asthma related inflammation and airways hyperreactivity.

e. Improve physical fitness

2. Barriers to exercise in asthma are clearly definable through questionnaire assessment

1.10 Aims

I aimed to

1. Recruit patients with symptomatic asthma (defined by an ACQ6 score ≥ 1.5) to a SRETP to demonstrate tolerability and feasibility of this exercise intervention in this patient group
2. Assess physical responses to acute exercise challenge and symptom and quality of life scores before and after the SRETP intervention with demonstration of:
    a. Improved symptom scores assessed by ACQ score, with a clinically significant improvement of >0.5
    b. Improved quality of life assessed by AQLQ with a clinically significant improvement of >0.5
    c. Improved physical fitness assessed through improvements in $\dot{V}O_2$ peak and AT, with clinically significant improvements of > 2ml/kg/min
3. Isolate serum, plasma and RNA from peripheral blood to identify possible mechanisms of symptom improvement via demonstration of changes in asthma specific cytokines, downstream markers of redox regulation and NRF2 and Keap1 expression
4. Ascertain barriers to exercise in the WATCH cohort of difficult asthma using a questionnaire

Chapter 2 Materials and Methods

Based on initial safety work undertaken at the Alfred Hospital, Melbourne, Australia, and utilising an exercise programme that has been demonstrated to have disease modulating properties in other diseases (308), I have developed and implemented an exploratory study of an exercise training programme for asthma at University Hospital Southampton.

2.1 Clinical Pilot Design

2.1.1 Ethical Approval

Volunteers with symptomatic asthma gave written informed consent and the study was approved by the South Central—Southampton B NRES Committee (17/SC/0256, 31st July 2017).

2.1.2 Experimental Design

Volunteers were recruited through media advertisement (such as posters and Hospital and University Intranet advertisments), established databases, outpatient departments and wards within the University Hospitals Southampton Trust. Volunteers were screened for suitability with full medical history and examination, pre and post bronchodilator spirometry, ECG and assessment of current exercise levels and asthma control ((Asthma Control Questionnaire) ACQ)(119) (Table 2.1)).

Table 2.1 Inclusion and exclusion criteria

Abbreviations: ACQ; asthma control questionnaire, CPET; cardiopulmonary exercise test

Inclusion Criteria	**Exclusion Criteria**
Male or female aged 18 to 80 years	Intercurrent exacerbation or exacerbation within 4 weeks prior to enrolment in study requiring treatment with oral corticosteroids and/or antibiotics
Able and willing to give informed consent	Current smoker (<6 months since cessation) or > 10 pack year history of smoking
Diagnosis of asthma determined by 1. Significant bronchodilator reversibility on spirometry or significant peak flow variability either historically or at enrolment 2. If either of above not demonstrable then evidence of airways hyperreactivity on challenge testing required	Change in regular asthma medication during trial period
Demonstration of suboptimal symptom control through an ACQ ≥ 1.5 at enrolment	Other medical conditions limiting ability to exercise or that may in the opinion of the study clinician carry inherent risk for exercise
Evidence of airway hyper responsiveness, diurnal variation >8% on peak flow diary or reversibility (>12% or 200 ml) on lung function testing (historically or at screening)	Contraindication to CPET
Normal lung function or mild/moderate airflow obstruction at baseline (FEV1>50% predicted)	Positive pregnancy test in females of childbearing age
Physically inactive as described by the American College of Sports Medicine's Guidelines (<60 minutes of structured or planned physical activity/week)	Other clinically significant (as determined by researcher) respiratory or inflammatory disease eg COPD/Crohn's disease/Rheumatoid arthritis
12 lead ECG without clinically significant abnormality	

Participants were randomised to either a personalised interval exercise training programme (Structured Responsive Exercise Training Programme or SRETP) alone or SRETP with strength training. The SRETP was developed at UHS, and allows adaptation of the programme as fitness improves. This SRETP showed increased tumour shrinkage in a sub-group of cancer patients concurrently undergoing chemotherapy (309), suggesting this exercise programme may influence disease processes, which is one of the reasons why this particular training programme was chosen for this research. Once randomised, participants attended a baseline sampling visit, where samples of saliva, exhaled breath condensate, induced sputum and blood were taken (Table 2.2.). Complex lung function testing was performed including exhaled fraction of nitric oxide (FeNO), spirometry, Multibreath Nitrogen Washout (MBNW) and Airwave Oscillometry as determined by forced oscillation technique (FOT). Body composition and quality of life were assessed (Asthma Quality of Life Questionnaire) (310). On a third visit, participants performed a baseline CPET (cardiopulmonary exercise test) to measure physical fitness and derive anaerobic threshold (AT) and peak oxygen uptake ($\dot{V}O_2$ peak), which was utilised to define the exercise program. Patients randomised to SRETP with strength training underwent a 1 repetition maximum (1RM) test to define the strength training programme.

Table 2.2 Sampling schedule

Abbreviations: CPET; cardiopulmonary exercise test, CRP; C-reactive protein, FBC; full blood count, PBMC; peripheral blood mononuclear cell

Sample	**Baseline**	**Week 3**	**Week 6**	**Week 12**
Questionnaires	X	X	X	X
Saliva	X	X	X	X
Exhaled breath condensate	X	X	X	X
Redox regulation bloods	Pre and post CPET samples	Pre and post CPET samples	Pre and post CPET samples	Pre and post CPET samples
FBC/CRP	X	X	X	X
Total IgE	X			X
PBMC isolation	X		X	X
Sputum	x		x	x
Plasma and serum for cytokine analysis	x		x	x

Both groups wore an activity monitor for periods of time at the beginning and end of the study to assess activity levels. Both exercise groups completed 3 x 30-minute sessions per week for 12 weeks, with additional warm up and cool down periods, to give a total exercise duration of 40 minutes, +/- strength training. Participants underwent interim assessment after 3 and 6 weeks' training, where saliva, exhaled breath, blood, sputum (6 weeks only) and symptoms and quality of life (ACQ and AQLQ) and complex lung function were assessed, and CPET was repeated to re-prescribe the training intensity at 3 and 6 weeks. A full re-assessment was undertaken after 12 weeks' training (see figure 2.1). The 12 week intervention period was chosen based on previous exercise interventions in asthma, which used exercise interventions of between 8-12 weeks(5, 6, 279, 311)

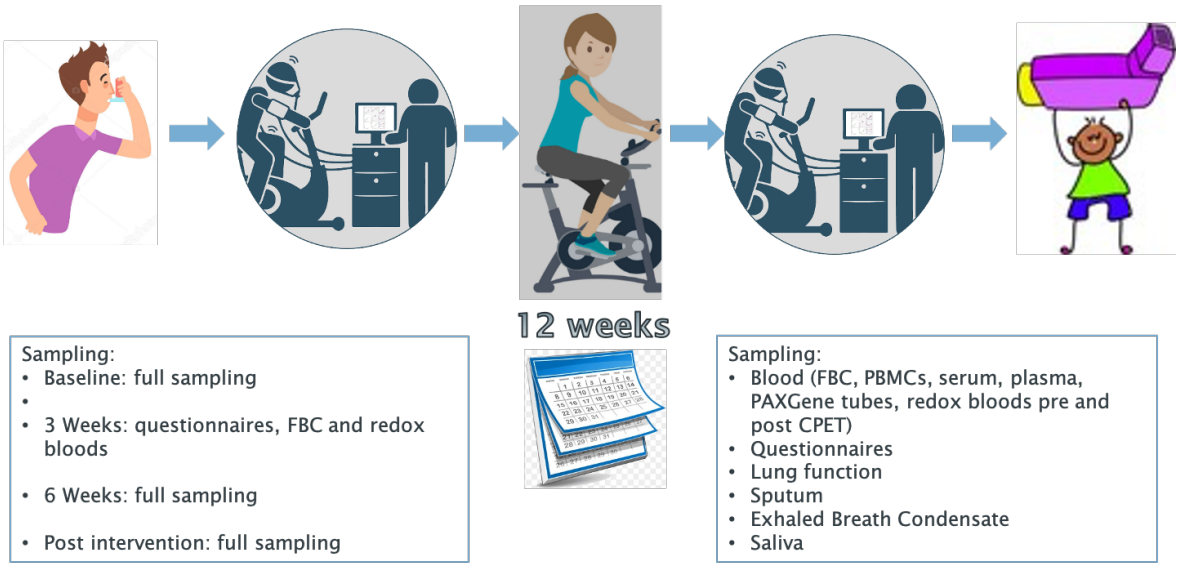


Figure 2.1 Study design including detailing of sampling processes and timeline, exercise prescription and training intervention

Abbreviations CPET; cardiopulmonary exercise test, FBC; full blood count; PBMCs; peripheral blood mononuclear cells

2.1.3 Quantification of baseline fitness and prescription of the exercise intervention

2.1.3.1.1 Cardiopulmonary Exercise Test

Exercise derived physiological parameters were assessed through symptom limited, incremental Cardiopulmonary Exercise Testing to the limit of tolerance. Test equipment comprised an electronically braked cycle ergometer, 12 lead ECG, pulse oximetry and a metabolic cart. The metabolic cart has oxygen and carbon dioxide analysers with a response time of 90 m/s and a gas flow sensor to enable breath-by-breath measurements. Calibration was made before each test of the flow sensor and the oxygen and carbon dioxide analysers. During the calibration of flow, adjustments for barometric pressure, humidity and temperature were made. The flow sensor is calibrated using a 3 litre calibration syringe over a range of flow rates. Since the accuracy of the values obtained during testing is directly determined by the accuracy of the gases used to calibrate the gas analysers, calibration gases are gravimetrically weighed to ensure concentration accuracy. Calibration accuracy is accurate to two decimal places (± 0.01%). The calibration used a two-point calibration system; these two points correspond to the equivalent of normal gas concentrations at sea level (room air) and exhaled gas concentrations (calibration mixture: 5% CO_2 and 15% O_2 in N_2 purchased from BOC

Special Gases). Gas calibration also included a measure of the delay between the change in gas concentration at the distal end of the sample line and the time it takes for this change to be measured by the gas analysers. This ensured that the data from the gas analysers were accurately aligned with measurements made by the flow sensor. The procedures allowed direct measurement of oxygen uptake ($\dot{V}O_2$), carbon dioxide output ($\dot{V}CO_2$), tidal volume (VT) and breathing frequency (RR) (amongst others) for each breath. From these other physiological variables were directly derived, with determination of the anaerobic threshold (AT) and $\dot{V}O_2$ peak used to calculate the SRETP. Changes in cardiovascular response to exercise at interim and final assessment were assessed, with a minimal clinically important difference (MCID) in oxygen uptake defined as improvement of 2ml/kg/min(179).

2.1.3.1.1.1 Setting up the test

ECG electrodes and leads were applied to the patient before getting on the stationary cycle ergometer (exercise bike). The patient was coached on facemask placement and instructions for communicating were given. The patient was then be instructed to give their “best effort” however they were told to stop if they felt dizzy or faint. The patient was instructed to report any other symptoms by using hand signals wherever possible. The patient was shown where their leg speed (RPM) was displayed and told to keep this at between 55 and 65 RPM for the duration of the test.

The incremental rise in work rate was predetermined using the equation below and physiologist expertise. This was done in an objective manner with the aim of test duration of approximately 8 - 12 minutes using the following equations:

Men: weight x ((50.72 – (0.372 x age =)) / 100 =

Women: (42.8 + weight) x ((22.78 – (0.17 x age)) / 100 =

[Weight in kg; age in yrs]

Once this was determined, this work rate ramp was used for all other CPET tests performed by that patient. The bike height was set to ensure consistency between each test, with a 5 degree bend in the leg at the lowest level of the pedal crank. When this was checked it was important to ensure that the patient was not extending at the hip in order to reach the pedals. Once the patient was comfortable on the bike the mask was fitted.

2.1.3.1.1.2 Test protocol

2.1.3.1.1.2.1 Rest:

Five minutes of resting data were recorded to ensure that the patient was comfortable with the facemask and baseline measurements of VE, $\dot{V}O_2$, HR, BP and end-tidal PO_2 and PCO_2 were stable. The Borg Score (Scale 0 to 10) for subjective rating of breathlessness and for leg fatigue was assessed after 4 minutes. BP was obtained at 2 and at 4 minutes of resting measurements.

2.1.3.1.1.2.2 Unloaded Cycling

The rest period was followed by a period of 3 minutes of unloaded cycling. This allowed for the $\dot{V}O_2$ 'cost' of just turning the legs against minimum external resistance to be evaluated, and provides minimum resistance for the particularly detrained or functionally limited.

2.1.3.1.1.2.3 Ramp

The ramp follows unloaded cycling in a continuous fashion. The gradient of the ramp was predetermined before testing started as detailed above. The patient and their cardiopulmonary variables (in particular the 12 lead ECG and S_pO_2) were monitored continuously for the duration of the test. Non-invasive blood pressure were taken approximately every 2 minutes during the test. The patients were given verbal feedback and encouragement throughout the test. The work load was terminated (test stopped) when the "Stopping Criteria" below were met.

2.1.3.1.1.2.4 Stopping Criteria

This incremental ramp test has a volitional end point; the test is stopped when the patient can no longer continue and therefore the stopping point is the limit of patient tolerance. The physiological aims of the study require the measurement of anaerobic threshold (AT) and peak oxygen uptake ($\dot{V}O_{2peak}$). The patient was encouraged to continue for as long as they were able. It was important to ensure that the patient understood he/she can stop at any point if they had symptoms or felt unable to continue. The reason for stopping the test was recorded (both the subject's reason and the operators' observations). Specific criteria for stopping were:

- The patient stopped pedalling due to fatigue and dyspnoea, pain or light headedness
- The patient failed to maintain an RPM of greater than 40 RPM for more than 1 minute and did not respond to encouragement

2.1.3.1.1.2.5 Recovery

Recovery data were collected for a period of up to 5 minutes. The ECG was monitored until any dysrythmia or ST changes reverted to pre-test levels, or until heart rate was within 10 bpm of the pre-test rate. Blood pressure was monitored until it returned to the pre-test level.

2.1.3.1.1.2.6 Interpretation

All tests were reported to define AT and $\dot{V}O_2$ peak contemporaneously, for prescription of the exercise intervention, by 2 individuals, one of whom was blinded, using Blue Cherry software (Geratherm Respiratory, Bad Kissingham, Germany). Formal reporting of the CPET data were undertaken by 2 CPET competent clinicians, blinded to patient data. If their analysis differed by > 4.8% for any outcome measure, and independent third interpreter was involved for additional analysis. This method of CPET reporting has been described and utilised by previous exercise intervention studies undertaken at UoS (308). The data were interpreted using standard CPET interpretation techniques, as per local standard operating procedure (SOP) (164). Examples of the 9 panel plots of data used for interpretation is displayed in figure 2.2.

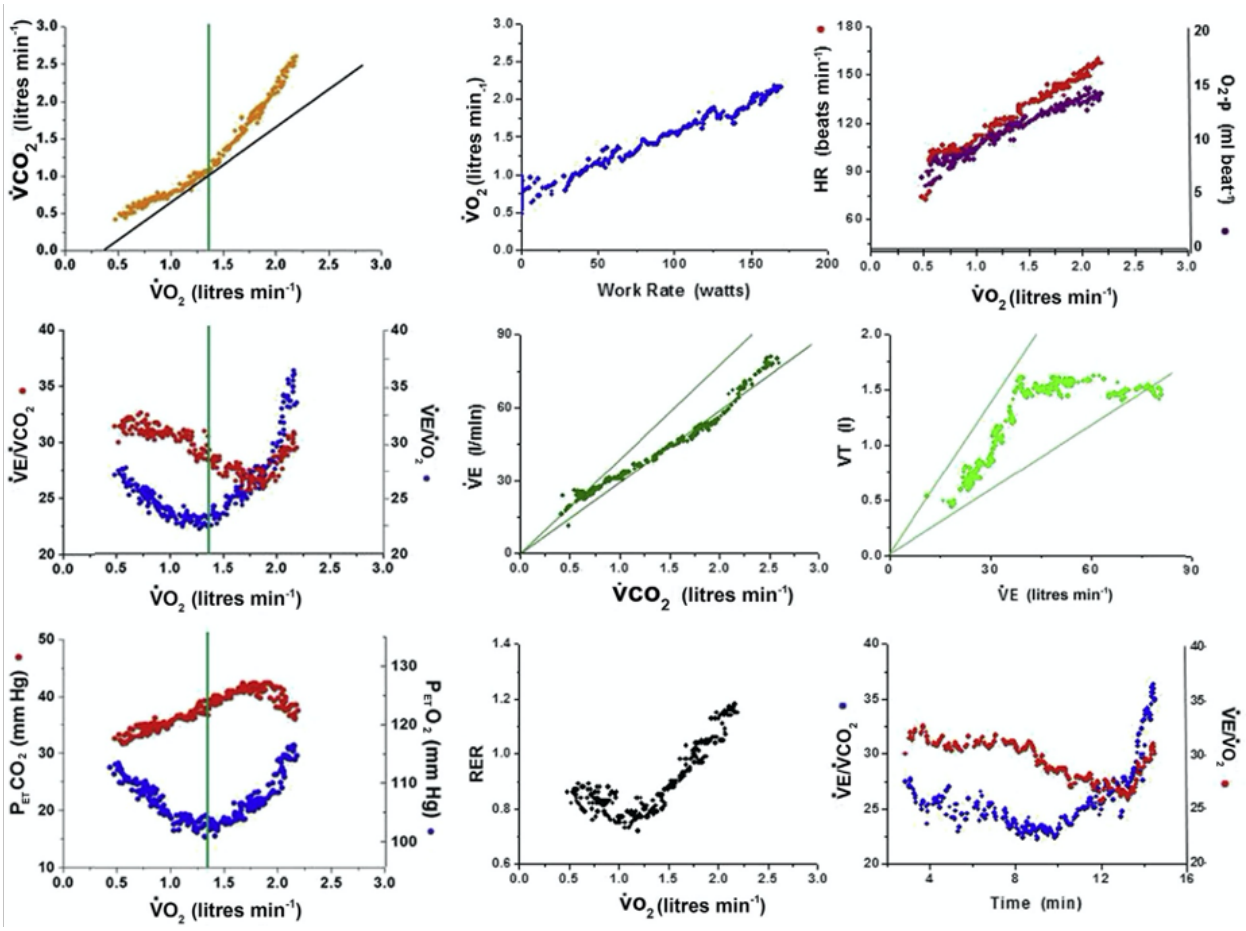


Figure 2.2 Example of a nine-panel CPET display for the normal individual:

TOP ROW: panel 1: $\dot{V}CO_2$ vs $\dot{V}O_2$; Panel 2: $\dot{V}O_2$ vs work rate; and Panel 3: : $\dot{V}O_2$, HR and O_2 pulse. SECOND ROW: Panel 4: VE=$\dot{V}CO_2$ and VE=$\dot{V}O_2$ vs $\dot{V}O_2$; Panel 5: VE vs $\dot{V}CO_2$; and Panel 6: VT vs VE. BOTTOM ROW Panel 7: $PETO_2$ and $P_{ET}CO_2$ vs V O2 (SpO_2 may be included); Panel 8: RER vs $\dot{V}O_2$; and Panel 9: unassigned, but here showing V E= $\dot{V}O_2$ and V E= $\dot{V}CO_2$ vs time.Suggested clusters for interpretation: AT estimation (green vertical line), [panels: 1,4,7 (and 9)] cardiovascular limitation, (panels: 2,3,4,5) respiratory limitation, (panels: 4,5,6 and 7). RER, Respiratory exchange ratio, copied from (164).

The Americal Thoracic Society/ American College of Chest Physicians (ATS/ACCP) recommend the use of the two most widely used sets of reference values (Jones et al(175) and Hansen et al(176)) in their statement on cardiopulmonary exercise testing, and therefore I have used Hansen et al calculations in this thesis (177), with the formulae for interpretation detailed in table 2.3.

Table2.3 Calculations for reference values for maximal incremental cycle ergometer symptom limited cardiopulmonary exercise test

Adapted from (176). Abbreviations AT; anaerobic threshold, V̇O2; oxygen uptake

Variable	Equation
$\dot{V}O_2$ ml/min (male)	weight $*$ (50.75 − (0.37 $*$ age))
$\dot{V}O_2$ ml/min (female)	(weight + 43) $*$ (22.78 − (0.17 $*$ age))
Maximum predicted heart rate	220- age
O_2 pulse ml/beat	Predicted $\dot{V}O_2$/predicted heart rate max
AT, ml/min	>40% predicted $\dot{V}O_2$ peak

Integrated interpretation of the features identified in each CPET was performed using the definitions in table 2.4 . These are copied from work by McNicholl et al(92), who investigated the utility of CPET for indentifying the cause of ongoing dyspnoea in difficult asthma, with the definitions adapted from the ATS/ACCP guidance(177).

Table 2.4 Definition of features identified using CPET

Copied from (92). Abbreviations AT; anaerobic threshold; CPET; cardiopulmonary exercise test; HR; heart rate; MVV; maximal voluntary ventilation; O_2; oxygen; $P_{ET}CO_2$; end-tidal P co 2 ; RER; respiratory exchange ratio; SpO_2; oxygen saturation; $\dot{V}E\dot{V}CO_2$; ventilatory equivalent for CO_2 ; $\dot{V}O_2$; oxygen uptake

Feature	**Interpretation**
Hyperventilation	$\dot{V}O_2$ peak: often normal (>83% predicted) or near normal (> 80% predicted) $\dot{V}E\dot{V}CO_2$at AT: increased (> 34) Highest $P_{ET}CO_2$: low (< 36 mm Hg) Respiratory frequency: increased (> 55/min) SpO_2: normal (> 95%, < 4% drop during exercise) Variable RER, especially at beginning of test Hyperventilation occurring below the respiratory compensation point
Ventilatory Limitation	$\dot{V}O_2$ peak < 83% predicted Breathing reserve < 20% of MVV $\dot{V}E\dot{V}CO_2$ at AT> 34 SpO_2> 4% drop during exercise RER at peak exercise: < 1
Cardiac Ischaemia	ST depression:>2 mm in > 1 lead $\dot{V}O_2$ peak: low (< 83% predicted) Breathing reserve:> 20% of MVV SpO_2 normal (> 95%, <4% drop during exercise) AT: normal or low (< 40% predicted peak$\dot{V}O_2$) or not detected
Physical deconditioning	$\dot{V}O_2$ peak: decreased (< 83% predicted) Peak HR: normal/slightly decreased (> 90% age predicted) O_2 pulse: decreased (< 80% predicted) Breathing reserve:> 20% of MVV $\dot{V}E\dot{V}CO_2$ at AT: normal (< 34) SpO_2: normal (> 95%, < 4% drop duringexercise)
Submaximal test	Early cessation of exercise $\dot{V}O_2$ peak:< 83% predicted AT: normal (> 40% predicted peak $\dot{V}O_2$) or unattained RER at exercise cessation: low (< 1) Peak HR <80% predicted Breathing reserve: substantial (> 40% of MVV)

2.1.4 Interventions

The initial study was designed around a combined aerobic and resistance exercise training programme versus the aerobic exercise intervention alone. The rationale for including additional strength training was that the strongest immunological signals in asthma patients were seen following an aerobic and resistance exercise intervention (5). However, recruitment proved difficult and interim assessment (table 3.4) suggested, despite small numbers, that benefit was apparent with the aerobic exercise intervention alone. This was considered, alongside the difficulty in recruitment which patient feedback suggested were in part due to time demands of the study. A decision was reached with my supervisory team to make the resistance training optional, alongside a relaxation of the inclusion criteria. Therefore, an amendment was submitted to REC in 2018 to allow optional inclusion of the resistance training programme, in addition to broadening of the inclusion criteria, in the context of an interim assessment (see table 3.4) and to optimise recruitment.

2.1.4.1 Structured Responsive Exercise Training Programme

The structured responsive aerobic interval exercise programme was based on the standard exercise protocol used and validated in the EMPOWER trial (312). The training programme was personalised as it is derived from the CPET variables (and the 1 rep maximum load for those in the strength training arm) of each volunteer being trained. The supervised in hospital training programme was performed using a computer controlled, electromagnetically braked, cycle ergometer (Ergoline 200, Germany). The training program was individually responsive to each patient following the initial CPET test (week 0) and all other CPETs. The intervention is described as per the ACSM's FITT VP principles.

2.1.4.1.1 Frequency

Thrice weekly in hospital training sessions were prescribed, with adequate attendance defined as > 75% of sessions attended

2.1.4.1.2 Intensity

The sessions were 4 to 6 repeats of: a) 2 minutes high/severe intensity Cycling immediately followed by b) 3 minutes of moderate intensity (20 to 30 minutes total),

defined based on baseline CPET, and modified following each interim CPET. Moderate intensity exercise was at a power output equivalent to 80% of AT and high intensity was set at a power output that is halfway between AT and $\dot{V}O_2$Peak (termed 50% Δ).

2.1.4.1.3 Training time

The sessions were 4 repeats in week 1, increased to 6 repeats from week 2 onwards of: a) 2 minutes high/severe intensity Cycling immediately followed by b) 3 minutes of moderate intensity (20 to 30 minutes total), defined based on baseline CPET, and modified following each interim CPET. Five minutes of unloaded cycling was performed at the start and end of all training sessions for a warm up and cool down.

2.1.4.1.4 Training type

The individualised training programme was placed on a chip card which was placed in the head of the Optibike and delivered the training programme automatically recording watts, RPM and HR. Compliance was assessed via the chip card on the training bike, and via session attendance. A log of the duration of training was also completed on each visit. Exercise sessions could be terminated by the patient at any time, or terminated by the supervisor of the test if any of the criteria to stop the test were met, based on the ATS CPET safety guidelines.

Patients were screened prior to each session to ensure that it was safe to perform exercise. Blood pressure was monitored before and after exercise and heart rate was recorded continuously from the R R interval of the ECG (Polar FT7, Warwick, UK). Sessions 9 and 18 (week 3 and 6) were a standard CPET test to monitor the progression of training induced improvements in physical fitness. The absolute power output for subsequent training sessions was adjusted according to the outcome of CPET test. CPET was repeated at 12 weeks to quantify any change in cardiovascular fitness from baseline.

2.1.4.2 SRETP with Strength Training

The SRETP with strength training included the SRETP programme as described above, with the addition of resistance training for upper and lower limbs, and is based on the programme devised by Freitas et al (284). Patients began the resistance training programme with 2 sets of 10 repetitions for each major muscle group at an intensity of 60% of 1 maximal test; increasing to 2 sets of 15 repetitions based on patient tolerance.

When the patient reached 15 repetitions, the load was increased from 1-3kg for upper limb exercises and 5-10kg for lower limb exercises depending on patient tolerance. Patients repeated a 1 repetition maximum test at final assessment.

Best treatment practice and optimisation of treatment regimens were followed at all times, although any changes in medication necessitated exclusion from the study to avoid confounding results

2.1.5 Environment and Safety Monitoring

All exercise sessions were supervised in the exercise laboratory of the NIHR Southampton Biomedical Research Centre at the University Hospital Southampton, and all participants were reviewed by a co-investigator prior to each exercise session. During the exercise training sessions, heart rate was monitored and the exercise training programme pre-programmed onto a chip and-pin card that executes the interval intensities automatically. This has been shown to be safe in cancer patients undergoing chemotherapy (312).

2.1.6 Randomisation

Participants were randomised to either the Structured Response Exercise Training Programme alone or the Structured Response Exercise Training and Strength Training Programme. The randomisation was computer generated, and carried out by a researcher blinded to the recruitment and evaluation of the patients, and concealed in sealed, numbered envelopes. It was not possible to blind the participants or the researchers to the exercise groups after randomization had occurred due to the design of the study

2.1.7 Outcome Measures

The primary outcome was improvement in ACQ6 score, with secondary and exploratory outcome measures grouped into physiogical outcomes, asthma outcomes and mechanistic outcomes.

2.1.7.1 Evaluation of symptom scores

Pre and post intervention asthma control was assessed using the ACQ, which has been validated for measuring changes in asthma control. International guidelines have suggested that the primary end point in asthma management should be symptom control and future risk assessment (313), and therefore asthma control is a sensible primary

outcome measure to assess response to an intervention for asthma control. The asthma control questionnaire (ACQ) is a well-established and validated tool in the assessment of symptom burden in asthma (120), with clear demarcation for well controlled and symptomatic asthma (119). The ACQ6 comprises 6 questions with answers on a 7 point scale, assessing symptom burden over the preceding week. The score was calculated by adding together the total of the 6 questions and then dividing by 6. It has been examined in the context of identifying well controlled and sub optimally controlled disease, based on international guidelines, and the cross over point identified as near to 1, with the optimal cut off points to be confident that a patient has well controlled asthma falling at 0.75 (negative predictive value 0.85) and the optimal cut off for sub optimally controlled asthma at 1.5 (negative predictive value 0.88) (119). A change ≥0.5 is regarded as clinically significant (119).

2.1.7.2 Secondary and exploratory outcome measures: Physiological Outcomes

Physiological measures focussed on body composition, cardiopulmonary exercise test outcomes and daily life activity levels.

2.1.7.2.1 Body Composition

Height and weight were measured. Body mass index was calculated from these values. Participants stood on the Seca mBCA (medical Body Composition Analyser) (Seca, Birmingham, UK); each foot on a pair of electrodes and each hand on electrodes within the hand rails. The mBCA measured impedance at a number of frequencies ranging from 1 kHz to 1 MHz. Bioelectical Impedance derived measures, such as Fat Free Mass (FFM) and Percentage Body Mass were generated according to manufacturer equations.

2.1.7.2.2 Quantification of physical fitness

This was assessed via repetition of a CPET at week 3, week 6 and following the intervention. The methods are described in 2.1.3.

2.1.7.2.3 Daily Life Physical Activity

The ‘MioSlice’ activity tracker was worn prior to and after the 12 week exercise training intervention to assess the effect of the intervention on daily physical activity. The MioSlice used an optical heart rate tracker, 3-axis accelerometer and a vibration motor to assess heart rate, resting heart rate, sleep, calories burned, steps, distance, and time. It

used a 'Personal Activity Intelligence' Algorithm, based on heart rate intensity as a measure of cardiorespiratory fitness, and was validated in the HUNT study as a risk predictor for cardiovascular disease mortality (314)

2.1.7.3 Secondary and exploratory outcome measures: Asthma Outcomes

2.1.7.3.1 Quality of life measures

The Asthma Quality of Life Questionnaire (AQLQ) evaluates the domains of symptoms, activity limitation, emotional function and environmental stimuli in assessing quality of life, with a better quality of life suggested by a higher score.(310) The overall score was calculated by adding together the answers for each question, then dividing the total by 32 (the total number of questions). The domain score was calculated by adding together the answers for each question in each domain separately, then dividing by the number of questions in that domain, to give an average for each domain. For overall asthma specific quality of life, the minimally important difference (MCID) has been shown to be 0.5 (310), with a higher score suggestive of better quality of life.

2.1.7.3.2 Spirometry with reversibility

For spirometry, if patients were able to withhold their regular inhaler therapy then reversibility testing to 400 micrograms of inhaled salbutamol was performed.

Restrictions before Reversibility Testing

- Withheld short acting inhalers such as the ß-agonist salbutamol or the anticholinergic drug ipratropium bromide for at least 4 hours
- Withheld long acting ß-agonist bronchodilators such as salmeterol for at least 12 hours. Oral therapy with aminophylline or slow release ß-agonist were also withheld for 12 hours

Spirometry was performed using either Carefusion® (Chatham, UK) or Inspire Health Ltd (Hertford, UK) equipment. If subjects were unable to withhold regular inhaler therapy then Spirometry results were recorded as "post-bronchodilator" scores and percentage predictive values were calculated using GLI (Global Lung function Initiative) look up tables.

2.1.7.3.3 Impulse Oscillometry

Impulse Oscillometry, as an indicator of small airways obstruction, was measured using the Jaeger Impulse Oscillation System, Carefusion® (Chatham, UK) and performed according to ERS/ATS guidelines. Subjects supported their cheeks with their hands and breathed normally until at least 90 seconds of data was recorded. This was either done in 3 trials of 30 seconds or one trail of 90 seconds. The breathing pattern was observed for consistency and to check it was free of artefacts. If 3 trials were recorded then the average of the trials was used for each index. Percentage predictive values were calculated using the Vogel 1994 equations.

2.1.7.3.4 Airway and Systemic Inflammation

2.1.7.3.4.1 Peripheral Blood Cell Counts

Clinically requested blood tests (table 2.2) were processed by the UHSFT Pathology Laboratory, which is fully UKAS accredited and compliant to ISO142819 standards. Results were stored on the hospital's local results server from which data is extracted (see data management).

2.1.7.3.4.2 Sputum Eosinophil Count

Spirometry with Reversibility was recorded prior to the the sputum induction procedure (See Lung Function Testing). Three 5-minute rounds of saline nebulisation, starting at 0.9% and escalating to 3% then 4.5% were performed with FEV1 measurements after each round. Patients underwent a modified protocol (2 minutes nebulisation) if their BD FEV1 <1.5L. If the participant needed to cough and expectorate during the nebulisation then they could do so,collecting any dislodged sputum into a petri dish.

Sputum was processed as soon as possible and within 2 hours of expectoration(315). Sputum plugs were visually identified, selected and condensed into one mass to remove contaminating saliva and homogenise the plug. Sputum was incubated on ice with 8 volumes of Phosphate Buffered Saline (PBS) and passed through a pasteur pipette, vortexed and then agitated for 30 minutes on a bench rocker. The sample was then centrifuged and 6 volumes of supernatant removed. The remaining sample from PBS processing was resuspended with 0.2% Dithiotreitol (DTT) (Calbiochem, San Diego, USA) giving a final concentration of 0.1% DTT. The samples was again passed through a pasteur

pipette, vortexed and then agitated for 30 minutes on a bench rocker. The sample was then centrifuged and the cell pellet re-suspended in PBS and counted using the trypan blue exclusion method to ascertain the number of cells and their viability. Cytospins were generated using a cytospin. 2 cytospins were stained using rapid Romanowsky stain and 400-800 respiratory cells plus squamous cells were counted for differential cell count and to ascertain the volume of squamous cell contamination.

2.1.7.3.4.3 Fractional Exhaled Nitric Oxide (FeNO)

Subjects were asked to refrain from the following if possible before lung function testing.

- No smoking for 24 hours
- No alcohol consumption for 4 hours
- No vigorous exercise for 30 minutes
- No tight fitting clothing that could restrict full chest and/or abdominal expansion
- No food for 2 hours
- If clinically acceptable, no supplemental oxygen for 10 minutes

Fraction of Exhaled Nitric Oxide (FeNO) was measured using the NIOX VERO® (Oxford, UK) at a flow rate of 50ml/s. Exhaled nitric oxide was measured **before** any other lung function test due to the influence of breathing manoeuvres on FeNO readings. A minimum of 2 technically acceptable tests were recorded with the two highest values within 10% of each other and the mean value reported.

2.1.7.4 Secondary and exploratory outcome measures: Mechanistic outcomes

2.1.7.4.1 Luminex Cytometric Bead Assay

Serum and Plasma samples were collected and stored at baseline, 6 and 12 weeks, prior to acute exercise challenge. Luminex analysis of plasma and serum collected from patients at baseline, at 6 weeks and post intervention was performed, according to manufacturer instructions (R and D systems, Oxford, UK), with a pre-mixed 8 plex panel, using a magnetic bead system (see table 2.3 for cytokine panel and limits of detection).

Table 2.5 Cytokine panel selected and limits of detection for each cytokine

Abbreviations: pg/ml; picogram/millilitre

Cytokine	Limits of Detection pg/ml
IL-5	21-927
IL-6	37.6-1679
IL-10	63.4-4600
IL-13	101-4536
IL-1ra	39.1-1732
CCL11/eotaxin	71.8-3182
TNF alpha	39.7-1637
IFN gamma	16.4-727

Briefly, 50 μl of standard, control or sample was added per well, with samples having undergone a 2 fold dilution with calibrator diluent. Diluted microparticle cocktail was added to the plate. The Luminex plate as then incubated for 2 hours at room temperature on a horizontal microplate shaker set at 800 ± 50 rpm. The plate was then washed three times with 100μl of wash buffer (R and D systems). A Bioplex Handheld Magnetic Washer (Biorad, California, USA) was used to hold the microparticles to the bottom of the plate during the wash steps. Next, 50μl of diluted Biotin Antibody Cocktail was added to all wells and the plate was incubated in the dark at room temperature for an hour on the shaker set to 800±50rpm.Following a further wash (as above), 50 μl of Streptavidin-PE was added to all wells. The plate was incubated as previously for 30 mins at room temperature in the dark. Following a final wash, the microparticles were resuspended by adding 100μl of Wash Buffer to each well and incubated for 2 mins on the shaker at 800±50rpm for 2 minutes. The plate was then read within 90 minutes using a Luminex analyser.

2.1.7.4.2 Redox regulation experiments

2.1.7.4.2.1 Nitrospecies analysis

Analysis of nitrospecies was performed as per the departmental SOP by our redox regulation collaborators (266). Briefly, venous blood was treated with 100mM NEM solution at point of collection and frozen at -80 °C until processing. Once defrosted, for

quantification of total nitroso species (RXNO), 10% (v/v) of 5% sulphanilamide in 1M HCl solution was added to the sample and incubated for 15 min in room temperature (RT).

After incubation, the sample was injected using a gas-tight Hamilton syringe into the reaction chamber containing Iodine/Iodide solution.

Samples were then analysed in a Chemiluminescence Detection System (depicted in figure 2.3)

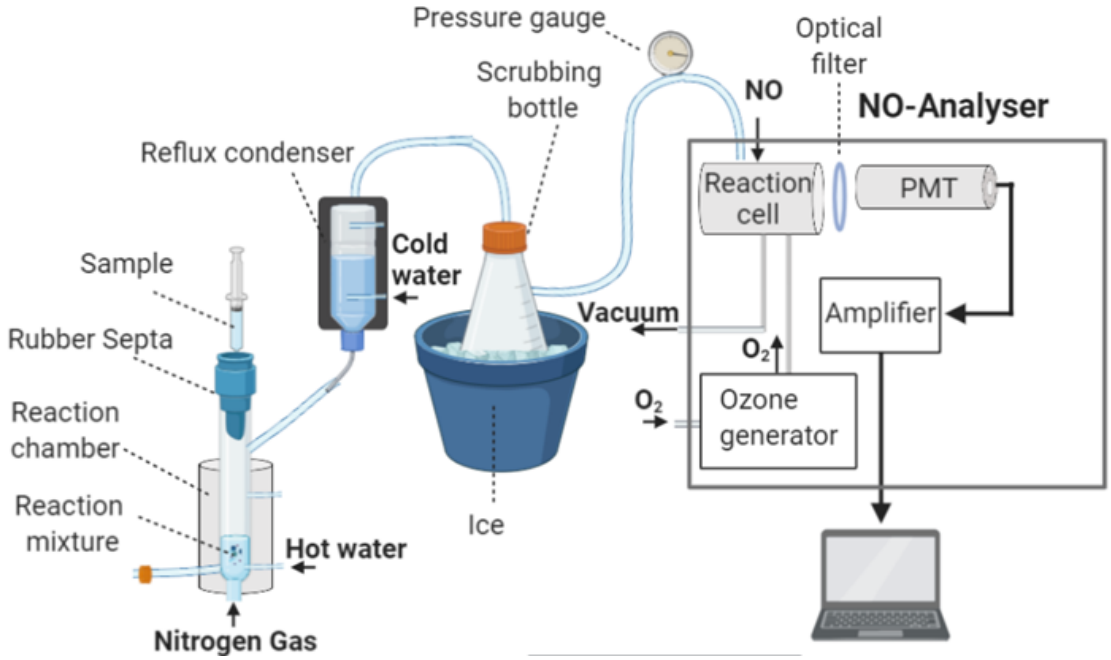


Figure 2.3 Chemiluminescence Detection System Schema

Within the reaction chamber, 13.5mL of glacial acetic acid was added into a rubber septum-sealed, water jacked reaction chamber and 1mL of Iodine/Iodide solution added. The reaction mixture was kept at the constant temperature of 60 °C and continuously bubbled with nitrogen. The temperature of the reaction chamber was controlled by the heating water bath. The reaction chambers were connected to efficient reflux condensers that were kept below 0 °C. The antifreeze coolant was added to the cooling bath to enable the cooling temperature to be below freezing point. Sufficient volume of 1M sodium hydroxide was added into the scrubbing bottle, and it was connected with the reaction chamber through reflux condenser and placed in the plastic beaker filled up with ice. The outlet of the gas from the reaction chamber was passed through the scrubbing bottle in order to trap traces of acetic acid and iodine before transfer into the detector (NO-Analyser). A pressure gauge was placed between the outlet of the scrubbing bottle and the detector inlet for continuous pressure monitoring and adjustment of purging gas

(nitrogen or argon) flow. The sample tube was connected to the inlet fitting on the rear panel of the analyser. The injection volume and the range were set up on the instrument dependant on the levels of analytes of interest in the samples. The data was saved using PowerChrom Software and integration values transferred into Microsoft Excel version 16.40 (London, UK) and Graphpad Prism version 8 (La Jolla, USA) for analysis.

2.1.7.4.2.2 Determination of Thionarbituric Acid Reactive Substances (TBARS)

TBARS levels were determined from a Malondialdehyde equivalence standard. The MDA-TBA addict was formed by the reaction of MDA and TBA under high temperature (90-100 °C) and acidic conditions were measured colourimetrically at 532nm and 750 nm (as a background correction). Unknown samples were compared to the standard curve. Briefly, the TBARS reagent was brought to room temperature. A blank sample was performed with the assay: deionized water that was used to prepare stock solution/reagents. To prepare Standards/ Blank, 100µLwas added 50µL of TBARS Colour Reagent. (2:1 ratio), with the same ratio used for the sample: TBARS reagent preparation. All standards, samples and blanks were then incubated for 0.5 hour on the block heater at 90 °C. After incubation, the samples were cooled down on ice for 10 min. Next, 50 µL of each of the samples (including standards and blanks) were added to each well (in duplicate) on the clear 384 well microplate for colourimetric method, and read at the absorbance of 532 and 750nm.

2.1.7.4.2.3 Total and Free Thiol Groups

Briefly, the concentrations of total free thiols (TFTs) in serum were determined spectrophotometrically (Molecular Devices SpextraMax M5 plate reader) using Ellman's reagent. A set of cysteine standards of different concentrations (from 0.977 µM to 500 µM) was prepared in 0.1M Tris 10mM EDTA pH 8.2 buffer. Serum samples were centrifuged at 16.1 rcf for 10 min and diluted 1:4 (v:v) with 0.1M Tris pH 8.2 buffer. 90 µL of each standard and sample was transferred to a microplate and background absorption was measured at 412 nm with a reference filter at 630 nm. Subsequently, 20 µL of 1.9mM 5,5'-dithio-bis-2-nitrobenzoic acid (DTNB) in 0.1M phosphate pH 7.0 buffer was added and plate was incubated in the dark, at room temperature for 20min, while shaking. Following the incubation, the absorption was measured again. The concentration of free thiol groups in the sample was determined by comparing the

corrected absorbance (absorbance post incubation minus absorbance pre incubation) of the sample to that of the cysteine standards. Since sulfhydryl groups attached to protein account for most of the free thiol groups in serum, free thiol concentrations are expressed per gram of protein.

2.1.7.4.2.4 Ferric Reducing Antioxidant Capacity of Plasma

The FRAP assay was used as a measure of total antioxidant capacity of plasma, as described previously (267). Briefly, standard concentrations of 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) and Iron(III) chloride ($FeCl_3$) were prepared on the day of analysis, according to local SOP. Plasma samples were centrifuged for 5 minutes at the maximum speed of the centrifuge. 10µL of centrifuged sample was then mixed with 30 µL of ultrapure water. 15 µL of ultrapure water was then added to a flat bottomed 96 well plate, followed by 5 µL of sample, standard and ultrapure water. 150 µL of FRAP solution was then added into each well. The plate was then incubated at 37C for 30 minutes, prior to measurement of absorbance at 593nm.

2.1.7.4.3 Peripheral blood mononuclear cell isolation

Peripheral Blood Mononuclear Cells (PBMC) were stored and frozen as per standard methods. Briefly ,54mls of venous blood was collected pre CPET at baseline, 6 weeks and final sampling. It was diluted 1:1 In Dulbecco's Phosphate Buffered Saline (D-PBS, Sigma-Aldritch, Poole, UK) prior to layering over 20ml of Ficoll-Pacque (GE Halthcare, Little Chalfont, UK). It was then centrifuged at 800g for 30 mins at 20°C to form a visible interface layer, which was pipetted into a fresh Falcon Tube as PBMCs. The cells were then washed twice in D-PBS prior to counting and resuspending in DMSO/FCS for freezing at -80° in a Mr Frosty. Once frozen to -80°C, samples were transferred to liquid nitrogen and stored until further analysis.

2.1.7.4.4 RNA isolation, cDNA synthesis and Real-Time PCR

RNA was extracted from stored PBMCs using an miRNAeasy (Qiagen, Manchester, UK). Briefly, PBMCs were defrosted on ice, before washing in 20 mls of RPMI, and centrifuging at 400g (1640 RCF) for 5 minutes at 4°C. Following this wash, 700 µl of QIAzol Lysis Reagent (Qiagen) was added and the samples incubated at 15-25°C for 5 minutes. 140µL of chloroform was then added and incubated at room temperature for a further 2-3 min, prior to centrifuging for 15 min at 12000g at 4 °C. The upper aqueous phase was then transferred to a new collection tube and 525µl of 100% ethanol added. The sample was

then pipetted into an RNeasy Mini Column (Qiagen) and centrifuged at 8000g for 15 sec at room temperature. Next, 700μl of Buffer RWT was then added to the RNeasy mini column, and centrifuged for 15 sec at 8000g. The flow through was discarded and then 500μl of Buffer RPE was pipetted on prior to centrifuging at 8000g for 15 seconds. The flow through was discarded again and the step repeated. The RNAeasy mini column was then transferred to another 2 ml collection tube and centrifuged at full speed for a further minute to dry the collection membrane. Finally, the RNeasy mini column was transferred to another 1.5ml collection tube and 50μl of RNase free water was added directly onto the column membrane. The sample was then centrifuged at 8000g for 1 minute to elute the sample.

Reverse Transcription was undertaken in 20μl reactions, with 250ng of RNA added to 2 μl 10x RT buffer, 2μl 10 xRT random primers, 0.8μl of dNT[mix, 1μl of MultiScribe Reverse Transcriptase and 1μl RNase Inhibitor (Applied Biosystems, Paisley, UK). The remaining volume was made up with RNAse free water (Sigma Aldrich, Gillingham, UK) All samples were then briefly vortexed and centrifuged. Reverse transcription was carried out using a DNA Engine Thermal Cycler (MJ Research, Cambridge, UK) at 25 °C for 10 min, followed by 37°C for 2 hours and then 85°C for 5 minutes.

Once cDNA had been synthesised, quantitative PCR (qPCR) was carried out in 5μl aliquots. 0.25 μl of primers (see table 2.5) and 1.25μl of RNAse free water (Sigma) were added to 2.5μl of buffer (Applied Biosystems) 1μL of cDNA was added to a 384 well plate along with 4μl of the master mix cocktail. Gene expression was normalised with the housekeeping gene actin beta, and the reactions performed using a 7900HT Fast Real-Time PCR System at 95°C for 10 min followed by 40 cycles of 95°C for 15 sec and 60°C for 1 min. Gene expression was quantified using the $2^{-\Delta}$cycle threshold (Ct) method, where cycle threshold is a logarithmic number to represent the number of cycles to reach the threshold for gene expression. Values are shown as the difference between Ct value of gene of interest and the Ct value of the housekeeping gene chosen between known stably expressed genes ($^{\Delta Ct}$) and then linearised ($^{2-\Delta Ct}$). $2^{-\Delta\Delta Ct}$ method is used to measure fold induction in gene expression compared to baseline where the Ct value difference between gene of interest and housekeeping gene at baseline is further subtracted from Ct value difference at every time point and finally linearised.

2.1.7.5 Data Analysis for the exercise intervention and associated laboratory experiments

This was an exploratory study so it was not powered for significant results. However, when determining the initial target sample size of 20, a number of variables were taken into consideration. In terms of detecting a significant improvement in quality of life scores as a primary outcome, previous exercise in asthma iterventions have calculated a MCID in ACQ would be detected by a sample size of between 21 (316)-24 (5). The EMPOWER trial based their sample size of 46, split between 2 groups, required to detect a difference in V̇O2 between groups of 2.0 ml/kg/min using a two-sample t-test at the 5 % significance level with 90 % power(168). Similarly, another group calculated a sample size of 20 intervention to 10 controls to demonstrate an improvement in AT of 2 ml O_2/kg/ min in the intervention group compared with the control at 80% power with allowance for attrition of 20%. (179). To detect differences in redox regulation parameters, exploratory work has been undertaken and published in a sample size of 4 (292). Taking all this into consideration, a pragmatic sample size of up to 20 patients was decided. A further, appropriately powered study is planned (see further work), based on power calculations for the data described in this thesis.

An analysis was performed after 6 patients had completed the intervention and sampling process, to review safety and tolerability of the intervention, and to look for initial suggestion of reduction in airways inflammation and symptoms as assessed by FeNO and ACQ scores. This number was chosen pragmatically in the context of challenging recruitment and retention rates, to assess whether this was an intervention that demonstrates benefits and would be worth pursuing. Given the significant improvements in symptoms, QoL, inflammation and antioxidant capacity, recruitment was halted at this stage to allow application for funding for a fully powered study. The results for this exploratory proof of concept study are presented herein.

All analysis was performed using IBM SPSS version 26 (NY, USA), with graphics generated in GraphPad Prism version 8 (La Jolla, California, USA). P values of <0.05 were considered statistically significant. However, given the small sample size and associated power limitations, the magnitude of any observed differences, and whether the differences are clinically important were considered in addition to statistical significance. The small sample size precluded assessment of normality, so non-parametric analyses were used. All analyses were two-tailed.

To assess whether subjects improved over time a Wilcoxon signed rank test was used to analyse the difference between baseline and post-intervention values. This analysis was conducted for the group as a whole. A Friedman test was used to assess whether there was a significant trend for improvement over the course of the study, including data from all assessment points.

To assess whether one intervention group improved more than the other, a Mann Whitney U Test was run on the difference (between baseline and post-intervention) for the two intervention groups.

To assess for correlations to link symptom and quality of life improvements to inflammatory and redox regulation measurements, a Spearman test was used. An r value of > 0.7 was considered a strong correlation, an r value of 0.4 to 0.6 is considered a moderate correlation and an r value of 0.1 to 0.3 considered a weak correlation (317)

Repeated measures analyses were not employed due to the small sample size, and difficulty in accurately assessing data of this size for normal or non normal distribution. It has been demonstrated that in small sample sizes of non-normally distribted data that these analyses are not robust, and may produce both overly liberal and conservative results (318). Instead, the absolute change in terms of median and interquartile range will be taken into consideration when interpreting outcomes.

2.2 Barriers to Exercise in Difficult Asthma

Assuming the hypotheses for exercise intervention in asthma is correct, the first step to engaging patients with asthma in an exercise intervention is to better understand the barriers they face in engaging with an exercise program. We therefore assessed this within a cohort of patients with difficult asthma using the Exercise Therapy Burden Questionnaire (ETBQ). The Exercise Therapy Burden Questionnaire (ETBQ) has been validated in French and Spanish for the assessment of barriers to physical activity in chronic illness, and consists of 10 questions graded from 0-10, with a higher score indicating higher perceived barriers to exercise ((319, 320)) and an English translation of this was used to better understand the barriers to exercise in difficult asthma.

2.2.1 The Wessex Asthma Cohort of Difficult Asthma (WATCH)

WATCH is a longitudinal clinical cohort of patients with Difficult Asthma (n = 501) based at University Hospitals Southampton NHS Foundation Trust (UHSFT), Southampton, United Kingdom (UK). All patients managed with British Thoracic Society Step "high dose therapies" and/or "continuous or frequent use of oral steroids" (84)in the Adult or Transitional Regional Asthma Clinic at UHSFT were invited to participate. Briefly, research data capture was aligned with the extensive clinical characterisation required of a commissioned National Health Service (NHS) Specialist Centre for Severe Asthma Data acquisition at enrolment included detailed clinical, health and disease-related questionnaires (ACQ6, St George's Respiratory Questionnaire (SGRQ) and EQ-5D-5L, Njimegen questionnaire for dysfunctional breathing, Sinonasal Outcome Test (SNOT22) for sinonasal symptom burden and Hospital Anxiety and Depression score (HADS) for anxiety and depression) , anthropometry, allergy skin prick testing (SPT), lung function testing, radiological imaging (in a subset) and collection of biological samples (blood, and urine). Brief longitudinal updates of data were obtained annually. A detailed outline of study protocol and methodology has previously been published(139). The study was approved by West Midlands – Solihull Research Ethics Committee (REC reference: 14/WM/1226), (see supplement for questionnaire). WATCH participants were asked to complete these questionnaires at their clinic and WATCH study follow up visits. Data were then extracted for the clinical correlates most temporally associated with the ETBQ completion

2.2.2 Data Analysis for the Barriers to Exercise in Difficult Asthma Study

Statistical analysis was performed using SPSS 24 (NY, USA), and GraphPad Prism 8 (La Jolla, California, USA). Non-parametric tests were used due to some of the data being non-Normally distributed. Quantitative variables are presented as median (mdn) and inter-quartile range (IQR). Mann-Whitney and Fisher's exact tests were used to compare the WATCH cohort as a whole with the ETBQ cohort. Kruskall Wallis and Independent Samples Median tests were used to look for differences between groups. The association between variables was tested using a Spearman's Rho test. A p value of <0.05 was considered statistically significant.

2.3 Discussion

This study design was based on previous exercise intervention studies in asthma (5) and on exercise intervention studies in other diseases using the SRETP exercise programme (168). Additionally, I incorporated redox regulation parameter assessments not previously employed in exercise interventions in asthma, in the context of the potential role of redox regulation in the pathogenesis and propagation of asthma, as reviewed in Chapter 1. The pragmatic and exploratory nature of the study resulted in some potential weaknesses that need to be taken into consideration when interpreting the results. These became increasingly apparent as the project progressed, and would be managed differently in future work based on the learning process of the exploratory work. The lack of control group raises concerns that outcome variables could be affected by extraneous factors such as change in compliance, which are known to be associated with study enrolment (321), or environmental impacts on outcomes such as FeNO, with demonstration of varibality across the seasons(322). Awareness of this as the study developed led to verbal discussions with patients surrounding compliance changes, as discussed in the results chapters in relation to specific outcome variables. On reflection, a control group would have been advantageous. To an extent, seasonal variability is controlled for by the recruitment of participants throughout the year; although this does not entirely control for potential confounding. The inclusion criteria specified that demonstration of airways hyperresponsiveness through either variability in peak flow, bronchodilator reversibility in spirometry, or through challenge testing needed to be demonstrated either at enrolment or historically to confirm a diagnosis of asthma. Consideration was made to removing the historical demonstration, with a requirement to assess airways hyperresponsiveness at baseline, but historical demonstration was retained. The reasoning for this decision was based on the exploratory nature of the study, to increase eligibility for recruitment, and that if patients were optimised in terms of their inhaled corticosteroids, they may lose this significant hyperreactivity (323) whilst still remaining symptomtic with disease modifiable through exercise intervention. In fact, all patients recruited were able to demonstrate concurrent airways hyperreactivity at screening either through significant bronchodilator reversibility or peak flow variability. Consideration was also made to demonstration and monitoring of bronchial smooth muscle hyperresponsiveness through methacholine challenge testing rather than the more pragmatic but less sensitive

bronchodilator reversibility or peak flow variability. It was deemed impractical in the context of sampling and training sessions, as well as incorporation of the interim CPET monitoring. However, methacholine challenge testing would be preferable in a fully powered study, with demonstration that it is superior to bronchodilator reversibility testing for asthma diagnosis (324).

In terms of exclusion criteria, excluding patients who required a change in their medication during the intervention period was necessary to avoid confounding of exercise intervention responses as a result of uptitration of medication. However, this does risk bias, in that non-responders to the exercise intervention are more likely to need to increase their medication, and therefore those that were able to complete the study are more likely to be responders to an exercise training intervention. The concept of responders to exercise has been investigated in a non-asthma context, in terms of improvements in cardiorespiratory fitness. In describing non-responders, a number of parameters have been assessed to define non-response. Those variables that demonstrate inter-individual variability include oxygen uptake at anaerobic thresgold and peak, resting and exercise heart rate, exercising blood pressure and muscle enzyme activity (325). Some of these, such as heart rate and blood pressure bear more relevance to cardiovascular disease outcomes, but the variability in muscle enzyme response may well impact on immunological and redox related responses to exercise (326). Whilst there is some debate as to whether non-responders truly exist, or if they just demonstrate differing responses to specific exercise training regimes (327), variability in response has not been specifically investigated in the context of exercise in asthma. The SRTEP used in this project has demonstrated increased disease modulation in those patients who respond to the exercise intervention in terms of improvement in their cardiorespiratory fitness when compared to those who do not (308). The association between differences in disease response to exercise training and gains in physical fitness suggest there may be a link between the two outcomes. The concept of exercise responders and the influence of this on disease modulation requires investigation in asthma, which would be included in a fully powered study beyond the exploratory work included in this thesis.

To avoid confounding from intercurrent treatment for exacerbations, participants requiring treatment for their exacerbations were excluded. An additional consideration is the impact of more minor exacerbations on changes in lung function and inflammatory and redox measurements. Given the variability of asthma symptoms and the difficulty in

defining a cut off for a minor exacerbation in the context of this variability (328), these patients were not excluded. However, a control group would have allowed greater confidence in interpretation of changes in the exercise group as a result of the intervention rather than just reflection of the normal variability in asthma symptoms.

In designing the study, a balance between recruitment of patients of working age to a study involvement a high level of time commitment and intensive sampling had to be balanced with perfecting scientific design, reflected in the study amendment submitted in 2018 as a result of recruitment difficulties. Prior to this, the inclusion criteria was more stringent, including demonstration of active T2 inflammation through FeNO levels above normal range or a peripheral blood eosinophil count greater than or equal to 0.3×10^9 cells/L. The amendment also included switching from randomisation to either SRETP or SRETP plus strength training to allow the addition of resistance exercises to be optional. The reasoning for the inclusion of a resistance training arm in the original study design was based on the Freitas study (284). Results from this study demonstrated the greatest inflammatory change as a result of exercise intervention and used a combination of aerobic and resistance exercises (5). However, recruitment proved challenging, and patient feedback highlighted that the additional time required by the strength training exercises was prohibitive to participation. A pragmatic decision was made in conjunction with my supervisory team to amend the protocol accordingly.

The exercise therapy burden questionnaire (ETBQ) was chosen to assess barriers to exercise within the WATCH difficult asthma cohort. There were a number of reasons for this. There are no specific questionnaires designed to assess perceived barriers to exercise in asthma patients. Whilst in depth qualitative interviews would provide greater depth to understanding of barriers to exercise than a questionnaire format, they are not practical on a wider scale. The ETBQ was utilised to assess whether clinically useful information could be obtained about the burden of exercise therapy in patients with asthma. For those patients with sub optimally controlled asthma, such as the majority of those in the WATCH cohort, the impact of their disease on daily activities is increased (329), and this group are therefore more likely to provide information regarding asthma specific disease limitations to exercise than those with milder disease. This greater severity could also be regarded as a limitation. The WATCH cohort are likely have more severe, potentially fixed airway disease than the relatively mild, although symptomatic at

baseline, group of patients enrolled in this exploratory study, and therefore their barriers to exercise may be different. The rationale for the exploration of barriers to exercise in the WATCH cohort rather than a milder cohort is two fold; difficult and severe asthma patients contribute a disproportionately high proportion of the morbidity and mortality that is associated with asthma overall, and therefore may be more likely to benefit from a disease modifying intervention. Secondly, the group enrolled in the exploratory exercise study described in this thesis may be more motivated than the asthma population as a whole, and therefore their perception of the burden of exercise therapy may be less broadly applicable to the overall asthma population than the WATCH group. Consideration of a validation study using the ETBQ and a more in depth, qualitative interview would be useful future work, which, if supportive of the utility of the ETBQ in this context, could then be followed by a study using the ETBQ within a milder, primary care cohort. Despite the limitations discussed, the data from this exploratory study presented in the following chapters, provides interesting initial data. These data can be used as a basis from which to explore the role of exercise intervention in asthma in greater depth, as discussed in section 7.4.

Chapter 3 Baseline Demographics, Safety and Tolerability and Physiological Response to Intervention

In this chapter, I present the enrolment data for the volunteers recruited to this proof of principle study. I then describe the results in the context of patient safety and tolerability. The changes in physiological parameters were assssed by the baseline, interim and final cardiopulmonary exercise tests, and the impact of the intervention on these outcomes are then presented and discussed.

3.1 Enrolment data

Twenty-four participants attended for a screening visit (1 rescreened following amendment and recruited on second screening), with 14 recruited and randomised. Table 3.1 details the reasons for exclusion at the screening stage.

Table 3.1 Patient screening numbers and study outcomes

n=24 (one re-screen); abbreviations ACQ; asthma control quesitoonaire, CPET; cardiopulmonary exercise test; FeNO; fractional exhaled nitric oxide

Screening number	**Study outcome**
001	Completed study; study ID 001
002	Failed screening-FeNO too low prior to amendment
003	Failed screening: ACQ too low
004	Failed screening: ACQ too low
005	Failed screening-FeNO too low prior to amendment
006	Completed study; study ID 005; rescreened following amendment
007	Failed screening, ACQ too low
008	Exacerbation at screening visit then declined rebook
009	Recruited; dropped out at week 3 due to work commitments. Study ID 002
010	Recruited but failed to start due to unrelated surgical procedure and work commitments. Study ID 003
011	Recruited, excluded week 3 for biologic switch
012	Completed study; study ID 006
013	Failed screening, too fit
014	Completed study; study ID 007
015	Recruited; withdrew after a few training sessions; interpersonal problems (cat died); study ID 008
016	Completed study; study ID 010
017	Recruited; withdrew after a few training sessions; work and family pressures
018	Failed screening; ACQ too low
019	Recruited; failed to start due to planned surgery; study ID 011
020	Completed study; study ID 012
021	Recruited; excluded baseline CPET; cardiac abnormalities with exercise; study ID 013
022	Failed screening, already physically active
023	Recruited, completed exercise intervention but last sampling visit out of protocol due to D and V
024	Failed screening-low ACQ

Of the 14 patients who were recruited and randomised, only 6 completed the study and all sampling visits (see figure 3.1). Two patients did not achieve baseline sampling due to recurrent treatment with antibiotics or steroids, other life events or commitments, or recent surgery. One was excluded at baseline CPET due to detection of bigeminy that did not settle with exercise, with subsequent referral onto cardiology. Two participants dropped out of the study prior to week 3 sampling, due to significant study independent life events. Nine participants have completed upto and including week 3 sampling, with one participant lost at this stage due to initiation of biological treatment for asthma and another lost due to work commitments. Seven patients have completed the exercise intervention. One of the seven participants had to be excluded from week 12 analysis due to gastroenteritis affecting their week 12 assessment. Therefore, I describe the demographic data and clinical data for the 9 patients with baseline and $\geq$1 additional sampling visits, and longitudinal data for the 6 patients who completed baseline, week 6 and final sampling. The subsequent mechanistic data for the 6 patients that completed the study with viable baseline, week 6 and final assessment and sampling visits. Due to the small number, patients have been analysed as a whole rather than separated into interval and weight training or interval training alone, with interim analysis, interpreted with the caveat that numbers were too small to draw firm conclusions, suggesting no significant benefit from additional strength training (see table 3.3 for data) .

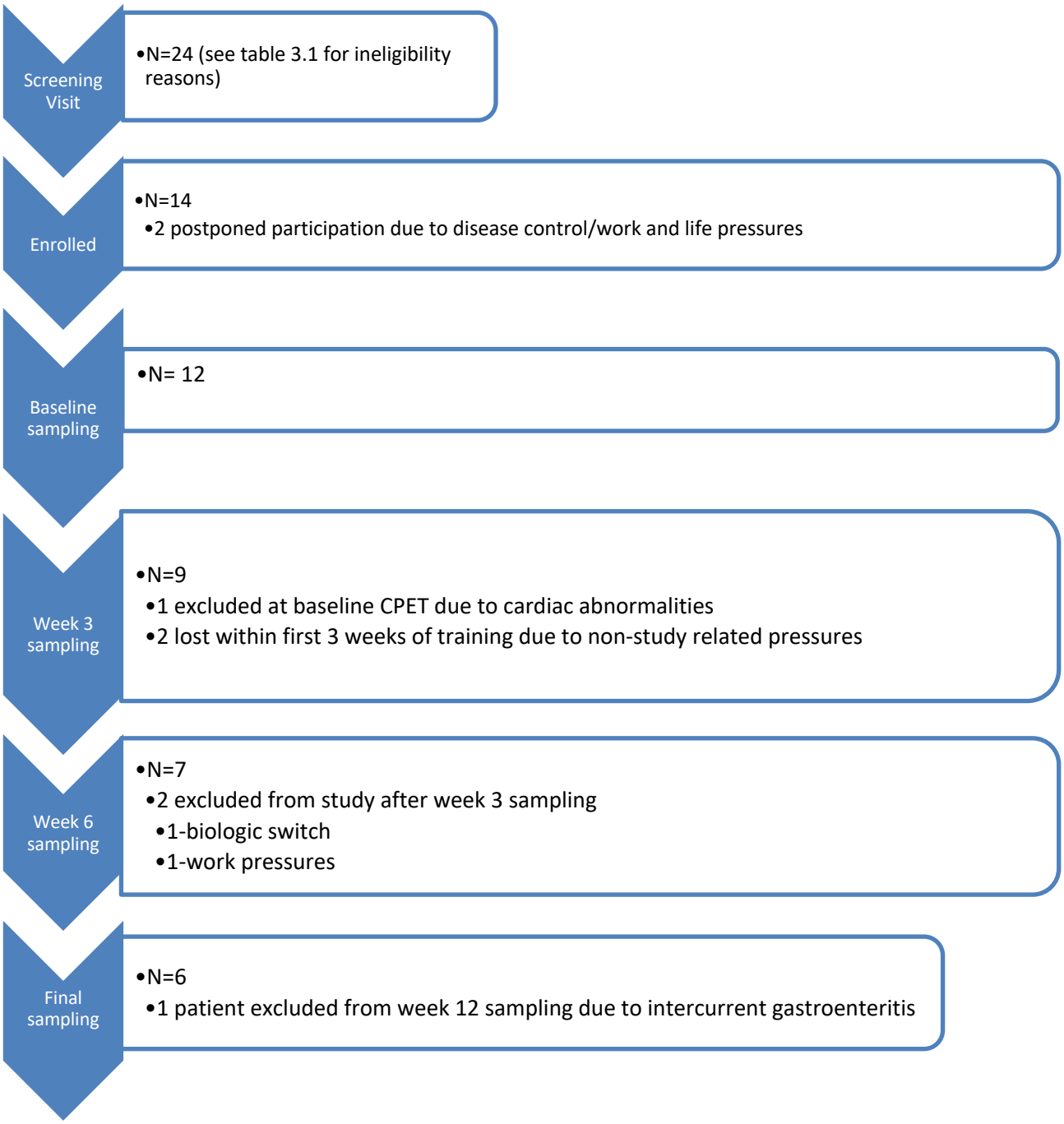


Figure 3.1 Flow chart of patient enrolment and sampling, including numbers and reasons for withdrawal/exclusion

3.2 Baseline Demographics

Baseline demographics for the 9 patients sampled at baseline and ≥1 point(s) during the training intervention are shown in table 3.2. Baseline spirometry is also shown in table 3.2. There is a non-significant amount of bronchodilator reversibility overall, but those patients who did not demonstrate bronchodilator reversibility of >200ml or 12% did demonstrate peak flow variability of > 8% to meet the inclusion criteria of the study.

Table 3.2 Baseline demographics

Baseline demographics, presented as number (%) or median (IQR). Column 2 n = 9 for those with baseline +1 sampling, column 3 n=6 for those with baseline and final sampling. Abbreviations BD; bronchodilator, BMI; body mass index, FeNO; fractional exhaled nitric oxide, FEF; forced expiratory flow, FEV1, forced expiratory volume in 1 second, FVC; forced vital capacity, GORD; gastroesophageal reflux disease

Characteristic	**Number (%) or median (IQR) for those with baseline +1 sampling (n=9)**	**Number (%) or median (IQR) for those with baseline + final sampling (n=6)**
Female sex	7 (77.8)	6 (100)
Age in years	40 (24.5, 47.5)	31.3±10
Never smoker	7 (87.5)	5 (83)
BMI (kg/m2)	27.6 (25.4, 31.5)	27±4.9
Peripheral blood eosinophil count	0.2 (0.2,0.5)	0.4 ± 0.36
FeNO (ppb)	44.5 (28, 87)	63.75 ±53
Co-morbidities	**Number (%) or mdn (IQR)**	**Number (%) or mdn (IQR)**
Atopy	8 (88.89)	5 (83%)
Anxiety and depression	4 (44%)	2 (33%)
GORD	1 (11%)	0 (0%)
Urticaria and angioedema	1 (11%)	1 (16%)
Anaphylaxis	1 (11%)	1 (16%)
Dysfunctional breathing	2 (22%)	1 (16%)
Pulmonary Function (pre bronchodilator)	**mdn (IQR)**	**mdn (IQR)**
FEV1 % predicted	85 (62.5, 93)	85.83±13.75
FVC % predicted	100 (82, 102)	95.67±14.10
FEV1/FVC	79.5 (72.3, 82.5)	75.87±5.76
FEF 25-75 % predicted	64 (25, 78)	58.54±32.08
% change FEV1 post BD	6 (3,13.5)	7.17±5.04

There was wide variability in levels of asthma treatment within the group (table 3.2), with 1 participant on step 1 treatment of the BTS guidelines and 1 patient on biological treatment, with all demonstrating suboptimal symptom control as assessed by an ACQ6 score of ≥ 1.5. Compliance was checked through verbal questioning when medication history recorded and re-confirmed at the end of the study. Whilst not all patients were regularly compliant with their medications, their compliance did not change over the intervention period.

Table 3.3 Asthma medications

Asthma medications presented as number of participants on that particular type of medication (%) and dose of medication presented as mdn (IQR); column 2 n = 9 for those with baseline +1 sampling, column 3 n=6 for those with baseline and final sampling. Abbreviations ICS; inhaled corticosteroid, LABA; long acting bronchodilator, LAMA; long acting antimuscarinic,

Asthma Medication	**Number (%) or median (IQR) for those with baseline +1 sampling (n=9)**	**Number (%) or median (IQR) for those with baseline + final sampling (n=6)**
On ICS	8 (88.9%)	5 (83%)
ICS dose (BDP equivalent µg/day)	650 (425, 1000)	500 (300, 900)
On Oral corticosteroids	1 (12.5%)	0
OCS dose (mg/day)	5 (56%)	NA
On LABA	5 (56%)	2 (33%)
LABA dose (formoterol equivalent µg/day)	24 (12, 62)	18 (12, 24)
On LAMA	1 (12.5%)	0
LAMA dose (µg day)	5 (NA)	NA
On Montelukast	2 (25%)	1 (17%)
On Omalizumab	1 (12.5%)	0
Omalizumab dose (mg/month)	600 (NA)	NA

3.3 Comparison between SRETP vs SRETP and resistance training

Given difficulty in recruitment, and feedback from potential participants that the time commitment of a resistance and aerobic intervention was too great, I considered revising the intervention to make the strength training optional. To inform this decision, whilst noting the difficulty in making any conclusive decisions from such a small data set, I assessed if there was any added benefit with the addition of resistance training. Comparison of the SRETP and STREP with resistance training for first six completed patients are detailed in table table 3.4. There were no significant differences between the 2 groups at baseline and 12 weeks, in terms of baseline demographics such as BMI and fitness as assessed through CPET variables (based on contemporaneous assessment values for prescription of exercise intervention). Basic analysis using a Mann Whitney test of the two groups suggest there was little difference in terms of improvements in outcome measures at baseline and week 12 between exercise training alone and exercise training with resistance exercises, although, as covered in the discussion, the low numbers render statistical interpretation of these data impossible.

Table 3.4 Comparison of SRETP (n=4) vs SRETP and resistance training (n=2)

Results compared using a Mann Whitney U Test between the two groups at baseline and after 12 weeks' training shows no significant differences between the two groups. Abbreviations: AT, anaerobic threshold, BMI; body mass index, ACQ; asthma control questionnaire, AQLQ; asthma quality of life questionnaire, FeNO; fractional exhaled nitric oxide, V̇O2, oxygen uptake

	SRETP (Median +IQR) (n=4)		**SRETP and resistance training (Median +IQR) (n=2)**		**P value**	
	Baseline	**Week 12**	**Baseline**	**Week 12**	**Baseline**	**Week 12**
BMI	29.6; 19.3-33.7	28.71; 21.4-32.7	25.4; 23.2-27.6	25.4; 22.9-27.9	0.5	0.8
AT	11.2; 9.3-24.9	12.4; 9.3-22.8	10.7; 10.5-10.9	13.2; 13-13.4	>0.9	>0.9
V̇O2 peak	20.8; 16.8-35.9	25.2; 20.3-37.6	22.9; 21.3-24.5	25.2; 21.1-29.2	0.8	>0.9
ACQ Score	2.1; 1.8-3.1	1.3; 1.2-2.0	1.9; 1.6-2.2	0.83; 0.83-0.83	0.53	0.13
AQLQ Score	5.1; 3.5-5.3	6.0; 5.2-6.4	4.5; 4.5-4.6	5.8; 5.8-5.9	0.46	>0.9
Peripheral blood eosinophil count	0.4; 0.2-1.1	0.25; 0.2-0.6	0.25; 0.2-0.3	0.15; 0.1 -0.2	0.5	0.27
FeNO	57; 23-141	54; 21-165	44; 21-57	22; 22-22	0.8	0.46

A minor amendment was subsequently approved by the ethics committee in August 2019 to change the strength training to optional, on a pragmatic basis to reduce patient burden in terms of the study intervention and associated time commitment. Improvements were seen in terms of symptom scores with SRETP alone, and therefore any patients recruited

after the amendment were enrolled in the SRETP only, with the option of additional strength exercises. Of note, in terms of PPI feedback, all chose SRETP alone.

3.4 Safety and Tolerability

Cardiopulmonary exercise testing is well validated and widely used to determine levels of fitness, and is used in this study to prescribe the exercise intervention(182).

To assess if this was extendable to this population of poorly controlled patients, the compliance with cardiopulmonary tests was reviewed. All patients were able to complete cardiopulmonary exercise tests without an acute exacerbation of their asthma symptoms, and to the level to determine anaerobic threshold and V̇O2 peak. This is consistent with feasibility data from previous work in Australia.

3.5 Compliance

To assess whether the intervention was acceptable to patients, the level of compliance with the exercise training sessions were assessed. All patients completed > 74% of sessions within their duration in the study. This cut off point for compliance was selected based on the compliance criteria for the EMBRACE and EMPOWER (168) studies of exercise training in cancer patients, as this has been demonstrated to be effective at obtaining clinically significant and immunomodulatory improvements(308). Patient 002 was excluded from the study after week 3 CPET as travel for work precluded compliance with the study protocol so only data for baseline and week 3 are available. Patient 004 was excluded from the study due to addition of mepolizumab to their medication regime, which is against study protocol, so only data for baseline and week 3 are available.

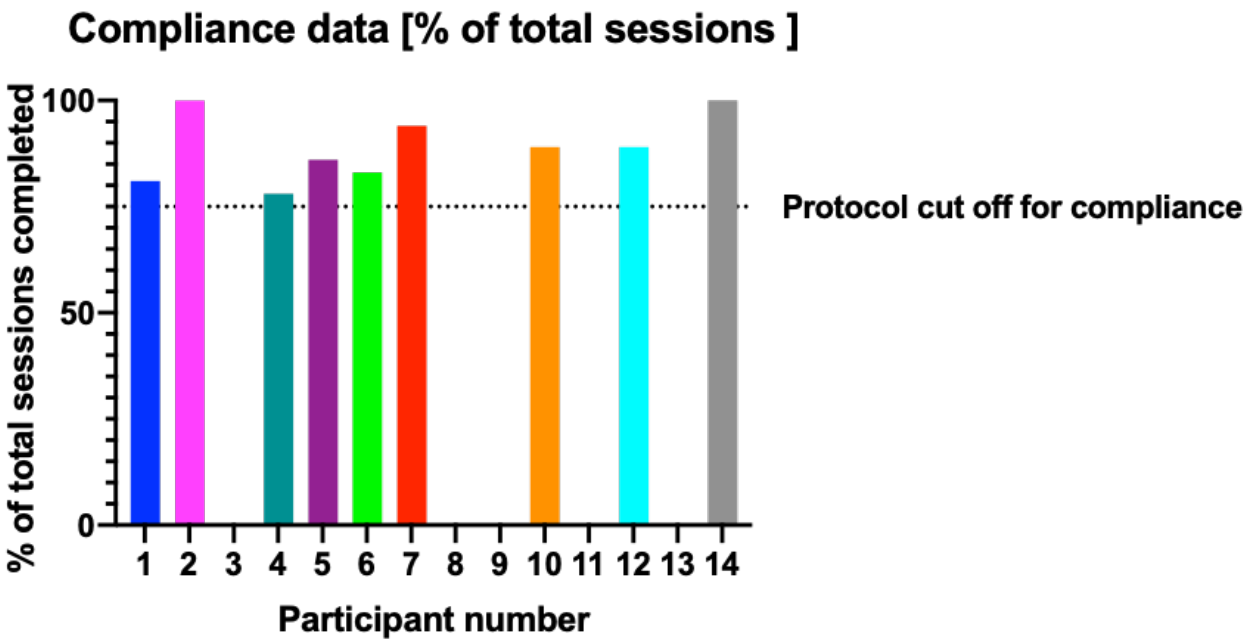


Figure 3.2 Compliance data

Percentage of total sessions to completion or withdrawal, with protocol cut off for compliance of ≥74% of the total available indicated by dotted line, n=9; each participant is represented by a different colour

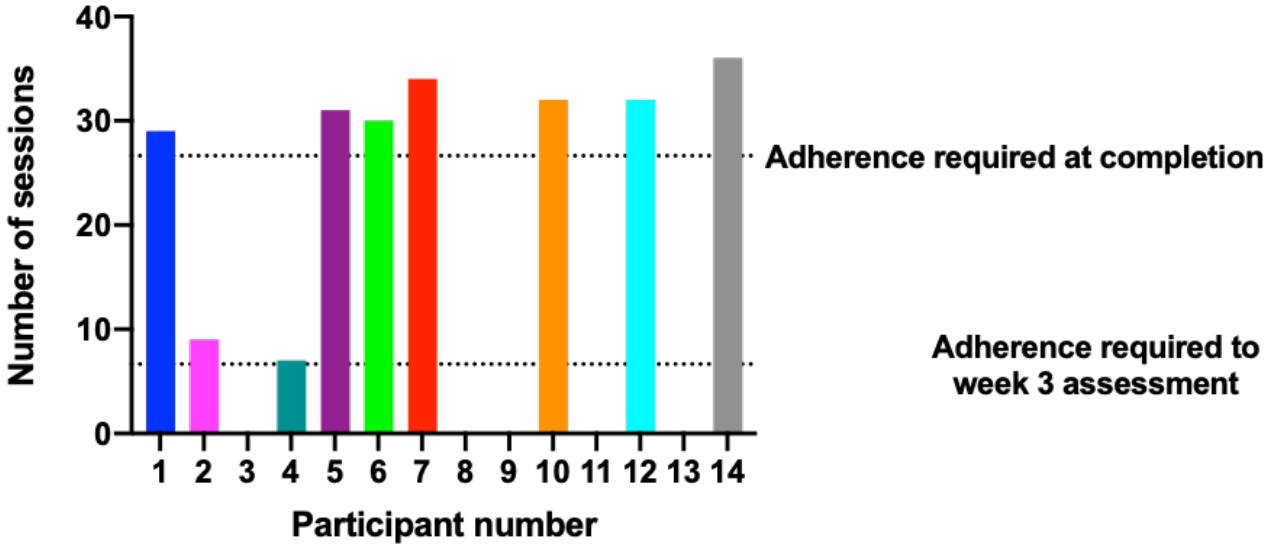


Figure 3.3 Compliance data

Number of sessions (maximum number of sessions for patients competing to 3 weeks assessment was 9 (n=6), and maximum number of sessions for those completing the 12 week intervention (n=7) was 36) , n=9; each participant is represented by a different colour

Median adherence to training sessions was 86% (IQR 81.5-92.75%) overall or 31 (IQR 29.75-32.5) of the 36 sessions available for those who completed the study.

3.6 Body Composition

To assess if any possible improvements in asthma symptoms could be associated with weight loss, BMI was assessed longitudinally. To further assess if a reduction in adiposity driven inflammation could explain any improvements in asthma symptoms and inflammation, fat free mass and fat mass, as a percentage of total body weight were assessed. To assess if visceral fat deposition was an explanation for the changes in symptom scores and inflammation, visceral adipose tissue was assessed. Finally, fat mass index and fat free mass index, measured in kg/m^2, was assessed. I present the data for n=6 (those who completed the study with valid initial and final sampling visits).

3.6.1 Body Mass Index

Body mass index did not significantly change between the beginning and end of the study, as assessed by a Friedman test (p=0.17) (Figure 3.4). When comparing body mass index for the participants who completed the exercise training to those who withdrew from the study

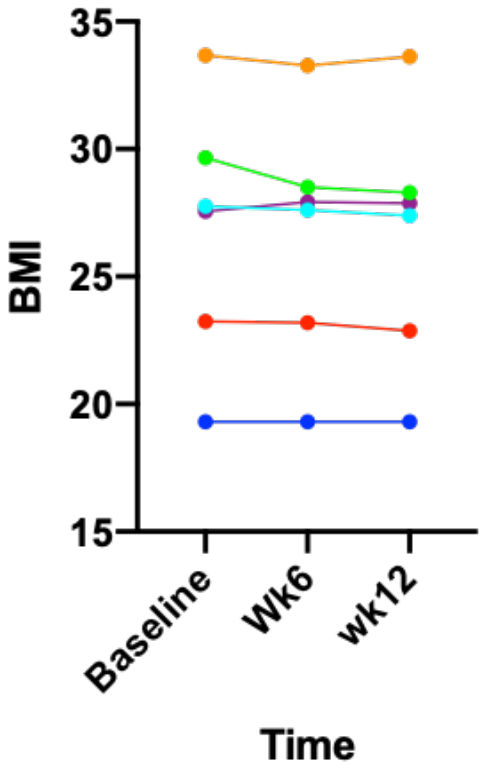


Figure 3.4 BMI in kg/m^2

(SECA values); n=6 for those participants who completed baseline, week 6 and week 12 sampling, as assessed Wilcoxon signed rank test p=0.17. Individual participants are represented by a different colour, with each point indicating a sampling event.

3.6.2 Fat Free Mass

There was no significant difference in fat free mass between baseline and 12 weeks, as assessed by Wilcoxon test (mdn =62.94 vs mdn = 60.71, p=1.0.) (figure 3.5)

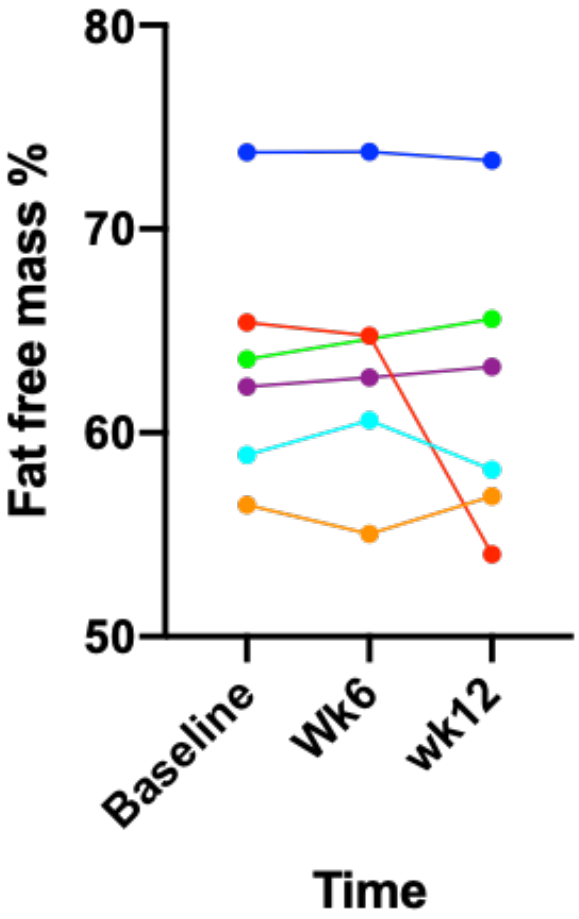


Figure 3.5 Fat free mass (%)

(SECA values); n=6 for volunteers who completed baseline, week 6 and week 12 sampling, as assessed Wilcoxon signed rank test p=1.0. Individual participants are represented by a different colour, with each point indicating a sampling event.

3.6.3 Fat Mass

There was no significant difference in fat mass assessed at baseline and 12 weeks, as assessed by Wilcoxon test (mdn =36.58 vs mdn = 36.85, p=.528.) (figure 3.6)

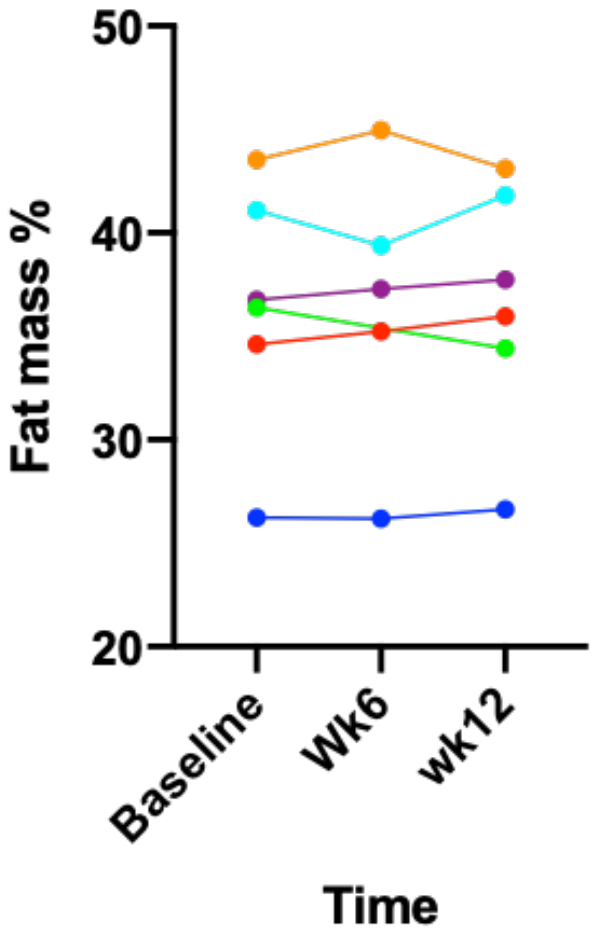


Figure 3.6 Fat mass (%)

(SECA values); n=6 for volunteers who completed valid baseline, week 6 and week 12 sampling, as assessed Wilcoxon signed rank test p=.528. Individual participants are represented by a different colour, with each point indicating a sampling event.

3.6.4 Visceral Adipose Tissue

There was no significant difference in visceral adipose tissue assessed at baseline and 12 weeks, as assessed by Wilcoxon test (mdn =0.83 vs mdn = 0.74, p=.465.) (figure 3.7)

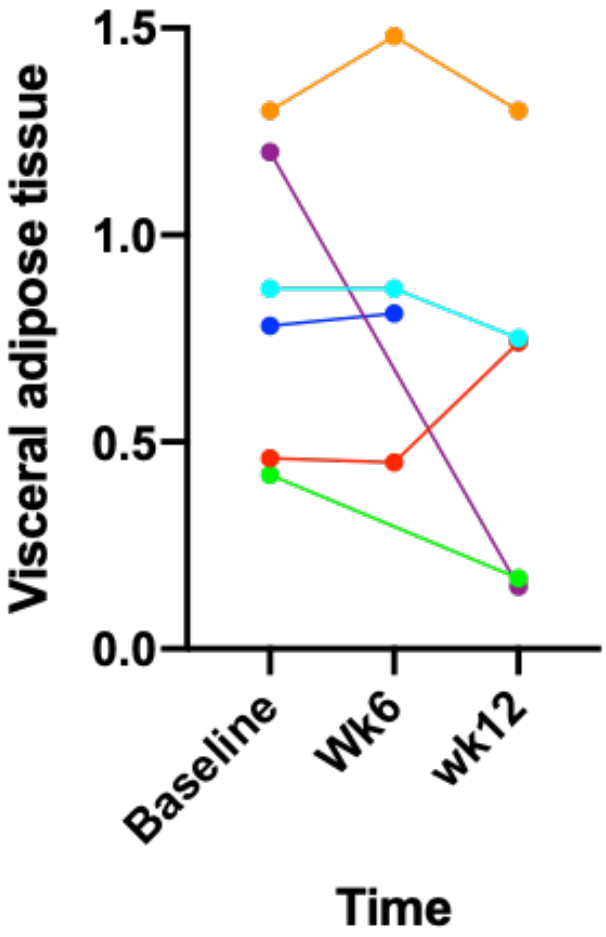


Figure 3.7 Visceral adipose tissue

(SECA values); n=6 for volunteers who completed valid baseline, week 6 and week 12 sampling, as assessed Wilcoxon signed rank test p=.465; data missing for 1 patient at 12 weeks. Individual participants are represented by a different colour, with each point indicating a sampling event.

3.6.5 Skeletal Muscle Mass

There was no significant difference in skeletal muscle mass assessed at baseline and 12 weeks, as assessed by Wilcoxon test (mdn =23.03 vs mdn = 22.9, p=.463.)(figure 3.8)

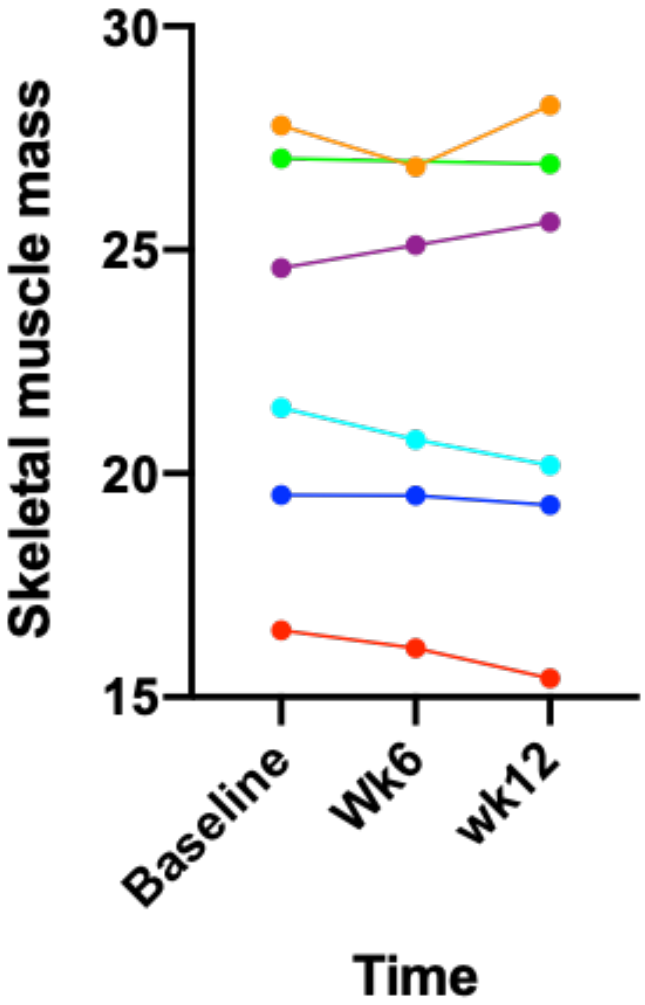


Figure 3.8 Skeletal muscle mass

(SECA values); n=6 for volunteers who completed valid baseline, week 6 and week 12 sampling, as assessed Wilcoxon signed rank test p=.463. Individual participants are represented by a different colour, with each point indicating a sampling event.

3.6.6 Fat Free Mass Index

There was no significant difference in fat free mass index assessed at baseline and 12 weeks, as assessed by Wilcoxon test (mdn =16.71 vs mdn = 16.78, p=.917.) (figure 3.9)

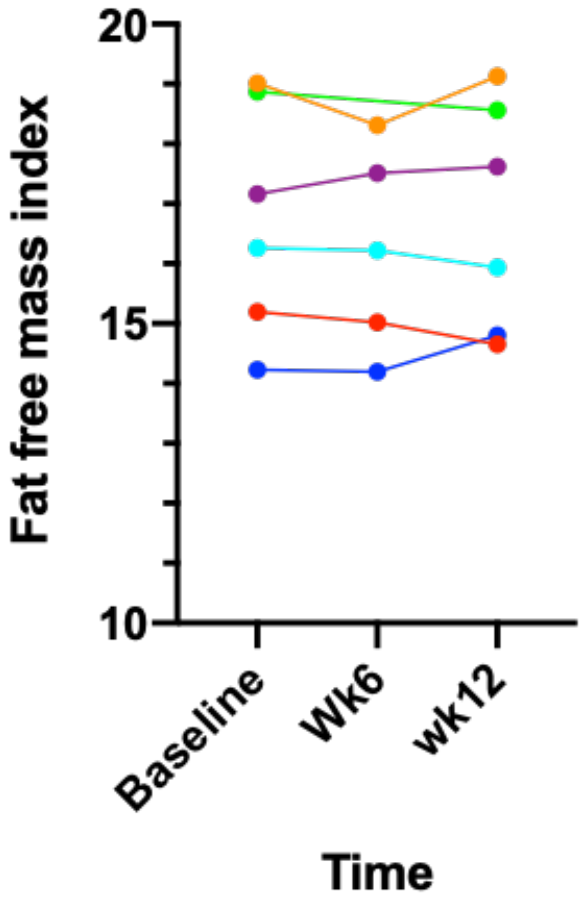


Figure 3.9 Fat free mass index

(SECA values); n=6 for volunteers who completed valid baseline, week 6 and week 12 sampling, as assessed Wilcoxon signed rank test p=.917. Individual participants are represented by a different colour, with each point indicating a sampling event.

3.6.7 Fat Mass Index

There was no significant difference in fat mass index assessed at baseline and 12 weeks, as assessed by Wilcoxon test (mdn =10.6 vs mdn = 10, p=.917.)

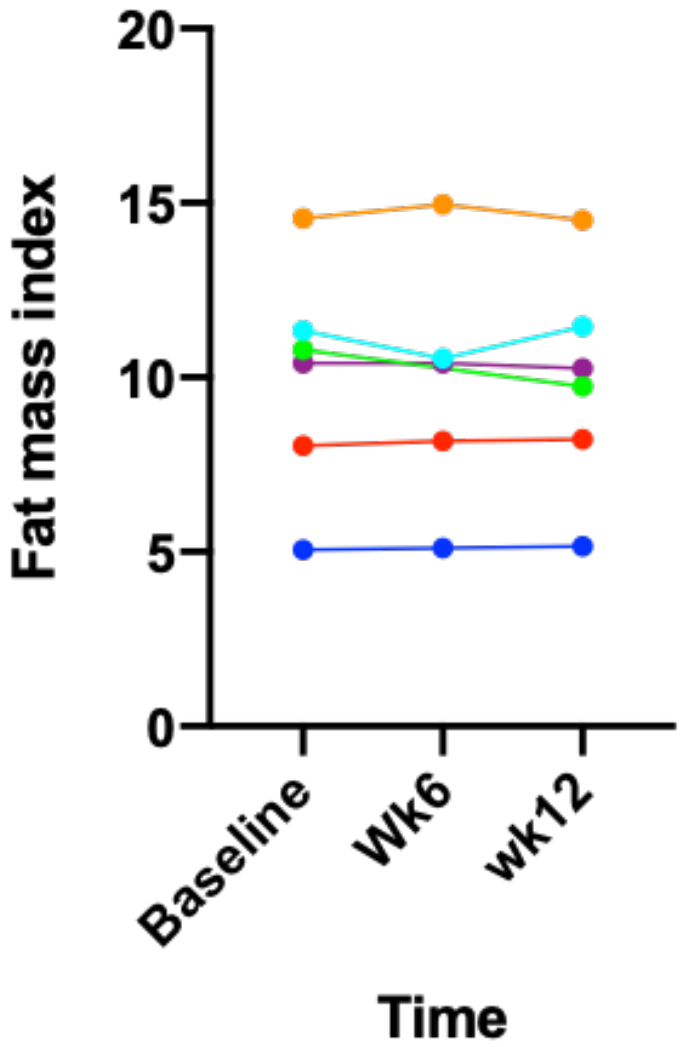


Figure 3.10 Fat mass index

(SECA values); n=6 for volunteers who completed valid baseline, week 6 and week 12 sampling, as assessed Wilcoxon signed rank test p=.917. Individual participants are represented by a different colour, with each point indicating a sampling event.

3.7 Physical Fitness

To assess if any possible improvements in asthma symptoms could be associated with changes in cardiopulmonary fitness, fitness levels were at baseline, week 3, week 6 and

after completion of 12 weeks training using a symptom limited maximal ramp cardiopulmonary exercise test (CPET). The incremental rise in work rate was predetermined using the equation described in 2.1.3.1.1. and physiologist expertise. The same ramp was used for each test to ensure comparability of results. The formal reporting of the CPET data, as presented herein, were undertaken by 2 CPET competent clinicians, blinded to the patient data, with the mean of each value presented. If their analysis differed by > 4.8% for any outcome measure, an independent third interpreter was involved for additional analysis, with the third reporter data presented in preference to the mean of the two initial reporters . This method of CPET reporting has been described and utilised by previous exercise intervention studies undertaken at UoS (287). Longitudinal data are presented for the n=6 who completed the training intervention with valid baseline and final assessments to provide an overall demonstration of change over time (see table 3.5). Data for n=9 presented in graphic form for those patients who completed baseline + ≥ 1 CPET to demonstrate heterogeneity of response. The data from week 3 CPET for patient 012 have been presented herein; however, it is noteworthy that the patient had an non-study related exacerbation in week 2 that did not reach criteria for trial exclusion but impacted on CPET outcomes at 3 weeks. This patient continued training at baseline prescription to 6 weeks rather than re-prescribing the training programme at a reduced level due to their poor performance in week 3 CPET. All CPETs completed were good quality tests, with adequate effort demonstrated by all tests achieving an RER of > 1.1 and near maximal heart rates achieved (177). Table 3.5 demonstrates CPET variables for the n=6 who completed the training intervention and baseline and final assessment visits.

Table 3.5 CPET outcome variables

For n =6 at baseline and post intervention CPET. Abbreviations: AT; anaerobic threshold, CPET; cardiopulmonary exercise test, FEV1; forced expiratory volume in 1 second, HR; heartrate; MVV; maximal voluntary ventilation; $P_{ET}CO_2$, end tidal carbon dioxide; RER; respiratory exchange ratio, RPE; rating of perceived exertion, $\dot{V}CO_2$, carbon dioxide output; $\dot{V}O_2$, oxygen uptake; $\dot{V}_E$, ventilatory equivalent; W, watts

		Baseline			**Week 12**			**P value (Wilcoxon)**		
	Patient	**Rest**	**AT (%pred $\dot{V}O_2$peak)**	**Peak (%pred)**	**Rest**	**AT**	**Peak (%pred)**	**Rest**	**AT**	**Peak**
$\dot{V}$O2 (ml/kg/min) (% predicted)	001	3.8	21.2 (67)	39.7 (126)	5.5	22.2 (70)	41 (130)	0.916	0.046*	0.058
	005	3.3	10.6 (37)	21.5 (74)	3	13.4 (46)	20.2 (70)			
	006	3	9.8 (37)	21.6 (81)	2.7	9.45 (35)	25.4 (95)			
	007	3.8	11.6 (44)	24.55 (94)	3.2	13.1 (50)	28.3 (108)			
	010	2.4	8.3 (38)	15.75 (71)	2.7	10.15 (46)	18.7 (85)			
	012	3.1	10.2 (35)	20.4 (69)	3.2	12 (41)	24.3 (82)			
	Mdn (IQR)	**3.2 (2.9,3.8)**	**10.4 (9.4, 14)**	**21.6 (19, 28)**	**3.1 (2.7,3.8)**	**12.6 (10, 15.6)**	**25 (20, 31.5)**			
$\dot{V}CO_2$ (ml/kg/min)	001	0.23	1.14	2.895	0.27	1.15	2.735	0.673	0.058	0.249
	005	0.24	0.7	2.245	0.23	0.94	2.08			
	006	0.28	0.75	2.47	0.23	0.74	2.835			
	007	0.18	0.6	1.81	0.17	0.65	2.12			
	010	0.22	0.78	1.915	0.27	0.945	2.315			
	012	0.24	0.63	1.83	0.26	0.885	2.23			
	Mdn (IQR)	**0.24 (0.21, 0.25)**	**0.73 (0.62, 0.87)**	**44 (38, 50)**	**0.25 (0.33,0.27)**	*0.91 (0.72, 1.0)*	**45 (40, 52)**			

		Baseline			Week 12			P value (Wilcoxon)		
	Patient	**Rest**	**AT (%pred $\dot{V}O_2$peak)**	**Peak (%pred)**	**Rest**	**AT (%pred $\dot{V}O_2$peak)**	**Peak (%pred)**	**Rest**	**AT**	**Peak**
Work load (W)	001		104	213		96	232		0.075	0.028*
	005		72	166		102	204			
	006		64	204		75	244			
	007		64	136		65	160			
	010		50	132		78	162			
	012		52	156		75	168			
	Mdn (IQR)		**64 (52, 80)**	**161 (135, 206)**		**77 (73,98)**	**186 (162, 235)**			
$P_{ET}CO_2$	001	29	37	38	31	35	33	0.671	0.197	0.416
	005	29	37	28	30	39	28			
	006	28	35	31	33	42.5	33			
	007	28	33	30	27	33	28.5			
	010	33	38	35.5	31	38	36			
	012	28	30	32	27	34	30			
	Mdn (IQR)	**28.5 (28, 30)**	**36 (32.3, 37.3)**	**31.5 (29.5, 36.1)**	**30.5 (27,31.5)**	**36.5 (33.8, 39.9)**	**31.5 (28.4, 33.8)**			
$\dot{V}E$ (L/min)	001	11	37	85.5	12	38	98	0.914	0.172	0.046*
	005	11	23	96.5	10	29	91			
	006	12	26	91.5	9	21	103			
	007	9	25	75.5	8	25.5	91			
	010	9	26	66.5	11	32	81			
	012	11	27	70	12	30.5	89			
	Mdn (IQR)	**11 (9, 11.25)**	**26 (24.5, 29.5)**	**80.5 (69.1, 92.8)**	**10.5 (8.75, 12)**	**29.8 (24.4, 33.5)**	**91 (87, 99.25)**			
Respiratory rate	001	22	24	35	21	23	47.5	0.068	0.225	0.028*
	005	16	17	41	16	21	48			
	006	11	20	34	9	14	37			
	007	19	26	40.5	12	26	46.5			
	010	22	28	42.5	18	25.5	44.5			
	012	11	27	32.5	11	17	41			
	Mdn (IQR)	**17.5 (11,22)**	**25 (19.3, 27.3)**	**37.8 (33.6, 41.4)**	**14 (10.5, 18.8)**	**22 (16.3, 25.6)**	**45.5 (40, 47.6)**			

		Baseline			Week 12			P value (Wilcoxon)		
	Patient	**Rest**	**AT**	**Peak (%pred)**	**Rest**	**AT**	**Peak (%pred)**	**Rest**	**AT**	**Peak**
HR (%MPHR)	001 (194)	88	128	178 (92)	80	114	183 (94)	0.027*	0.833	0.4
	005 (197)	89	115	177 (90)	79	120	180 (91)			
	006(192)	79	96	160 (83)	69	97	166 (86)			
	007 (175)	82	106	164(94)	74	102	171 (98)			
	010 (177)	85	111	163 (92)	84	114	162 (92)			
	012 (197)	97	122	181 (92)	92	121	174 (88)			
	Mdn (IQR)	**87 (81, 91)**	**113 (104, 124)**	**171 (162, 179)**	**80 (73, 86)**	**114 (101, 120)**	**173 (165, 181)**			
FEV1 (% predicted)	001	3.13 (84)			2.83 (77)			0.463		
	005	3.45 (95)			3.47 (96)					
	006	3.89 (107)			4.11 (113)					
	007	2.98 (109)			2.95 (108)					
	010	2.20 (66)			2.24 (67)					
	012	3.39 (98)			3.90 (113)					
	Mdn (IQR)	**3.26 (2.79, 3.56)**			**3.21 (2.68, 3.95)**					
MVV	001	125.2			115.6			0.249		
	005	138			148					
	006	155.6			164.4					
	007	119.2			118					
	010	88			89.6					
	012	135.6			156					
	Mdn (IQR)	**130.4 (111.4, 142.4)**			**133 (109.1, 158.1)**					
Breathing reserve	001			38 (30)			24 (21)			0.046*
L/min (% MVV)	005			26.5 (19)			30 (20)			
	006			29 (19)			17.5 (11)			
	007			22.5 (19)			7 (6)			
	010			45.5 (52)			30.5 (34)			
	012			49 (36)			30 (19)			
	Mdn (IQR)			**33.5 (25.5, 46.38)**			**27 (14.9, 30.1)**			

		Baseline			Week 12			P value (Wilcoxon)		
	Patient	**Rest**	**AT**	**Peak (%pred)**	**Rest**	**AT**	**Peak (%pred)**	**Rest**	**AT**	**Peak (%pred)**
$\dot{V}E/\dot{V}CO_2$	001	35.2	29.8	34.6	38.4	31.2	39.6	0.116	0.141	0.138
	005	40.4	30	52.75	37.4	28.2	51.6			
	006	39.6	32.1	45.55	33.9	26.65	43.7			
	007	41.4	36.8	49.4	42.9	35.4	53.65			
	010	30.2	29.8	38.95	33	30.15	39.4			
	012	40.8	39.4	42.8	41.4	32.8	46.3			
	Mdn (IQR)	**40 (34,41)**	**31 (30,37)**	**36 (31,40)**	**38 (34,37)**	**31 (28,33)**	**37 (33,31)**			
$\dot{V}E/\dot{V}CO_2$ slope	001		25.60			33.25			0.400	
	005		32.85			32.5				
	006		27.65			30.4				
	007		34.00			31.55				
	010		29.35			28.15				
	012		30.70			33.15				
	Mdn (IQR)		**30 (27,33)**			**32 (30,31)**				
RER	001	1.02	0.9	1.24	0.87	0.9	1.17	0.462	0.223	0.917
	005	0.88	0.82	1.285	0.94	0.84	1.25			
	006	1.02	0.83	1.28	0.97	0.875	1.25			
	007	0.8	0.88	1.25	0.91	0.87	1.31			
	010	0.89	0.94	1.22	0.97	0.93	1.24			
	012	1	0.83	1.165	1.08	0.955	1.21			
	Mdn (IQR)	**0.95 (0.86, 1.02)**	**0.86 (0.83, 0.91)**	**1.25 (1.21, 1.28)**	**0.96 (0.9, 1)**	**0.89 (0.86, 0.94)**	**1.25 (1.2, 1.27)**			
O_2 pulse (% predicted)	001	2.5	9.8	13.15 (137)	3.9	11	12.8 (134)	0.223	0.046	0.116
	005	3	7.5	9.85 (83)	3.2	9.3	9.3 (79)			
	006	3.5	8.9	12 (97)	3.5	8.7	13.65 (110)			
	007	2.7	6.4	8.85 (100)	2.4	7.35	9.45 (108)			
	010	2.9	7.4	9.7 (78)	3.3	8.85	11.6 (93)			
	012	2.4	6.4	8.55 (75)	2.6	7.6	10.6 (93)			
	Mdn (IQR)	**2.8 (2.48, 3.13)**	**7.45 (6.4, 9.13)**	**9.78 (8.78, 12.29)**	**3.25 (2.55, 3.6)**	**8.78 (7.54, 9.73)**	**11.1 (9.41, 13.01)**			

		Baseline			Week 12			P value (Wilcoxon)		
	Patient	**Rest**	**AT**	**Peak (%pred)**	**Rest**	**AT**	**Peak (%pred)**	**Rest**	**AT**	**Peak (%pred)**
Reason for stopping	001 005 006 007 010 012	Legs Legs L/B Fatigue L/B Legs			Fatigue Legs NR NR Legs legs					
BORG RPE breathing	001 005 006 007 010 012	0 1 1 NR 0 NR		4 5 3 NR 3 NR	0 1 NR NR 3 0		7 7 NR NR 9 2			
BORG RPE legs	001 005 006 007 010 012	0 3 0 NR 0 NR		5 10 3 NR 3 NR	0 2 NR NR 0 0	7 9 NR NR 9 7				

Table 3.6 Oxygen uptake at all sampling points

for those patients with baseline and ≥1 CPET (n=9), displayed as individual patient values and median and IQR. Abbreviations AT; anaerobic threshold, CPET; cardiopulmonary exercise test, V̇O2; oxygen uptake

V̇O2 (ml/kg/min)	**Baseline**		**Week 3**		**Week 6**		**Week 12**	
	AT	Peak	AT	Peak	AT	Peak	AT	Peak
001	21.20	39.70	25.65	39.25	28.25	42.60	22.20	41.00
002	11.85	26.10	13.15	25.65				
004	10.35	12.80	9.25	13.65				
005	10.60	21.50	12.30	22.30	13.00	24.40	13.40	20.20
006	9.80	21.60	10.35	22.75	8.70	21.20	9.45	25.40
007	11.60	24.55	14.85	28.65	14.65	27.35	13.10	28.30
010	8.30	15.75	8.70	15.60	7.10	19.40	10.15	18.70
012	10.20	20.40	12.00	23.10	11.50	23.90	12.00	24.30
014	10.80	20.40	11.00	20.20	11.80	19.40		
Mdn (IQR)	10.6 (10,11.7)	21.5 (18.1, 25.3)	12 (9.8, 14)	22.8 (17.9, 21.2)	11.8 (8.7, 14.65)	23.9 (19.4, 27.4)	12.6 (10, 15.6)	25 (20, 31.5)

3.7.1 Oxygen uptake

Oxygen uptake ($\dot{V}O_2$; ml/kg/min) measured at anaerobic threshold (AT) significantly improved between baseline and post intervention CPETs (mdn= 10.4 vs mdn 12.6) (see table 3.5). Peak oxygen uptake ($\dot{V}O_2$ peak; ml/kg/min) showed a trend to significant improvement between baseline and post intervention CPETs (mdn=21.6 vs mdn 25), as assessed by Wilcoxon signed rank test. Including all those participants with baseline and ≥ CPET, there was a significant improvement between baseline and week 3 AT (n=9, mdn 10.6 vs mdn 12 p=0.028, see table 3.6 and figure 3.11). There was a trend for significant improvement in oxygen uptake between baseline and week 6 (n=7, mdn 21.5 vs 23.9, p= 0.063, see table 3.6 and figure 3.11). Due to the unexpected decrease in oxygen uptake at anaerobic threshold for participant 001 between weeks 6 and 12, additional review of these data were undertaken. The week 12 test demonstrated a >4.8% discrepancy between the initial two blinded assessors (21.2 ml/kg/minute and 26.5ml/kg/minute) and therefore the third assessor interpretation of oxygen uptake at AT was used

(22.2ml/kg/min). For the week 6 assessment, a mean of the two initial interpretors was used.

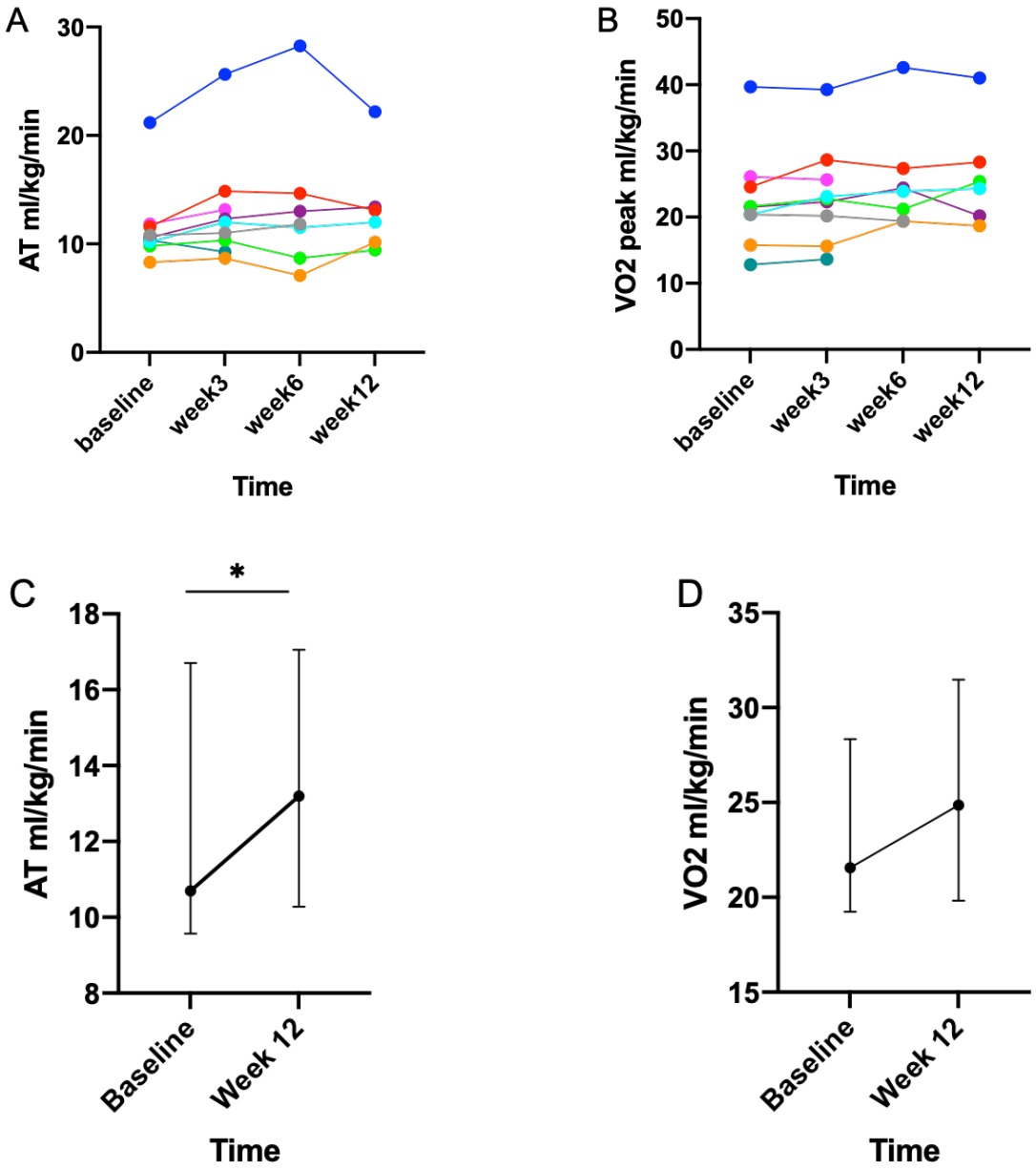


*Figure 3.11 Oxygen uptake in ml/kg/min at A) anaerobic threshold and B) peak exercise, n=9 for those patients with valid baseline and week 12 CPETs; individual participants are represented by a different colour, with each point indicating a sampling event. Line diagram showing median oxygen uptake at C) anaerobic threshold and D) peak exercise as assessed by Wilcoxon Test for n=6 with valid baseline and week 12 CPETs, *= p<0.05 .*

3.7.2 Maximum workload

All participants were able to significantly increase their maximum workload following the training intervention (figure 3.13).

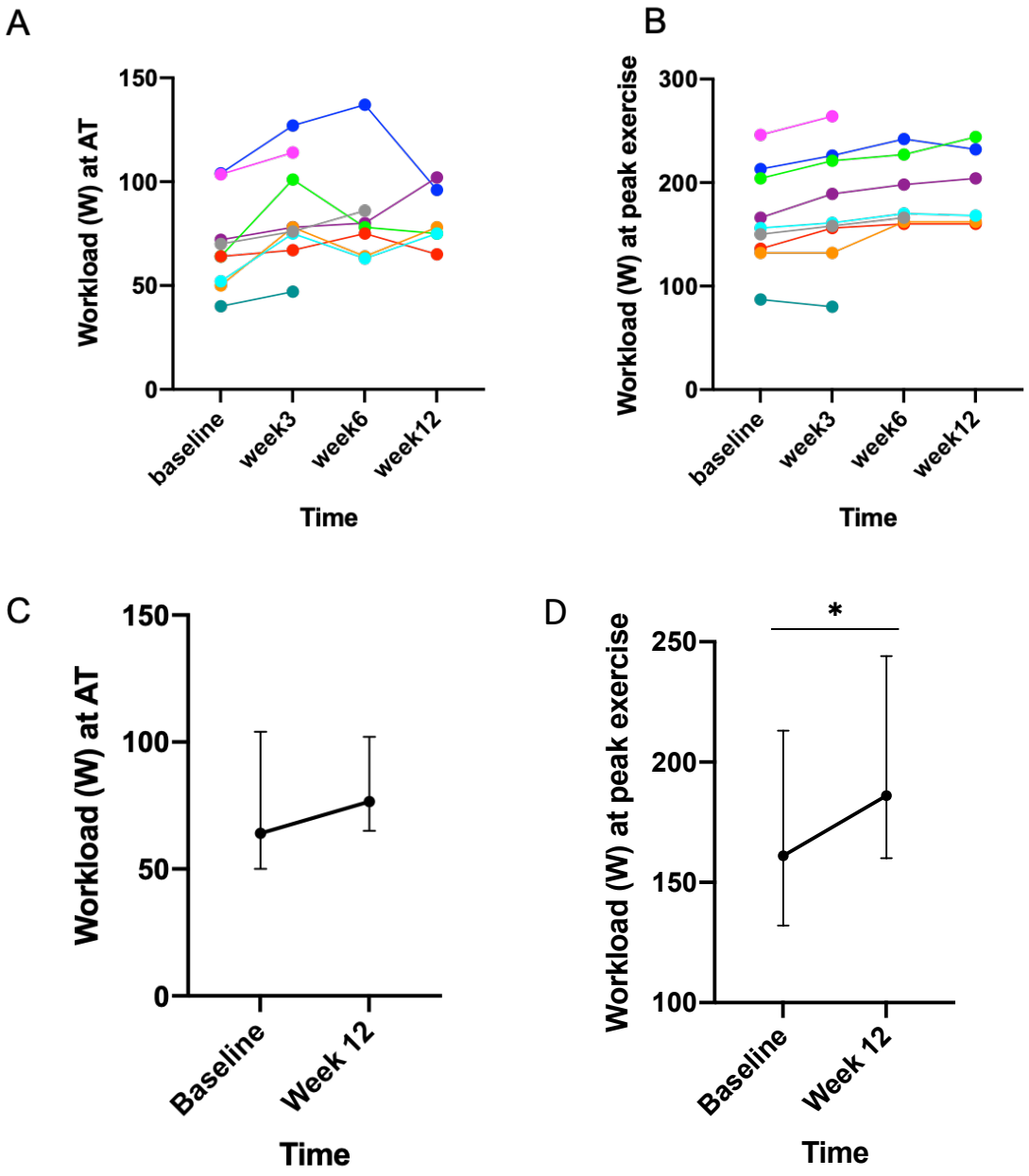


Figure 3.12 Workload in Watts

*at A) anaerobic threshold and B) peak exercise, n=9 for those patients with valid baseline and week 12 CPETs; individual participants are represented by a different colour, with each point indicating a sampling event. Line diagram showing median workload at C) anaerobic threshold and D) peak exercise as assessed by Wilcoxon Test for n=6 with valid baseline and week 12 CPETs, *= p<0.05*

3.7.3 O_2 pulse

Assessment of the O_2 pulse response to incremental exercise provides insight into the cardiovascular function of an individual. There was a significant increase in O_2 pulse at AT (mdn 7.45 vs 8.78, p=0.046, see table 3.5).

3.7.4 Ventilatory CPET parameters

To assess whether there was ventilatory limitation to the performance of participants as a result of their asthma, I looked at the respiratory specific CPET variables.

3.7.4.1 Respiratory rate and Ventilatory Equivalent

Respiratory rate (RR) and ventilatory equivalents ($\dot{V}E$) were measured at rest, at anaerobic threshold and at peak exercise (see table 3.5 for individual figures and median (IQR) at baseline sampling and post intervention). There was a trend for significant reduction in RR at rest between baseline and post intervention (mdn 17.5 vs 14, p=0.068), and a significant increase in RR at peak exercise (mdn 37.8 vs 45.5, p=0.028). There was also a significant increase in $\dot{V}E$ at peak exercise (mdn 80.5 vs 91, p=0.046).

3.7.4.2 Breathing reserve

Normal values for breathing reserve at $\dot{V}O_2$ peak are greater 11-15% of MVV, or greater than 11L/min (176), although it is noted that McNicholl et all used a value of >20% MVV to indicate normality(92). Breathing reserve (BR) was measured in L/min exercise (see table 3.5). BR demonstrated a significant decrease between baseline and post intervention CPET (mdn =33.5 vs 27, p=0.046), although only 1 patient demonstrated a lower than normal % value for BR. FEV1 was unchanged over the course of the intervention, and so the reduction in breathing reserve is a result of the increase in $\dot{V}E$. Given the increase in exercise capacity, demonstrated by increased workrates and oxygen uptake resulting in an increase in ventilation, this is an explainable and expected finding.

3.7.4.3 Partial pressure of End Tidal CO_2

Partial pressure of end tidal CO2 ($P_{ET}CO_2$) was measured at baseline, AT and peak. In health, $P_{ET}CO_2$ at rest runs between 36 and 44 mmHg, with an increase in $P_{ET}CO_2$ between rest and AT of between 5-8mmHg is expected, with a slight decrease thereafter (170). At baseline, all participants demonstrated low $P_{ET}CO_2$ values, which coupled with the

respiratory rate data and baseline RER data (see table 3.5), is suggestive of hyperventilation. By week 12 of the intervention, $P_{ET}CO_2$ levels at rest had increased towards normal range , suggesting a reduction in acute hyperventilation during the course of the intervention as particiapants became familiar with the CPET testing process.

$P_{ET}CO_2$ assessed at AT showed a normal increase from baseline for all participants across all sampling points. There was no significant change in $P_{ET}CO_2$ levels at AT between baseline and week 12. $P_{ET}CO_2$ at peak (mmHg)did not significantly change between baseline and week 12

3.7.4.4 $\dot{V}E/\dot{V}CO_2$ Gradient

The $\dot{V}E/\dot{V}CO_2$ reflects gas exchange and pulmonary dead space, and should be ≤ 32 at AT, and as a gradient (slope) (164, 170). $\dot{V}E/\dot{V}CO_2$ at AT at baseline was high for 3 of the 6 participants and reduced for each of these participants post intervention (see table 3.5). One participant had a high $\dot{V}E/\dot{V}CO_2$ slope at baseline, with three of the six participants demonstrating a $\dot{V}E/\dot{V}CO_2$ slope >32 at final sampling. Given the discrepancy between $\dot{V}E/\dot{V}CO_2$ values at AT and overall slope, the individual tests were further interrogated. Whilst both interpreters excluded data beyond RCP when interpreting $\dot{V}E/\dot{V}CO_2$ slope, there were individual variations in analysis of the same test in terms of lower limit of data used to assess the slope gradient. Therefore, the cut off point for hysteresis at the start of the ramp varied slightly between interpreters, which may account for the variation between subjective assessment of $\dot{V}E/\dot{V}CO_2$ slope and $\dot{V}E/\dot{V}CO_2$ at AT.

3.7.4.5 Interpretation of features identified at CPET

Interpretation of CPET is an integrative process, taking into consideration clinical evaluation of exercise data collected, comparison of these responses iwht normal reference values and correlation of these results with clinical information available. McNicholl et al investigated the utility of CPET in difficult asthma for assessment of dyspnoea, with definitions of features present derived mainly from the ATS/ACCP guidelines(177). I have used these guidelines to asses the CPETs integratively, with definitions of features identified presented in table 3.7.

Table 3.7 Definition of features identified using CPET

Copied from (92). Abbreviations AT; anaerobic threshold; CPET; cardiopulmonary exercise test; HR; heart rate; MVV; maximal voluntary ventilation; O2; oxygen; PETCO2; end-tidal P co 2 ; RER; respiratory exchange ratio; SpO2; oxygen saturation; V̇EV̇CO2; ventilatory equivalent for CO2 ; V̇O2; oxygen uptake

Feature	**Interpretation**
Hyperventilation	$\dot{V}O_2$ peak: often normal (>83% predicted) or near normal (> 80% predicted) $\dot{V}E\dot{V}CO_2$at AT: increased (> 34) Highest $P_{ET}CO_2$: low (< 36 mm Hg) Respiratory frequency: increased (> 55/min) SpO_2: normal (> 95%, < 4% drop during exercise) Variable RER, especially at beginning of test Hyperventilation occurring below the respiratory compensation point
Ventilatory Limitation	$\dot{V}O_2$ peak < 83% predicted Breathing reserve < 20% of MVV $\dot{V}E\dot{V}CO_2$ at AT> 34 SpO_2> 4% drop during exercise RER at peak exercise: < 1
Cardiac Ischaemia	ST depression:>2 mm in > 1 lead $\dot{V}O_2$ peak: low (< 83% predicted) Breathing reserve:> 20% of MVV SpO_2 normal (> 95%, <4% drop during exercise) AT: normal or low (< 40% predicted peak $\dot{V}O_2$) or not detected
Physical deconditioning	$\dot{V}O_2$ peak: decreased (< 83% predicted) Peak HR: normal/slightly decreased (> 90% age predicted) O_2 pulse: decreased (< 80% predicted) Breathing reserve:> 20% of MVV $\dot{V}E\dot{V}CO_2$ at AT: normal (< 34) SpO_2: normal (> 95%, < 4% drop during exercise)
Submaximal test	Early cessation of exercise $\dot{V}O_2$ peak:< 83% predicted AT: normal (> 40% predicted peak $\dot{V}O_2$) or unattained RER at exercise cessation: low (< 1) Peak HR <80% predicted Breathing reserve: substantial (> 40% of MVV)

The pattern of results identified at baseline and post intervention are categorised using the criteria described by McNicholl et al(92) and defined in table 3.6, and are detailed in table 3.7. Overall, there was a high level of hyperventilation at rest, with results suggestive of either normal physiology or deconditioning. Participants did not appear to be ventilatory limited in their exercise capacity. It is noteworthy that the limit of normality for breathing reserve as a % of MVV used by McNicholl et al is greater than values more commonly used and referenced throughout this thesis.(164)

Table 3.8 Features identified at CPET for participants at baseline and post intervention based on criteria detailed in table 3.6.

Patient	Baseline	Post intervention
001	Normal	Normal
005	Physical deconditioning	Physical deconditioning
006	Submaximal test/deconditioning	Physical deconditioning
007	Hyperventilation	Hyperventilation
010	Physical deconditioning	Normal
012	Hyperventilation/ deconditioning	Normal

Discussion

The patient group appears to be a mainly atopic, eosinophilic group with treatment ranging from only short acting bronchodilator to biological treatment. Whilst severity as assessed through FEV1 is relatively mild for the cohort as a whole, they are all suboptimally controlled or symptomatic, as assessed by an ACQ6 score of ≥1.5. One patient was on BTS step 4/5, thereby meeting the criteria for difficult or severe asthma (84,). The initiation of a biologic switch that had been approved prior to enrolment in the study necessated exclusion of this participant at 3 weeks. Symptom scores had improved

at this point, and whilst one can speculate on the reason for their decision to take up the biologic switch prior to completing the study, there are no data to clarify one way or another. This does, however, provide early exploratory data that support the concept that exercise intervention may be beneficial across the range of disease severity. The group is similar to other asthma exercise intervention studies in terms of demographics, which provides some support for these exploratory findings (5). The majority of subjects fall into the overweight category, with demonstration of the additional co-morbidities common to difficult and severe asthma (330), and are relatively reflective of the asthma patient group as a whole (331). The inclusion of patients with dysfunctional breathing and sensitisation to clinically relevant airway allergens may have led to confounding of results. An improvement in dysfunctional breathing may have resulted in the improvements in symptomatology described in Chapter 4, that are not attributable to the mechanistic hypothesis. The lack of control group makes it impossible to determine whether an improvement in dysfunctional breathing was contributary, and whether the improvement in dysfunctional breathing was as a result of the exercise intervention. Certainly, there has been demonstration of symptomatic improvement with breathing retraining in asthma (332, 333). However, another group used a sham group of breathing retraining to compare to a combination of breathing retraining and aerobic intervention. This group report benefit in terms of reduction in asthma symptoms were only apparent in the aerobic intervention arm (334). A recent study aimed to compare a breathing retraining programme with an aerobic exercise intervention (335). The report from this concluded that whilst both breathing exercises and aerobic training improve asthma in terms of symptom control, aerobic training was 2.6 times more likely to experience clinical improvement at the three month follow up, with a greater proportion of participants in the aerobic training group also presenting a reduction in the number of days without rescue medication use compared with the breathing group (336). Whilst the protocol of this study included Njimegan questionnaire for assessment of dysfunctional breathing, the published report did not comment on this. Assessment of Njimegan score would be useful in understanding whether an aerobic intervention imparts some of its affect on symptom scores via modulation of dysfunctional breathing. Inclusion of a Njimegan questionnaire would be something to consider for further work.

The prevalence of patients with sensitisation to inhaled airway allergens is reflective of, or slightly higher, than the broader asthma population (14, 337), which allows greater

confidence with regards to the generalisability of these exploratory results in this context. However, there is also a greater risk of confounding; the levels of aeroallergen exposure may differ across the course of the intervention, and association has been demonstrated between ambient concentrations of common aeroallergens and exacerbations requiring hospitalisation for asthma(338). Again, inclusion of a control group would have been beneficial in this context.

There were not any acute exacerbations as a result of the sampling, testing or intervention process, and adherence to the study protocol was good amongst those patients who completed the intervention. There were no study related adverse events or serious adverse events. These observations suggest that the sampling and intervention process appear to be safe and well tolerated within this patient cohort, although this needs interpretation in the context of the group consisting of patients who did not report exercise induced asthma symptoms. It is more likely that participants would become symptomatic as a result of the maximal volitional CPET testing than a submaximal exercise training programme. There is suggestion in children that an aerobic intervention may reduce exercise induced bronchospasm (93). If this aerobic intervention did reduce exercise induced bronchospasm, then it would be of relevance in the context of interpreting CPET outcomes. If exercise induced bronchospasm were present at baseline, and improved as a result of the exercise intervention, then this reduction in exercise induced bronchospasm may have led to an increase in exercise capacity. We did not perform repeated flow volume loops during and after CPET to assess whether exercise induced bronchospasm was present, which would have been useful in understanding whether the exercise intervention improved exercise induced bronchospasm. However, the CPETs did not demonstrate significant respiratory limitation at baseline and therefore improvements in oxygen uptake are more likely to be a result of increased fitness rather than reduced exercise induced bronchospasm. In a group of difficult asthma patients who underwent diagnostic CPET investigation, the rate of exercise induced bronchospasm was only identified as 21% (92).The design of the training programme (inside, on a cycle ergometer) is protective against exercise induced bronchospasm, with the addition of a warm up and cool down period shown to reduced exercise induced bronchospasm by up to 50%, through initiation of a refractory period induced by airway smooth muscle tachyphylaxis to mediators of bronchoconstriction (339).

The comparison of SRETP with SRETP plus additional strength training is not powered for significance, even if recruitment were to have proceeded as planned, which is reflective of the learning curve travelled during this project. The low numbers in the two groups make the data statistically uninterpretable; it is presented to provide an overview of the candidates in the two arms and to initiate reflection in the discussion around the inclusion of the strength training protocol. The change to the protocol to allow optional inclusion of strength training was pragmatic to address difficulties with recruitment. The data from this study do not allow determination as to whether strength training added additional benefit or not. However, if improvements can be demonstrated with SRETP alone, as discussed in the following Chapters, then patient burden would be reduced by limiting the intervention to SRETP alone.

Despite a demanding intervention, adherence of those who completed the exercise training intervention was good at 86%. The caveat to this is that recruitment rates overall were low. Further to this, the group of patients who completed the intervention are likely to be biased towards the potential benefits of an exercise intervention with baseline motivation to engage with exercise related research. Additionally, adherence to the exercise intervention was slightly lower than seen in prehabiliation for cancer patients (312). Verbal feedback from participants suggests that this was due to the difficulty in arranging sessions around work and family commitments. The mean age of participants in this cohort is 34.75 (range 23-50) and most participants were employed during their participation in the study. In contrast, patients in the rectal cancer study were older with a mean age of 64 (range 45-82) and less likely to be employed during their cancer treatment. Participant and Public Involvement during the study design process would have been helpful, although unlikely to influence the choice of an in -hospital exercise programme. The in-hospital aspect of the training intervention allowed accurate delivery of the exercise treatment, which was important in this exploratory work. Nontheless, all patients enrolled in the present study achieved protocol compliance. Good compliance is important in interpreting the outcome data; all completing participants received an equivalent ‘dose’ of exercise. The difficulty in recruitment and high rate of drop out require discussion. Verbal feedback suggests that commitment to an in-hospital training programme within working hours and multiple sampling visits are not feasible for many participants within this demographic. Following on from this exploratory study, consideration of a supervised out of hospital option for patients is important for further

work. This has particular relevance in the context of the COVID 19 pandemic, where in hospital and large group interventions are not advised for groups of potentially vulnerable patients.

The cohort presented herein have maintained a steady BMI throughout the training period and so any improvement in asthma symptoms or inflammatory parameters that may be observed is not likely to be subject to confounding from this weight loss effect. The stability of BMI may be due to the particpants in this study having a lower baseline BMI to other comparative studies (5) (340), which also employed a dietary intervention alongside the exercise prescription. The combination of dietary intervention and exercise prescription adds a confounder. It becomes difficult to determine whether the impact on symptoms and inflammation seen in these studies are due to a reduction in adipose tissue driven inflammation, or an improvement in asthma related inflammation as a result of disease modifying exercise.

I will now move on to discuss the impact of exercise training on exercise related outcomes. All CPETs demonstrated good patient effort with RER greater than 1.1 and nearing maximal predicted heart rate for all tests, although it is noteworthy that 1 of the baseline tests, and 2 of the final tests did not achieve the > 90 % of their maximal age predicted heart rate required to define a maximal test (177). These results suggest that even patients with symptomatic asthma (as assessed subjectively by an enrolment ACQ6 score ≥1.5) are able to exercise to peak capacity without exacerbation or respiratory compromise, which was a concern as uncontrolled asthma is a relative contraindication to CPET(164). This is supported by work by McNicholl et al, who demonstrated that CPET was safe in difficult asthma patients and can also be a useful diagnostic adjunct for persistent dyspnoea in this group of patients (92).

Physical fitness as assessed by change in oxygen uptake at anaerobic threshold has statistically significantly improved, with a trend to improvement at peak exercise. These results are in line with utilisation of the same exercise training programme in cancer patients after chemotherapy and prior to surgery (308). There was suggestion of improvement in physical fitness at both week 3 and week 6 timepoints, with AT demonstrating significant improvement between baseline and week 3, and $\dot{V}O_2$ peak showing a trend for significant improvement between baseline and week 6 (see table 3.6 and figure 3.11). The $\dot{V}O_2$ peak in this relatively young group of patients are lower than

expected, with the exception of 1 outlier. This interpretation is based on normal reference value equations described by Hansen et al (176), as recommended for use by the ATS/ACCP statement on Cardiopulmonary Exercise Testing (177). The reduced oxygen uptake demonstrated in this group is likely reflective of deconditioning at baseline, when interpreted using criteria detailed by McNicholl et al (92). Deconditioning at baseline is not unexpected in a cohort of patients with a chronic inflammatory disease who do not partake in regular exercise (341). With regards to the outlier demonstrating higher oxygen upake, whilst all patients met the inclusion criteria of physically inactive as described by the American College of Sports Medicine's Guidelines (<60 minutes of structured or planned physical activity/week), the outlier reported that they had previously been physically active before their asthma symptoms had limited them for the preceding few months prior to enrolment in the study. Absolute values for oxygen uptake at anaerobic threshold are also lower than expected (excepting the one outlier), in line with the integrative assessment of deconditioning. The lower than expected $\dot{V}O_2$ peak and AT absolute values are likely to reflect the untrained nature of the cohort at baseline. Reasons for not participating in regular exercise may be a result of perceived exercise limitation due to their asthma or anxiety surrounding exercise as a potential trigger for an exacerbation, and are addressed in Chapter 6. Demonstration of deconditioning in this group highlights the importance of regular physical activity to maintain fitness even in this relatively young group. With the exception of 1 participant, there are improvements in oxygen uptake at AT and peak exercise, suggesting the exercise intervention improved physical fitness as expected, with a non-responder within the group. This is reflective of an exercise and weightloss intervention in obese asthma patients, which similarly demonstrated an improvement in $\dot{V}O_2$ following exercise training. (5). However, one would expect a significant longitudinal improvement in both anaerobic threshold and peak oxygen uptake as markers of physical fitness with a training intervention (293). The lack of longitudinal improvement in peak oxygen uptake is likely to simply be reflective of the small numbers, although the volitional aspect of peak exercise capacity is always an important consideration in the interpretion of peak oxygen uptake. The values for RER and peak heart rate provide reassurance in addressing volitional considerations, that these tests are maximal, or close to maximal. Anaerobic threshold values have been demonstrated to be more sensitive measure of improvements in physical fitness that $\dot{V}O_2$ peak (342), which could explain the significant improvement in AT demonstrated in this

group, with only a trend to improvement in oxygen uptake at peak exercise capacity. It is also important to reflect on the subjective nature of interpretation of oxygen uptake at anaerobic threshold when interpreting these results. This is particularly relevant in assessing the oxygen uptake at peak exercise in patient 001 at final sampling, where further interrogation of the data were undertaken due to the unexpected reduction in oxygen uptake at AT between week 6 and week 12. In this case, due to the >4.8% discrepancy between the two initial intepretors for the week 12 test, the third assessor result was used. For the week 6 result, the mean of the two initial interpretors was used, with individual variation in interpretation a likely explanation for the perceived fall in AT. Despite the consideration of the the subjective nature of interpretation of oxygen uptake at AT demonstrated in the interpretation protocol for CPET as described in section 2.1.13.1.2.6, with the use of 2 blinded reporters and an adjudication process if interpretation differed beyond 4.8% mitigate for any subjectivity in interpretation, the data herein suggest these methods may not fully address this. An additional moderation step for CPET analysis may need to be considered for further work. Other potential explanations considered are that the training programme itself was not of a great enough intensity or length to induce improvements. However, the same programme has been utilised in prehabilitation of cancer patients and demonstrated improvement in $\dot{V}O_2$ peak and AT (308). Additionally, improvement in exercise capacity was seen in obese asthma patients with a similar length of training, although it is noteworthy this group also demonstrated significant weight loss which could compound improvements in $\dot{V}O_2$ when assessed in ml/kg/min (5). Of consideration in a chronic inflammatory disease is whether inflammation limits improvements in AT. It has been demonstrated that there are responders and non-responders to exercise training in the context of improvement in AT and $\dot{V}O_2$ peak, with non-responders demonstrating a higher level of inflammatory cytokines than responders both pre and post exercise (343). It may be that the exercise training must first address the systemic inflammation that could be potentially disrupting acquisition of muscle mass prior to demonstration of any improvement in fitness. This has potential to impact on exercise training gains in asthma as a whole, but in this relatively mild but symptomatic at enrolement group, it is unlikely to have had a huge impact. It is also of note that whilst the change in oxygen uptake at peak exercise were not statistically significant, in absolute values, peak oxygen uptake increased from a median of 21.6 to 25 ml/kg/min, demonstrating an increase of 14.6% or 3.4ml/kg/min. A rise of

3.5ml/kg/min resulting in a 10-25% improvement in survival from cardiovascular adverse outcomes and a minimal clinically important difference has been identified as >2ml/kg/min (344). A clinically significant improvement in oxygen uptake was defined in our hypothesis and aims as > 2ml/kg/min. Workload also increased significantly over the course of the training intervention, demonstrating improvement in physical fitness in the cohort, given that ramp remained the same for each participant across the intervention.

In terms of the respiratory specific parameters, breathing reserve was normal in the majority of participants throughout, although did demonstrate reduction over the course of the intervention that, with FEV1 remaining stable, is due to an increase in maximum $\dot{V}E$. Given the increase in exercise capacity, demonstrated by increased workrates and oxygen uptake resulting in an increase in ventilation, this is an explainable and expected finding that is reflective of an increase in fitness. Previously published exercise intervention studies in asthma patients have not described baseline ventilatory CPET variables or the role of an exercise intervention on these parameters. There is suggestion in the literature of a reduced BR with limited spirometric changes; symptomatic smokers with borderline spirometry had a greater reduction in breathing reserve at peak exercise in comparison to controls (345). In CPET in a difficult asthma group in Belfast, ventilatory limitation was only identified in 18% of patients, with CPET demonstrated to have utility in identifying the cause of dypnoea in this group (92). Of note, exercise induced bronchospasm was identified in 21% of the difficult asthma patients in the Belfast study, and inclusion of flow volume loops during exercise would have provided additional useful information in this cohort with regards to the impact of the exercise training intervention on exercise induced bronchospasm. This will be considered for future work. Typical CPET patterns in symptomatic asthma patients in response to exercise intervention have not been described in the literature to my knowledge, and a better understanding of this would facilitate more targeted interventions for shortness of breath.

The baseline $P_{ET}CO_2$ for the majority of participants is suggestive of hyperventilation, that settles on exercise appropriately across all time points(170). This is interesting in a number of contexts. Firstly, the results described herein suggest that there is a high proportion of hyperventilation or dysfunctional breathing within patients with suboptimally controlled asthma, which is in line with current literature (337). Secondly, and possibly of more clinical relevance, is that any dysfunctional breathing or hyperventilation appears to settle both acutely with exercise, but also longer term

following the exercise intervention, given the normalisation and rise of $P_{ET}CO_2$ at rest following the exercise training period. This again, is reflective of the literature, with a review supporting the role for exercise in the treatment of hyperventilation (346). An exercise intervention study in asthma patients collected data on Njimegan scores, but has not published these data(335). Another consideration is that the low baseline $P_{ET}CO_2$ may simply be a reflection of anxiety on initial testing that settles with increasing familiarity with the exercise testing procedures as patients progressed through the study.

The abnormality of the $\dot{V}E/\dot{V}CO_2$ slope in some patients is also of interest. Although non significant, a greater number of patients demonstrate raised values for $\dot{V}E/\dot{V}CO_2$ slope at week 12 than at baseline. Interpreted in the context of an increase in $\dot{V}E$, these results are likely reflective of hyperventilation rather than new abnormality in perfusion or increase in pulmonary dead space . The stability of the FEV1 provides further reassurance of this. In contrast, the results for $\dot{V}E/\dot{V}CO_2$ at AT reduced in those participants who had abnormal values at baseline. The values for $\dot{V}E/\dot{V}CO_2$ slope and $\dot{V}E/\dot{V}CO_2$ should be numerically similar (164), and as there were some variability in these numerical data, the raw CPET data were further interrogated. Whilst all reporters excluded data beyond RCP when interpreting $\dot{V}E/\dot{V}CO_2$ slope, there were individual variations in analysis in terms of the lower limit of data used to assess the slope gradient, with cut off point for hysteresis at the start of the ramp varying slightly between interpreters. This may account for the differences in results for $\dot{V}E/\dot{V}CO_2$ slope and $\dot{V}E/\dot{V}CO_2$ at AT. Low $\dot{V}E$ in the context of low $P_{ET}CO_2$ have previously been reported in patients with asthma, although response of these variables to exercise intervention has not (92, 347).

Exclusion of ventilatory limitation of exercise capacity through diagnostic CPET may be clinically useful in those patients with ongoing dyspnoea despite optimisation of medication, both to clinicians and patients. For clinicians, there may be support for decisions of non-escalation or withdrawal of unnecessary treatment in the absence of asthma related exercise limitation, as demonstrated by McNicholl et al (92). For patients, the benefit may come from increasing their confidence to exercise, which could be capitalised upon by an exercise intervention such as SRETP.

The limitations to the results presented in this chapter are extrapolatable to the broader thesis, and reflected on throughout. The number of participants are small, and therefore the study is not sufficiently powered to draw any firm conclusions from any of the results.

This is particularly relevant to the exercise physiology outcomes, with demonstration in the literature of the non-responder rate to exercise intervention varying between 20-30%m (348, 349), and allowance for this non-response rate would have to be incorporated into power calculations for future studies. Further to this, multiple analyses are performed on the data, in the context of the exploratory nature of the study. However, given this, and the associated increased risk of type 2 errors, these data need to be interpreted as only exploratory in nature, with appropriate caution (350). Furthermore, the degree to which the results are extrapalatable to the broader population are limited by the relatively mild cohort, with equivocal reversibility of their airways obstruction. Whilst the PEF variability of > 8% was met by all, a more useful and accurate measure of airways hyperreactivity would have been methacholine challenge. This was not chosen for this study due to the complex nature of sampling and exercising, and the subsequent demand on patient time. However, for further work, ways of including a methacholine challenge as part of the sampling process, without significantly increasing the burden of research for participants, need to be revisited. Throughout the thesis, there is the consideration of confounding from poor compliance with prescribed medication and/or poor inhaler technique, with adherence with asthma medications demonstrated to be as low as 30-35% (351). There is potential further confounding from poor inhaler technique, which, even if patients are compliant, can affect drug delivery, with only approximately a third of patients demonstrating correct inhaler technique in a recent meta analysis (352). A control group would have increased understanding of the impact of these confounders on the outcome variables and will need to be included in a further, appropriately powered study.

Having demonstrated exploratory data that suggests that exercise intervention may be safe and feasible, does not impact significantly on body habitus of patients with suboptimally controlled asthma and can increase physical fitness when assessed by $\dot{V}O_2$ at AT and peak exercise, this thesis will go on to describe the impact of the intervention on disease related parameters, before investigating the mechanism driving any change.

Chapter 4 Effects of SRETP on asthma symptoms and associated inflammation

4.1 Symptom Burden and Quality of Life Parameters

Chapter 3 demonstrated that SRETP and cardiopulmonary exercise testing is well tolerated in this group of symptomatic asthma patients, with some effect on physical fitness, without result in a significant reduction in body mass index. This chapter focuses on the symptom burden and inflammatory responses of the cohort over the exercise training intervention to address whether SRETP can improve symptom burden in these symptomatic patients, and whether this is accompanied by a reduction in inflammatory markers associated with asthma control. Data for the patients who have baseline and ≥ 1 interim assessment are presented to demonstrate validity of the smaller dataset for which there are valid baseline and final sampling visits. Longitudinal data are presented for the n=6 who completed the training intervention with valid baseline, week 6 and final assessments to provide an overall demonstration of change over time (see figure 3.1).

4.1.1 Asthma Control Questionnaire

To assess if the training intervention had any effect on symptom burden in the participants, asthma control questionnaire (ACQ) scores were assessed at baseline, week 3, week 6 and week 12. A clinically significant improvement in symptoms has been defined as a reduction in ACQ score ≥ 0.5, with suboptimal control demonstrated by scores ≥ 1.5 (119). All participants completed the questionnaire on all occasions. The ACQ score improved significantly from baseline to week 3 (Figure 4.1A) (mdn=2.0 vs mdn=1.6, p=0.018), baseline to week 6 (Figure 4.1B) (mdn=2 vs mdn=1.16, p=0.018), and baseline to week 12(Figure 4.1C) (mdn=2 vs mdn=1.2, p=0.028). There was also a statistically significant improvement over the course of the training period when assessed by Friedman test (P=0.012) (Figure 4.1D)

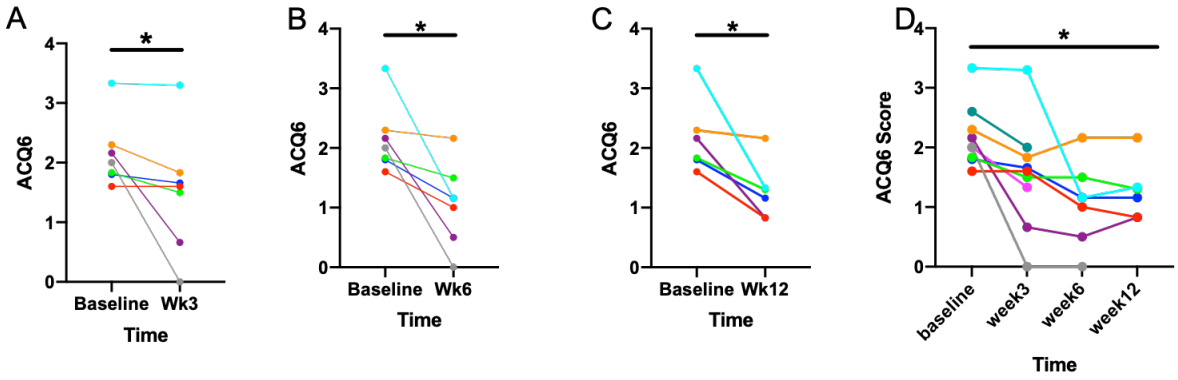


Figure 4.1 ACQ Score

*for A) Baseline to week 3; n=9 for those patients with valid baseline and week 3 scores B) Baseline to week 6; n=7 for those patients with valid baseline and week 6 scores, C) Baseline to week 12; n=6 for those patients with valid baseline and week 12 scores , as assessed by Wilcoxon Test and D) longitudinally; n=6 for those patients with valid baseline, week 3, week 6 and week 12 scores, as assessed by Friedman test. Individual participants are represented by a different colour, with each point indicating a sampling event. *=statistically significant result; p<0.05*

4.1.2 Asthma Quality of Life Questionnaire

To assess if quality of life improved as a result of the training intervention, Asthma Quality of Life Questionnaire (AQLQ) scores were assessed at baseline, 3, 6 and 12 weeks. All

participants completed the questionnaire on all occasions; see figure 3.1 for number of patients at each time point. The minimally clinically important increase in asthma quality of life score has been defined as ≥ 0.5 (310). The AQLQ score did not improve significantly from baseline at week 3 (mdn=4.97 vs mdn=5.38, p=0.123) (figure 4.2A). However, there was significant improvement from baseline to week 6 (mdn=4.97 vs mdn=6, p=0.018) (figure 4.2B), and week 12 (mdn=4.79 vs mdn=5.83, p=0.046) (figure 4.2C). Similarly to the ACQ data, there was a significant improvement in AQLQ over the training period (p=0.03)(figure 4.2D).

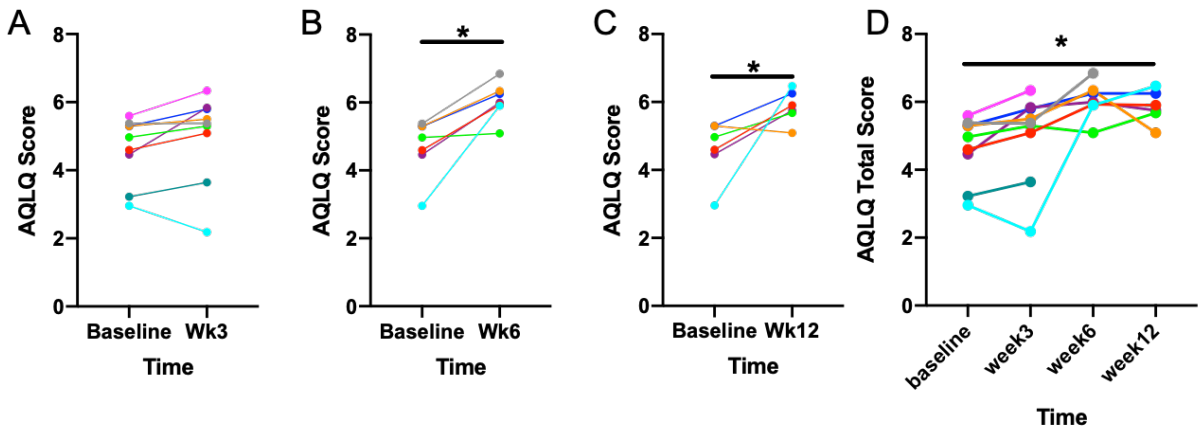


Figure 4.2 Total AQLQ Score

*for A) Baseline to week 3; n=9 for those patients with valid baseline and week 3 scores B) Baseline to week 6; n=7 for those patients with valid baseline and week 6 scores, C) Baseline to week 12; n=6 for those patients with valid baseline and week 12 scores , as assessed by Wilcoxon Test and D) longitudinally; n=6 for those patients with valid baseline, week 3, week 6 and week 12 scores, as assessed by Friedman test. Individual participants are represented by a different colour, with each point indicating a sampling event *=statistically significant result; p<0.05*

The scores from the different domains of the AQLQ were then assessed independently. AQLQ symptom score trends to significant improvement from baseline at week 3 (mdn=4.8 vs mdn=5.4, p=0.050 (Figure 4.3A), with significant improvement at week 6 (mdn=4.8 vs mdn=6.3, p=0.018) (Figure 4.3B) and week 12 (mdn=4.5 vs mdn=5.8, p=0.046) Figure 4.3C). There was a significant trend towards improvement over the course of the study (p=0.07) (Figure 4.3D).

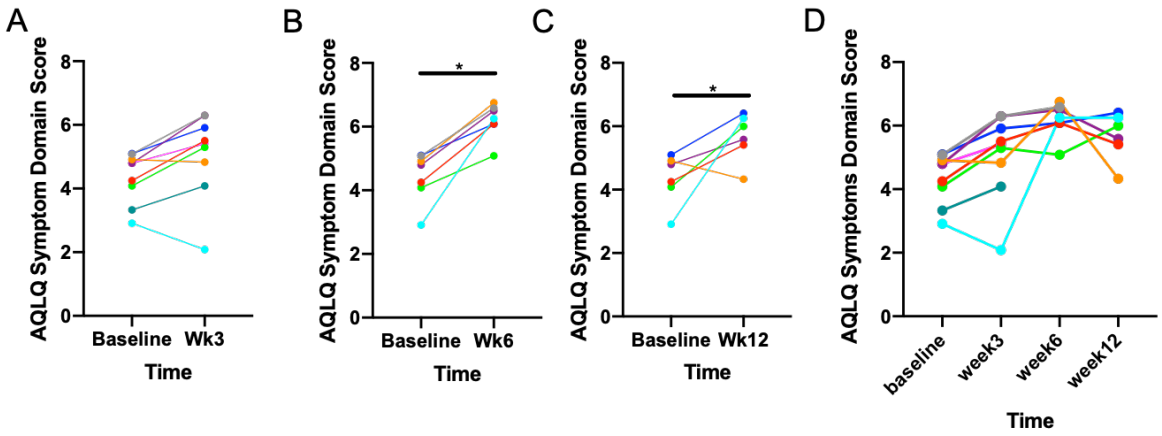


Figure 4.3 AQLQ Symptom Domain

*for A) Baseline to week 3; n=9 for those patients with valid baseline and week 3 scores B) Baseline to week 6; n=7 for those patients with valid baseline and week 6 scores, C) Baseline to week 12; n=6 for those patients with valid baseline and week 12 scores , as assessed by Wilcoxon Test and D) longitudinally; n=6 for those patients with valid baseline, week 3, week 6 and week 12 scores, as assessed by Friedman test. Individual participants are represented by a different colour, with each point indicating a sampling event. *=statistically significant result; p<0.05*

Activity scores did not improve significantly between baseline and week 3 (mdn=5.34 vs mdn=5.7, p=0.260 (Figure 4.4A)), week 6 (mdn=5.34 vs mdn=5.8, p=0.5 (Figure 4.4B)), week 12 (mdn=5.32 vs mdn=6.1, p=0.116 (Figure 4.4C)) or over the course of the study (p=0.515) (Figure 4.4D).

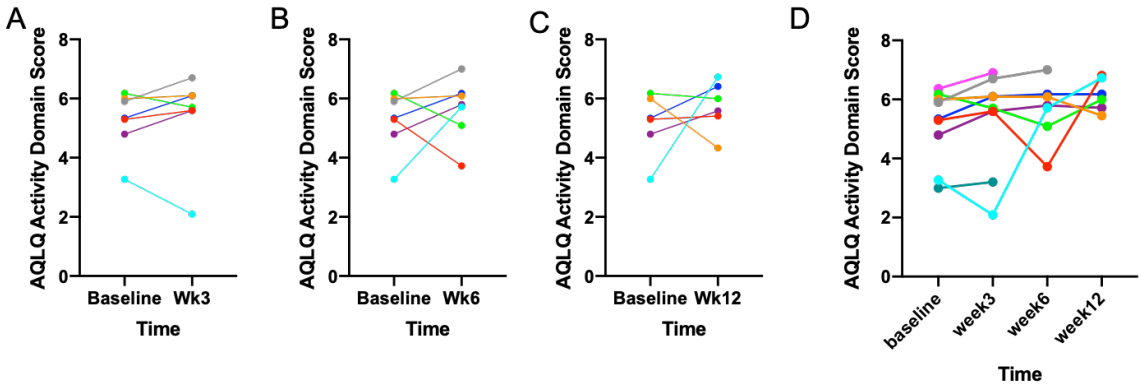


Figure 4.4 AQLQ Activity Domain

for A) Baseline to week 3; n=9 for those patients with valid baseline and week 3 scores B) Baseline to week 6; n=7 for those patients with valid baseline and week 6 scores, C) Baseline to week 12; n=6 for those patients with valid baseline and week 12 scores , as assessed by Wilcoxon Test and D) longitudinally; n=6 for those patients with valid baseline, week 3, week 6 and week 12 scores, as assessed by Friedman test. Individual participants are represented by a different colour, with each point indicating a sampling event

Emotion scores assessed by Wilcoxon signed rank showed a trend to significant improvement at week 3 (mdn=4.8 vs mdn=5.8, p=0.66) (Figure 4.5A), with significant improvement at week 6 (mdn=4.8 vs mdn=6.0, p=0.018) (Figure 4.5B), that was sustained at week 12. (mdn=4.8 vs mdn=6.1, p=0.027) (Figure 4.5C). There was also a significant improvement over the course of the study, as assessed by Friedman Test (p=0.007) (Figure 4.5D)

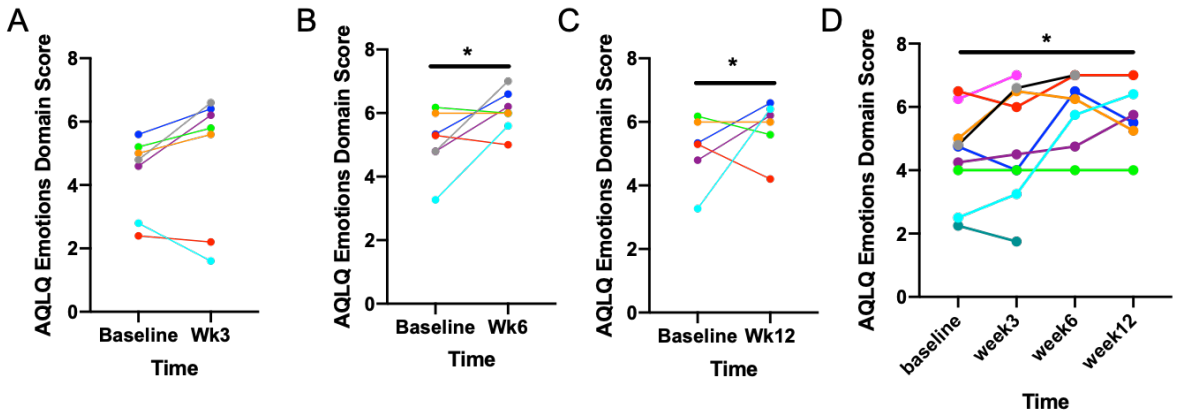


Figure 4.5 AQLQ Emotions Domain

*for A) Baseline to week 3; n=9 for those patients with valid baseline and week 3 scores B) Baseline to week 6; n=7 for those patients with valid baseline and week 6 scores, C) Baseline to week 12; n=6 for those patients with valid baseline and week 12 scores , as assessed by Wilcoxon Test and D) longitudinally; n=6 for those patients with valid baseline, week 3, week 6 and week 12 scores, as assessed by Friedman test. Individual participants are represented by a different colour, with each point indicating a sampling event. *=statistically significant result; p<0.05.*

There was not a significant improvement in the environmental domain between baseline and at week 3 (mdn=4.75 vs mdn=4.5, p=0.260) (Figure 4.6A). However, baseline to week 6 (mdn=4.75 vs mdn=6.25, p=0.027) and 12 (mdn=4.5 vs mdn=5.6, p=0.043) showed significant improvement (figure 4.6B-C), with significant improvement over the course of the study (p=0.039) (Figure 4.6D).

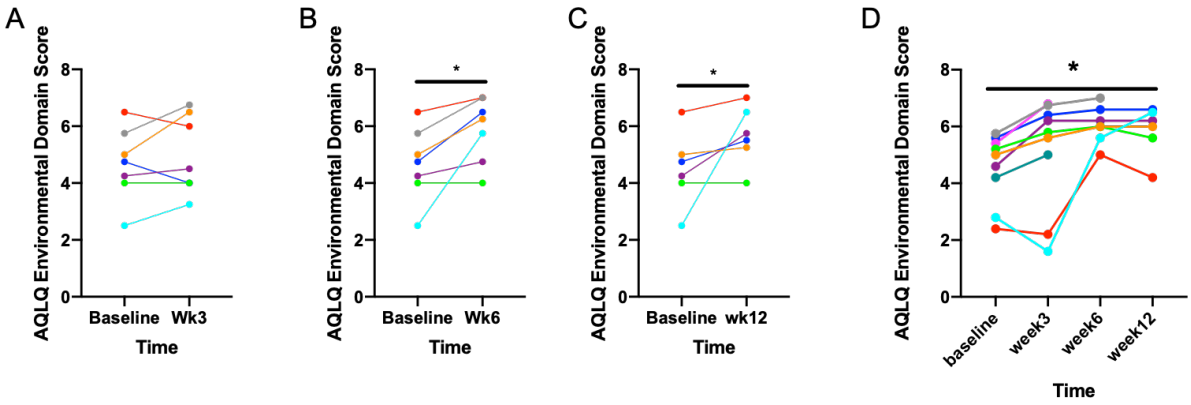


Figure 4.6 AQLQ Environmental Domain

*for A) Baseline to week 3; n=9 for those patients with valid baseline and week 3 scores B) Baseline to week 6; n=7 for those patients with valid baseline and week 6 scores, C) Baseline to week 12; n=6 for those patients with valid baseline and week 12 scores , as assessed by Wilcoxon Test and D) longitudinally; n=6 for those patients with valid baseline, week 3, week 6 and week 12 scores, as assessed by Friedman test. Individual participants are represented by a different colour, with each point indicating a sampling event. *=statistically significant result; p<0.05.*

Table 4.1 Summary of AQLQ change over the course of the intervention for comparison of changes between AQLQ domains

*Data presented as median and p value: * = significant; Abbreviations AQLQ; asthma quality of life questionnaire, Mdn; median*

AQLQ Domain	Week 3 Mdn and p value *=significant n=9	Week 6 Mdn and p value *=significant n=7	Week 12 Mdn and p value *=significant n=6	Longitudinal (Friedman) P value *=significant n=6
Total	4.97 to 5.38 P=0.123	4.97 to 6 P=0.018*	4.79 to 5.83 P=0.046*	P=0.03*
Symptoms	4.8 to 5.4 P=0.050	4.8 to 6.3 P=0.018*	4.5 to 5.8 P=0.046*	P=0.07
Activity	5.34 to 5.7 P=0.260	5.34 to 5.8 P=0.499	5.32 to 6.1 P=0.116	P=0.515
Emotions	5 to 6 P=0.066	4.8 to 6 P=0.018*	4.8 to 6.1 P=0.027*	P=0.007*
Environment	4.9 to 5.3 P=0.260	4.75 to 6.25 P=0.027*	4.5 to 5.6 P=0.043*	P=0.039*

4.2 Clinically Assessed Objective Parameters of Asthma Control

Whilst it has been long known that exercise makes one feel better, feeling better does not necessarily correlate with an objective measure of asthma control or demonstrate an anti-inflammatory effect of exercise. A better measure of the response of asthma to exercise training is to measure the lung function and inflammatory parameters routinely employed in asthma assessment and management, and track their change across the course of the exercise training period.

4.2.1 Spirometry

Pre-bronchodilator spirometry was assessed, with change between pre and post bronchodilatory spirometry assessed as a measure of airway hyperreactivity. Pre bronchodilator spirometry demonstrated a significant longitudinal improvement in FVC (Figure 4.7B) (p=0.016) but not in FEV1, FEV1/FVC ratio or FEF 25-75 (figure 4.7 A,C,D). There was no improvement between baseline and week 12 for any of the pre bronchodilator measures, as assessed by Wilcoxon test.

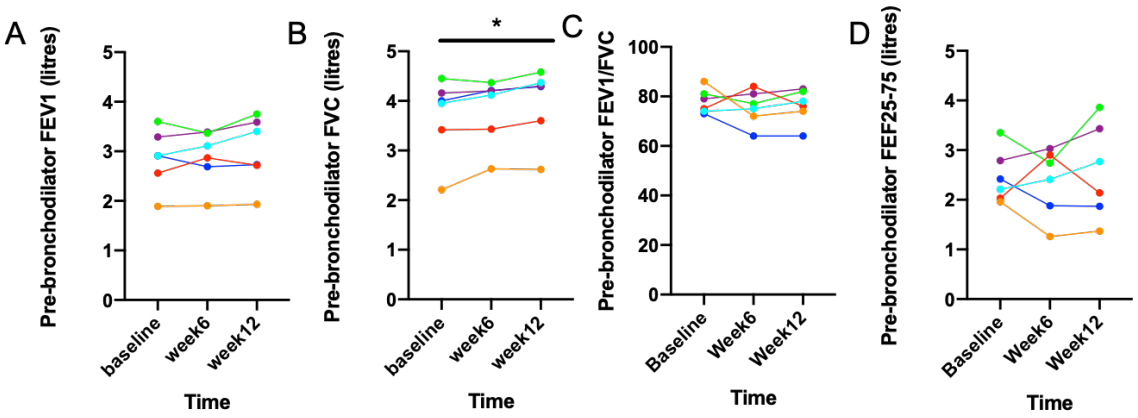


*Figure 4.7 Pre-bronchodilator spirometry over the course of the exercise intervention as assessed by Friedman test n=6 for those patients with valid baseline, week 6 and week 12 data. A) FEV1, B) FVC, C) FEV1/FVC ratio and D) FEF 25-75%. Individual participants are represented by a different colour, with each point indicating a sampling event. *=statistically significant result; p<0.05.*

Post bronchodilator spirometry was assessed as a measure of improvement in lung function after optimisation with a short acting bronchodilator, and did not show significant improvement in FEV1, FVC FEV1/FVC or FEF 25-75 (Figure 4.8 A-D). One participant was excluded from this analysis as there was no post bronchodilator dilator spirometry available.

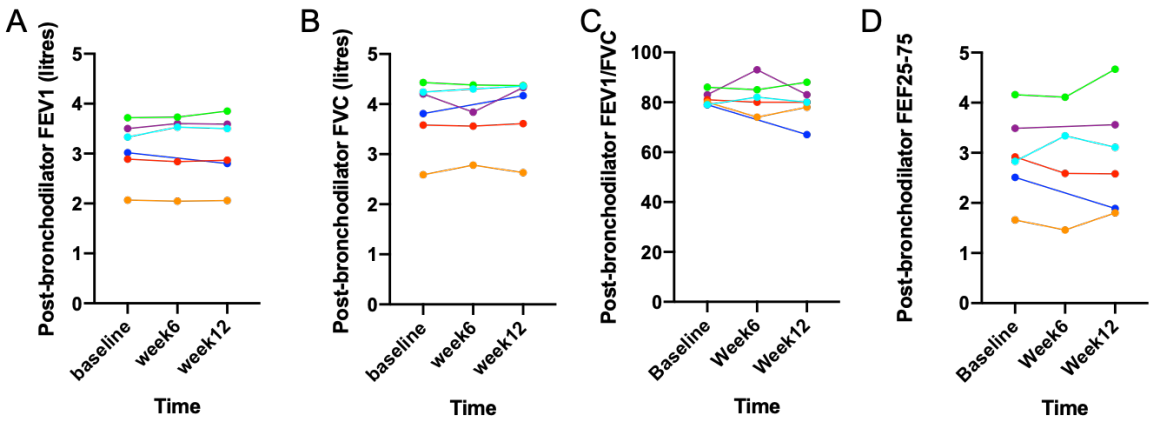


Figure 4.8 Post bronchodilator spirometry over the course of the exercise intervention as assessed by Friedman test n=6 for those patients with valid baseline, week 6 and week 12 data. A) FEV1, B) FVC, C) FEV1/FVC ratio and D) FEF 25-75%. Individual participants are represented by a different colour, with each point indicating a sampling event

FEV1 bronchodilator reversibility, expressed in litres, as a measure of airways hyperreactivity(figure 4.9A) , showed a significant reduction when assessed by Wilcoxon signed rank test (mdn 0.2 vs mdn 1 , p=0.028), and and as a percentage (mdn = 8 vs mdn 3, p= 0.043, figure 4.9B). FVC bronchodilator reversibility, expressed in litres, as a measure of airways hyperreactivity, showed a trend to significant reduction between baseline and week 12 when assessed by a Wilcoxon signed rank test (figure 4.10A) (mdn = 0.1 vs mdn 0.0 p= 0.75), but when expressed as a percentage, did not reach statistical significance (p=0.138) (figure 4.10B)

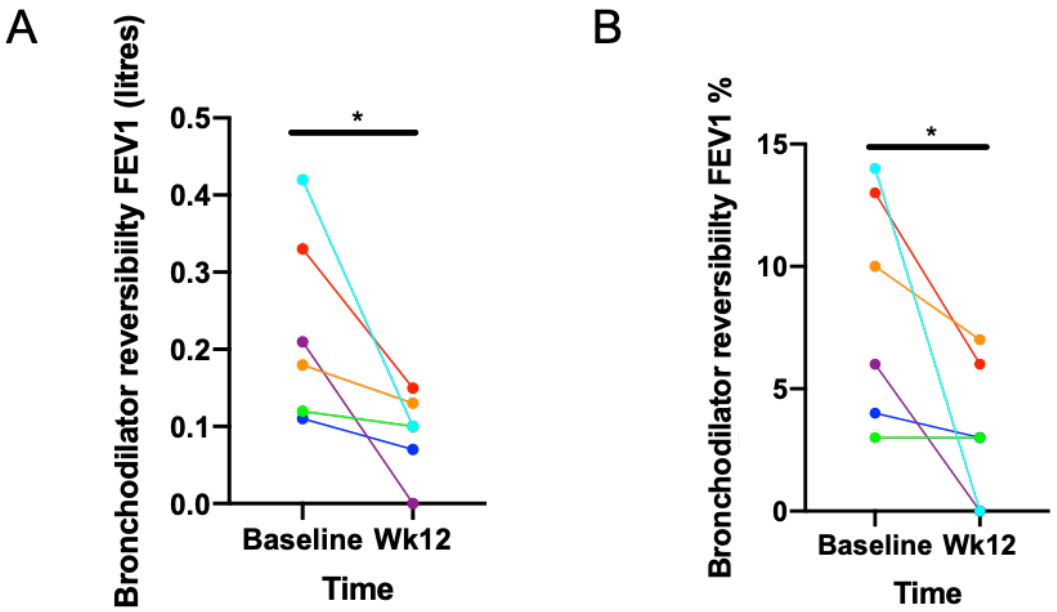


Figure 4.9 Bronchodilator reversibility FEV1

*n=6 for patients with valid baseline and week 12 data for (A)litres and B)%; baseline to week 12 as assessed by Wilcoxon signed rank test. Individual participants are represented by a different colour, with each point indicating a sampling event. *=statistically significant result; p<0.05*

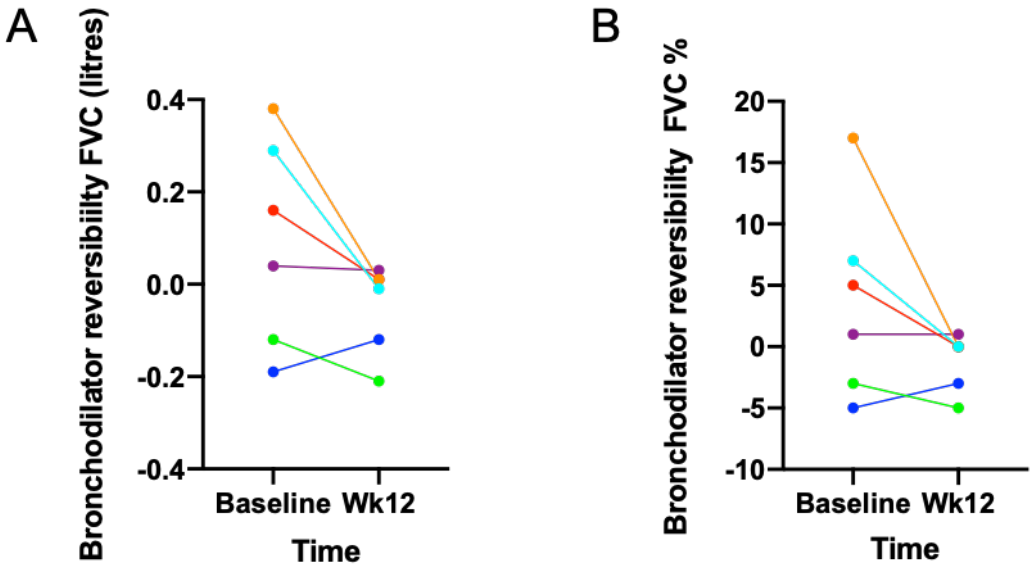


Figure 4.10 Bronchodilator reversibility FVC

n=6 for patients with valid baseline and week 12 data for (A)litres and B)%) baseline to week 12 as assessed by Wilcoxon signed rank test. Individual participants are represented by a different colour, with each point indicating a sampling event.

4.3 Clinically Assessed Inflammatory Parameters

Objective assessment of inflammation in asthma is also important in determining treatment escalation and responsiveness. One participant was excluded from baseline to week 3 statistical analysis of inflammation due to a non-study related exacerbation of her asthma the week prior to week 3 sampling. For all results, baseline to week 3 (n=8); baseline to week 6 (n=7) and 12, and over course of intervention (n=6)

4.3.1 Peripheral Blood Total White Cell Count

Total white cell counts significantly reduced over the duration of the intervention period (p=0.02, figure 4.11D). There ws no statistically significant difference between white cell count at baseline to week 3 (figure 4.11A) (mdn=7.4 vs mdn=8.3, p=0.17) or baseline to week 6 (figure 4.11B) (mdn=7.4 vs mdn=7.4, p=0.13), but there was a significant reduction between baseline and 12 weeks (figure 4.11C) (mdn=7.2 vs mdn=6.7, p=0.46).

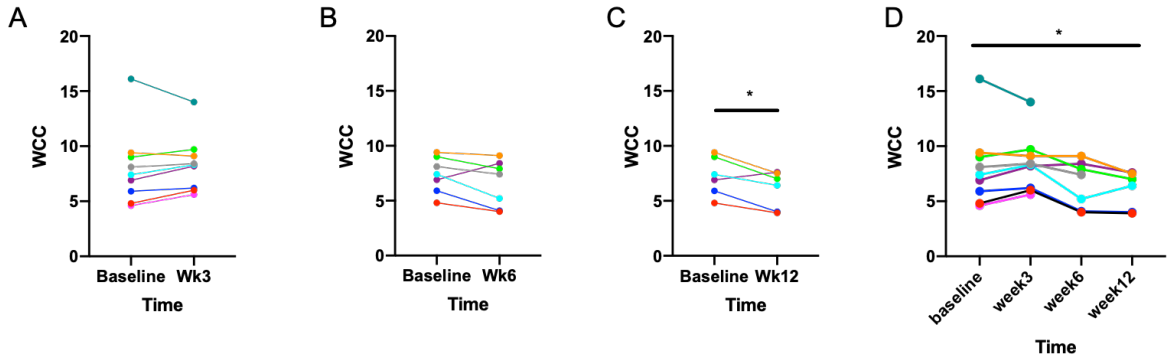


Figure 4.11 Peripheral Blood Total White Cell Count

*for A) Baseline to week 3; n=9 for those patients with baseline and week 3 data B) Baseline to week 6; n=7 for those patients with valid baseline and week 6 data, C) Baseline to week 12; n=6 for those patients with valid baseline and week 12 data , as assessed by Wilcoxon Test and D) longitudinally; n=6 for those patients with valid baseline, week 6 and week 12 data, as assessed by Friedman test. Individual participants are represented by a different colour, with each point indicating a sampling event. *=statistically significant result; p<0.05*

4.3.2 Peripheral Blood Neutrophil Count

There was a trend to significant reduction in neutrophil count over the duration of the intervention period (p=0.054, Figure 4.12D). There was no statistically significant difference between neutrophil count at baseline to week 3 (figure 4.12A) (mdn=4.3 vs mdn=4.6, p=0.34), week 6 (figure 4.12B) (mdn=4.3 vs mdn=4.8, p=0.18), or week 12 (figure 4.12C) (mdn=3.9 vs mdn=3.7, p=0.17).

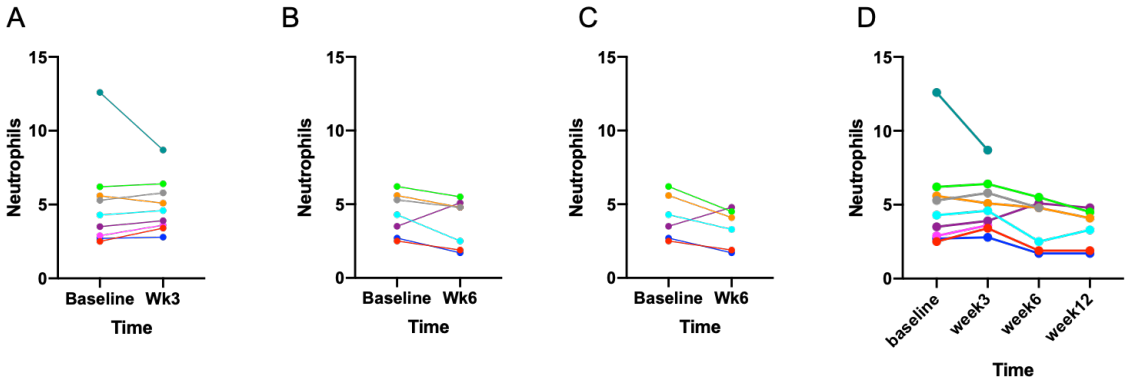


Figure 4.12 Peripheral Blood Neutrophil Count

for A) Baseline to week 3; n=9 for those patients with baseline and week 3 data B) Baseline to week 6; n=7 for those patients with valid baseline and week 6 data, C) Baseline to week 12; n=6 for those patients with valid baseline and week 12 data , as assessed by Wilcoxon Test and D) longitudinally; n=6 for those patients with valid baseline, week 6 and week 12 data , as assessed by Friedman test. Individual participants are represented by a different colour, with each point indicating a sampling event.

4.3.3 Peripheral Blood Eosinophil count

Peripheral Blood Eosinophil Count significantly reduced over the duration of the intervention period (Figure 4.13D, p=0.007). There was no statistically significant difference between peripheral blood eosinophil count at baseline (mdn=0.2 vs week 3 (mdn=0.3, p=0.33) (figure 4.13A), with a trend to significant reduction between baseline and week 6 (mdn=0.2 [IQR=0.2,0.6] vs mdn=0.2 [IQR=0.2,0.3], p=0.066) and week 12 (mdn=0.25 [IQR=0.2,0.7] vs mdn=0.2 [IQR=0.18,0.4], p=0.063) (figures 4.13 B and C).

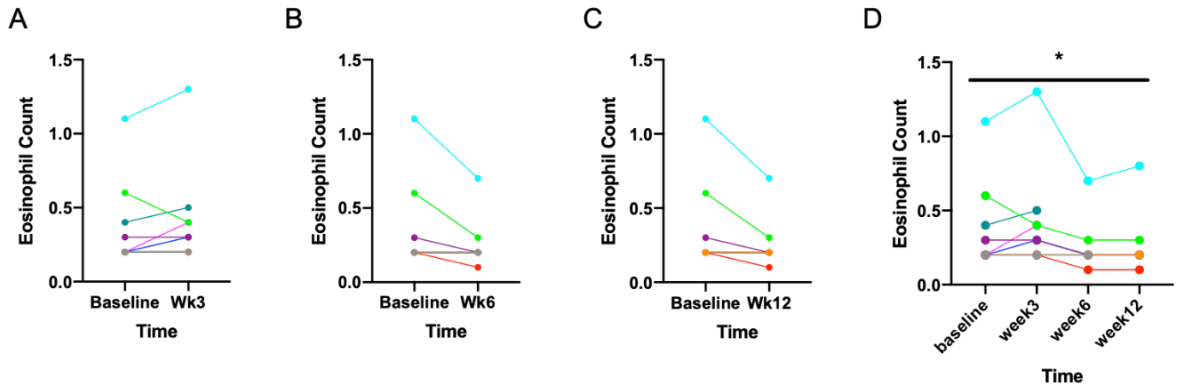


Figure 4.13 Peripheral Blood Eosinophil Count

*for A) Baseline to week 3; n=9 for those patients with baseline and week 3 data B) Baseline to week 6; n=7 for those patients with valid baseline and week 6 data, C) Baseline to week 12; n=6 for those patients with valid baseline and week 12 data , as assessed by Wilcoxon Test and D) longitudinally; n=6 for those patients with valid baseline, week 6 and week 12 data , as assessed by Friedman test. Individual participants are represented by a different colour, with each point indicating a sampling event. *=statistically significant result; p<0.05*

4.3.4 Peripheral Blood Lymphocyte count

Peripheral Blood Lymphocyte Count also significantly reduced over the duration of the intervention period (Figure 4.14D, p=0.049). There was a significant increase between peripheral blood lymphocyte count at baseline and week 3 (figure 4.14A) (mdn=1.7 vs mdn=2.3, p=0.028), with no significant change between baseline and week 6 (figure 4.14B) (mdn=1.7 vs mdn=1.7, p=0.47) or week 12 (figure 4.14C) (mdn=2.2 vs mdn=1.8, p=0.34)

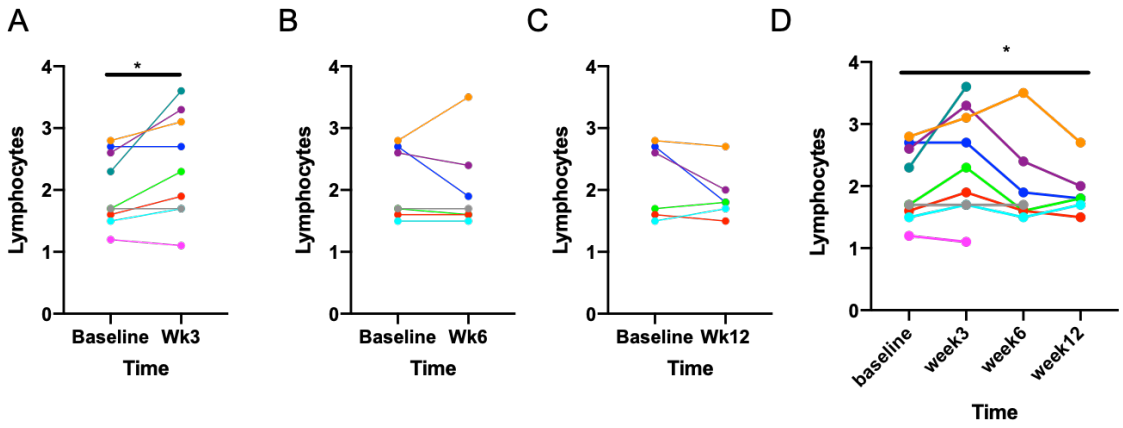


Figure 4.14 Peripheral blood Lymphocyte count

*for A) Baseline to week 3; n=9 for those patients with baseline and week 3 data B) Baseline to week 6; n=7 for those patients with valid baseline and week 6 data, C) Baseline to week 12; n=6 for those patients with valid baseline and week 12 data , as assessed by Wilcoxon Test and D) longitudinally; n=6 for those patients with valid baseline, week 6 and week 12 data , as assessed by Friedman test. Individual participants are represented by a different colour, with each point indicating a sampling event. *=statistically significant result; p<0.05.*

4.3.5 Peripheral Blood Monocyte Count

Peripheral Blood Monocyte Count did not significantly change over the duration of the intervention period (Figure 4.15D) (p=0.28). There were no significant changes between peripheral blood monocyte count at baseline and week 3 (figure 4.15A) (mdn=0.5 vs mdn=0.7, p=0.136), week 6 (figure 4.15B) (mdn=0.5 vs mdn=0.45, p=1.0) or week 12 (figure 4.15C) (mdn=0.5 vs mdn=0.45, p=0.41)

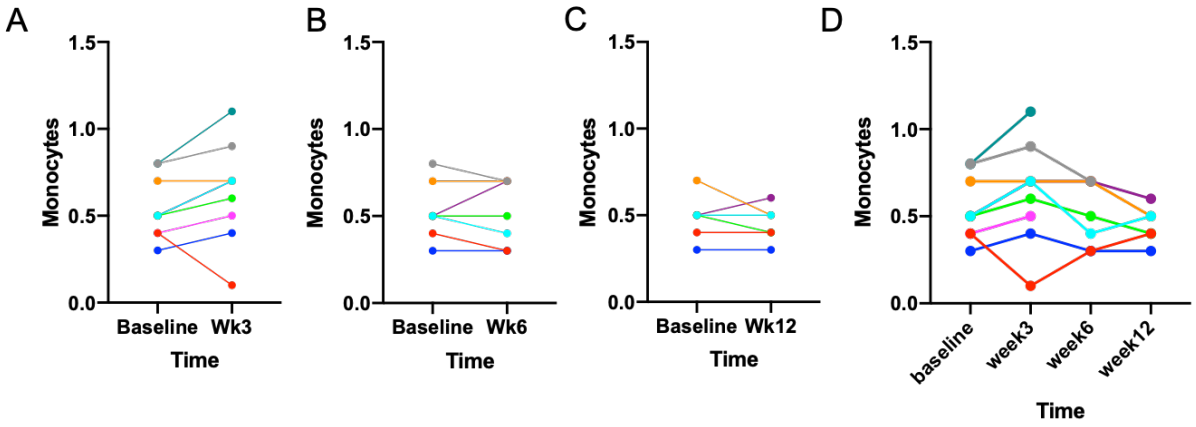


Figure 4.15 Peripheral Blood Monocyte Count

for A) Baseline to week 3; n=9 for those patients with baseline and week 3 data B) Baseline to week 6; n=7 for those patients with valid baseline and week 6 data, C) Baseline to week 12; n=6 for those patients with valid baseline and week 12 data , as assessed by Wilcoxon Test and D) longitudinally; n=6 for those patients with valid baseline , week 6 and week 12 data , as assessed by Friedman test. Individual participants are represented by a different colour, with each point indicating a sampling event.

4.3.6 Total IgE

Due to lab processing errors, baseline and week 12 total IgE results are only available for n=4 (figure 4.16). There was no significant change in these patients from baseline to week 12, as assessed using a Wilcoxon signed rank test (mdn=273.5 vs mdn=340.8, p=0.25)

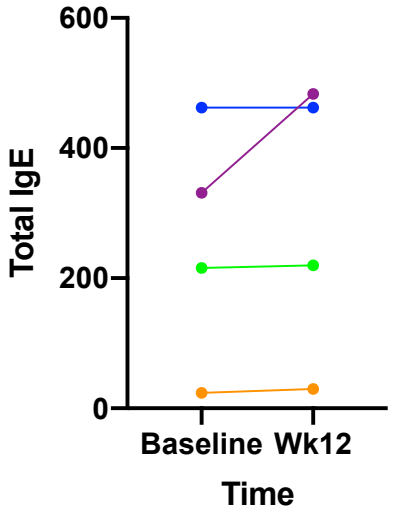


Figure 4.16 Total IgE baseline to week 12

(n=4)for those patients with valid data, as assessed by a Wilcoxon signed rank test). Individual participants are represented by a different colour, with each point indicating a sampling event.

4.3.7 C-Reactive Protein

CRP did not significantly change between baseline and week 3 (figure 4.17A) (mdn=2 vs mdn=2, p=0.89) , baseline to week 6 (figure 4.17B) (mdn=2 vs mdn=2, p=0.28), week 12 (figure4.17C) (mdn=3 vs mdn=1, p=0.46), or throughout the training intervention, (p=0.93, figure 4.17D).

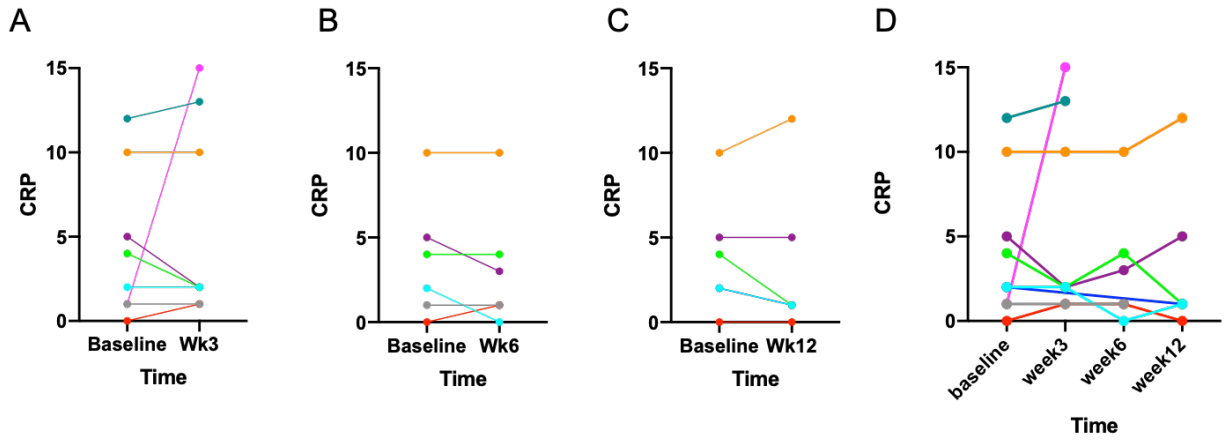


Figure 4.17 C-Reactive Protein

for A) Baseline to week 3; n=9 for those patients with baseline and week 3 data B) Baseline to week 6; n=7 for those patients with valid baseline and week 6 data, C) Baseline to week 12; n=6 for those patients with valid baseline and week 12 data , as assessed by Wilcoxon Test and D) longitudinally; n=6 for those patients with valid baseline, week 3, week 6 and week 12 data , as assessed by Friedman test. Individual participants are represented by a different colour, with each point indicating a sampling event.

4.3.8 Sputum cell count

Sputum was only obtained for 6 patients, of whom only 3 completed the intervention (data not shown for patient with only a single sputum sample at baseline). There was no significant reduction in sputum eosinophil percentage (p=0.94) and count (p=0.89) over the course of the intervention (figure 4.18). There was no change in sputum neutrophil percentage (p=0.94) or count (p=0.19) over the course of the intervention (Figure 4.19) and no significant change in sputum macrophage percentage (p=0.94) or count (p=0.94) over the course of the intervention (figure 4.20)

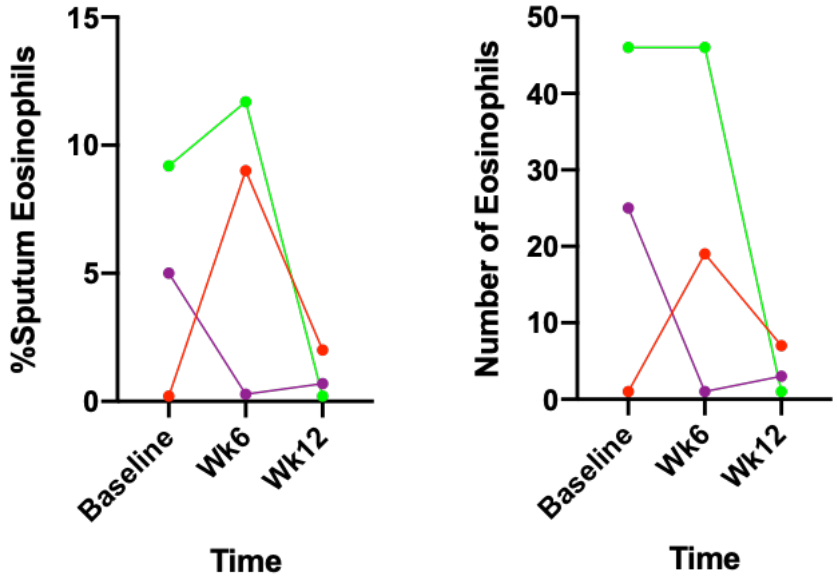


Figure 4.18 Sputum Eosinophil percentage and cell count over the course of the intervention, as assessed by Friedman test; n=3 for those patients with valid data. Individual participants are represented by a different colour, with each point indicating a sampling event

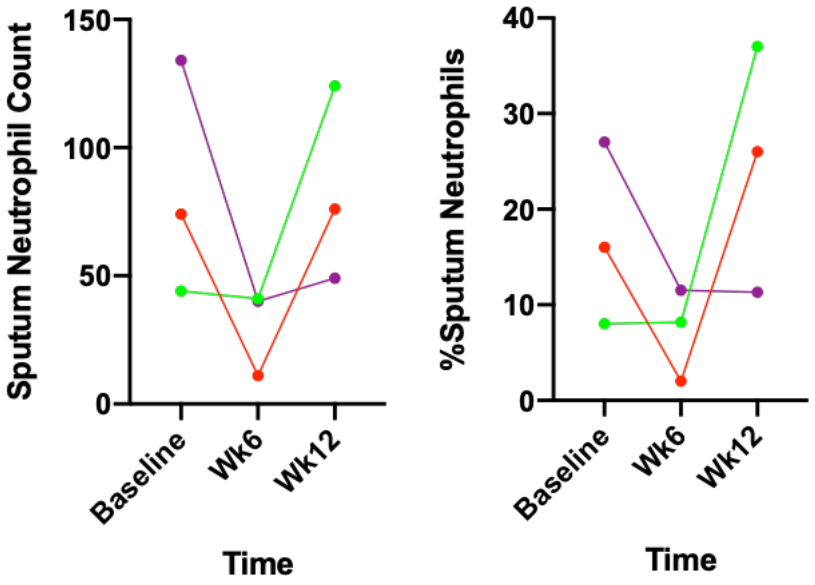


Figure 4.19 Sputum neutrophil percentage and cell count

over the course of the intervention, as assessed by Friedman test; n=3 for those patients with valid data. Individual participants are represented by a different colour, with each point indicating a sampling event

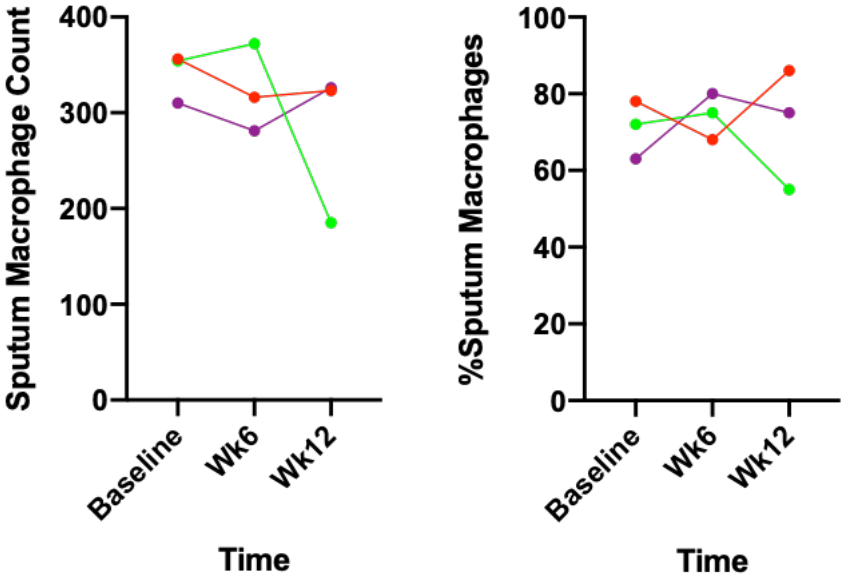


Figure 4.20 Sputum macrophage percentage and cell count

Data presented over the course of the intervention, as assessed by Friedman test; n=3 for those patients with valid data. Individual participants are represented by a different colour, with each point indicating a sampling event

4.3.9 FeNO

FeNO is increasingly used as an indirect measure of lung eosinophilic inflammation, and is a less invasive, less time-consuming measure than sputum cell count and percentages. There was no significant trend for reduction in FeNO across the course of the intervention as assessed by a Friedman Test (p=0.86) (figure 4.21D), and there was no statistically significant change in FeNO between baseline and week 3 (mdn=44.5 vs mdn=36, p=0.52) (figure 4.21A), week 6 (mdn=44.5 vs mdn=34.5, p=0.75) (figure 4.21B) or week 12 (mdn=50.75 vs mdn=35.25, p=0.75) (figure 4.21C), as assessed by Wilcoxon Signed Rank test.

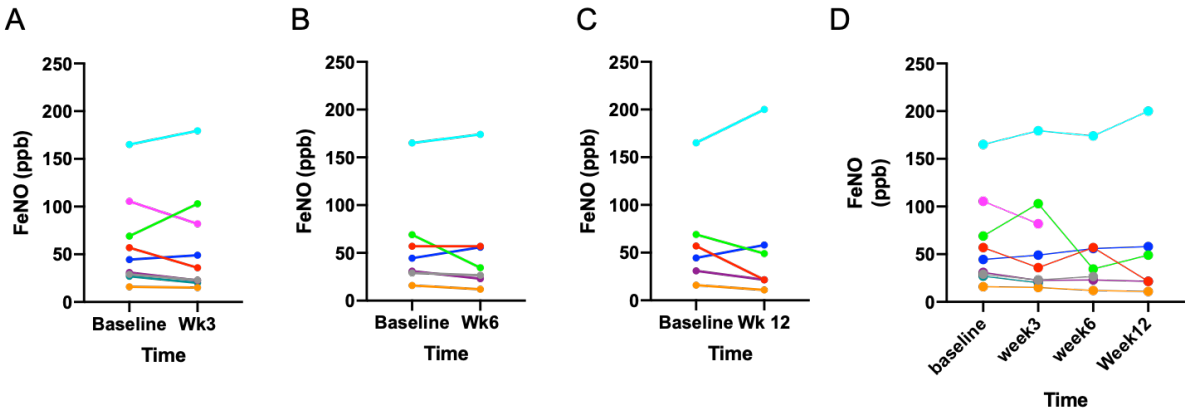


Figure 4.21 FeNO (ppb)

for A) Baseline to week 3; n=9 for those patients with valid baseline and week 3 datas B) Baseline to week 6; n=7 for those patients with valid baseline and week 6 data, C) Baseline to week 12; n=6 for those patients with valid baseline and week 12 data , as assessed by Wilcoxon Test and D) longitudinally; n=6 for those patients with valid baseline, week 3, week 6 and week 12 data , as assessed by Friedman test. Individual participants are represented by a different colour, with each point indicating a sampling event

4.4 Discussion

Asthma related outcomes of symptom control and quality of life scores both improved significantly over the course of the training intervention, further suggesting safety and tolerability of this intervention; symptoms did not worsen with exercise training. These exploratory results may not be translatable to patients with exercise induced

bronchospasm, and investigation of exercise intervention for patients with exercise induced bronchospasm is a consideration for future work. There are no published studies in adults investigating exercise intervention in patients with exercise induced bronchospasm. A better understanding of the mechanism through which exercise exerts its beneficial effect in asthma generally would allow more accurate assessment of potential benefits and risks associated with designing an exercise intervention study specifically for patients with exercise induced bronchospasm. In children, there is some suggestion that an aerobic exercise intervention can reduce exercise induced bronchospasm but this is not linearly associated with increase in fitness (93). However, if exercise induced bronchospasm is thought, for a large part, to reflect suboptimally controlled asthma as per the BTS/SIGN guidance (84), then there is some support from the work discussed herein. We have early exploratory data that suggests that exercising patients with symptomatic asthma (as defined by an ACQ score of ≥ 1.5), does not result in acute bronchospasm. Assessment of control through methacholine challenge rather than ACQ score would have provided a more objective assessment of baseline control, and therefore be more extrapolatable to patients with exercise induced asthma. CPETs are more likely to result in exercise induced bronchospasm than an exercise intervention, as airways hyperreactivity has been shown to increase with increasing exercise load, as reviewed in (86). The exercise intervention in its design is relatively protective against exercise induced bronchospasm. Cold air and exposure to environmental irritants such as chlorine exacerbate exercise induced bronchospasm, and neither are a consideration with an indoor and cycle ergometer based intervention (86). Exercise induced bronchospasm aside, the findings presented in this chapter are supported in the literature, which demonstrate improvements in symptom control scores in other exercise intervention studies (5, 6, 336). However, the Freitas study involved a dietary as well as an exercise intervention, and the results of the Freitas study have the potential to be confounded by significant weight loss (5). The Toennesen study also only demonstrated significant improvements in asthma control score with a combined diet and exercise intervention (280). The data presented herein are the first demonstration of improvements in ACQ after such a short duration of training, as demonstrated in the significant results after only 3 weeks of training in the ACQ results and 6 weeks for significant improvement in the AQLQ results. However, when interpreting these results, a number of caveats need to be taken into consideration. The majority of the improvement seen in ACQ score occurred

during the first 3 weeks of the intervention, which detracts from the hypothesis of gradual improvement in response to an exercise training intervention. The rapid improvement in symptom scores raise the question as to whether these improvements are more likely attributable to increased confidence and potential increased compliance with medication rather than the exercise intervention, although AT did also demonstrate significant improvement at 3 weeks (figure 3.11). It is noteworthy that in other disease areas, the SRETP has shown greatest improvements in physical fitness within the first 6 weeks of training, followed by a plateau; however, this is in the context of post chemotherapy recovery therefore not directly applicable to this cohort (308). A control group would have been useful in determining the degree of any placebo effect.

When interpreting the separate AQLQ domain results, it is interesting to see that the emotional parameter demonstrated a trend towards significant improvement at 3 weeks (figure 4.5A), with a significant improvement at 6 and 12 weeks (figure 4.5B and C). Over the duration of the study, this translated into a significant improvement (Figure 4.5D). This could be explained by an initial improvement in participants' emotional state from the positive effects that study participation (353) and exercise (354) independently confer, with this impact persisting throughout the course of the study. The benefits of study participation and exercise are well known, and an important consideration when interpreting the results from this study as disease modifying. A control group, again, would have been useful in interpreting these data. However, in the context of the other objective clinical improvements, the emotional benefits of exercise may not fully explain the overall improvement in ACQ and AQLQ. The improvement in the environmental domain is also interesting (Figure7D). A potential explanation for this is that a reduction in inflammation and increased tolerance of oxidative stressors, assuming the hypothesis for this study is correct, allow participants to better tolerate environmental conditions that previously have had detrimental effects on their asthma symptom burden. This may in part explain the reduction in symptom scores over the course of the intervention. The laboratory work presented in chapter 5 provides additional support for this hypothesis. There were not significant improvements in the symptom domain of the AQLQ over the course of the intervention. The most plausible explanation for this is due to underpowering, and supported by the significant improvement in the symptom domain of the AQLQ between baseline and 6 weeks and baseline and 12 weeks. This interpretation would explain the incongruity between the significant improvement in ACQ score and the

lack of significant improvement in symptom domain of the AQLQ score, where other work has demonstrated a strong correlation between the two (355). The lack of improvement in the activity domain of the AQLQ score is potentially a reflection of underpowering. However, it could also be reflective of the relatively mild asthma in this cohort, in that their symptoms, whilst being suboptimally controlled at baseline, may not interfere with their day to day activity. This explanation may translate to a lack of demonstrable improvement in the activity domain of the AQLQ.

There is significant improvement (increase) in pre-bronchodilator FVC, which is supported in the literature (5), with a trend for an improvement (reduction) in bronchodilator reversibility of FVC. FEV1 demonstrated significant reduction in bronchodilator reversibility. It may be that the number of patients are too small to demonstrate an improvement in other lung function parameters. However, interpretation of this analysis may also be confounded by wide baseline variability of these measurements (356) and by potential variability in medication compliance,. Both confounders would have been partly addressed by inclusion of a control group. The timing of dosing of inhaled corticosteroids and bronchodilator therapy may also impact on variability of lung function results, and inhaler technique may also contribute to and could impact on lung function variability (357, 358). Whilst the sampling sessions and CPETs were undertaken at roughly the same time of day to mitigate this impact, and bronchodilator therapy was consistently held prior to lung function testing, inclusion of a control group would have been useful in better understanding any temporal confounding.

Desgin of a control arm for an exercise intervention is difficult. Some studies have used a breathing retraining arm as a control arm. This has the advantage of mitigating, to some extent, the addition placebo benefit that enrolment in a study can confer. However, breathing retraining can also improve symptom scores, and in some studies, bronchial hyperreactivity. In this context, breathing retraining as a control arm has potential to additionally confound (332, 333). Whether the improvement demonstrated is linked to a reduction in inflammation is hard to elucidate, but the correlations discussed in Chapter 5 are suggestive of an association between the two. Higher lung function is associated with physical fitness in childhood and adolescence (359), with higher FEV1, FVC and PEF values correlating with increased physical fitness in healthy males (360). This association between lung function and physical fitness may partially explain the increase in FVC demonstrated in our cohort. In Chapter 3, we demonstrated that there was a clinically

and statistically significant increase fitness as assessed by oxygen uptake at AT, with a trend to improvement in oxygen uptake at peak exercise. However, it is unlikely the that the increase in physical fitness is fully responsible for the improvement in FVC, in the context of reduction in FEV1 bronchodilator reversibility. This reduction in FEV1 bronchodilator reversibility reflects improvement in pre bronchodilator FEV1 to the levels of post bronchodilator FEV1 post intervention, with associated reduction in bronchodilator reversibility. There was also a non significant trend for reduction in FVC bronchodilator reversibility. Similar has been demonstrated in the literature, with other exercise intervention studies also demonstrating an improvement in bronchial hyperreactivity (6) and lung function (5) following exercise intervention in asthma patients. This reduction in airways hyperreactivity as assessed by reduced bronchodilator reversibility demonstrates association with mechanistic results, as discussed in Chapter 5, and suggests support for the hypothesis around which this thesis has been constructed. However, in the context of normal spirometry or only mild obstruction at enrolment, it is more difficult interpret these changes as a reduction in airways hyperreactivty, which could have been more accurately assessed through serial methacholine challenge testing. Post bronchodilator spirometry did not change, and this could be a reflection of the relatively normal post bronchodilator lung function at baseline. These are patients who would have been expected to demonstrate a significant decrease in lung function on challenge testing, and a reduction in this decrease following the exercise intervention may have been a more sensitive way to demonstrate this.

There is demonstration of a reduction in asthma associated markers of inflammation, with a significant reduction in peripheral blood total white cell count, peripheral blood eosinophil count and peripheral blood lymphocyte count over the course of the intervention (Figure4.10D-figure 4.14D), with a trend for reduction in peripheral blood neutrophil count. The initial baseline to week 3 increase in lymphocyte count is reflective of the literature, as discussed in Chapter 1, where an initial blood lymphocytosis is seen with exercise, often translating to an observed lymphopaenia later in post exercise recovery. These changes may be representative of an improved state of immune surveillance and lymphocyte redeployment to peripheral tissues (361).

The strongest signal that is seen demonstrating the potential impact of exercise on the peripheral blood eosinophil count (figure 4.13D), although it is noteworthy that this was

partly a result of one patient demonstrating a particularly large decrease between weeks 3 and 6. The median change in peripheral blood eosinophil count is not greater than that expected with normal variation (362). As with any interpretation of these data, without a control group, there is no way to ascertain that the exercise intervention was instrumental in any observed changes. These changes may still, however, be of clinical significance and associated with the reduction in symptom scores demonstrated in (4.1.1 and 4.1.2), and demonstrative of a reduction in systemic inflammation, particularly in peripheral blood eosinophil count with SRETP. Patients with severe asthma are more likely to demonstrate a higher peripheral blood eosinophil count than those with milder disease (35) and those patients with higher blood eosinophil counts experience more severe exacerbations and demonstrating poorer asthma control (37). The improvements in blood eosinophils are also tracking ACQ scores which supports this previous finding. There is clinical utility in peripheral blood eosinophil count in the prescription of anti-IL-5 treatments for asthma (363). Clarification of these results will come with a larger, powered study that includes a control group. Monocytes, CRP and IgE did not significantly change. This may in part be a result of the small data set available. The lack of improvement may also be a reflection of both exercise (364) and asthma (365, 366) independently affecting monocyte levels in opposing directions, with a combined impact of no change. Additionally, the exercise effect on monocytes occurs rapidly over the course of minutes (367), with our sampling protocol designed to detect longer term impacts. CRP levels did not significantly change, and in addition to the small data set, there is the variability of CRP as a confounder to any impact of exercise to consider (368). The Freitas study, investigating a combined exercise and dietary intervention in asthma, did not demonstrate a reduction in CRP in an appropriately powered study of exercise intervention in asthma (5). A review of the impact of exercise interventions on CRP concluded that whilst exercise interventions produced inconsistent results, dietary and lifestyle interventions more consistently demonstrate reductions in CRP (369). The numbers of patients with IgE data available make these results uninterpretable.

In addition, there may also be a reduction in sputum eosinophil count in this cohort (Figure 4.15A). However, this conclusion is limited by the low number of participants who could produce sputum and it is not possible to interpret the small data set available. Sputum eosinophil count is not routinely used in clinical practice in asthma management, due to the difficulty in obtaining and processing samples; however, asthma control has

been shown to be associated with fluctuations in sputum eosinophil count over time (44). Sputum induction itself is an inflammatory procedure and may therefore not be the most appropriate sampling method to assess change in inflammation within the lungs in response to exercise. It was chosen as it was less invasive than bronchoscopy, but bronchoscopic sampling should be considered again when planning further work. The reduction in white cell count and trend to reduction in neutrophil count are reassuring in the context of adherence to inhaled corticosteroids. Had the clinical improvements been a result of increased compliance in the context of study participation, then one would expect to see an increase in white cell and neutrophil count as opposed to the reduction demonstrated in this cohort (370). However, the lack of control group mean these data are difficult to interpret, and could potentially also only represent normal variation over time (371) (372).

The lack of significant improvement in FeNO alongside the improvement in symptoms and systemic inflammation may be explained through its variability. FeNO is known to fluctuate and has not been demonstrated to be useful for tailoring asthma treatment (373, 374), although there may a role for this in patients who frequently exacerbate (374). FeNO suppression tests are used as a measure of compliance with inhaled corticosteroids (375). The lack of improvement in FeNO across the study period lends support to the improvements in symptoms and quality of life not being related to an increase in inhaled corticosteroid compliance. Addition of an exercising healthy and non-exercising asthma control group in a fully powered study would further address the concept of increased compliance to therapy as an explanation for improvements in symptom scores, quality of life and inflammatory parameters.

As noted in Chapter 3, the limitations of the study in terms of sample size remain. There is also the consideration of those participants who dropped out of the study after week 3 sampling and the question of whether they dropped out because they were non-responders. However, the reasons given by these two participants were not study related. Furthermore, these participants demonstrated improvement in their symptom and quality of life scores at 3 weeks, although it is noteworthy that their eosinophils and other inflammatory markers had not demonstrated a fall. The exclusion of 1 patient from week 3 statistical analysis due to a non-study related exacerbation and another at week 12 due to gastroenteritis, also require comment. Whilst study exclusion criteria were clearly

defined, on reflection, criteria for exclusion from a sampling point should also have been pre-defined, and this is a reflection of the learning curve travelled over the course of this project. Statistically, there is the question of confounding from repeated measures. This is rationalised through the study being exploratory in its design and therefore the repeated measures are used for hypothesis testing to inform and power further work, as detailed in Chapter 7. An additional reason for not adjusting the results for repeated measures, is due to the small sample size. Oberfeld and Franke (318) investigated the use of repeated measures analysis for small sample sizes in both normally distributed and non-normally distributed data, and concluded that none of the approaches they tested could be considered robust in the context of small sample size and non-normally distributed data. However, despite the lack of controlling for repeated measures testing, the improvement in symptom scores, Quality of Life Scores and inflammatory parameters together raise the question that the results presented herein are not purely a result of repeated analyses, and instead reflect improvements as a result of the intervention that would be sustained in a fully powered study. These improvements could be validated in a fully powered further study, with inclusion of a control group to address many of the questions these data are not able to.

Even in this small cohort, there are statistically significant reductions in clinically relevant markers of asthma control and in inflammatory parameters, providing some early support for the hypothesis of this thesis. The following chapter will investigate the potential mechanism through which these changes may be effected.

Chapter 5 Investigating the mechanism: cytokine and redox regulation changes with exercise intervention

We have demonstrated in Chapters 3 and 4 that an interval exercise intervention is safe and tolerable, and may be beneficial to patients with asthma in terms of symptoms, quality of life, lung function and routine clinical markers of systemic inflammation. In this chapter, we present results in support of our hypothesis as to the mechanism behind these clinical improvements. Firstly, we investigated whether the exercise intervention had any impact on expression of asthma relevant cytokines. We then looked more mechanistically at whether downstream markers of redox regulation were affected by the intervention, and whether expression of NRF2 and Keap1 in PBMCs changed with this. Finally, we looked for associations to link our mechanistic hypothesis to the clinical outcomes demonstrated in Chapeters 3 and 4.

5.1 Luminex Cytometric Bead Assay

Asthma specific cytokines were analysed using a commercially prepared magnetic bead assay in plasma and serum before, mid and post intervention prior to acute exercise challenge in the form of a cardiopulmonary exercise test. The results presented here are for the 6 participants who completed the exercise training intervention with baseline and final sampling. The cytokine panel selected included CCL11/eoxtaxin, which is a major eosinophil chemoattractant , and has been demonstrated to be important in driving allergic inflammation and asthma (376). IL-5 is central to eosinophilic asthma, with anti Il-5 treatments revolutionising the management of this endotype(377). TNF alpha and IFN gamma have been included as Th1 proinflammatory cytokines that have been demonstrated to induce corticosteroid resistance, with corticosteroid resistant asthma presenting a particularly challenging endotype of asthma to manage clinically (378). Murine studies demonstrate that exercise can increase steroid responsiveness via increased glucocorticoid receptor binding and subsequent nuclear translocation in airway epithelial cells (287). The same study then used the GR antagonist RU486 to demonstrate that the GR was involved in mediating previously demonstrated, exercise mediated

decreases in NF-κB nuclear translocation and DNA binding in the OVA-sensitized lung (271, 287). Il-10 and IL-1ra expression are increased by exercise in murine models, with IL-10 increases also seen in a human exercise intervention study, and are known for their anti-inflammatory effects (5, 291). IL-6 is central to the acute response to exercise, and has also been implicated as a useful biomarker as a useful biomarker associated with severe asthma, notably neutrophilic endotypes (379). Treatment of asthma with the anti IL-13 Dupilumab has been demonstrated to be effective (380), with exercise in murine models shown to reduce BAL levels of Il-13 (381). Both serum and plasma cytokines of all eight cytokines were measured, as the changes expected in cytokine levels were small based on results from previous human exercise studies (5), and small differences have been demonstrated between cytokine levels in simultaneous serum and plasma samples (382). Results are presented for the eight cytokines that were detected in samples of plasma and for the five cytokines that were detected in the samples of serum analysed. The cytokines IL-5, IL-13 and IFNγ were below the detectable range for the assay in serum, and so there are no data to present for these. This exploratory study was not powered for these experimental outcomes, and therefore data must be interpreted in this context.

5.1.1 Plasma Cytokine Results

There were significant reductions in plasma CCL11/eotaxin between baseline (mdn=173; and 12 weeks (mdn=154) (p=0.046) (see figure 5.1A). There were also significant reductions between baseline and week 12 in plasma IL-5 (mdn=7.24 vs mdn=5.34, =p=0.046) , TNFα(mdn=15.63 vs mdn=9.96, p=0.046) and IFNγ (mdn=17.89 vs mdn=11.9, p=0.046) although these readings fell below the line of quantification for the assay and so need to be interpreted with some caution (see figure 5.1 B-D).

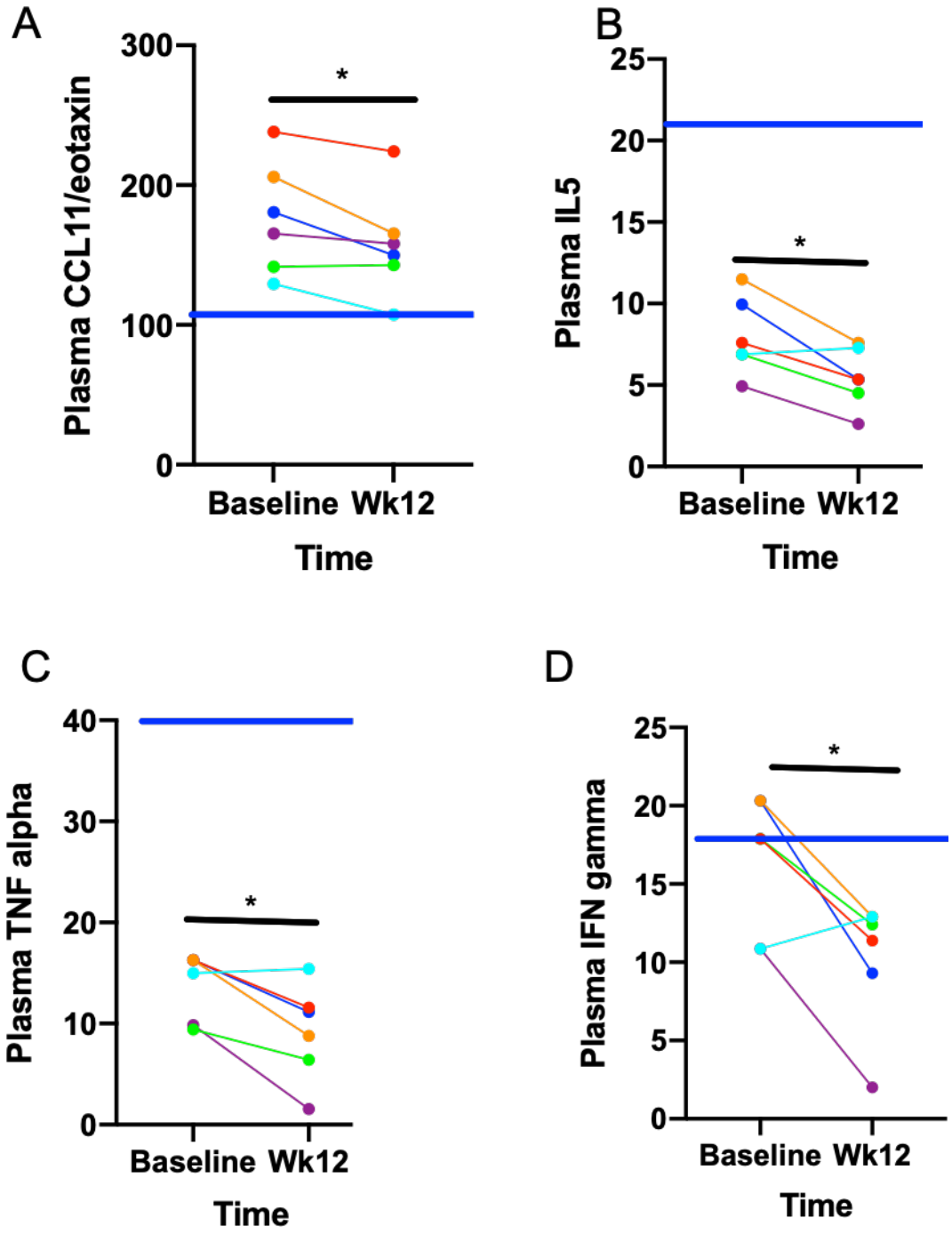


Figure 5.1 Plasma CCL11, IL-5, TNFα and IFNγ between baseline and post intervention

*Assessed by Wilcoxon Test; n=6 for patients with valid baseline and final data. Individual participants are represented by a different colour, with each point indicating a sampling event. Units are pg/ml. *=statistically significant result; p<0.05, blue line indicates lower limit of quantification*

There were no significant changes in plasma IL-1ra (mdn=644.3 vs mdn=469.9, p=0.116), IL-6 (mdn=8.32 vs mdn=6.6,p=0.115), IL-10 (mdn=79.2 vs mdn=72.9, p=0.173) or IL-13(mdn=66.8 vs mdn=55.73, p=0.141) (see figure 5.2A-D)

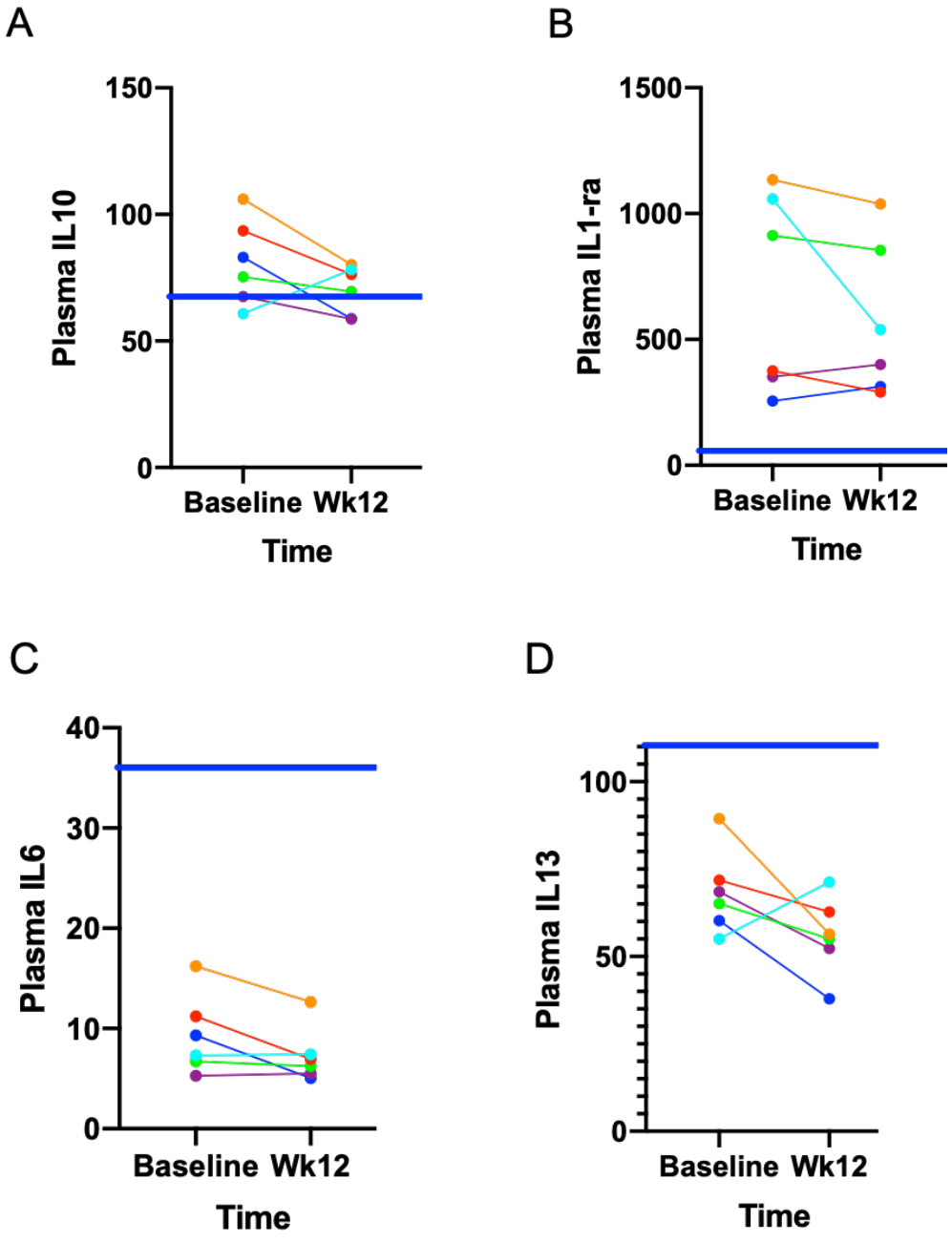


Figure 5.2 Plasma IL-10, IL-1ra, IL-6 and IL-13 between baseline and post intervention as assessed by Wilcoxon Test. n=6 for patients with valid baseline and final data. Individual participants are represented by a different colour, with each point indicating a sampling event, blue line depicts lower limit of quantification. Units are pg/ml

5.1.2 Serum Cytokine Results

There were no significant changes in serum CCL11/eotaxin (mdn=270.1 vs mdn=251.8, p=0.753), IL-1ra (mdn=1109.5 vs mdn=928.3, p=0.249), IL-6 (mdn=2.66; vs mdn=2.54, p=0.458), IL-10 (mdn=24.24 vs mdn=23.23, p=0.893) and TNFα,(mdn=2.89 vs mdn=3.11, p=1.0) (see figure 5.3 A-E). Serum IL-5, IL-13 and IFNγ were below the lowest detectable range and so were not able to be assessed.

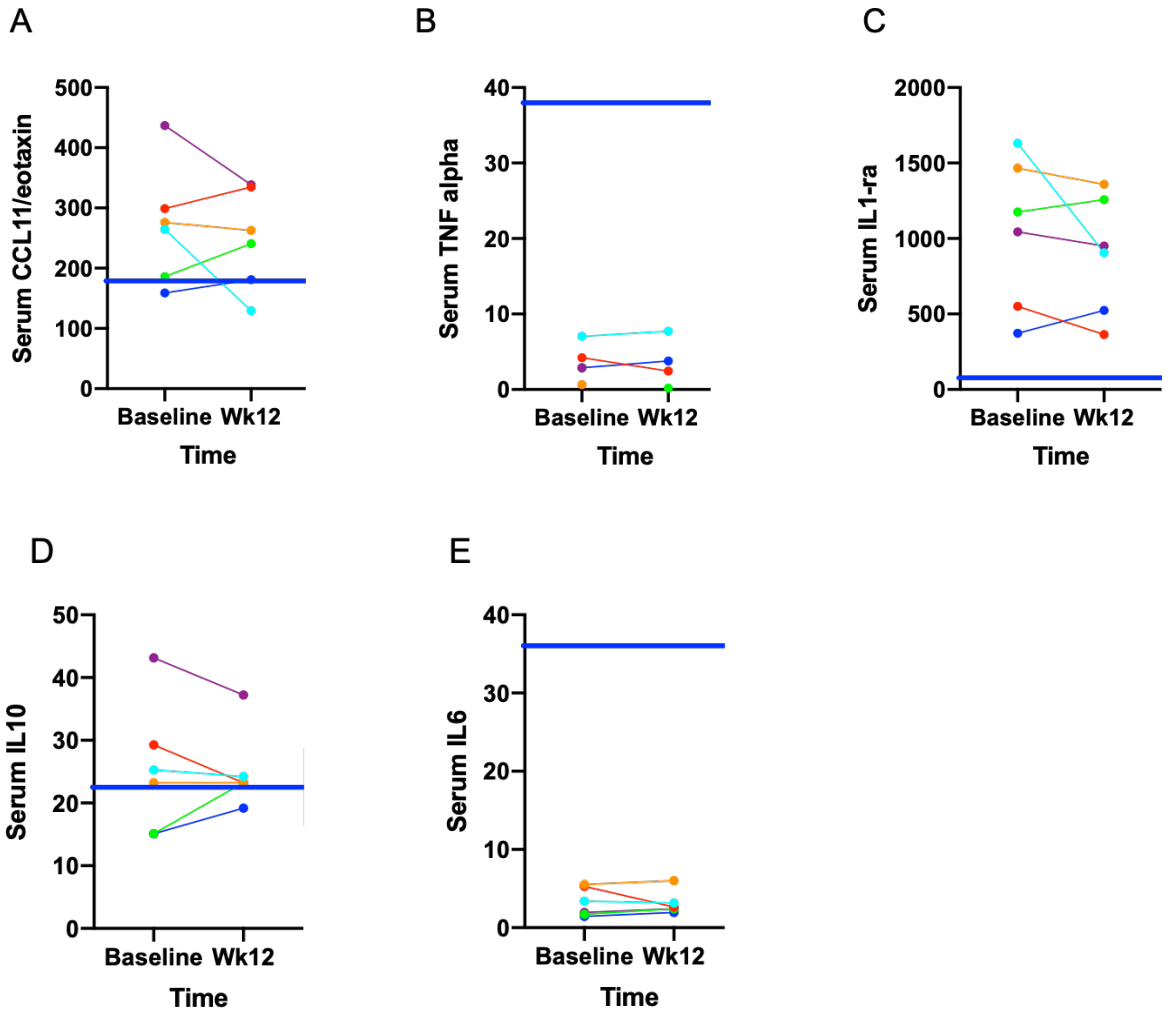


Figure 5.3 Serum CCL11/eotaxin, IL1-ra, IL-6, IL-10 and TNF α between baseline and post intervention

Assessed by Wilcoxon Test. n=6 for patients with valid baseline and final data. Individual participants are represented by a different colour, with each point indicating a sampling event. Blue line depicts lower limit of quantification. Units are pg/ml.

5.2 Redox regulation

We hypothesised that the improvement in symptom scores and inflammation following exercise intervention and demonstrated in Chapters 3 and 4 are as a result of improved redox regulation via optimisation of NRF2 expression. Therefore, downstream markers of NRF2 activity and redox regulation changes were assessed pre and immediately post CPET at baseline, 3, 6 and 12 weeks. Included in the statistical testing were the 6 participants to complete the exercise intervention without intercurrent illness at baseline and final sampling. A number of analytes were measured because NRF2 has widespread effects on redox metabolism, and it was important, in a hypothesis generating study, to capture these potential changes. Figure 5.8 demonstrates the marked variation in responses in all the redox regulation parameters. All change refers to increase or decrease over the course of the intervention period. At all sampling points, samples were taken both before and after an acute exercise challenge in the form of a maximal, symptom limited incremental CPET, and this is specified for individual results.

5.2.1 Nitrite and nitrate

Nitrite (NO^{2-}) can be interpreted as a non-specific marker of NRF2 activity, with nitric oxide propised to contribute to redox signalling through activation of the NRF2/Keap1 pathway(383). Nitrite (measured in µM) significantly increased overall between baseline (mdn=2.65) and 12 weeks (mdn=5.75) (p=0.028), as assessed by Wilcoxon Test before CPET and post CPET(mdn=2.7) and 12 weeks (mdn=5.7) (p=0.028). Longitudinal change both pre and post CPET, as assessed by Friedman test, also showed significant increases (p=0.001 and 0.009 respectively) (see figure 5.4).

Nitrate (measured in µM) did not change significantly overall either pre (mdn=41.54 vs mdn=47.45, p=0.116) or post CPET (mdn=48.09 vs mdn=49.15, p=0.6) between baseline and week 12, or over the course of the intervention, as assessed by Wilcoxon and Friedman tests (p=0.392 and 0.989 pre and post respectively) (see figure 5.4).

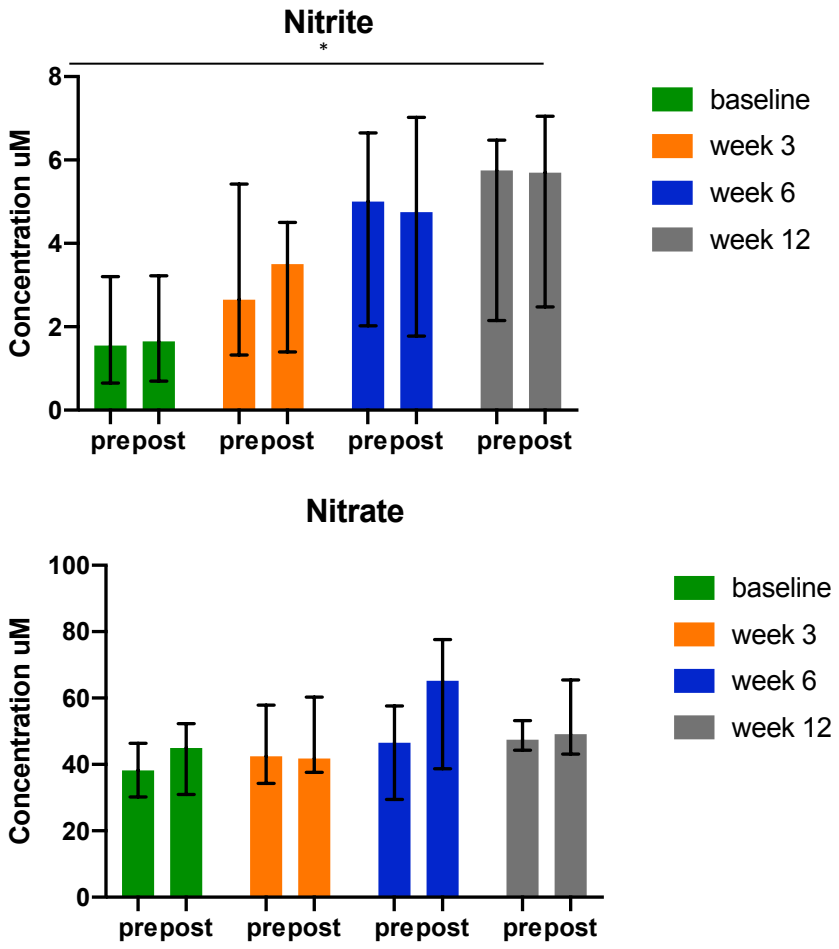


*Figure 5.4 Overview of nitrite and nitrate levels pre and post CPET for baseline (green; n=8), week 3 (orange; n=8), week 6 (blue, n=6) and week 12 (grey, n=6). Bars =median; error bars =IQR. Assessed for statistical significance (n=6) between baseline and week 12 using Wilcoxon signed rank test, and for longitudinal change using a Friedman test. *=statistically significant result; p<0.05.*

5.2.2 Other nitroso species (RXNO)

To complete the measurement of nitrate and nitrite, other nitrosospecies (RXNO) were also measured at baseline, week 3, 6 and post intervention before and after an acute exercise stimulus in the form of a maximal CPET.

There were no significant changes between RXNO (measured in µM) either pre (mdn=9.5 vs mdn=12, p=0.104) or post CPET (mdn=7.35 vs mdn=7.7, p=0.715) between baseline and week 12, or over the course of the intervention, as assessed by Friedman test see (p=0.568 and p= 0.117 pre and post respectively) (figure 5.5).

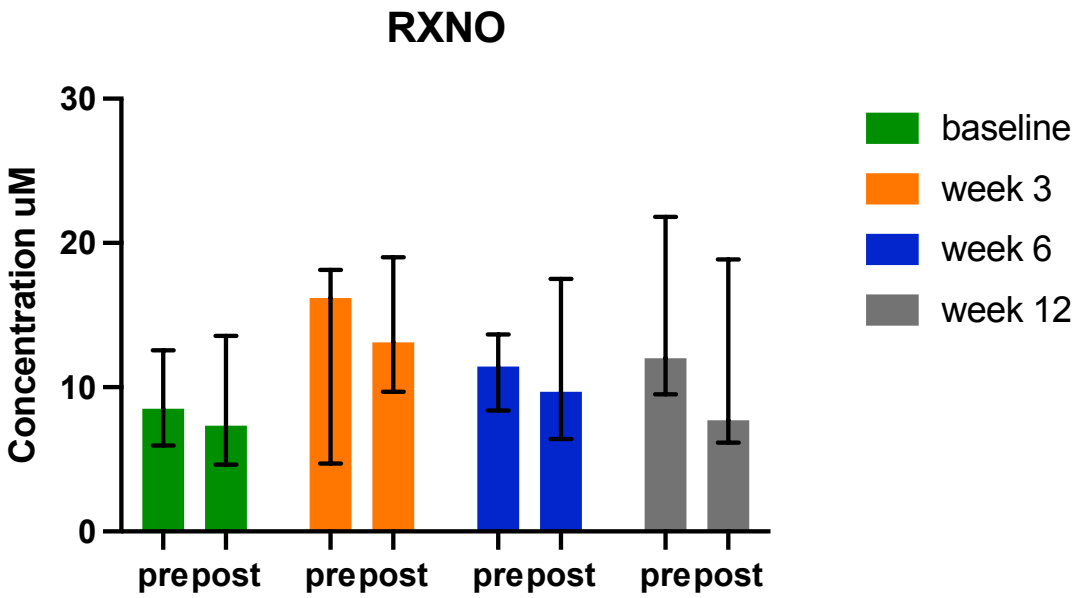


Figure 5.5 Overview of RXNO levels pre and post CPET

Overview of RXNO levels pre and post CPET for baseline (green; n=8), week 3 (orange; n=8), week 6 (blue, n=6) and week 12 (grey, n=6). Bars =median; error bars =IQR. Assessed for statistical significance (n=6) between baseline and week 12 using Wilcoxon signed rank test, and for longitudinal change using a Friedman test.

5.2.3 Thiobarbituric acid reactive substances (TBARS) assay

The TBARS assay was used to measure lipid peroxidation. Lipid peroxidation is of potential interest because accumulation of products of lipid oxidation are important in the pathogenesis of age related and oxidative stress related diseases. Assessment of thiobarbituric acid reactive substances (TBARS) is widely used to measure this and provide an assessment of lipid peroxidation as a contributor to redox reserve(266). There were no significant changes between TBARS (measured in µM) either pre (mdn=4.6 vs mdn=6.1, p=0.104, or post CPET (mdn=5.95 vs mdn=6.45, p=0.686) between baseline and week 12, or over the course of the intervention, as assessed by Friedman test (p=0.323 and p=0.277 pre and post respectively) see (figure 5.6).

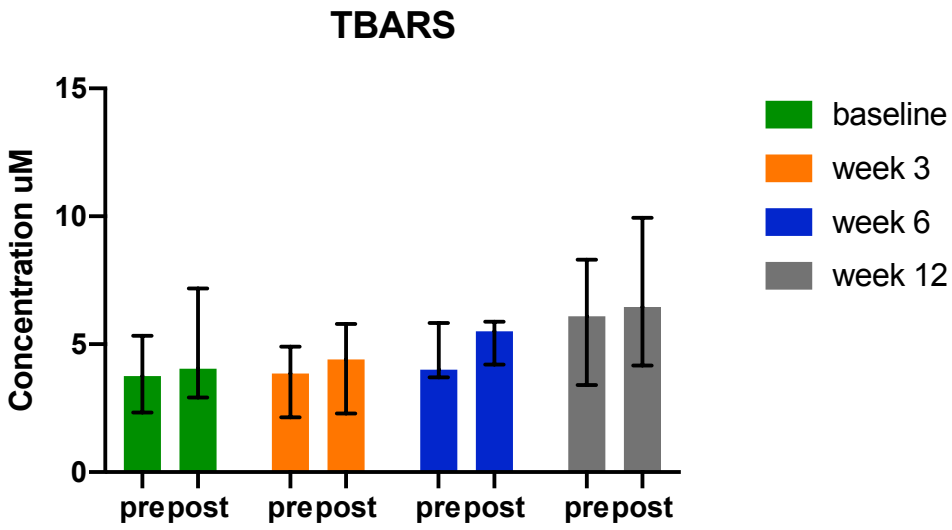


Figure 5.6 Overview of TBARS levels pre and post CPET

Overview of TBARS levels pre and post CPET for baseline (green; n=8), week 3 (orange; n=8), week 6 (blue, n=6) and week 12 (grey, n=6). Bars =median; error bars =IQR. Assessed for statistical significance (n=6) between baseline and week 12 using Wilcoxon signed rank test, and for longitudinal change using a Friedman test.

5.2.4 Ferric reducing antioxidant capacity of plasma

Ferric reducing antioxidant capacity of blood (FRAP) is a non-specific assessment of the antioxidant capacity of blood. It provides a putative index of the reducing (or antioxidant) power of a biological fluid. The level of ferric to ferrous ion reduction in acidic conditions results in differing absorbances, which are then compared to reaction mixtures with known concentrations of ferrous ions (267).

There were significant increases in FRAP (measured in µM) pre (mdn=860.5 vs mdn=966) and post CPET (mdn=847.5 vs mdn=1004) between baseline and week 12, as assessed by Wilcoxon test (p=0.028 (figure 5.7). There was marked variation in responses between individual participants (see figure 5.8).

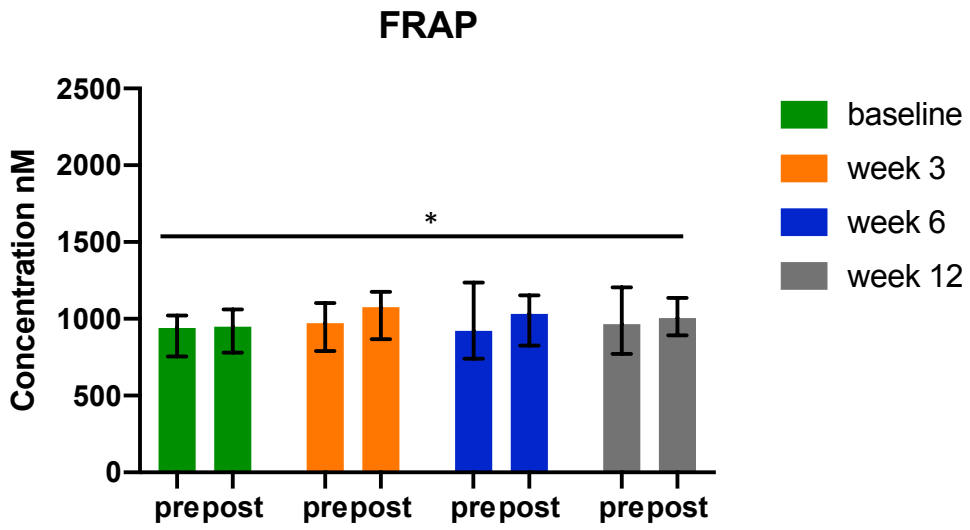


Figure 5.7 Overview of FRAP pre and post CPET

*Overview of FRAP pre and post CPET for baseline (green; n=8), week 3 (orange; n=8), week 6 (blue, n=6) and week 12 (grey, n=6). Bars =median; error bars =IQR. Assessed for statistical significance (n=6) between baseline and week 12 using Wilcoxon signed rank test, and for longitudinal change using a Friedman test. *=statistically significant result; p<0.05*

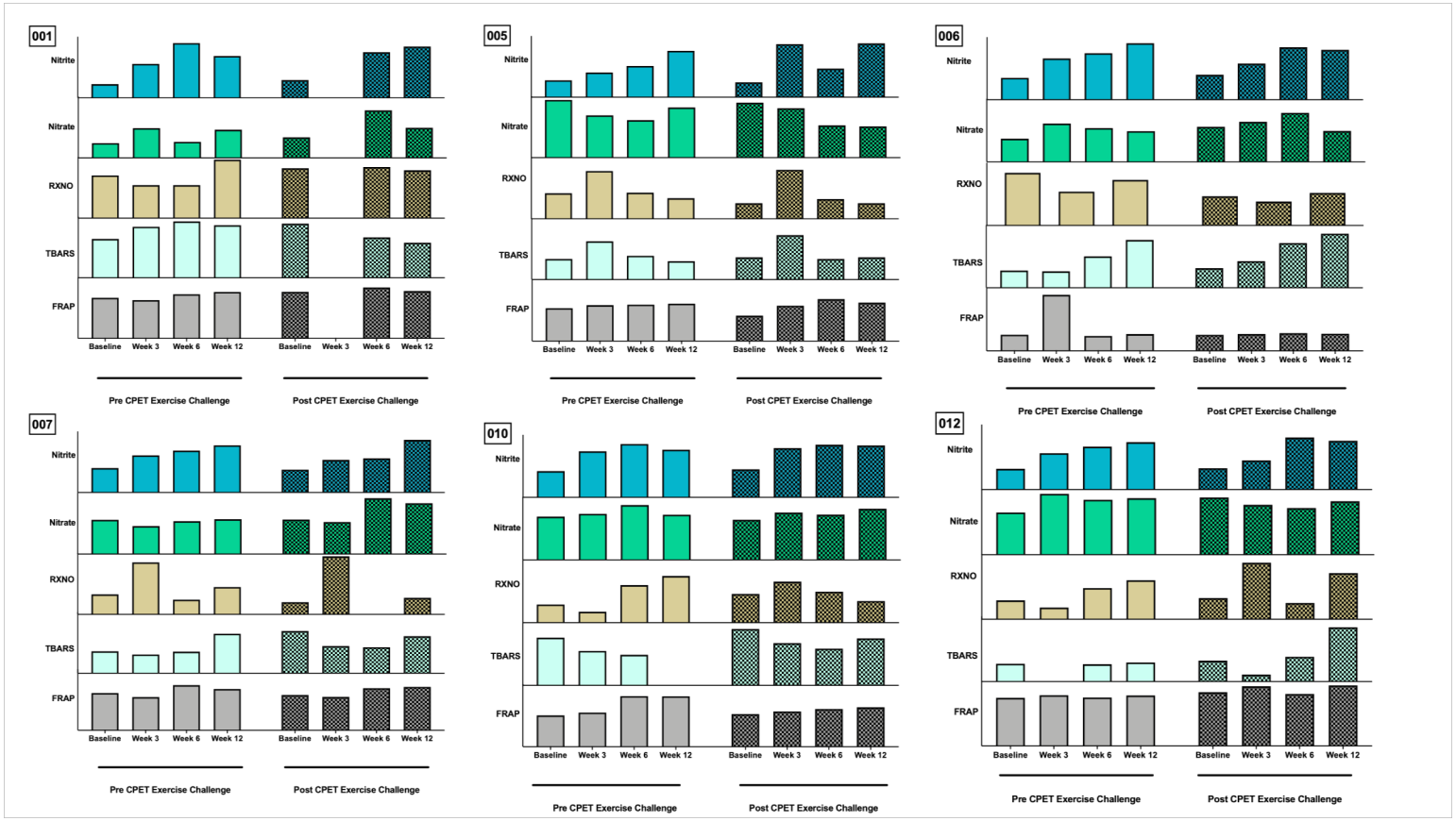
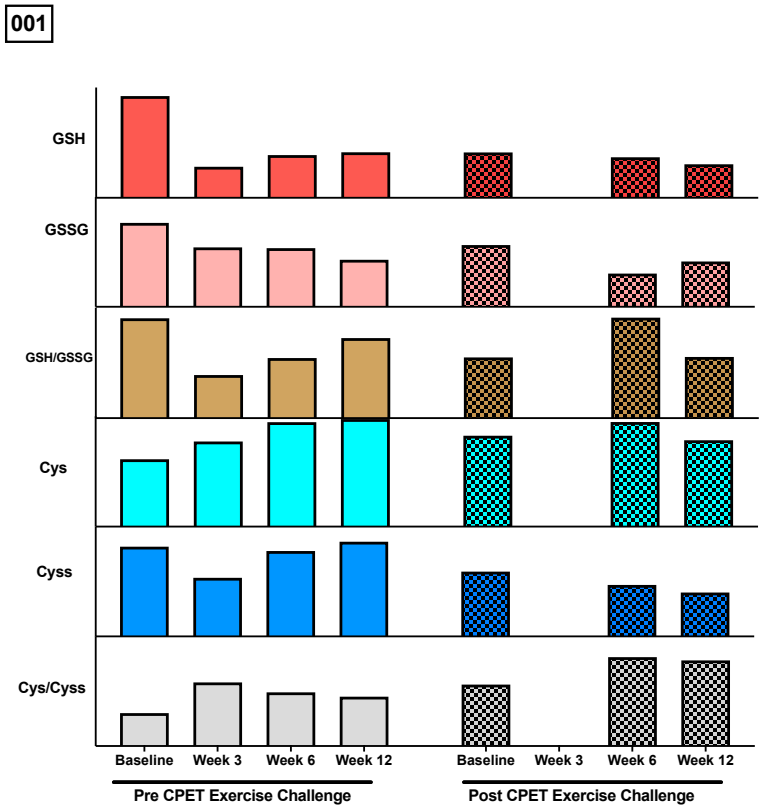
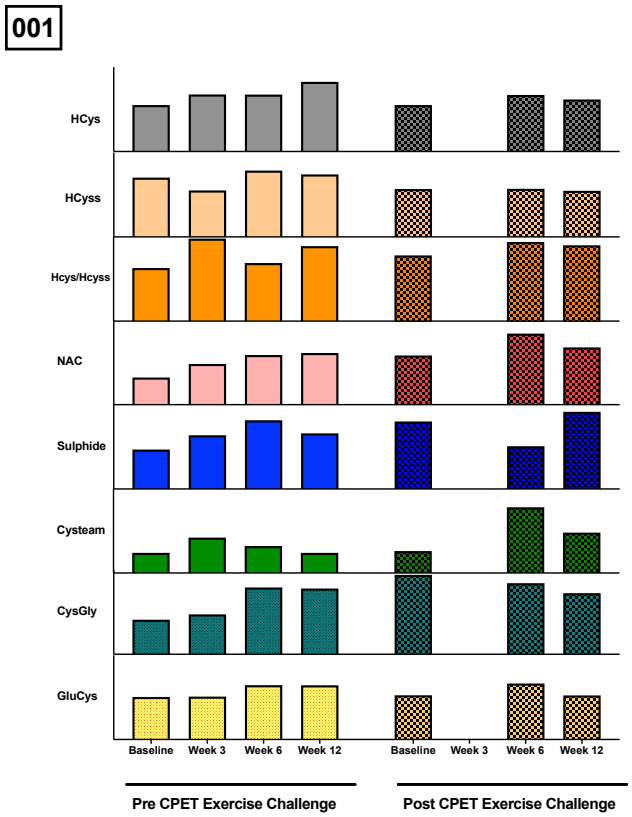


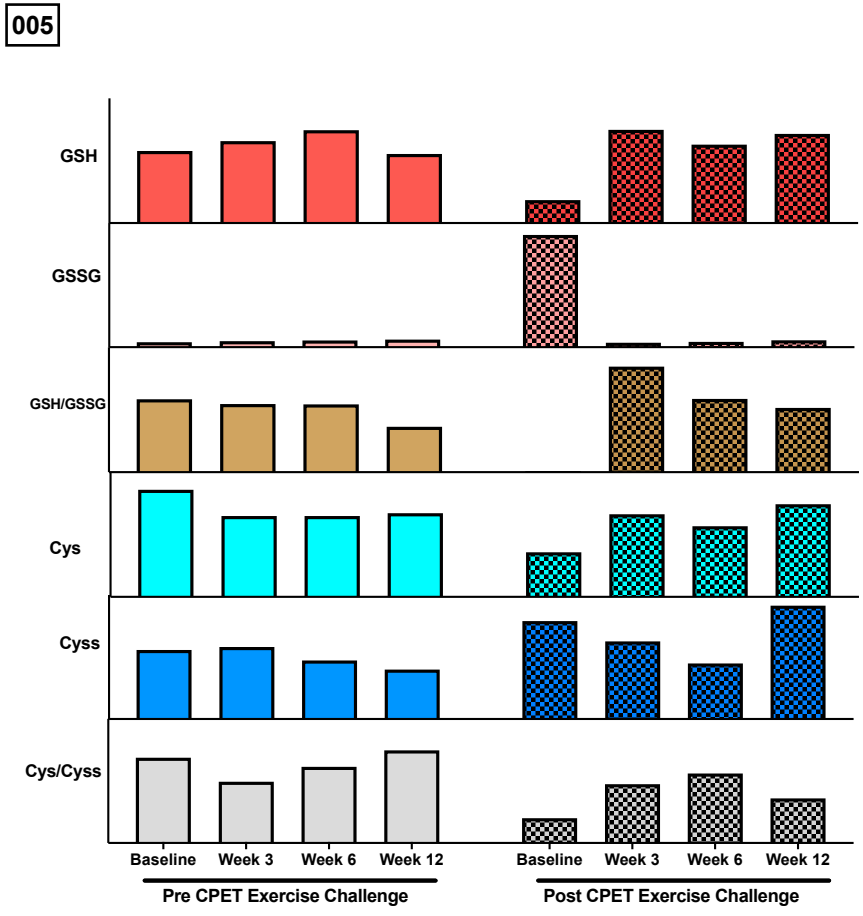
Figure 5.8 Pattern of redox responses to pre and post acute exercise challenge at each sampling point in the study for nitrite (µM), nitrate (µM), other nitrosospecies (µM), TBARS (µM) and Ferric Reducing Antioxidant Capacity of plasma (µM) charted for each individual participant. Units omitted for the sake of clarity. Abbreviations: CPET; cardiopulmonary exercise test; RXNO; other nitrosospecies; TBARS; thiobarbituric acid reactive substances, FRAP; Ferric reducing antioxidant capacity of plasma

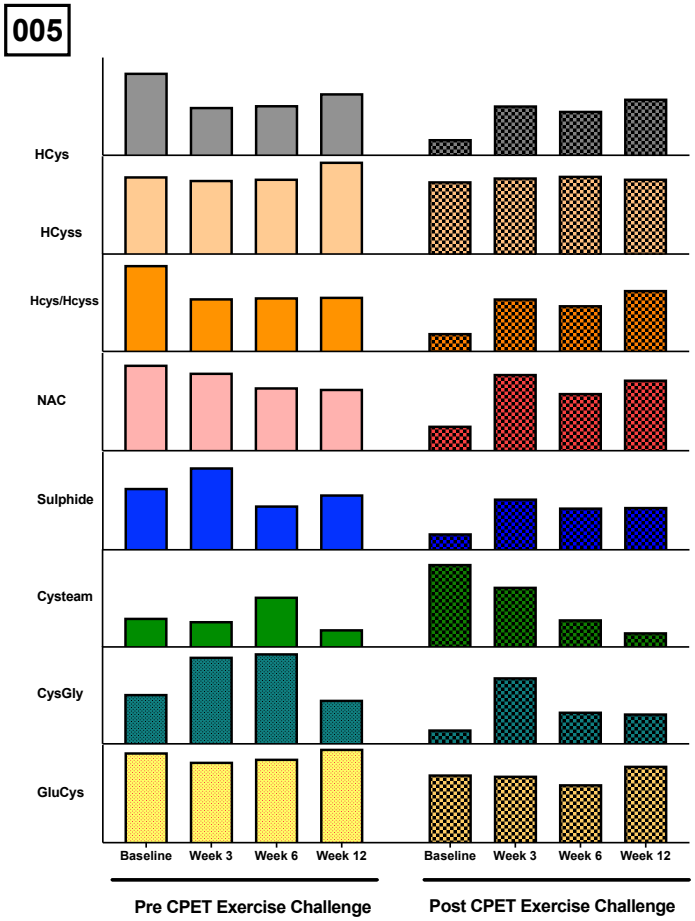
5.2.5 The thiol redox metabolome

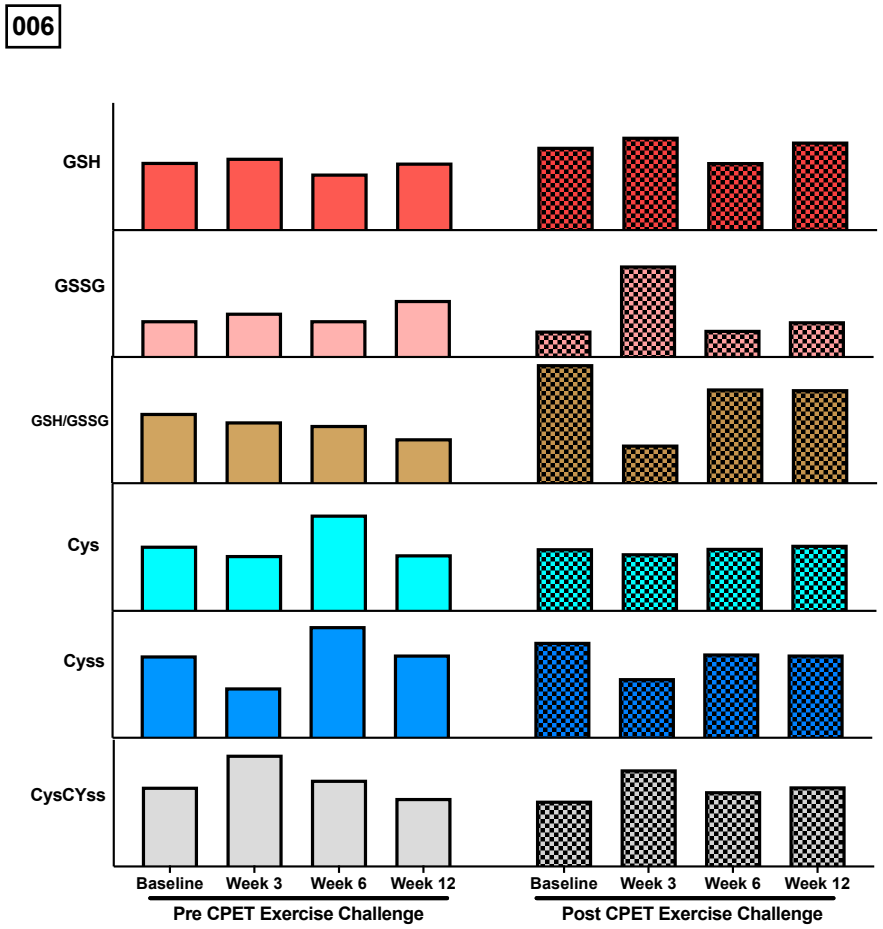
A number of assays were used to assess the status of the thiol redox metabolome. A thiol is a compound carrying a sulfhydryl group. They are classified into low molecular weight thiols (e.g. cysteine, homocysteine, and glutathione) and high molecular weight thiols such as protein thiols. Free thiols are those that are in their reduced state, and bound thiols exist in their oxidised state. Dependent on their relative concentrations, thiols define the redox status and reserve of a cell (266). Total thiol status of plasma, especially thiol groups, is the major determinant of to the plasma antioxidant status of the body. All thiols were measured in nM. GSH is the reduced form of glutathione, and GSSG the oxidised form. GSH/GSSG is the ratio of the reduced and oxidised versions of glutathione. Cys is the reduced form of cysteine with CYSS the oxidised version, with the ratio illustrated as CYS/CYSS, and similarly for homocysteine with HCYS for the reduced version, HCYSS for the oxidised version and HCYS/HCYSS describing the ratio. N-acetylcyesteine (NAC), cysteinylglycine (CysGly) and L-Glutamyl-L-Cysteine (GluCys) are all intermediaries in the gluthathione synthesis pathway. The results of the the pattern of response of the thiol redox metabolome were variable between participants, with each participant demonstrating a unique pattern of response to an acute exercise challenge that varied during the course of the exercise intervention (see figure 5.9 for an overview of thiol redox metabolome changes for each patient).

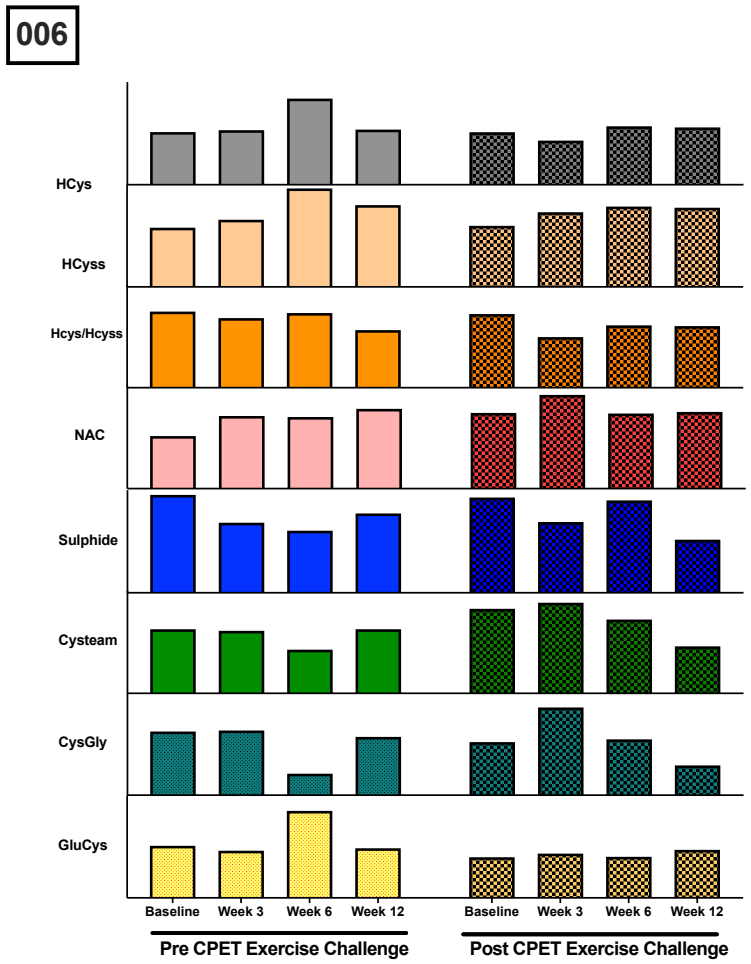

001
GSH
GSSG
GSH/GSSG
Cys
Cyss
Cys/Cyss
Baseline
Week 3
Week 6
Week 12
Baseline
Week 3
Week 6
Week 12
Pre CPET Exercise Challenge
Post CPET Exercise Challenge

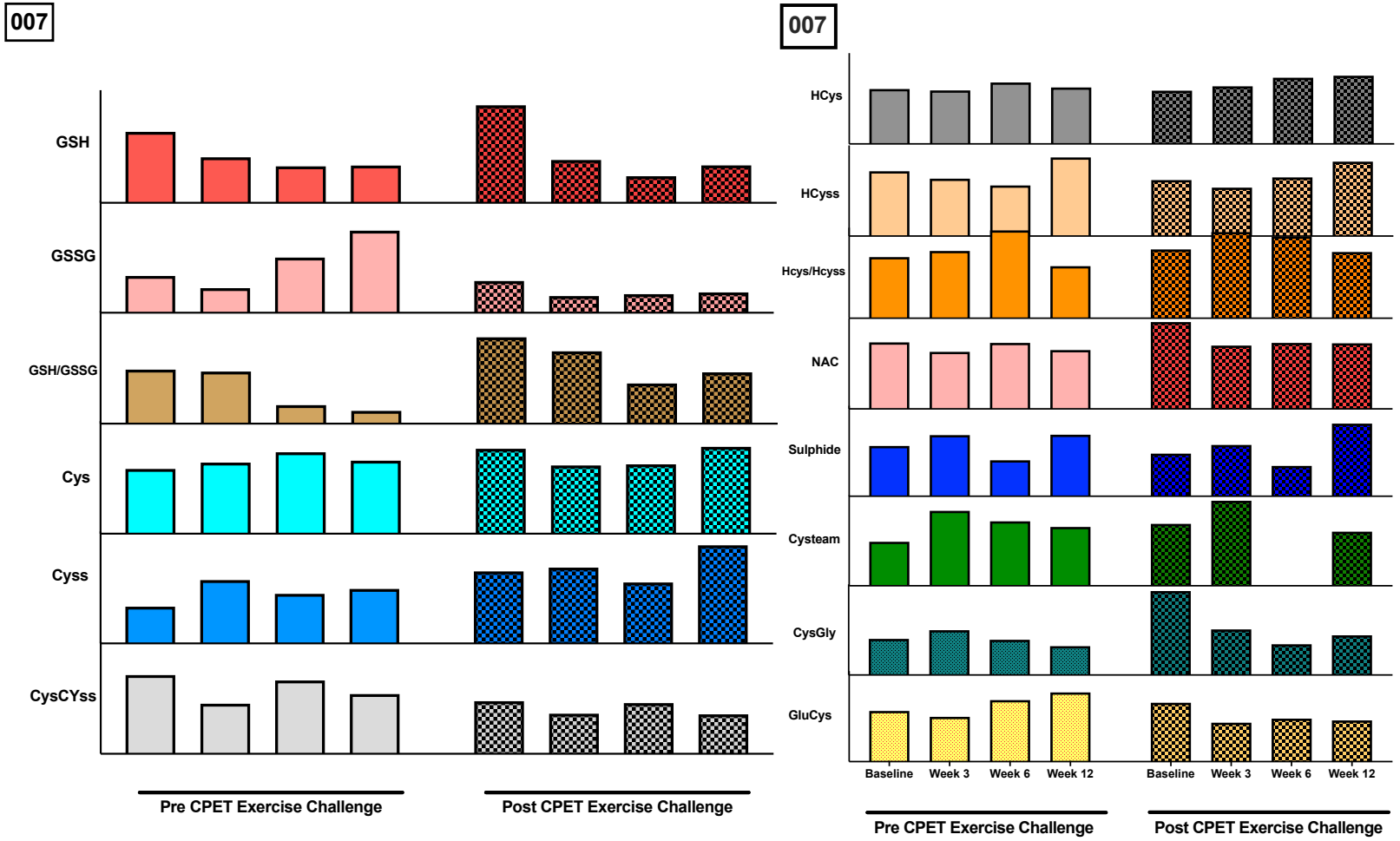

001
HCys
HCyss
Hcys/Hcyss
NAC
Sulphide
Cysteam
CysGly
GluCys
Baseline
Week 3
Week 6
Week 12
Baseline
Week 3
Week 6
Week 12
Pre CPET Exercise Challenge
Post CPET Exercise Challenge

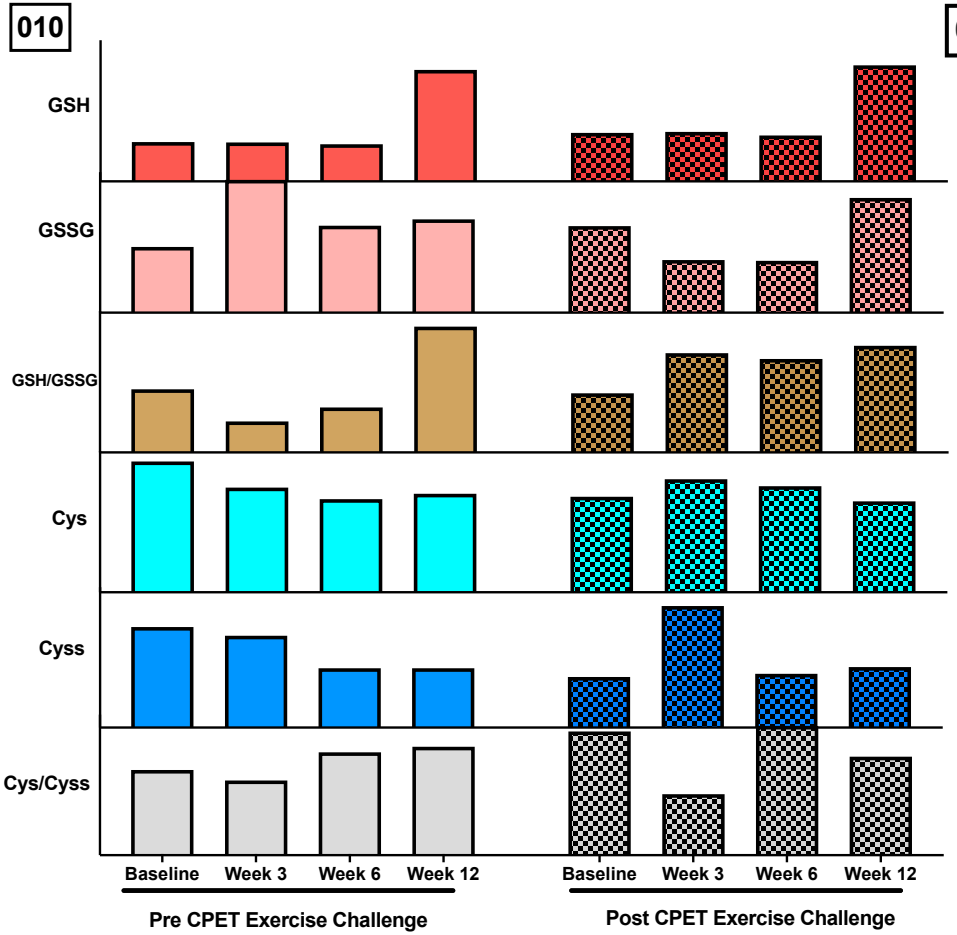

005
GSH
GSSG
GSH/GSSG
Cys
Cyss
Cys/Cyss
Baseline
Week 3
Week 6
Week 12
Baseline
Week 3
Week 6
Week 12
Pre CPET Exercise Challenge
Post CPET Exercise Challenge

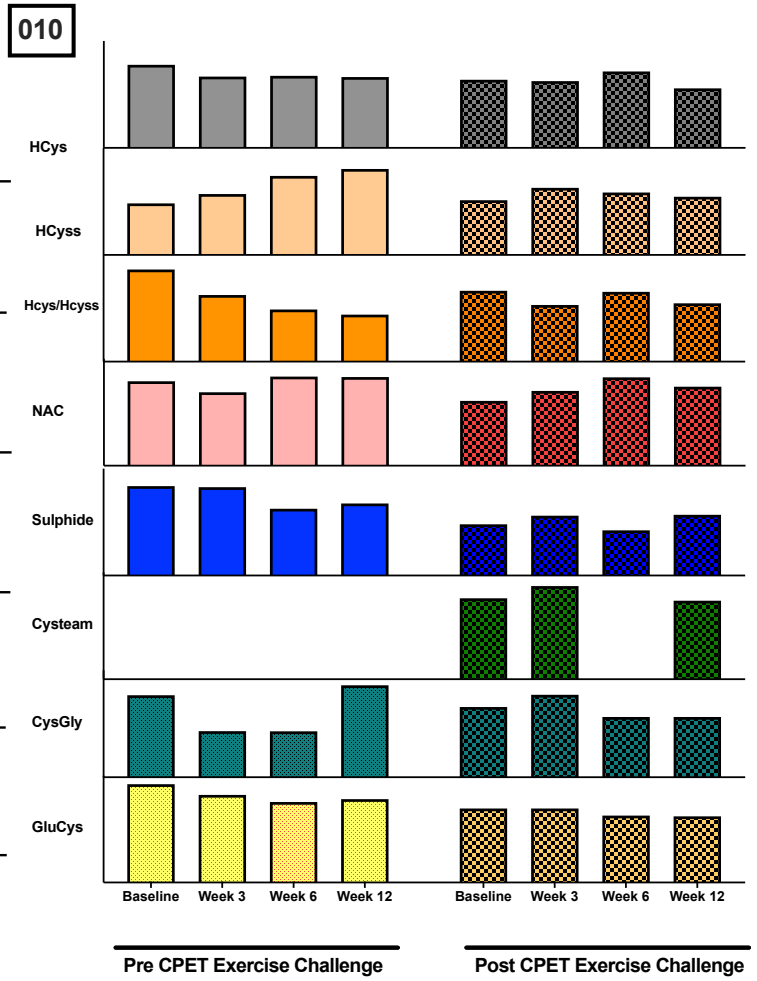

005
HCys
HCyss
Hcys/Hcyss
NAC
Sulphide
Cysteam
CysGly
GluCys
Baseline
Week 3
Week 6
Week 12
Baseline
Week 3
Week 6
Week 12
Pre CPET Exercise Challenge
Post CPET Exercise Challenge


006
GSH
GSSG
GSH/GSSG
Cys
Cyss
CysCYss
Baseline
Week 3
Week 6
Week 12
Baseline
Week 3
Week 6
Week 12
Pre CPET Exercise Challenge
Post CPET Exercise Challenge


006
HCys
HCyss
Hcys/Hcyss
NAC
Sulphide
Cysteam
CysGly
GluCys
Baseline
Week 3
Week 6
Week 12
Baseline
Week 3
Week 6
Week 12
Pre CPET Exercise Challenge
Post CPET Exercise Challenge


007
GSH
GSSG
GSH/GSSG
Cys
Cyss
CysCYss
Pre CPET Exercise Challenge
Post CPET Exercise Challenge
007
HCys
HCyss
Hcys/Hcyss
NAC
Sulphide
Cysteam
CysGly
GluCys
Baseline
Week 3
Week 6
Week 12
Baseline
Week 3
Week 6
Week 12
Pre CPET Exercise Challenge
Post CPET Exercise Challenge


010
GSH
GSSG
GSH/GSSG
Cys
Cyss
Cys/Cyss
Baseline
Week 3
Week 6
Week 12
Baseline
Week 3
Week 6
Week 12
Pre CPET Exercise Challenge
Post CPET Exercise Challenge


010
HCys
HCyss
Hcys/Hcyss
NAC
Sulphide
Cysteam
CysGly
GluCys
Baseline
Week 3
Week 6
Week 12
Baseline
Week 3
Week 6
Week 12
Pre CPET Exercise Challenge
Post CPET Exercise Challenge

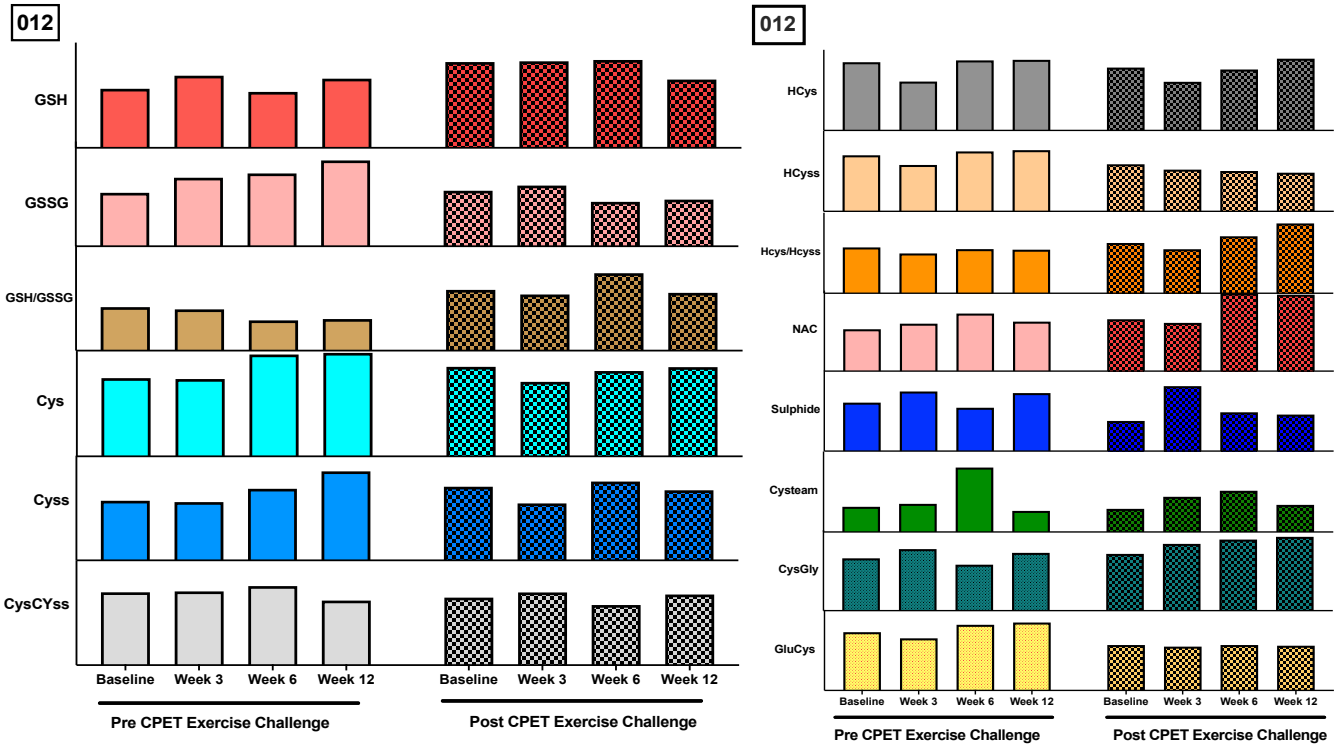


Figure 5.9 Individual changes in the pattern of responses of the thiol redox metabolome

Data presented for before and after acute physiological challenge of a cardiopulmonary exercise test at each sampling point during the training intervention. Units (nM) omitted for sake of clarity. Abbreviations: GSH: reduced gluthathion; GSSG: oxidised glutathione; cys:cysteine, cyss: cystine, hcys: homocysteine, hcyss:homocystine, NAC: N-acetylecysteine, Cysteam: cysteamine, CysGly: Cysteinylglycine, GluCys: L-Glutamyl-L-Cysteine

There were no significant changes between GSH either pre (mdn=8243 vs mdn=10473) or post CPET (mdn=9253.5 vs mdn=10103.5) between baseline and week 12, or over the course of the intervention, as assessed by Friedman test see (figure 5.10).

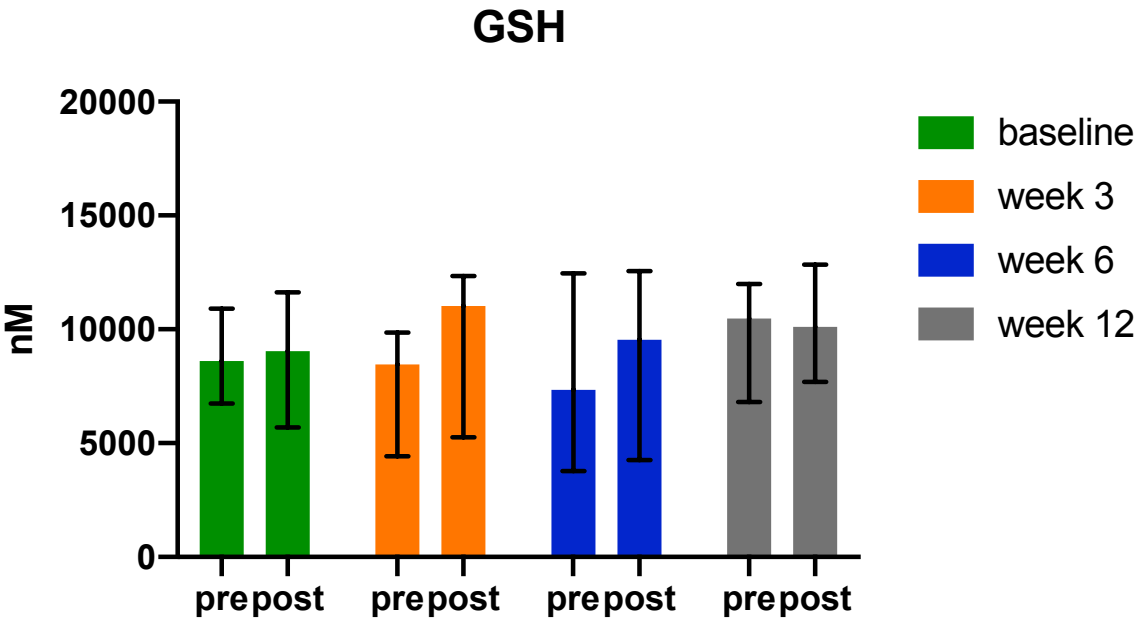


Figure 5.10 Overview of GSH levels pre and post CPET

Overview of GSH levels pre and post CPET for baseline (green; n=8), week 3 (orange; n=8), week 6 (blue, n=6) and week 12 (grey, n=6). Bars =median; error bars =IQR. Assessed for statistical significance (n=6) between baseline and week 12 using Wilcoxon signed rank test, and for longitudinal change using a Friedman test.

There were no significant changes between GSSG either pre (mdn=72.5 vs mdn=106) or post CPET (mdn=83.8 vs mdn=82.15) between baseline and week 12, or over the course of the intervention, as assessed by Friedman test see (figure 5.11).

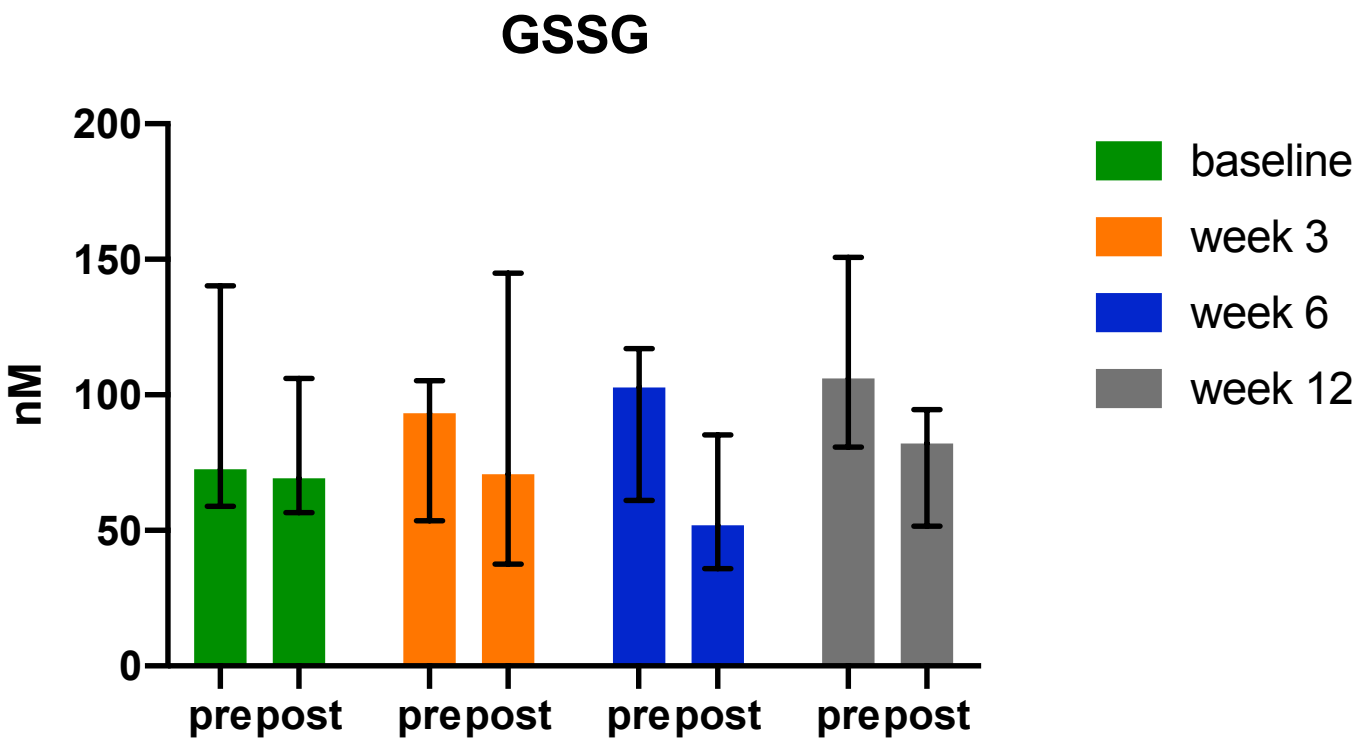


Figure 5.11 Overview of GSSG levels pre and post CPET

Overview of GSSG levels pre and post CPET for baseline (green; n=8), week 3 (orange; n=8), week 6 (blue, n=6) and week 12 (grey, n=6). Bars =median; error bars =IQR. Assessed for statistical significance (n=6) between baseline and week 12 using Wilcoxon signed rank test, and for longitudinal change using a Friedman test.

There were no significant changes between GSH/GSSG ratio either pre (mdn=117.15; vs mdn=89.65) or post CPET (mdn=112.59mvs mdn=119.75) between baseline and week 12. Pre CPET GSH/GSSG ratio significantly decreased over the course of the intervention, as assessed by Friedman test (p=0.029) but post CPET GSH/GSSG ratio did not significantly change (figure 5.12).

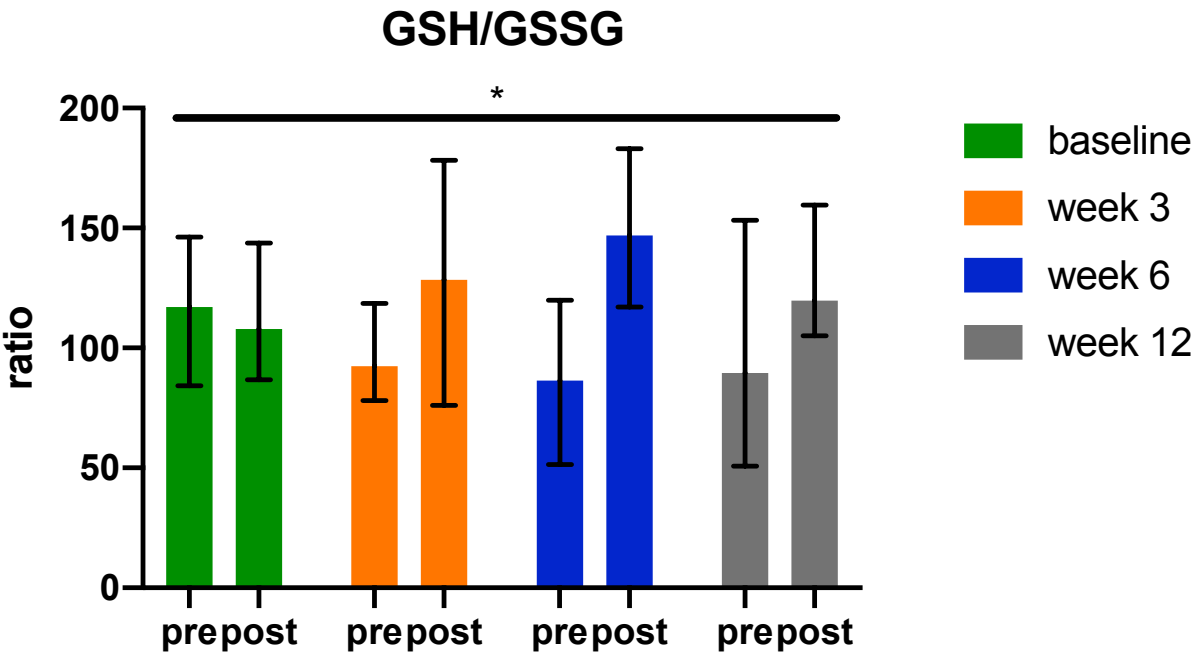


Figure 5.12 Overview of GSH/GSSG ratio pre and post CPET

*Overview of GSH/GSSG ratio pre and post CPET for baseline (green; n=8), week 3 (orange; n=8), week 6 (blue, n=6) and week 12 (grey, n=6). Bars =median; error bars =IQR. Assessed for statistical significance (n=6) between baseline and week 12 using Wilcoxon signed rank test, and for longitudinal change using a Friedman test. *=statistically significant result; p<0.05.*

There were no significant changes in CYS either pre (mdn=9458.5 vs mdn=9738.5) or post CPET (mdn=9059 vs mdn=100047) between baseline and week 12, or over the course of the intervention, as assessed by Friedman test see (figure 5.13).

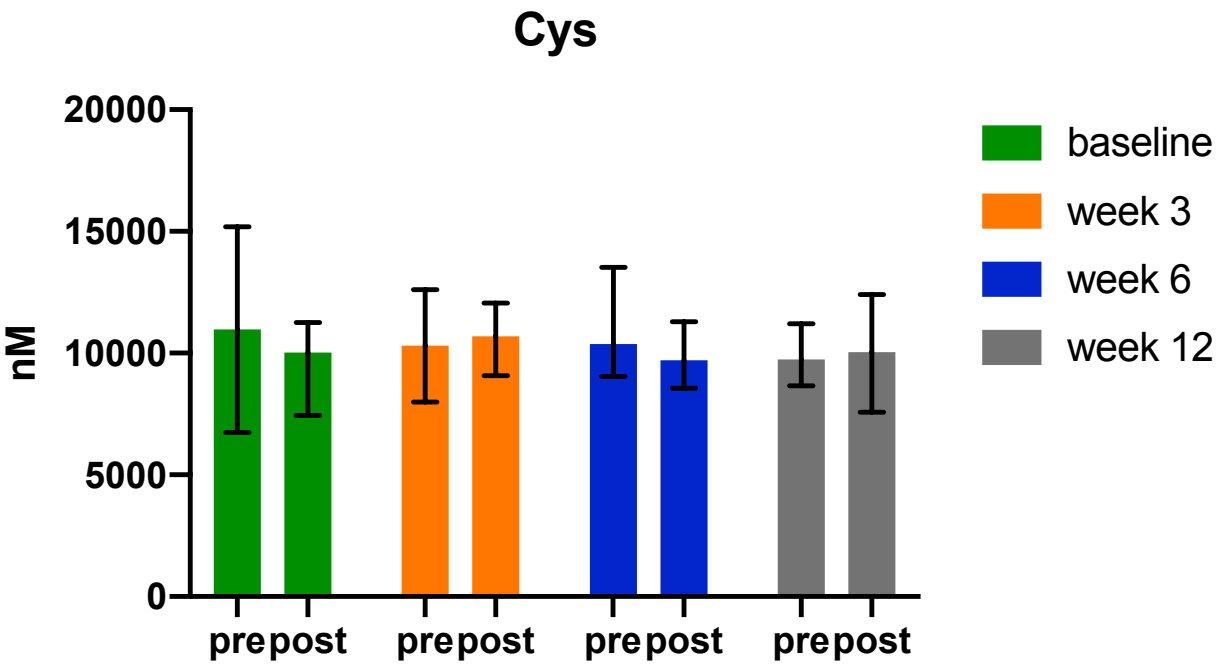


Figure 5.13 Overview of Cys levels pre and post CPET

Overview of Cys levels pre and post CPET for baseline (green; n=8), week 3 (orange; n=8), week 6 (blue, n=6) and week 12 (grey, n=6). Bars =median; error bars =IQR. Assessed for statistical significance (n=6) between baseline and week 12 using Wilcoxon signed rank test, and for longitudinal change using a Friedman test.

There were no significant changes in CYSS either pre (mdn=164959 vs mdn=.143229.5) or post CPET (mdn=154249 vs mdn=168627) between baseline and week 12, or over the course of the intervention, as assessed by Friedman test see (figure 5.14).

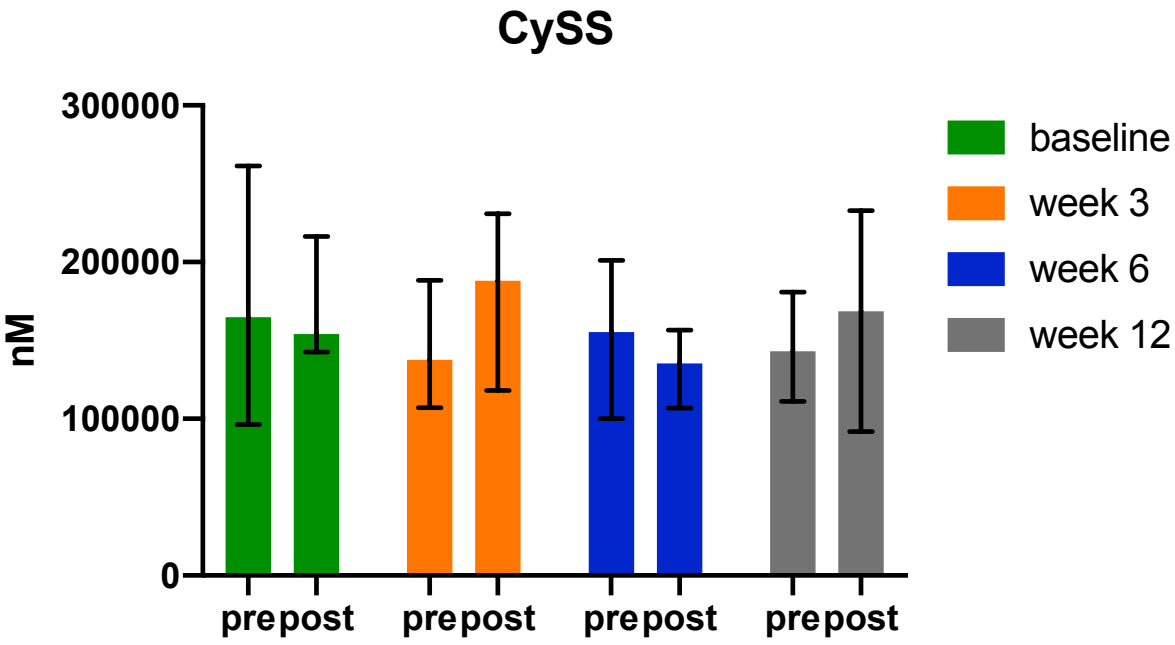


Figure 5.14 Overview of Cys levels pre and post CPET

Overview of Cyss levels pre and post CPET for baseline (green; n=8), week 3 (orange; n=8), week 6 (blue, n=6) and week 12 (grey, n=6). Bars =median; error bars =IQR. Assessed for statistical significance (n=6) between baseline and week 12 using Wilcoxon signed rank test, and for longitudinal change using a Friedman test.

There were no significant changes in CYS/CYSS ratio either pre (mdn=.0831 vs mdn=.0709) or post CPET (mdn=.0633 vs mdn=.0597) between baseline and week 12, or over the course of the intervention, as assessed by Friedman test see (figure 5.15).

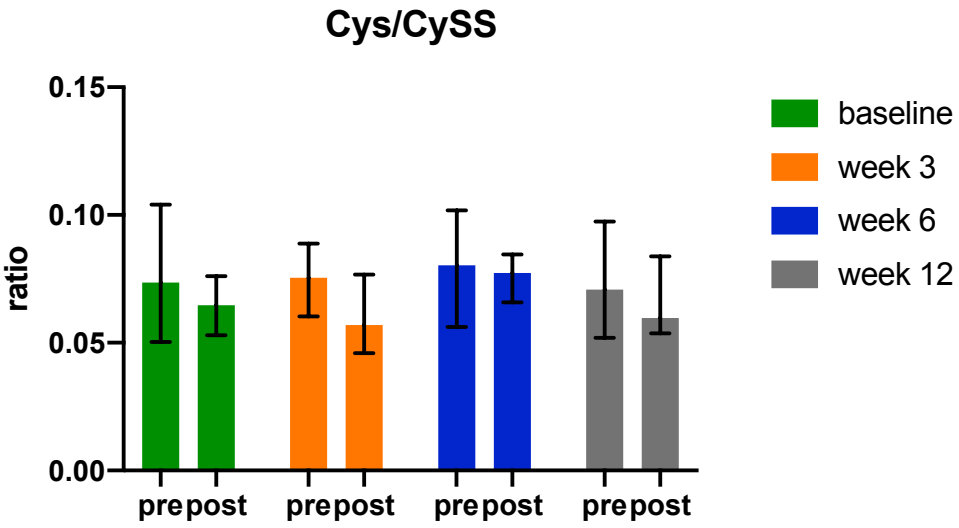


Figure 5.15 Overview CYS/CYSS ratio pre and post CPET

Overview of Cys/Cyss ratio pre and post CPET for baseline (green; n=8), week 3 (orange; n=8), week 6 (blue, n=6) and week 12 (grey, n=6). Bars =median; error bars =IQR. Assessed for statistical significance (n=6) between baseline and week 12 using Wilcoxon signed rank test, and for longitudinal change using a Friedman test.

There were no significant changes in HCYS either pre (mdn=255.5 vs mdn=267) or post CPET (mdn=234.5 vs mdn=273) between baseline and week 12, or over the course of the intervention, as assessed by Friedman test see (figure 5.16).

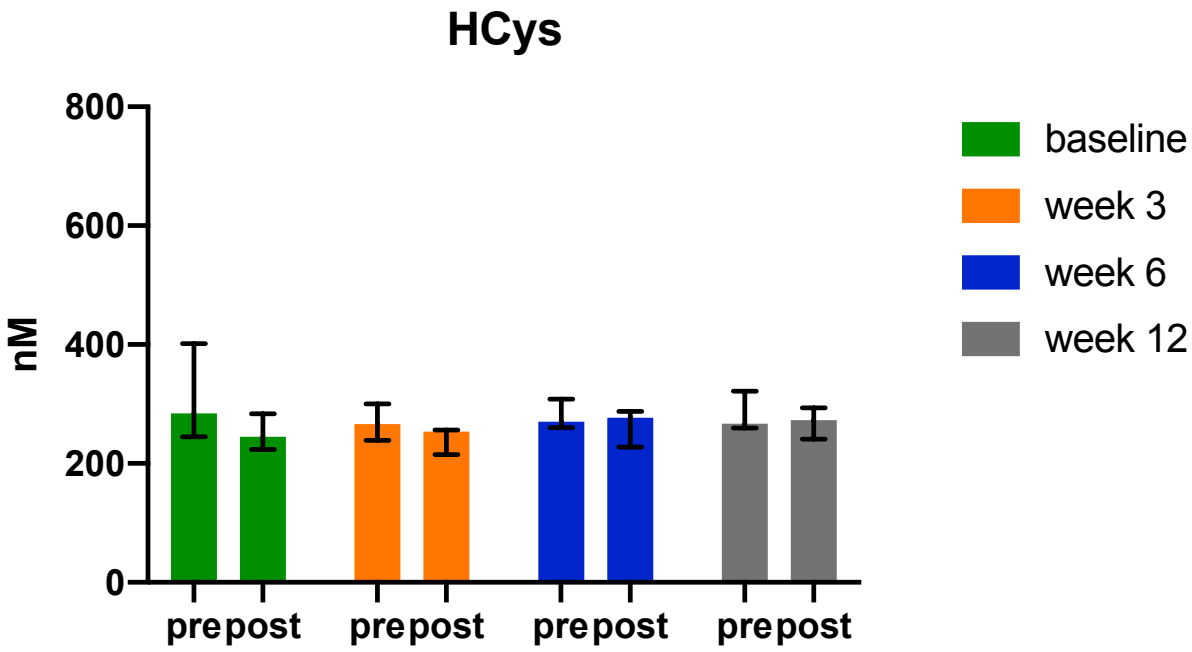


Figure 5.16 Overview of HCys levels pre and post CPET

Overview of HCys levels pre and post CPET for baseline (green; n=8), week 3 (orange; n=8), week 6 (blue, n=6) and week 12 (grey, n=6). Bars =median; error bars =IQR. Assessed for statistical significance (n=6) between baseline and week 12 using Wilcoxon signed rank test, and for longitudinal change using a Friedman test.

There was a significant increase in HCYSS pre CPET (mdn=29.65 vs mdn=38.65) as assessed by Wilcoxon (p=0.028) and Friedman (p=0.02). There was no significant change in HCYSS post CPET (mdn=27 vs mdn=29.15) between baseline and week 12, or over the course of the intervention, as assessed by Friedman test see (figure 5.17).

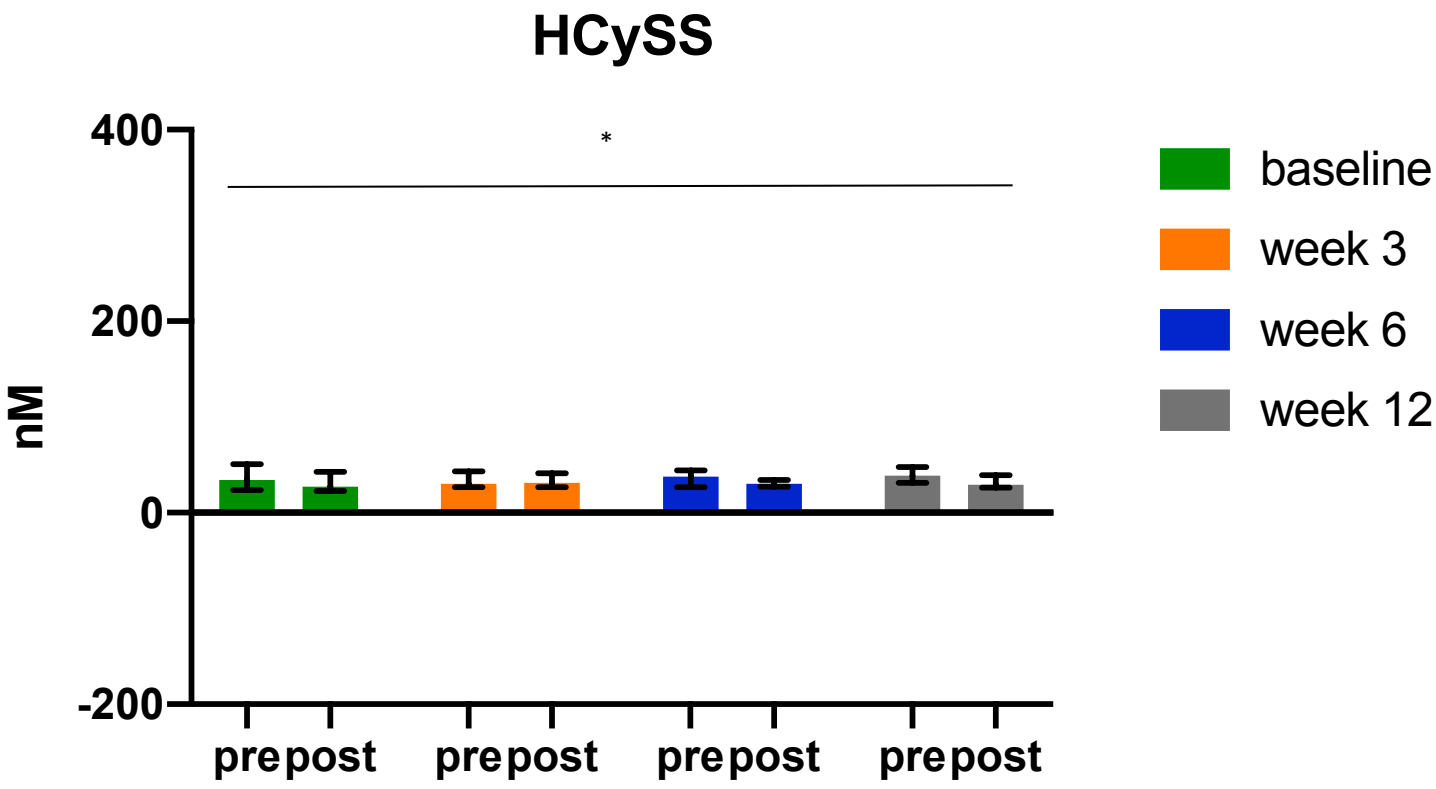


Figure 5.17 Overview of HCyss levels pre and post CPET

*Overview of HCyss levels pre and post CPET for baseline (green; n=8), week 3 (orange; n=8), week 6 (blue, n=6) and week 12 (grey, n=6). Bars =median; error bars =IQR. Assessed for statistical significance (n=6) between baseline and week 12 using Wilcoxon signed rank test, and for longitudinal change using a Friedman test. *=statistically significant result; p<0.05.*

There were no significant changes in HCYS/HCYSS ratio either pre (mdn=8.95 vs mdn=7.52) or post CPET (mdn=7.7 vs mdn=8.59) between baseline and week 12, or over the course of the intervention, as assessed by Friedman test see (figure 5.18).

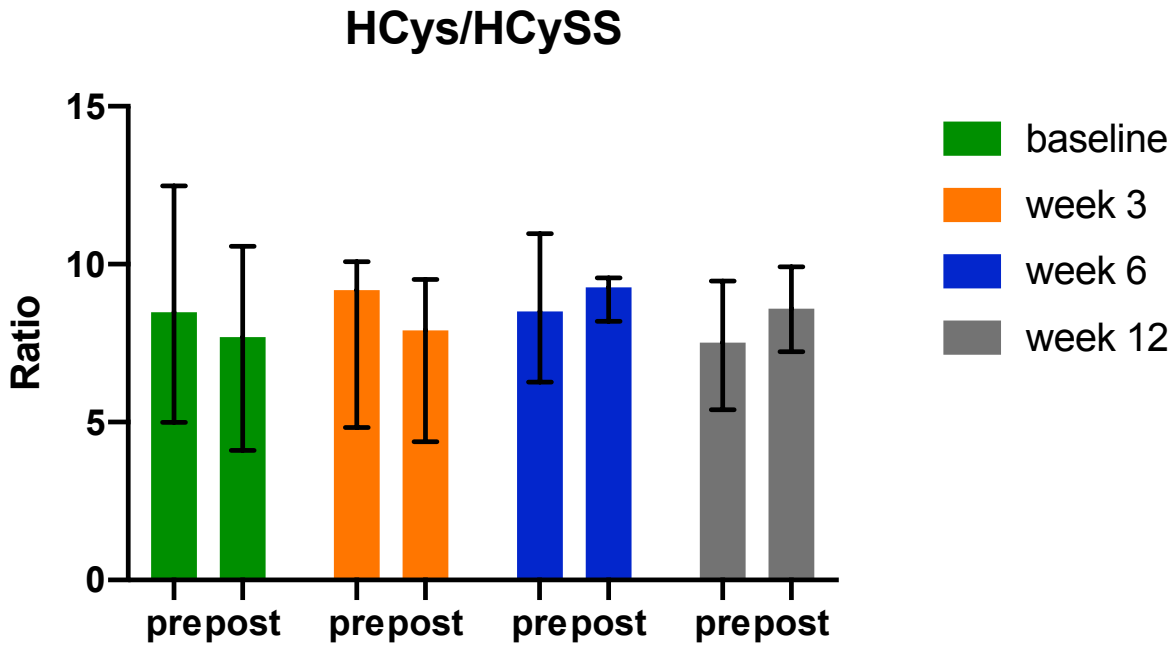


Figure 5.18 Overview of HCys/HCyss ratio pre and post CPET

Overview of HCys/HCyss ratio pre and post CPET for baseline (green; n=8), week 3 (orange; n=8), week 6 (blue, n=6) and week 12 (grey, n=6). Bars =median; error bars =IQR. Assessed for statistical significance (n=6) between baseline and week 12 using Wilcoxon signed rank test, and for longitudinal change using a Friedman test.

There were no significant changes in N-acetylcysteine (NAC) either pre (mdn=12.55 vs mdn=18.7) or post CPET (mdn=16.1 vs mdn=19.7) between baseline and week 12, or over the course of the intervention, as assessed by Friedman test see (figure 5.19).

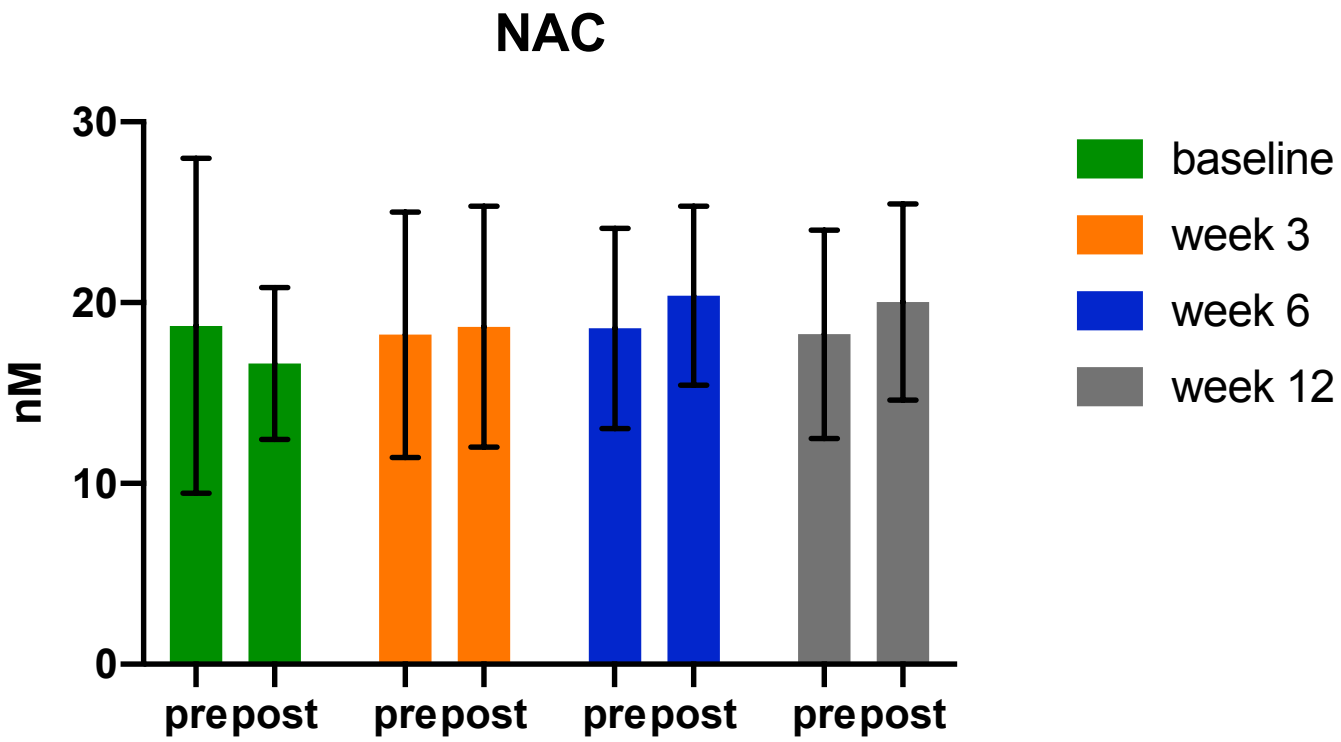


Figure 5.19 Overview of NAC levels pre and post CPET

Overview of NAC levels pre and post CPET for baseline (green; n=8), week 3 (orange; n=8), week 6 (blue, n=6) and week 12 (grey, n=6). Bars =median; error bars =IQR. Assessed for statistical significance (n=6) between baseline and week 12 using Wilcoxon signed rank test, and for longitudinal change using a Friedman test.

There were no significant changes in sulphide either pre (mdn=3017.5 vs mdn=2907)or post CPET (mdn=2138.5 vs mdn=2741) between baseline and week 12, or over the course of the intervention, as assessed by Friedman test see (figure 5.20).

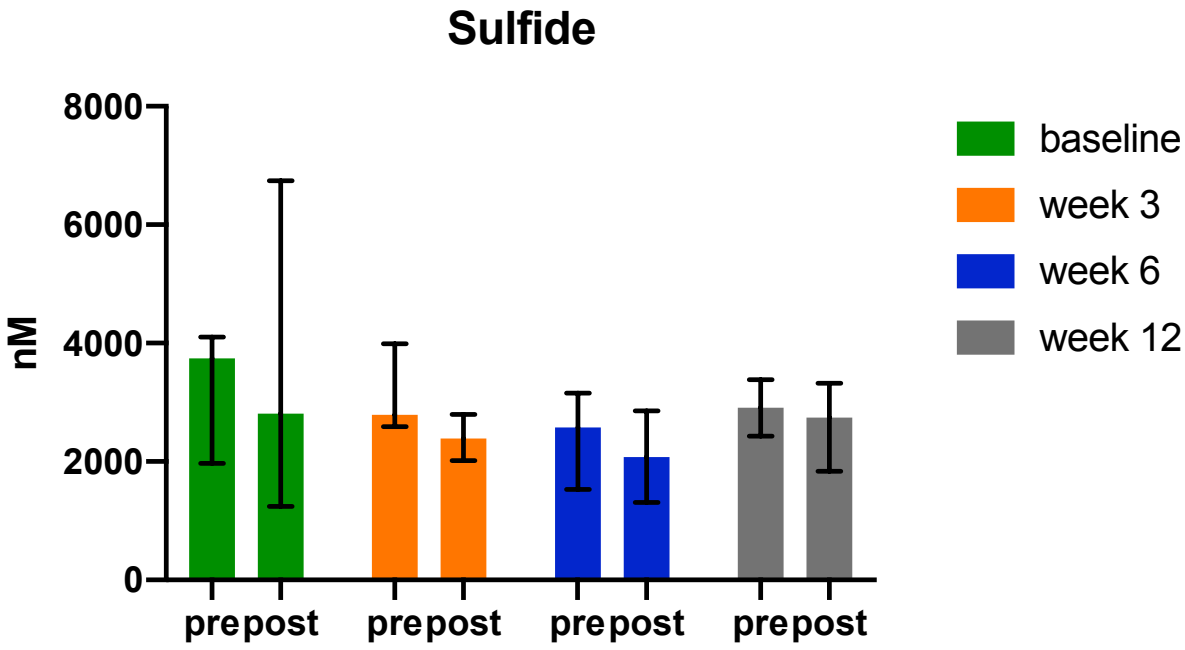


Figure 5.20 Overview of Sulphide levels pre and post CPET

Overview of sulphide levels pre and post CPET for baseline (green; n=8), week 3 (orange; n=8), week 6 (blue, n=6) and week 12 (grey, n=6). Bars =median; error bars =IQR. Assessed for statistical significance (n=6) between baseline and week 12 using Wilcoxon signed rank test, and for longitudinal change using a Friedman test

There were no significant changes in cysteamine either pre (mdn=2.43 vs mdn=2.1) or post CPET (mdn=3.23 vs mdn=2.55) between baseline and week 12, or over the course of the intervention, as assessed by Friedman test see (figure 5.21).

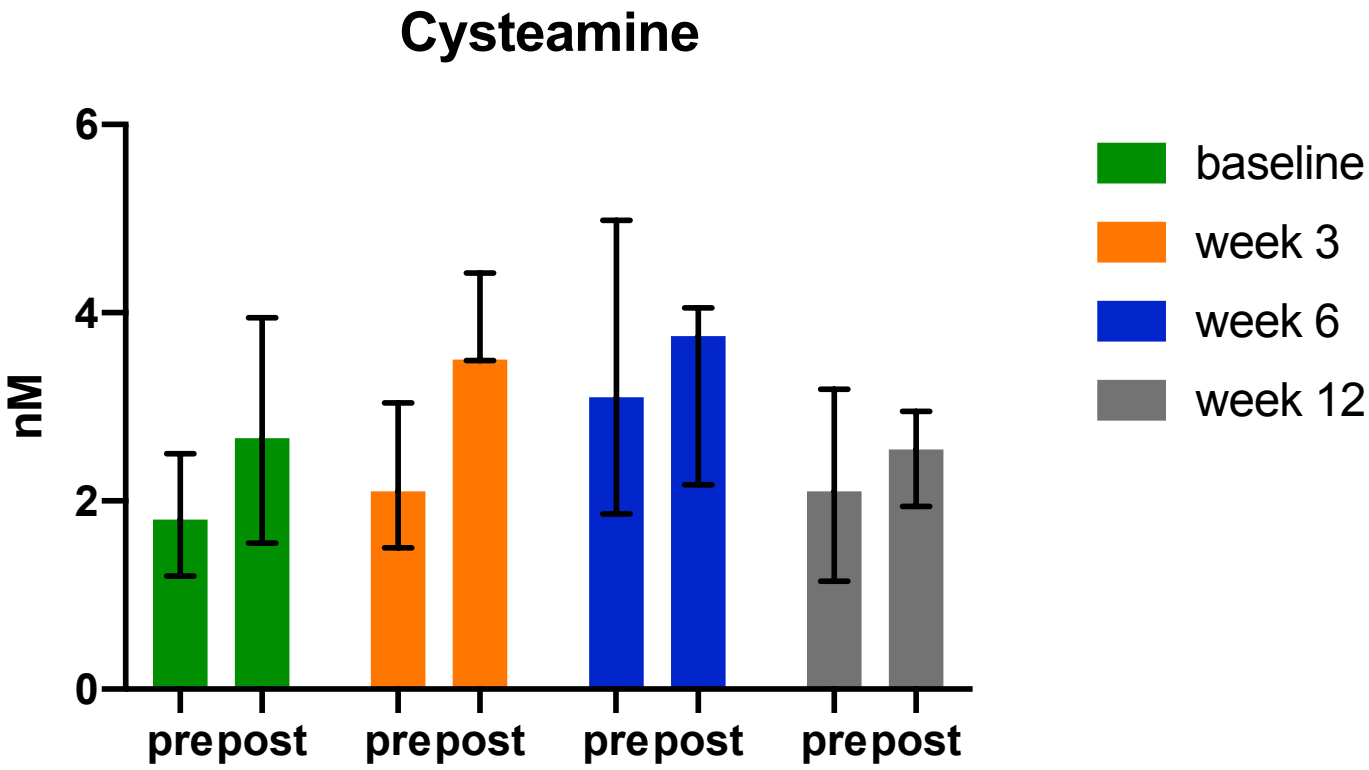


Figure 5.21 Overview of Cysteamine levels pre and post CPET

Overview of cysteamine levels pre and post CPET for baseline (green; n=8), week 3 (orange; n=8), week 6 (blue, n=6) and week 12 (grey, n=6). Bars =median; error bars =IQR. Assessed for statistical significance (n=6) between baseline and week 12 using Wilcoxon signed rank test, and for longitudinal change using a Friedman test.

There were no significant changes in cys/gly ratio either pre (mdn=6839.5 vs mdn=7294.5) or post CPET (mdn=9650 vs mdn=6908.5) between baseline and week 12, or over the course of the intervention, as assessed by Friedman test see (figure 5.22).

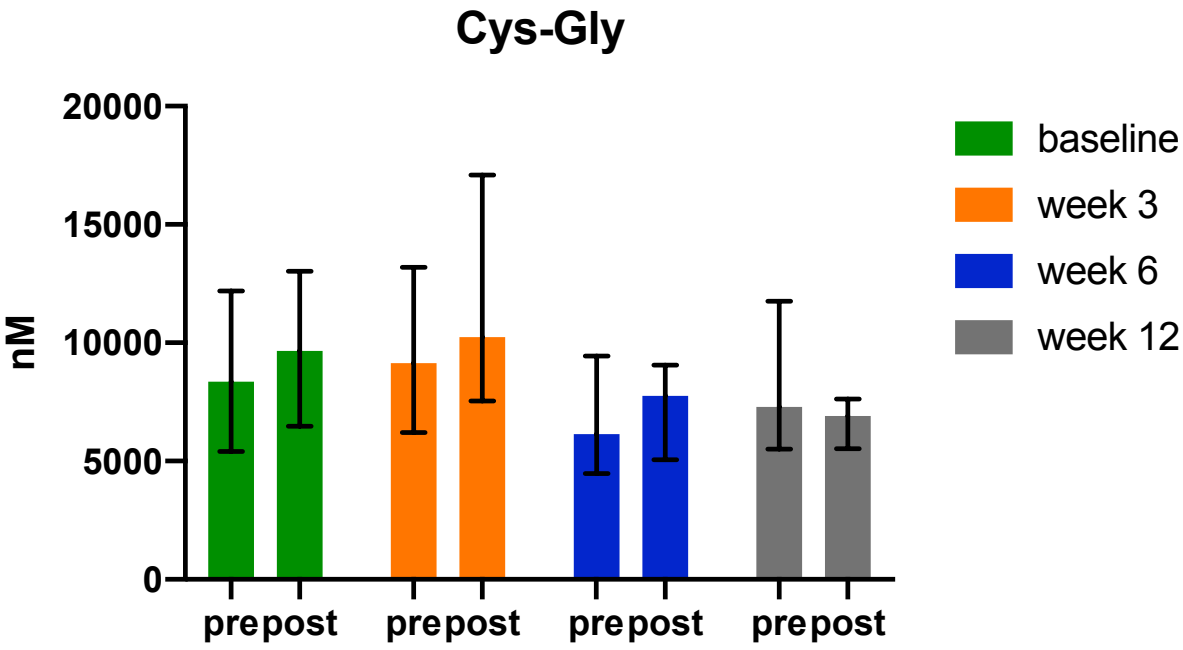


Figure 5.22 Overview of Cys/Gly ratio pre and post CPET

Overview of Cys/Gly ratio pre and post CPET for baseline (green; n=8), week 3 (orange; n=8), week 6 (blue, n=6) and week 12 (grey, n=6). Bars =median; error bars =IQR. Assessed for statistical significance (n=6) between baseline and week 12 using Wilcoxon signed rank test, and for longitudinal change using a Friedman test.

There were no significant changes in glu/cys ratio either pre (mdn=132.5 vs mdn=137) or post CPET (mdn=105 vs mdn=6908.5) between baseline and week 12, or over the course of the intervention, as assessed by Friedman test see (figure 5.23).

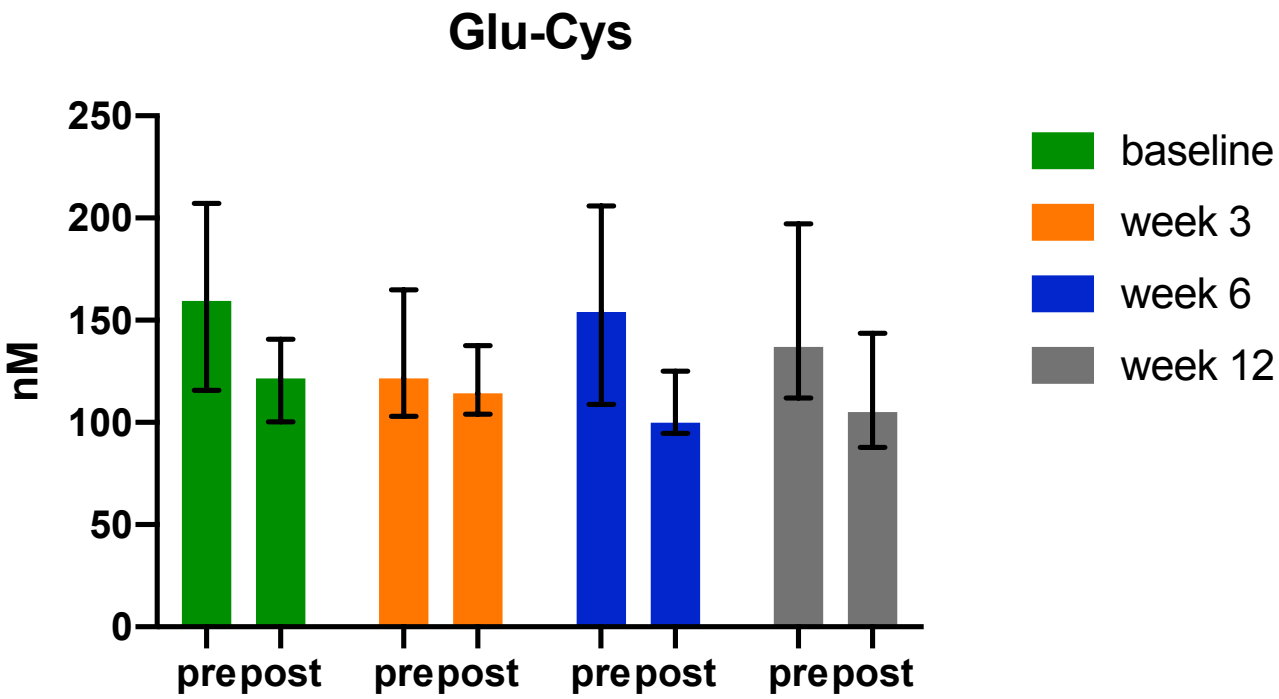


Figure 5.23 Overview of Glu/Cys ratio pre and post CPET

Overview of Glu/Cys ratio pre and post CPET for baseline (green; n=8), week 3 (orange; n=8), week 6 (blue, n=6) and week 12 (grey, n=6). Bars =median; error bars =IQR. Assessed for statistical significance (n=6) between baseline and week 12 using Wilcoxon signed rank test, and for longitudinal change using a Friedman test

5.3 NRF2 and KEAP1

I have so far demonstrated that a personalised interval training programme is safe and feasible for patients with symptomatic asthma, has some impact on physical fitness and improves symptom scores and quality of life measures. Furthermore, I have demonstrated a reduction in asthma specific inflammatory markers and related cytokines, with changes in markers of redox specific compounds. I hypothesised that these changes would be a result of upregulation of NRF2, a 'master antioxidant' gene. I therefore went on to quantify NRF2 and Keap1, an NRF2 repressor protein, expression in PBMCs taken pre CPET at baseline, 6 weeks and 12 weeks of training via PCR. Gene expression was

quantified using the $2^{-\Delta Ct}$ method, where values are shown as the difference between Ct value of gene of interest and the Ct value of the housekeeping gene Actin beta chosen from known stably expressed genes ($^{\Delta Ct}$) and then linearised ($2^{-\Delta Ct}$). $2^{-\Delta\Delta Ct}$ method is used to measure the fold induction in gene expression compared to baseline where the Ct value difference between gene of interest and housekeeping gene at baseline is further subtracted from Ct value difference at every time point and finally linearised.

5.3.1 NRF2 expression in PBMCs

NRF2 expression was measured in PBMCs prior to CPET at baseline, week 6 and week 12. NRF2 expression did not significantly increase between baseline and week 6 (mdn 0.11841 vs mdn 0.01229, p=0.463), baseline and week 12 (mdn 0.11841 vs mdn 0.01229, p= 0.6) as assessed by Wilcoxon, or across the course of the intervention as assessed by Friedman test (p=0.846) (see figure 5.24). Looking at the fold change in NRF2 expression, there were no significant changes (see figure 5.24).

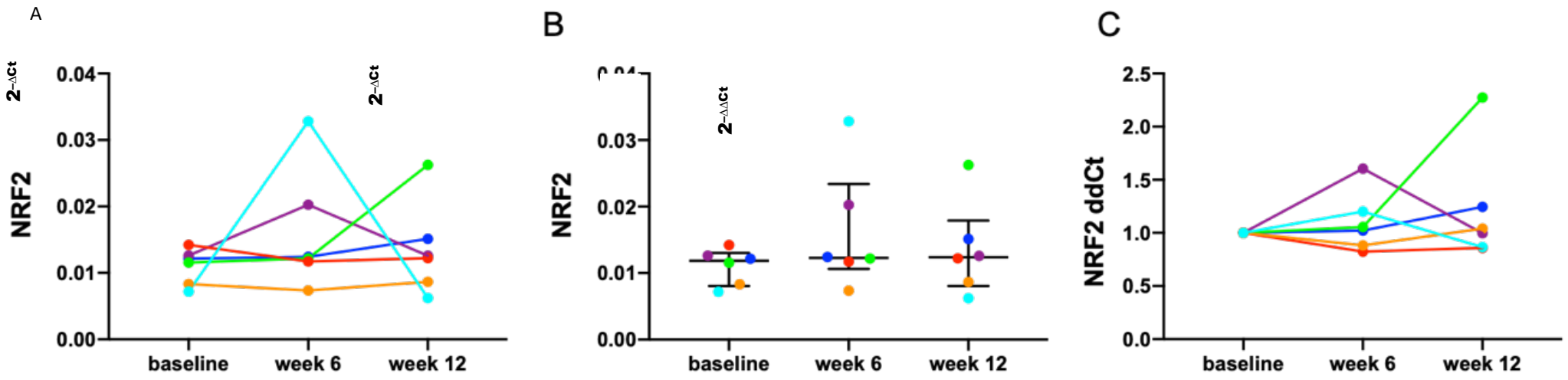


Figure 5.24 NRF2 RNA expression in PBMCs

depicted as A) line graph for $2^{-\Delta Ct}$ values for individual patients, B) scatter plot for $2^{-\Delta Ct}$ values (lines demonstrate median and IQR) and C) line graph $2^{-\Delta\Delta Ct}$. n=6 for patients with valid baseline and final data. $2^{-\Delta Ct}$ demonstrates the difference between Ct value of gene of interest and the Ct value of the housekeeping gene Actin beta, which is then linearised. $2^{-\Delta\Delta Ct}$ method is used to measure the fold induction in gene expression compared to baseline where the Ct value difference between gene of interest and housekeeping gene at baseline is further subtracted from Ct value difference at every time point and finally linearised. For all graphs, n=6 for participants with valid baseline, week 6 and week 12 PBMC sampling; individual participants are represented by a different colour, with each point indicating a sampling event. Abbreviations Ct; cycle threshold

5.3.2 Keap1 expression in PBMCs

Keap1 expression was measured in PBMCs prior to CPET at baseline, week 6 and week 12. Expression did not significantly increase between baseline and week 6 (mdn 0.00504 vs mdn 0.00590, p=0.075), baseline and week 12 (mdn 0.00504 vs mdn 0.00483, p= 0.75) as assessed by Wilcoxon, or across the course of the intervention as assessed by Friedman test (p=0.223) (see figure 5.25) .

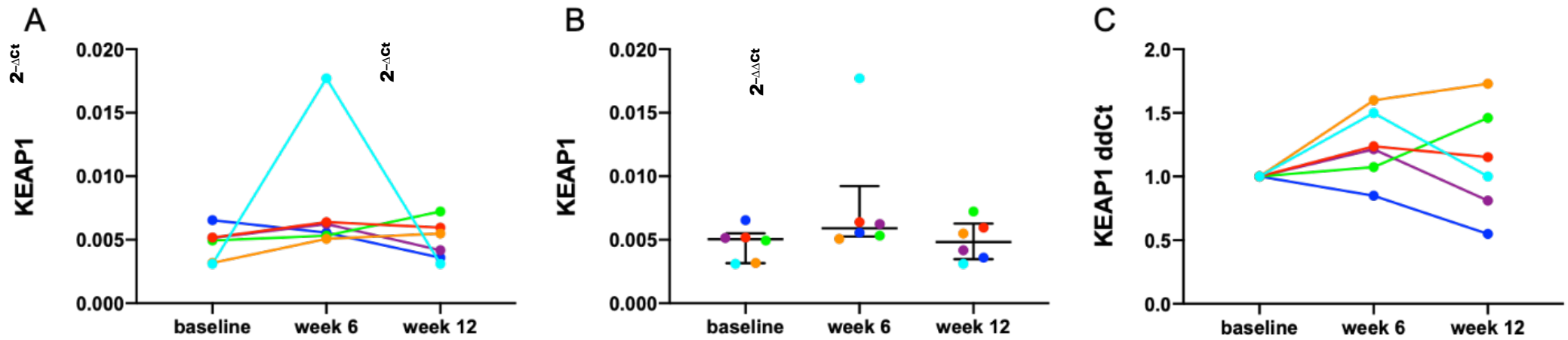


Figure 5.25 Keap1 RNA expression in PBMCs

depicted as A) line graph for $2^{-\Delta Ct}$ values, B) scatter plot for $2^{-\Delta Ct}$ values (lines demonstrate median and IQR) and C) line graph $2^{-\Delta\Delta Ct}$. $2^{-\Delta Ct}$ demonstrates the difference between Ct value of gene of interest and the Ct value of the housekeeping gene Actin beta, which is then linearised. $2^{-\Delta\Delta Ct}$ method is used to measure the fold induction in gene expression compared to baseline where the Ct value difference between gene of interest and housekeeping gene at baseline is further subtracted from Ct value difference at every time point and finally linearised. n=6 for patients with valid baseline and final data. For all graphs, n=6 for participants with valid baseline, week 6 and week 12 PBMC sampling; individual participants are represented by a different colour, with each point indicating a sampling event. Abbreviations Ct; cycle threshold

5.3.3 Association between physical fitness, redox regulation, inflammation and clinical outcomes

In earlier chapters, I have presented data to support exercise intervention in improving clinical symptoms, quality of life, lung function and inflammation. In this chapter I have presented data showing a reduction in asthma related cytokines and and change in markers of redox regulation. The key to demonstrating support for the hypothesis on which this thesis is based lies in demonstrating a link between asthma symptoms and quality of life through clinical markers of asthma control to redox regulation changes and improvements in physical fitness. I therefore looked to demonstrate association between these variables. Spearman correlations were used, due to the small number of patients which make assessment of normality tests unreliable (384). All correlations are 2 tailed.

Whilst NRF2 expression did not significantly change over the course of the intervention, with potential reasons for this considered in the discussion of this chapter, there is suggestion in the literature that NRF2 expression is involved in regulation of inflammatory responses in murine models of asthma (106, 385, 386), and demonstration in human studies that NRF2 expression in severe asthma is disrupted (99).Therefore, I looked for association between markers of asthma and NRF2 expression at baseline in my patient group. Baseline ACQ6 score and baseline NRF2 expression in PBMCs were significantly negatively correlated (r=-0.829, p=0.042), whilst other markers of QoL, inflammation and lung function did not.

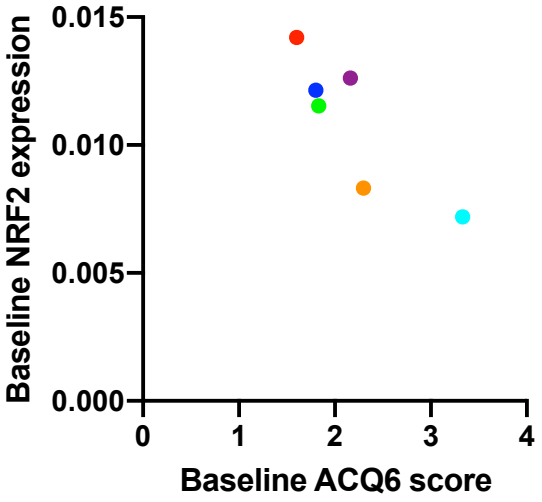


Figure 5.26 Associations (Spearman's Test) between Baseline ACQ6 Score and Baseline NRF2 expression in PBMCs

n=6 for patients with valid baseline and final data, r=-0.829, p=0.042. Individual participants are represented by a different colour. An r value of > 0.7 is considered a strong correlation.

Given the hypothesis that exercise increases redox resilience, which is responsible for downstream reduction in asthma related inflammation and improved symptom control, I looked for association to link changes in QoL scores and inflammation, through to changes in redox regulation markers and improvements in physical fitness (see figure 5.27). Significant associations (Spearman's Test) were demonstrated between improvements in physical fitness and changes in redox regulation through to improvements in QoL from baseline to post intervention. This provides early, exploratory support that increases in redox resilience may be linked to exercise related improvements in asthma symptoms and inflammation. Throughout, a 'change' is defined as the difference between baseline value and post intervention value. On the top row of figure 5.27, a greater increase in maximum oxygen uptake is associated with a greater increase in pre CPET nitrite (r=.943, figure 5.27A). A greater increase in pre CPET nitrite significantly correlates with a greater reduction in eosinophil levels (r=-.837, figure 5.27B); the larger the increased in reducing capacity (i.e in nitrite) is associated with a greater reduction in eosinophil count. A greater reduction in eosinophils following the exercise intervention correlates with a greater increase in pre-BD FEV1 (r=-717, figure 5.27C); a larger reduction in eosinophil count is associated with a greater increase in pre bronchodilator FEV1. A greater increase in FEV1 from baseline to post intervention

correlates with a greater increase in AQLQ total score (r=.771, figure 5.27D) and AQLQ environmental score (r=.600, figure 5.27E) following the intervention. On the bottom row, a greater increase in oxygen uptake at AT is associated with a larger increase in FRAP (r=.886, figure 5.27F). A greater reduction in FEV1% BD reversibility as a marker of reduction in airways hyperreactivity, negatively correlates with a greater increase in AQLQ total and environmental scores (r=-.829, figure 5.27G and -.714, figure 5.27H). To link the two rows, a greater reduction in airways hyperreactivity as assessed by reduction in %FEV1 BD reversibility negatively correlates with a greater increase in FEV1 in litres (r=-.771; data not shown).

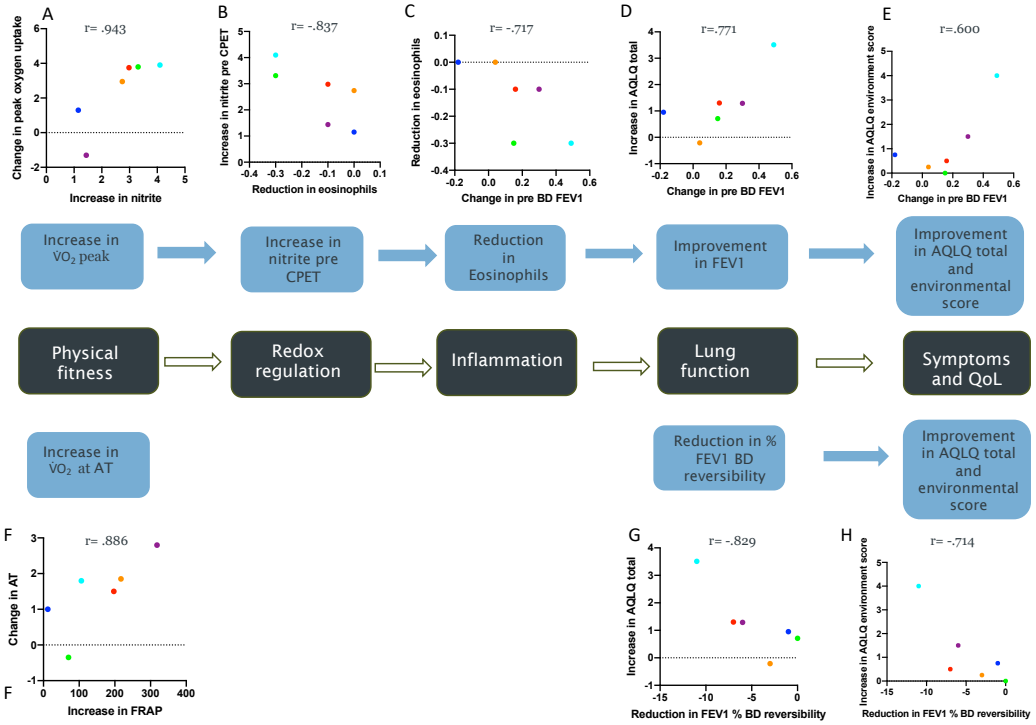


Figure 5.27 Significant associations (Spearman's Test) between physical fitness and clinical asthma symptoms

On the top row,A) a greater increase in maximum oxygen uptake is associated with a greater increase in pre CPET nitrite from baseline (r=.943). B) A greater increase in pre CPET nitrite is significantly associated with a greater the reduction in eosinophil levels (r=-.837). C) A greater reduction in eosinophils is associated with a greater increase in pre-BD FEV1 (r=-717), and a D) greater increase in FEV1 correlates with a greater increase in AQLQ total score (r=.771) and E) AQLQ environmental score (r=.600). On the bottom row, F) a greater increase in oxygen uptake at AT is associated with a larger increase in FRAP. G and H) A greater reduction in FEV1% BD reversibility negatively correlates with a greater increase in AQLQ total and environmental scores (r=-.829 and -.714). To link the two rows, a greater reduction in airways hyperreactivity as assessed by reduction in %FEV1 BD reversibility negatively correlates with a greater increase in FEV1 in litres (r=-.771; data not shown) Abbreviations: AT; anaerobic threshold, CPET; cardiopulmonary exercise test; BD; bronchodilator, FEV1; forced expiratory volume in 1 second, FRAP; ferric reducing antioxidant capacity of plasma. An r value of > 0.7 is considered a strong correlation

5.3.4 Redox regulation gene expression and downstream markers of redox activity

The relationships between gene expression of master antioxidant genes and the changes in clinical outcomes, inflammation, downstream markers of redox activity and redox regulation gene expression were then assessed to investigate the final link in our hypothesis chain. The change in the ratio of NRF2/Keap1 is highly significantly correlated with the change in FVC % bronchodilator reversibility r=0.886 p=0.019, with a greater reduction in bronchodilator reversibility significantly correlating with a reduction or smaller increase in NRF2 ratio (see figure 5.28) . Similarly, the absolute change in NRF2 significantly positively correlates (Spearman's Test) with the change in FEV1 %BD reversibility, with greater reduction in BD reversibility as a measure of airways hyperreactivity significantly correlated with a smaller increase in NRF2 (r=-0.943, p=0.005)

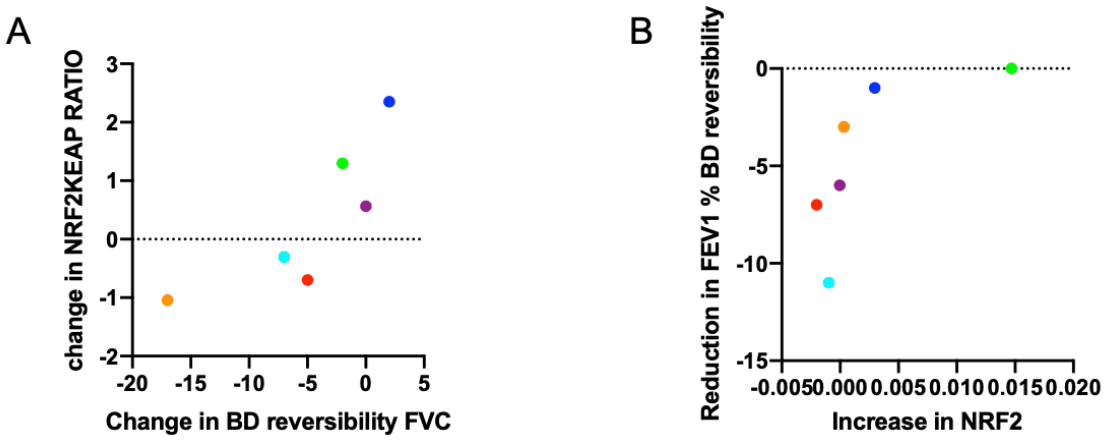


Figure 5.28 Associations (Spearman's Test) between NRF2 and lung function

*between A) change in NRF2:Keap ratio and change in bronchodilator reversibility of FVC: the change in the ratio of NRF2/Keap1 is highly significantly associated with the change in FVC bronchodilator reversibility (BD) r=0.886 p=0.019, with a greater reduction in bronchodilator reversibility significantly correlating with a reduction or smaller increase in NRF2 ratio and B) change in NRF2 levels and reduction in FEV %BD reversibility: the absolute change in NRF2 significantly positively correlates (Spearman's Test) with the change in FEV1 %BD reversibility, with greater reduction in BD reversibility as a measure of airways hyperreactivity significantly associated with a smaller increase in NRF2 (r=-0.928, p=0.008) ; n=6 for patients with valid baseline and final data. For all graphs, individual participants are represented by a different colour. An r value of > 0.7 is considered a strong correlation; *=statistically significant result; p<0.05.*

5.3.5 Longitudinal analysis of NRF2 expression

I hypothesised that the improvement in NRF2 will pre-date the improvement in clinical asthma markers. To investigate this, I took the level of NRF2 RNA expression in PBMCs at 6 weeks to assess for association with overall change and week 12 clinical outcomes. All analyses were exploratory and must be interpreted in that context, with an awareness that NRF2 expression did not significantly change throughout. It is also noteworthy that in terms of longitudinal change, the timepoints of week 6 and week 12 are arbitrary, but were selected as the only available longtidunal assessment points following initiation of the exercise intervention. NRF2 expression at week 6 was significantly negatively

correlated with reduction in ACQ6 score at 12 weeks (r=-.-943, p=0.005) (figure 5.29A); a higher level of NRF2 at 6 weeks is significantly correlated with a greater reduction in ACQ score over the course of the intervention. Improvement in AQLQ total score and NRF2 levels at 6 weeks showed a strong correlation (Spearman's Test, r=0.657, p=0.156), and C) there was significant positive association between week 6 NRF2 and a greater improvement over the course of the intervention in AQLQ environmental domain score (Spearman's Test, r=0.829 p=0.042). As above, these time point choices were arbitrary and therefore have to be interpreted in the context of exploratory work.

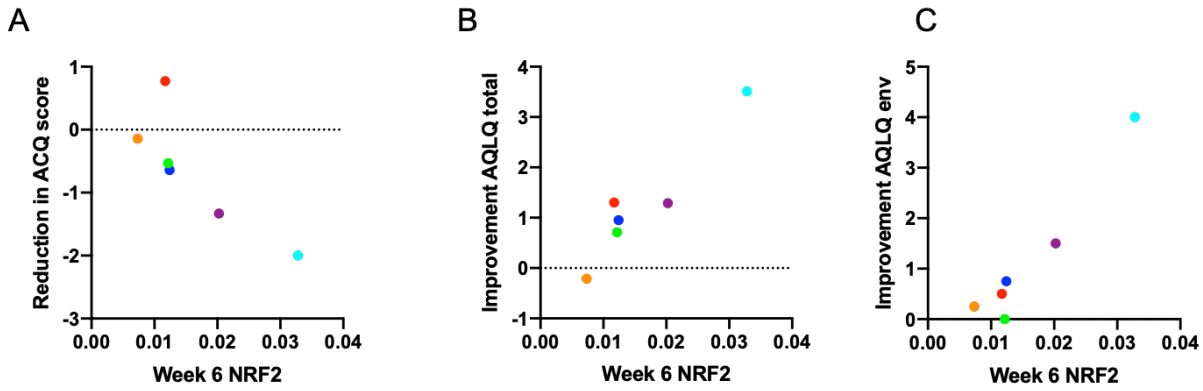


Figure 5.29 Associations (Spearman's Test) between Week 6 NRF2 expression in PBMCs and symptom and quality of life scores

*Week 6 NRF2 RNA expression in PBMCs and A) reduction in ACQ6 score: NRF2 expression at week 6 was significantly negatively correlated with reduction in ACQ6 score at 12 weeks (Spearman's Test, r=-.943, p=0.005); a higher level of NRF2 at 6 weeks is significantly associated with a greater reduction in ACQ score over the course of the intervention, B) Improvement in AQLQ total score and NRF2 at 6 weeks demonstrated moderate positive association (Spearman's Test, r=0.657, p= 0.156), C) There was significant positive association between week 6 NRF2 and a greater improvement over the course of the intervention in AQLQ environmental domain score (Spearman's Test, r=0.829, p=0.042, n=6 for patients with valid baseline and final data. For all graphs, individual participants are represented by a different colour. *=statistically significant result; p<0.05; An r value of > 0.7 is considered a strong correlation and 4-6.9 is considered moderate.*

I then went on to look at associations between the inflammatory markers of asthma and week 6 NRF2 levels. I demonstrated a strong negative association between week 6 NRF2

levels and week 12 CCL11/eotaxin levels; with higher levels of NRF2 at week 6 correlating with lower levels of CCL11/eotaxin at week 12 (r=-.714, p=.111; see figure 5.30A). There was moderate negative association between the reduction in eosinophils over the course of the intervention and levels of NRF2 at week 6 (r=-478, p=0.338) (figure 5.30B), and improvement in pre bronchodilator FEV1 and week 6 NRF2 levels (r=.600, p=0.208); (figure 5.30C).

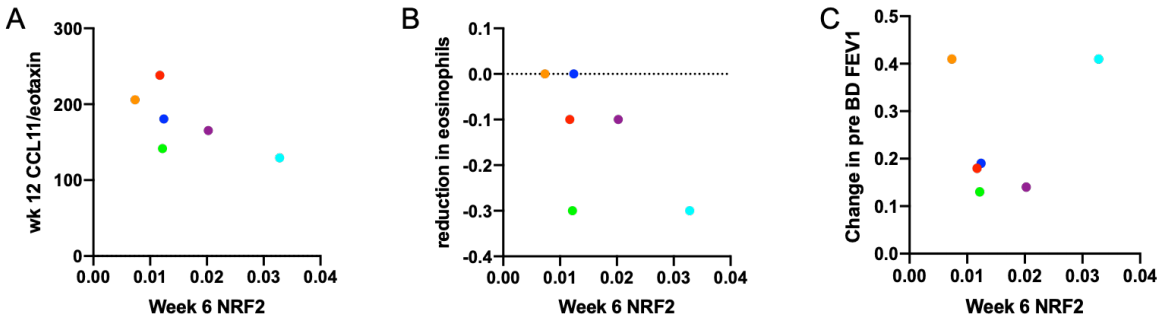


Figure 5.30 Associations (Spearman's Test) between week 6 NRF2 PBMC expression and markers of inflammation and lung function

Week 6 NRF2 PBMC expression and A) week 12 eotaxin levels in plasma, expressed as pg/ml, Spearman's Test r=-.714, p=.111 B) reduction in eosinophils in blood, expressed as cells/L, Spearman's Test r=-478, p=0.338 , and C) improvement in pre bronchodilator FEV1 in litres, Spearman's Test r=.600, p=0.208 ; n=6 for patients with valid baseline and final data. For all graphs, individual participants are represented by a different colour. An r value of > 0.7 is considered a strong correlation and 4-6.9 is considered moderate

5.4 Discussion

There were significant reductions in CCL11/eotaxin, IL-5, TNF α and IFNγ between baseline and week 12, as assessed by cytometric bead assay. CCL11/eotaxin is the most noteworthy, as the results fell within the limitations of quantification for the assay. CCL11/eotaxin has been demonstrated to be an NRF2 regulated gene in human bronchial epithelial cells (387), and whilst we were not able to demonstrate significant changes in NRF2 expression in PBMCs, as discussed later in this chapter, it is noteworthy that we were able to demonstrate a reduction in an NRF2 regulated chemokine. The presence of a control group would have allowed more accurate understanding as to whether these

changes were as a result of the exercise intervention. The reduction in IL-5, TNF α and IFNγ need to be interpreted with caution as they are outside the limits of quantification for the assay. There is also the consideration that, without an appropriate control group, these changes may just reflect normal variation in these cytokines in patients with asthma. However, in the context of the other laboratory and clinical results, specifically the reduction in eosinophilic inflammation, these add early suggestion of support for our hypothesis. For example, IL-5 is known to stimulate bone marrow eosinophilic proliferation and differentiation and thus a reduction in both IL-5 and circulating eosinophil numbers would be expected (388).

The redox regulation experiment results are interesting and demonstrate significant variability between participants. The changes in nitric oxide metabolism are noteworthy. Firstly, levels of nitrite throughout the intervention are higher than we have seen in healthy individuals in response to an acute exercise challenge (292). This is consistent with other work, demonstrating higher levels in patients with both stable and exacerbating asthma compared to healthy controls (389, 390). However, the conclusion that the authors of this work reached was that the increase in nitrite was reflective of an increase in oxidative stress and inflammation. Our data suggest that the relationship between NO metabolism and inflammation may be more complex. This relationship is further complicated by the impact of exercise on the nitric oxide pathway, with exercise shown to increase nitrite in both young and older adults (391, 392). An alternative explanation to understand these complexities may be found in the multifactorial role nitrite plays in redox chemistry in the lung (393). Asthmatic airways present a more acidic environment, which encourages nitrite protonation to nitric oxide, with slower formation of nitrite from nitric oxide (393). A reduction in systemic inflammation following the exercise intervention may result in reduced airways acidity, with a shift in balance towards nitrite formation. Additionally, nitrite is consumed by leukocyte peroxidases, including eosinophil peroxidase. Eosinophil peroxidase is secreted from activated eosinophils (394). If exercise intervention reduces the numbers of eosinophils, then secretion of eosinophil peroxidase may also be reduced, with subsequent increases in nitrite levels. Thirdly, nitrite is also required to form nitrosoglutathione (GSNO), an endogenous bronchodilator, which is catabolised by GSNO reductase to release GSSG (393). The increase in available nitrite in may drive formation of GSNO and facilitate improved bronchodilation, and also contribute to the demonstrated increase in ratio of

GSSG:GSH.. There is, however, consideration that without an appropriate control group, these changes may reflect natural variation.

There are also significant increases in FRAP both pre and post CPET following the intervention, with FRAP offering an index of the antioxidant or reducing capacities of a biological fluid (267). A control group would have allowed improved understanding as to whether the increased nitrite and FRAP across the course of the study was a result of the exercise intervention. With these caveats, the results do provide early support of our hypothesis, that following our exercise intervention, there is a greater ability to deal with environmental or disease related oxidant challenges in this group of symptomatic asthma patients. There is some support found in a study demonstrating plasma total antioxidant capacity of blood to be reduced in acute exacerbation of asthma (395). Given there are reductions in antioxidant capacity with exacerbation, it follows that protection from exacerbation may be conferred by increased antioxidant capacity. This requires further investigation in the form of a fully powered study with a suitable control group.

The first comment to make with regards to thiol redox metabolome is that despite the training programme being designed to apply comparable metabolic stress to each participant, the individual responses to this were strikingly different between each individual. These data are reflective of work looking at response to exercise at varying altitudes, where, in a similar number of participants, very different responses were seen. The authors suggested that better characterisation of the redox metabolomic profile of individuals may help with greater personalisation of medicine (292). These data also provide an interesting area of study into the investigation as to they varying responses seen to exercise intervention, and may have relevance in understanding the concept of 'non-responders' As discussed in 2.3, the concept of non-responders to exercise interventions has been investigated. Greater disease modification was seen in cancer patients in the those who demonstrated increased improvements in physical fitness (308). The association demonstrated in figure 5.27 suggests there may be a similar relationship between disease related improvements and increases in physical fitness, although this is caveated with the dataset being only small and exploratory. The relationship between increases in physical fitness and disease related improvement could be further investigated in future work. The significant reduction in GSH/GSSG ratio is contradictory to what has been demonstrated in bronchial samples in relation to asthma severity and control, with these studies demonstrating an increase in disease severity

with increased levels of oxidation of glutathione (111, 112). However, these studies looked at GSH and GSSG within BAL, which may provide an explanation for our contradictory findings in the context of improvement in inflammation and asthma symptoms. Within any system, there is a need for balance within the reactive species interactome. Perhaps, if exercise acts to increase tolerance to reactive species stressors within the lungs, the compensatory balance to this within plasma is the counterintuitive changes demonstrated within our cohort (256). There is some evidence to support the need for balance of the reactive specied interactome within the literature, with demonstration of opposing changes in glutathione states within plasma and RBCs following an exercise intervention (396). Within this cohort, there are not the samples to further investigate this hypothesis but understanding and investigation of compartmental change could be included in a further study. Investigation of compartmental change discussed in the further work section. The final limitation follows on from the previous points. Many compartmental questions could be answered if this study had a viable lung compartment sample to compare the blood results to. Sputum supernatant was the intended sample for investigation of compartmental change. However, not all participants were able to produce induced sputum. Bronchoalveolar lavage would be another option for consideration in a future study, with the caveat that this is an invasive test for participants to go through twice for pre and post samples. An alternative explanation is that the increase in GSSG may be reflective of increased enzymatic lung antioxidant activity, and a greater ability to detoxify superoxide to water (393). In this detoxification pathway, superoxide can be detoxified to hydrogen peroxide by superoxide dismutases. The resultant hydrogen peroxide is then further detoxified to water by catalase, thioredoxin and glutoredoxin, or gluthathione peroxidase. Thioredoxin reductase, glutoredoxin and gluthathione peroxidase all use gluthathione as a co-factor, which result in an increase in oxidised glutathione (393).

The significant increase in oxidised homocysteine (HCyss) is similarly not in the direction expected, demonstrating a reduction in oxidised stress following exercise intervention. However, the compartmental explanation may come into play again with support from animal models in the literature. Oxidised cysteine residues were measured in rats bred for high and low aerobic capacity. In rats bred for high aerobic capacity, there were higher levels of oxidised cysteine residues in the skeletal muscle, with the opposite demonstrated in the heart muscle (397). We demonstrated in Chapter 3 that the aerobic

capacity of volunteers increased in terms of maximal exercise capacity following the exercise intervention, and therefore it would follow that skeletal muscle oxidised cysteine residues might rise, with associated leak into the circulatory system, which is then quantifiable in PBMCs. There may be, as with the GSH/GSSG ratio change, a paired, opposing change in the lung compartment which demonstrates a shift towards a more reduced balance, with associated increase in capacity to buffer oxidative stress.

Contrary to the hypothesis for this project, I did not demonstrate significant changes in NRF2 or Keap 1 expression in peripheral blood mononuclear cells in response to the exercise intervention. The lack of significant change may be for a number of reasons. The small numbers may not be sufficient to assess this. There are also no asthma or healthy control groups to assess for either asthma related changes or response to exercise intervention, which may have demonstrated between group differences. Finally, these changes may be compartmental, and therefore, as discussed above, what is happening in the peripheral circulation may not be reflective of what is happening in the lungs. All these limitations are demonstrative of the learning curve experienced during the course of this research, and ways to address are discussed in future work (section 7.4). We have, however, demonstrated a link between improved asthma control as assessed by lower ACQ score at baseline and higher expression of NRF2 RNA in PBMCs at baseline, suggesting that a worse level of asthma control is associated with a lower level of expression of the master antioxidant gene NRF2 . This association is reflective of the literature, where downregulation of NRF2 expression is seen in pulmonary macrophages of older current smokers and those with COPD (398) .There is also evidence of NRF2 involvement in a mechanistic context, with NRF2 upregulation shown to be protective against oxidative stress in animal models of asthma (103, 399). However, the associations between NRF2 gene expression and clinical and inflammatory outcomes are not as would be anticipated by our hypothesis. We demonstrate that reduced levels of NRF2 expression are associated with greater improvements airways hyperreactivity as measured by a larger reduction in % bronchodilator reversibility of FEV1. A lower NRF2: Keap1 ratio is asociated with a greater reduction in FVC % bronchodilator reversibility as a marker of airways hyperreactivity. Firstly, it is debatable whether a larger reduction in bronchodilator reversibility is reflective of reduced airways hyperreactivity (400), and consideration of a more accurate measure, such as methacholine challenge is important when planning future work. Secondly, NRF2 expression did not significantly change across

the course of the intervention, and therefore the relevance of any association with NRF2 expression is questionable. A fully powered study with a control group would better assess the impact of an exercise intervention on NRF2 expression. However, as part of the exploratory work, I sought to investigate relationships between these parameters in the hope of providing insight into potential mechanisms with the awareness of the limitations of any findings. Whilst bearing these limitations in mind, potential explanations that are considered for the inverse association between NRF2 expression and lung function, include downregulation of NRF2 at the RNA level due to higher levels of NRF2 protein. I may need to look further downstream to NRF2 protein levels in lungs and blood to demonstrate a link between changes in NRF2 expression with exercise and improvement in asthma symptoms and inflammation. Another theory centres on the idea that redox regulation is a finely balanced process that is compartmentally based. and the levels in the lung need to be balanced (256). It may be that the levels falling in PBMCs with improvement in clinical asthma outcomes are reflective of a matched increase in NRF2 levels in the lungs. However, there do not appear to be any published data directly comparing NRF2 expression in PBMCs and the lung. This is something to be considered for future work. Also to be taken into consideration is that to activate downstream antioxidant processes, NRF2 needs to dislocate from Keap1 and translocate from the cytoplasm to the nucleus (260). Therefore, analysis of overall PBMC NRF2 expression does not allow evaluation of this activation and translocation process. The relevance of this is illustrated in a study by Fitzpatrick et al, that demonstrated increased NRF2 expression in PBMCs and airway lavage cells in children with severe asthma vs mild-moderate asthma, but evidence of dysfunctional NRF2 at the protein level (99). In the Fitzpatrick study, the dysfunctional NRF2 protein appeared unable to bind downstream targets. The final explanation considered for the inverse associations demonstrated was that that NRF2 RNA expression only remains elevated with ongoing inflammation. This point has been proposed as an explanation for their findings by the authors of a study that looked at NRF2 expression in PBMCs derived from ex-smokers with persistent oxidative stress (401). Their paper was reflective of our data, in that they demonstrated an inverse association between lung function and PBMC expression of NRF2 and increased macrophage expression of oxidative stress markers, with the proposal that it was an appropriate response to ongoing oxidative imbalance.

I also looked for associations between physical fitness and markers of redox activity, and inflammatory, lung function and clinical outcomes in asthma. The significance of these associations are questionable given the lack of significant change in some of the individual lung function parameters, and the sample size, as throughout, remains small. However, there is suggestion of association between improvements in physical fitness, increases in redox reserve and reduction in inflammation that may warrant further investigation. Many of the time points chosen for longitudinal assessment were arbitrary, and must be interpreted in an exploratory context. Despite these limitations, it is interesting to see an association between increases in physical fitness through to markers of redox regulation, inflammation, lung function and symptom scores. However, due to the subjective nature of symptom scores in addition to already stated limitations of these analyses, it is impossible to conclude anything from these results other than that further work is needed. Mechanistic modelling may be required to fully understand the relationships, alongside a fully powered intervention study. Levels of NRF2 expression and also downstream markers in our redox data seem to peak at 6 weeks for some patients (non significant change, see figure 5.24). This increase would fit with our hypothesis that increases in levels of NRF2 and upstream redox regulation markers would predate the reduction in inflammatory parameters and improvement in clinical markers of asthma control. However, this interpretation needs to be considered in the context of non-significant change in NRF2 levels over the course of the intervention that could be reflective of underpowering or of the exercise intervention not affecting NRF2 expression. Adequate powering of the study to investigate this effect and a control group would have been helpful in this assessment. I hypothesised that NRF2 expression at week 6 may be more reflective of the clinical outcomes at week 12. Possibly, levels of NRF2 expression were already falling at week 12 with restoration of the oxidative buffering capacity to environmental stressors following exercise intervention. The arbitrary choice of week 6 NRF2 expression requires laboratory work to support, although there is some support of this in murine models that NRF2 levels rise prior to further downstream inflammatory change(402). However, we were able to demonstrate a strong negative association between higher levels of NRF2 expression at 6 weeks and a low (good) ACQ6 score at 12 weeks (see figure 5.29A), and a greater increase (good) in AQLQ total and environmental domain scores (figure 5.29B and C). The environmental association particularly supports our hypothesis that an increase in NRF2 and asscociated redox buffering capacity enables

greater buffering of external allergenic and irritant stressors. Questions in the AQLQ domain reflect asthma symptoms on exposure to environmental stressor and are therefore a reflection of exposure to and tolerance of environmental stressors. In defence of our timeline selection, similar timelines have been demonstrated in a mouse model of the protective effects of NRF2 against oxidative damage in the lungs following diesel exhaust particle exposure(402). Here, ROS peaked along with lung damage prior to the induction of NRF2, which then declined following resolution of oxidative stress at gene and protein levels. We demonstrate a comparable timeline, with a moderate association demonstrated between week 6 NRF2 levels and the improvement in FEV1 over the course of the intervention.There are similar associations demonstrable for longitudinal reduction in eosinophils, and for the week 12 levels of CCL11/eotaxin, with a moderate and strong association as assessed by Spearman's rho correlation respectively, suggesting that NRF2 levels increase with exercise prior to a demonstration of reduction in inflammation. However, these associations must be interpreted with the following caveats. Firstly, NRF2 levels or FEV1 did not significantly change across the course of the intervention, and therefore the significance of any association is questionable. Without a control group, there is no evidence to suggest that, even if changes in the level of NRF2 expression were related to changes in symptomatology or inflammation, any change was a result of the exercise intervention. The most that can be concluded from these analyses are that further investigation of interesting hypotheses are needed.

Also noteworthy, and reflected on throughout the discussion of this Chapter, are the differences in redox regulation overall between the participants. This variability highlights the personal nature of the redox balance within a system or patient, and the interaction of factors that likely contribute (256). Whilst exercise may act to reduce symptom burden and inflammation in many patients and diseases, the biochemical mechanisms through which this is mediated appear to differ from patient to patient, reiterating the importance of personalised medicine as we move forward in the treatment of airways diseases and beyond (403). An improved understanding of the biochemical nature of individualised responses of the redox metabolome through the clinical paradigm of exercise interventions may provide novel opportunity for drug discovery and targeting of the right therapy to each patient.

Chapter 6 Barriers to Exercise in Difficult Asthma

We have demonstrated in Chapters 3-5 that an interval exercise intervention in patients with sub optimally controlled symptoms and evidence of airways hyperresponsiveness are associated with in improved symptoms and quality of life. We have additionally demonstrated that these symptom improvements may be a result of improved lung function, reduced airways and systemic inflammation, all of could be associated with improved resilience of the redox buffering system. Based on these preliminary results, it seems that exercise intervention may provide a beneficial and disease modifying treatment for patients with asthma. One of the major limitations to the intervention study is the small number of participants, that is partly explained by the high drop out rate amongst those who were successfully recruited. Reasons for drop out were variable, and included work and home pressures. Given this, it seems important to understand the barriers to exercise and the burden of exercise therapy in this cohort, prior to the design of a fully powered intervention study. In a broader context, assuming successful demonstration of the hypothesis in a fully powered study, compliance will be important to the successful wider adoption of exercise The first step to facilitating this is the understanding of barriers to exercise in general, and any specific disease related barriers within the target patient group. We therefore asked patients within the WATCH Cohort of Difficult Asthma to complete the Exercise Therapy Burden Questionnaire, and in this chapter we present the results of this.

6.1 Demographic data

The sub cohort of patients who completed the ETBQ were comparable in most core characteristics to the wider WATCH cohort (see table 6.1). Demographic and co-morbidites of the ETBQ group in the context of the broader WATCH cohort are shown in table 6.1.

Table 6.1 Demographic and disease related data for the Barriers to Exercise Cohort within the WATCH Cohort of Difficult Asthma

ns=non-significant, p value quoted for statistically significant differences. Mann-Whitney-U test used to compare continuous data, Fisher's exact test for categorical data throughout. Abbreviations BMI; body mass index, ETBQ; exercise therapy burden questionnaire, GORD; gastroesophageal reflux disease, WATCH; Wessex Asthma Cohort of Difficult Asthma

	WATCH Cohort as a whole (n)	**Median [IQR]**	***N (%)***	***EBTQ Cohort Baseline Data (n)***	**Median (IQR)**	***N (%)***	***P value***
Demographics							
Female	501		65.3%	62		69.4%	ns
Age at Study Enrolment (years)	501	52 [38.5, 63.0]		62	53.5 [35.75, 65.25]		ns
Age at asthma diagnosis	479	19 [4, 40]		62	23 [3.0, 40.35]		ns
BMI	495	29.7 [25.6, 35.3]		60	29.25 [25.5, 36.23]		ns
Obese	*495*		48.3%	62		48.3%	ns
Current or Ex Smokers	*500*		47.6%	62		31.1%	ns
Co-Morbidities							
Rhinitis	446		67.5%	62		58.1%	ns
Eczema	495		26.1%	62		25.8%	ns
Bronchiectasis	493		6.9%	62		16.1%	ns
GORD	495		14.1%	61		50%	ns
Depression	486		64.8%	62		17.7%	ns
Anxiety	454		36.8%	62		19.4%	ns
Dysfunctional Breathing	451		48.7%	61		41%	ns
Intermittent Laryngeal Dysfunction	476		14.5%	59		10.2%	ns
Sulphite Sensitivity	447		7.7%	62		4.8%	ns
Salicylate Sensitivity	493		25.1%	62		21%	ns
Sleep Apnoea			7.2%	62		6.5%	ns

There were some significant differences between the broader WATCH cohort and the ETBQ group in terms of healthcare utilisation, with the cohort as a whole demonstrating a higher use of rescue oral corticosteroids (OCS) (mean 3.60; 95%CI 3.24,3.96 vs 1.93; 95%CI 1.24,2.62, p<0.0001) and a higher rate of hospitalisation in the previous 12 months (mean 0.76; 95% CI 0.59,0.93 vs 0.24; 95%CI 0.01,0.47 p=0.0025). Biologic use was higher in the ETBQ group than the WATCH cohort overall (39% vs 18%, p=0.0016) (see table 6.2)

Table 6.2 Healthcare utilisation related data for the Barriers to Exercise Cohort within the WATCH Cohort of Difficult Asthma

ns=non-significant, p value quoted for statistically significant differences. Mann-Whitney-U test used to compare continuous data, Fisher's exact test for categorical data throughout. Abbreviations ETBQ; exercise therapy burden questionnaire, ICU; intensive care unit, WATCH; Wessex Asthma Cohort of Difficult Asthma

Healthcare Utilisation					
	WATCH Cohort as a whole (n)	***N (%)***	***EBTQ Cohort Baseline Data (n)***	***N (%)***	***P value***
≥1 Asthma Related ICU Visits ever	500	28.2%	60	1.7%	ns
≥1 Asthma Hospital Admission (last 12 months)	497	29.0%	62	11.3%	P=0.0025
≥3 Rescue Oral Corticosteroids (last 12 months)	448	43.6%	60	31.7%	ns
Biological treatment in last 12 months	495	17.6%		39%	P=0.0016

In terms of biological markers of disease severity, the WATCH cohort overall had a lower FeNO (mean 31.1; 95%CI 27.5,34.8 vs 48.55; 95% CI 16.5,80.6)p=0.03) than the ETBQ subcohort (see table 6.3). Post bronchodilator spirometry was chosen as the majority of the WATCH participants are unable to withhold their inhalers to provide true pre-bronchodilator results.

Table 6.3 Bioligical markers of disease severity related data for the Barriers to Exercise Cohort within the WATCH Cohort of Difficult Asthma

ns=non-significant, p value quoted for statistically significant differences. Mann-Whitney-U test used to compare continuous data, Fisher's exact test for categorical data throughout. Abbreviations: BD; bronchodilator, ETBQ; exercise therapy burden questionnaire, FeNO; FEV1; forced expiratory volume in 1 second, Fractional exhaled nitric oxide, FVC; forced vital capacity, WATCH; Wessex Asthma Cohort of Difficult Asthma

Blood Test Results							
	WATCH Cohort as a whole (n)	**Median [IQR]**	***N (%)***	***EBTQ Cohort Baseline Data (n)***	**Median (IQR)**	***N (%)***	***P value***
Eosinophil Count	405	0.2 (0.1, 0.3)			0.2 [0.1, 0.4]		ns
Lung Function Test Results							ns
FeNO50 (ppb)	329	19.7 [10.0, 38.7]		62	22[14, 45.5]		P=.03
Post BD FEV1 (%)	341	75 [59.3, 92.1]		57	73.4 [59.5, 86.6]		ns
Post BD FEV1/FVC (ratio)	340	68 [58, 78]		57	72 [56.5, 78]		ns
Skin Prick Tests							
Positive to any Aeroallergen	391		68.0 %	52		75%	ns
Positive to Aspergillus	355		15.8 %	47		17%	ns

Subjective disease and co-morbidity perception was comparable between the overall WATCH cohort and the ETBQ subcohort, other than the WATCH cohort demonstrated a higher HADS-D score than the ETBQ subcohort (mean 5.4; 95%CI 5,5.8 vs 4; 95%CI 0.1,5, p=0.04) (see table 6.4).

Table 6.4 Subjective disease and co-morbidity perception related data for the Barriers to Exercise Cohort within the WATCH Cohort of Difficult Asthma

ns=non-significant, p value quoted for statistically significant differences. Mann-Whitney-U test used to compare continuous data, Fisher's exact test for categorical data throughout. ACQ6; asthma control questionnaire, EQ-5D-5L; Euroqol 5 level questionnaire, ETBQ; exercise therapy burden questionnaire, HADS; hospital anxiety and depression questionnaire, SGRQ; St George's Respiratory Questionnaire, SNOT22; sinonasal outcome test 22, WATCH; Wessex Asthma Cohort of Difficult Asthma

Questionnaires	***WATCH Cohort as a whole (n)***	***Median [IQR]***	***N (%)***	***EBTQ Cohort Baseline Data (n)***	***Median (IQR)***	***N (%)***	***P value***
ACQ6 Score	467	2.5 [1.5, 3.5]		62	2.4 [1.28, 3.2]		ns
Epworth Score	424	8 [4, 12.75]		55	8 [3, 11]		ns
HADS Total Score	418	10.5 [6, 18]		53	8 [4.0, 15.5]		ns
HADS A Score	425	6 [3, 10]		55	5 [3, 9]		ns
HADS D Score	426	4 [2, 8]		53	3 [1, 6]		P=.04
Hull Cough Score	378	25 [14, 36]		48	30 [14.25, 41.75]		ns
Nijmegen Score	373	21 [12, 31]		47	21 [13, 26]		ns
SNOT22 Score	324	31.5 [20, 50]		40	36.5 [23.25, 48.75]		ns
EQ_5D_5L Index value	170	0.72 [0.53, 0.83]		62	0.72 [0.54, 1.00]		ns
SGRQ Total Score	381	51.1 [35.25, 67.34]		49	59.6 [37.1, 63.4]		ns
SGRQ Symptoms Score	411	67.73 [50.72, 81.31]		53	68 [53, 81.7]		ns
SGRQ Activity Score	389	66.1 [43.7, 85.7]		50	66.2 [41.8, 73.8]		ns
SGRQ Impacts Score	396	38.71 [22.76, 55.74]		52	36 [25.4, 54.1]		ns

6.2 Barriers to Exercise Results

Ninety patients were approached to complete an ETBQ, either as part of their WATCH enrolment, or whilst they were attending a routine clinic follow-up visit. A total of 62 patients fully completed the questionnaire. No significant differences in the demographics, co-morbidities, healthcare utilisation, blood test results or questionnaire results described in tables 6.1-6.4 were seen between those patients who completed the questionnaire and those who did not. Forty nine (79%) of the patients who fully completed the questionnaire took part in some physical activity, with 18 (29%) stating that they played sports, 11 (17.7%) attending physiotherapy sessions and 20 (32.3%) undertaking a home-based exercise programme. There was a median total score of 25.5 (IQR 11.25, 42.75) out of a possible total score of 100.

Median results for the specific questions within the ETBQ are shown in table 6.5. Motivation (mdn 3, IQR 0,5), discomfort (mdn 4 (IQR 0,6), fatigue (mdn 5 IQR 2, 7.25) and being reminded of their asthma (mdn 5 IQR 0,7) were the most limiting factors to exercise programmes within this group.

Table 6.5 Scores for individual questions in the ETBQ

median and range (min-max) results for each of the ten questions comprising the ETBQ; n=62

Question	***Median***	***Range***
Q1 (Pain or discomfort)	4	0-10
Q2 (Fatigue)	5	0-10
Q3 (Boredom)	1	0-10
Q4 (Too Difficult)	2	0-9
Q5 (Wastes Time)	0	0-9
Q6 (Reminds of Condition)	5	0-10
Q7 (Lacks Support)	0	0-10
Q8 (Lacks Motivation)	3	0-10
Q9 (Inappropriate)	0	0-9
Q10 (Not Efficient)	0.5	0-10

There were no significant differences for individual questions or overall score when grouped by gender. There were no significant differences across age range or body mass index in total scores.

However, when the individual question scores were analysed by body mass index, there was a significant difference (p=0.017) in scores for question 1 (The exercise causes me pain) among the BMI categories (see figure 6.1). However, post-hoc pairwise comparisons were not significant once adjusted for multiple testing, raising the question as to the generalisability of this result to the broader asthma group.

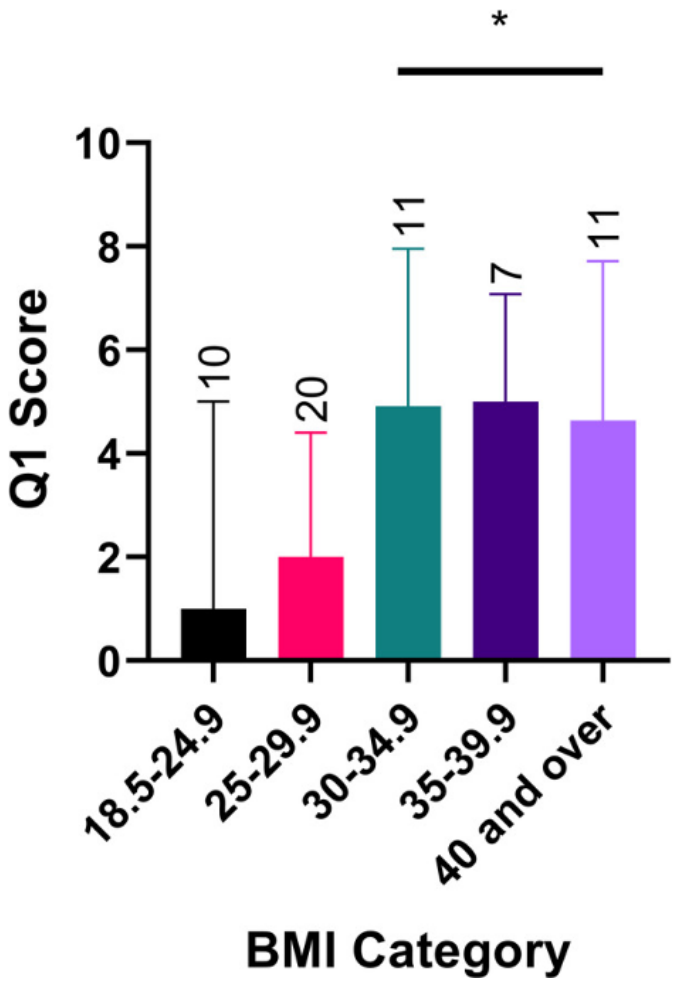


Figure 6.1 Q1 (The exercise causes me pain): results for comparison using Kruskal Wallis Test.

*Comparison of ETBQ scores for question 1 when grouped by BMI category (mdn and IQR), with significantly higher scores noticed in those overweight (p=0.017). *=p<0.05. Abbreviations BMI; body mass index*

When individual question scores were analysed for differences across the age range at diagnosis (not age on completion of the ETBQ questionnaire), there were significant differences in scores for question 6 (Exercising reminds me of my condition), with diagnosis in the age 6-11 group scoring significantly higher than 5 years and under (see figure 6.2).

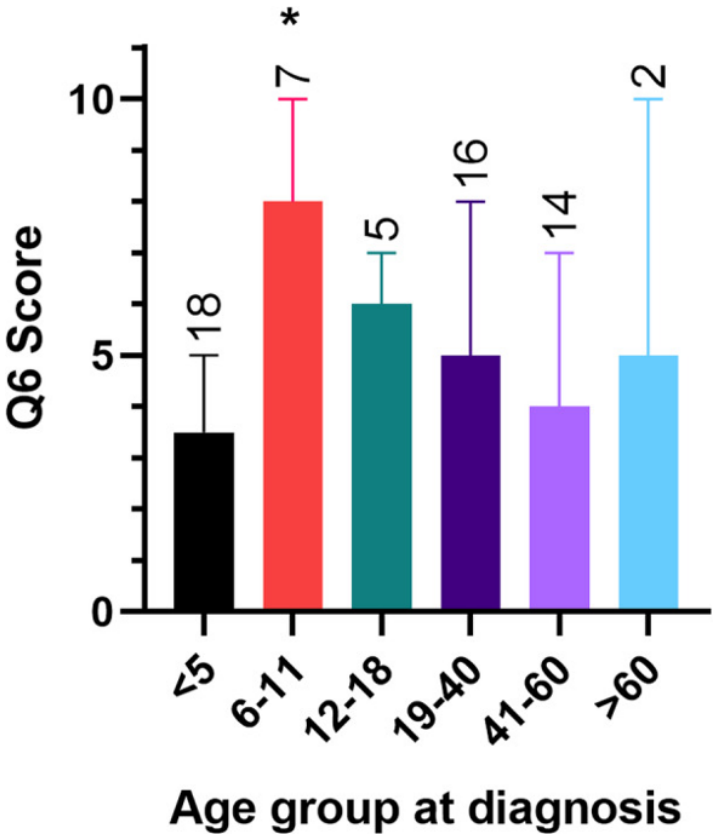


Figure 6.2 Q6 (Exercising reminds me of my condition): Independent samples Median Test results

*for comparison of ETBQ scores for question 6 when grouped by age at diagnosis (mdn and IQR), with significant differences in the age 6-11 group (p=0.03). *=p<0.05*

6.3 Relationships between ETBQ score and asthma related assessments

We then looked at associations between a high total ETBQ score and markers of asthma severity and symptom burden. High perceived barriers to exercise scores were significantly associated with asthma symptoms as measured by the Asthma Control Questionnaire (ACQ6) (r=.452, p=<.0001) and number of rescue OCS uses in the past 12 months (r=.257, p=.048) (See figure 6.3).

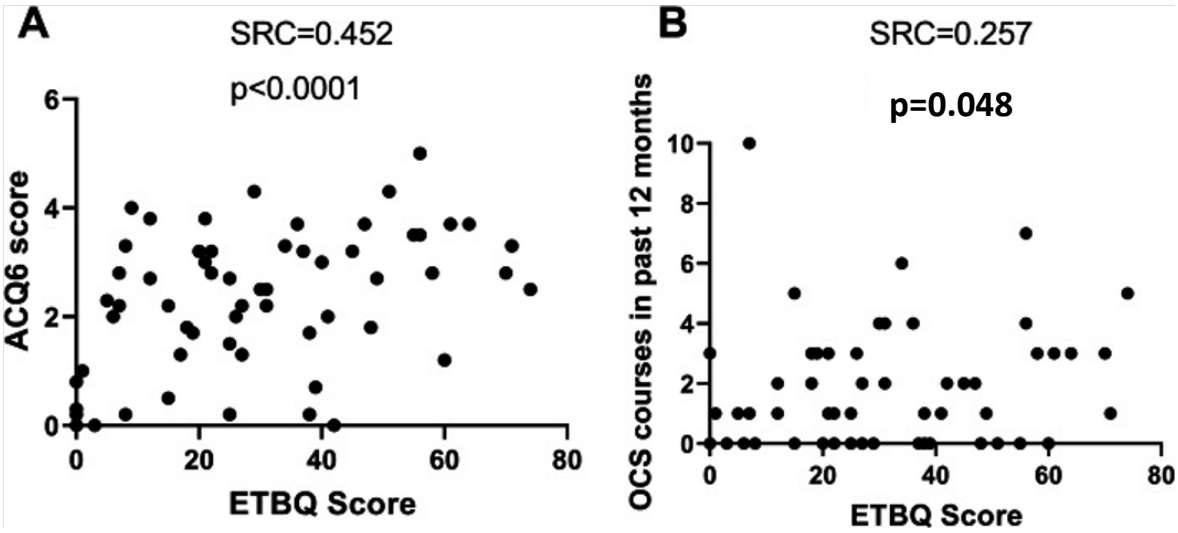


Figure 6.3 Association between ETBQ scores and symptom scores and rescue OCS (ACQ6, figure 6.3A) and rescue OCS (figure 6,3B) as assessed by Spearman Rank Correlation with r and p values. Abbreviations ACQ; asthma control questionnaire, ETBQ; exercise therapy burden questionnaire, OCS; oral corticosteroids

Psychological co-morbidities in the form of anxiety and depression were assessed by the HADS questionnaire. There was significant correlation between high perceived barriers to exercise therapy and high HADS scores, both for anxiety (r=.363, p=.008) and depression independently (r=.375, p=.002) and as a total score (R=.389, P=.004) (see figure 6.4).

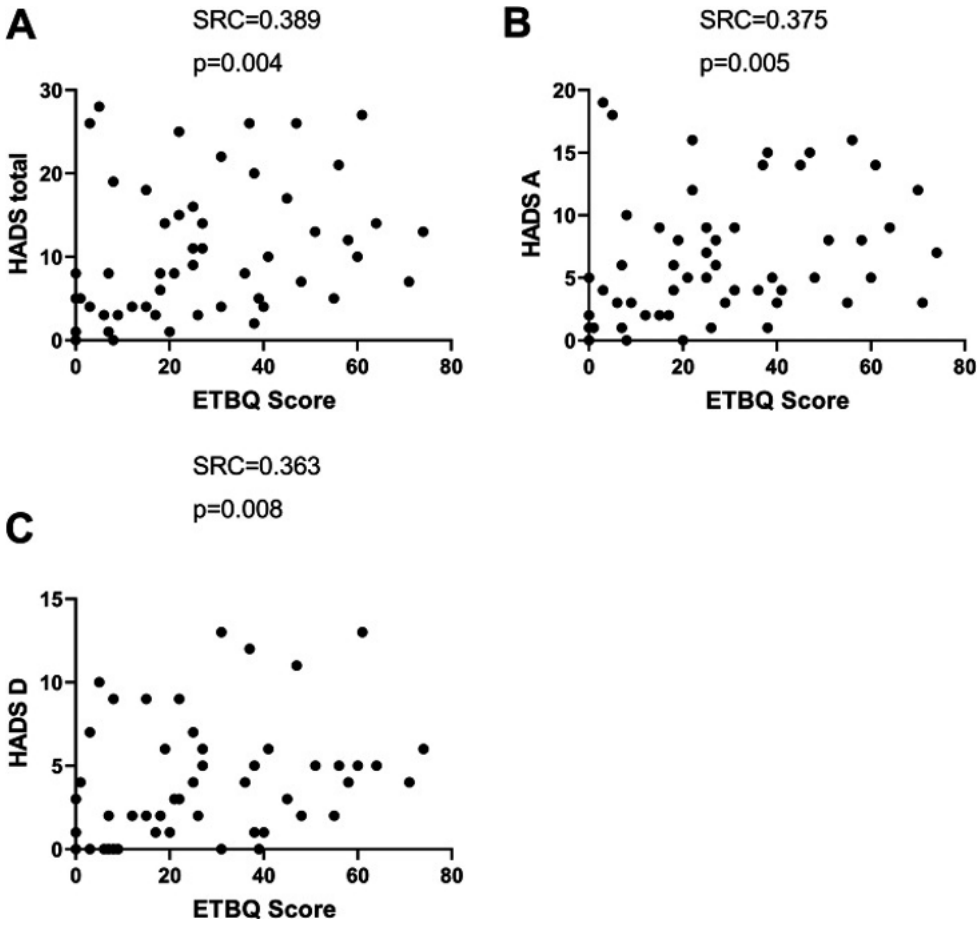


Figure 6.4 Associations between ETBQ and psychological comorbidity for anxiety and depression

(HADS total, figure 6.4A), anxiety (HADSA, figure 6.4B), depression (HADSD, figure 5C), as assessed by Spearman Rank Correlation with r and p values. Abbreviations ETBQ; exercise therapy burden questionnaire, HADS; hospital anxiety and depression score

Low perceived quality of life scores were assessed by the EQ-5D-5L and the SGRQ and correlated with a higher perceived barriers to exercise (see figure 6.5).

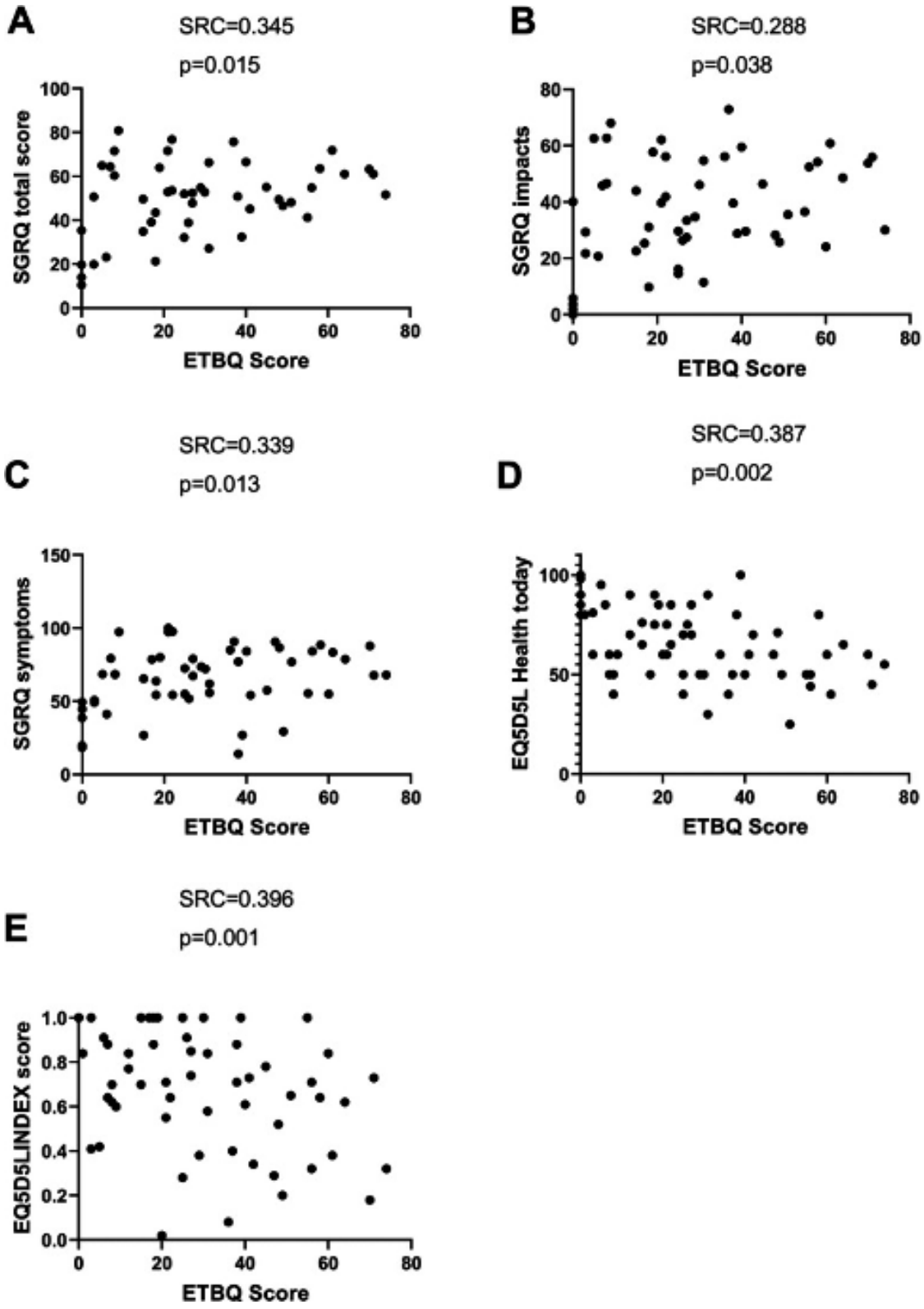


Figure 6.5 ETBQ and Quality of Life Scores

for SGRQ total (6A), impacts (6B) and symptoms (6C), and EQ-5D5L heath today (6D), and EQ-5D-5L Index (6E) as assessed by Spearman correlation, with r and p value. Abbreviations: ETBQ; exercise therapy burden questionnaire, SGRQ; St George's Respiratory Questionnaire

Lung function, eosinophil count, FeNO, Nijmegen and SNOT22 scores, BMI and hospitalisations in the previous year were not significantly associated with ETBQ score (see table 6.6).

Table 6.6 Asthma disease variables that did not demonstrate significant (p<0.05) association with ETBQ score

Abbreviations: BD; bronchodilator, BMI; body mass index, FeNO; fractional exhaled nitric oxide, SNOT-22; sinonasal outcome test 22

Variable	***Association (Spearman's rho)*** ***R value (p value)***
Post BD FEV1	-.177 (.187)
Post BD FVC	-.153 (.255)
Eosinphil Count	.154 (.235)
FeNO	.031 (.808)
Nijmegan score	.213 (.151)
SNOT-22 score	.261 (.104)
BMI	.180 (.168)
Hospitalisations in previous 12 months	-.078 (.548)

There were no statistically significant differences in total ETBQ score, or individual question scores when participants were divided by biologic use or not in the last 12 months. To assess whether the results for lung function results, eosinophil counts and FeNO were compounded by biologic use masking the degree of biological markers of disease, results for these variables were compared using an independent samples Mann Whitney test, which demonstrated no significant differences in results for those on and not on biological treatments.

6.4 Discussion

We assessed the perceived barriers to exercise in patients with difficult asthma under the care of a tertiary clinical service to create a better real-life understanding of relevant limiting factors. To the best of our knowledge, this is the first study to explore this in patients with difficult asthma. Our patient group was generally comparable to the WATCH Cohort as a whole, and representative of a typical group of patients with difficult asthma. It is noteworthy that some of the patients approached to complete the questionnaire did not complete it fully. This is likely to be a result of the methodology of the project, where patients were asked to complete the questionnaires unsupported whilst awaiting their clinic appointments, and is reflective of the real-life nature of the WATCH cohort. This could be addressed in future work with additional support during questionnaire completion or with a supplementary explanation sheet.

Patient perceptions of barriers to exercise in difficult asthma were high; the median score within our cohort were comparable to those found in patients with cardiovascular disease, and much higher than those seen in patients with cancer (319). The distribution of scores was wide throughout the cohort, suggesting that the perceived barriers to exercise are patient specific, possibly reflective of the heterogeneity of difficult asthma. Heterogeneity within the wider general population is also a consideration, with attitudes towards and perceptions of exercise very variable. Inclusion of a healthy control group would have allowed improved understanding of how much of the variation demonstrated was asthma specific.

There did not appear to be significant differences between sex of the patient and perceived barriers to exercise. The lack of significant sex difference contrasts with a previous study which investigated perceived barriers to exercise in a cohort of university students with disabilities. This study demonstrated that the most significant barriers to exercise were interpersonal and that females were more likely to experience higher interpersonal barriers (404). It may be that within our difficult asthma patient group, the disease related barriers to exercise were great enough to balance out any sex specific barriers, and a matched control group without asthma would have been useful to understand this better.

The lack of association between frequency of hospitalisations and perceived barriers to exercise is interesting, suggesting that exacerbations on a background of reasonable day to day control may impact less on perceptions of barriers to exercise than a constant level of poor control with few exacerbations. This may be of relevance to prescribing criteria for biological treatments, which, at present focus on exacerbation frequency (405).

There was a significant effect of BMI categories on Q1 score, as identified by the overall Kruskall Wallis test. The post-hoc pairwise comparisons were not significant, but this is may be due to a lack of statistical power due to small group sizes. However, the importance of adjusting for multiple comparisons in the context of investigating multiple different outcomes, as in this study, is less than in the context of repeated measures for the same outcome (406). This effect of BMI on perceived barriers to exercise is noteworthy, but not a wholly unexpected finding as differences between BMI and barriers to physical activity have previously been demonstrated in young Australian males(407). Patients with asthma who are obese have a greater symptom burden and lose more days to illness than non-obese patients with asthma (65). This population are more likely to benefit from exercise interventions to address both obesity and asthma driven inflammation (5, 408), with a meta-analysis concluding that exercise intervention is effective at lowering body fat (409), and a recent review concluding that exercise constitutes an indispensable tool in the management of obesity (410). It is therefore important to adapt current exercise interventions to make them more accessible to this group of patients. Understanding that perceived barriers to exercise differ for obese patients with asthma is the essential first step in doing this. Further work investigating the specific causes of pain in these patients is now important.

In this present study, significantly different perceptions in the effect of asthma on barriers to exercise were demonstrated between groups based on age of diagnosis. Those whose disease was diagnosed between the ages of 6-11 were more likely to see their disease as a barrier to exercise than those diagnosed under the age of 5. This age appears to be a key stage for engagement in sport in later life, with a report from The Women in Sport Research group showing that if children start to drop out of sporting activities at this age then they tend not to re-engage as adults (411), whereas exercise levels in children at age 7 are not reduced in those with a diagnosis of asthma (412). It may be that diagnosis at this age compounds the effects of this transition point. Diagnosis at this age may result in a higher dropout rate from physical activity that continues into adulthood, and data

regarding levels of sport undertaken by this cohort during childhood would have been useful in interpretation of this. This could partly explain some of the lower levels of activity seen in patients with asthma compared to the general population. Targeted interventions in this age group may go some way to ameliorating this effect (295).

A high perceived symptom burden as assessed through the symptom scores (ACQ6) and number of rescue courses of OCS were found to significantly correlate with a higher perceived barrier to exercise. These associations between a perceived high barrier to exercise therapy and disease specific assessments were reflective of the literature, with those with more severe disease previously shown to view exercise as more likely to be detrimental (301). However, in this present research, objective asthma specific markers of severity such as lung function and markers of T2 high disease did not correlate with perceived barriers to exercise. Similarly, a cross sectional analysis of physical activity in the UK millennium cohort demonstrated that activity levels in children with asthma were not affected by the severity of their disease (412). The lack of association between disease severity and activity levels in children is a clinically relevant finding which suggests that severity of disease is not necessarily a barrier to exercise. These findings have been supported by our pilot work (413), and that of others (5), investigating exercise intervention in asthma patients, suggesting that high levels of biological disease are not necessarily a barrier to adoption of exercise for some patients. These data are of use for reassuring both patients and clinicians that exercise intervention is safe in asthma regardless of disease severity.

Psychological co-morbidity in the form of a high HADS A, D and total score also associated with higher perceived barriers to exercise scores. A meta-analysis has identified low mood and stress as one of the most significant barriers to exercise in mental illness (414), yet conversely, exercise has also been demonstrated to improve mood associated with reduction in depression-associated inflammation in COPD (415) and in health (416). A similar pattern has been seen with QoL where exercise specific self-efficacy has been shown to correlate with health related QoL in COPD(417). Therefore, our results which show that a higher barrier to exercise is associated with a lower QoL score are not unexpected. Exercise is, however, known to improve health related QoL in asthma(79, 305) and therefore interventions to address this paradox need investigating.

There are limitations to this study. Many of the questions could be of relevance to the broader population and therefore the ETBQ does not necessarily assess disease specific barriers to exercise. Inclusion of an age, BMI and socioeconomically comparable control group would have allowed greater understanding of disease specific barriers. Additionally, asthma symptoms can fluctuate, and clinical data were not necessarily collected at the same time as the ETBQ. However, the clinical data which most closely temporally aligned with the ETBQ data were extracted from the database to reduce any inaccuracies. Also, questionnaires were completed at different stages of enrolment in the WATCH study; some at baseline, and others at follow up visits. Similarly, perceived patient barriers to exercise may change depending on the day of the exercise, this may not be captured by a single time point questionnaire. There were a few significant differences between the WATCH cohort and the ETBQ sub-cohort, including number of rescue courses of OCS in the last 12 months. These differences may partly explain the only borderline significance of the association between ETBQ total score and OCS rescue courses. Besides the differences discussed above, the ETBQ cohort was representative of the wider WATCH population and there was no difference between those who completed the questionnaire compared to those who did not, suggesting any differences between groups were not a bias to taking part in the ETBQ study. The ETBQ focuses on a prescribed activity and yet some patients within the cohort were not prescribed any activity. If participants did not have a prescribed activity, then they were asked to complete the questionnaire from the perspective of what prevents them from exercising rather than the burden of any prescribed exercise. With any self-reported questionnaire-based research, there is always the concern of responder bias. However, patients were asked to complete the questionnaire regardless of whether they undertook regular exercise. This removed any expectation that they should be taking part in exercise. Finally, the lack of control group means it is impossible to determine if the barriers to exercise identified in the WATCH cohort are specific to a diagnosis of asthma, and it has been demonstrated that in rheumatoid arthritis, pain is similarly a barrier to physical activity (418). Further exploration of barriers to exercise in asthma via a combination of qualitative interview and use of a control group would help to clarify this.

In summary, patient perceived barriers to exercise therefore appear to be more related to symptom burden and psychological morbidity than to specific disease severity indicators, but it is noteworthy that an age, sex and BMI compatible control group would have been

useful in clarification of this. Many of the questions are generalisable to a healthy population and a control group would have allowed improved understanding as to disease specific barriers. However, the findings in this chapter are still of relevance to the demonstration of tolerability of the exercise intervention that are reported and discussed in Chapter 3, where, despite high levels of subjective disease, exercise testing and exercise intervention did not result in any adverse events and were well tolerated. We further demonstrated in Chapters 4 and 5 that the exercise intervention may have an impact on inflammation, via improved redox regulation capacity, which is reflected in improvement in the symptom burden that this chapter has identified as a barrier to exercise. Understanding barriers to exercise in asthma is important for further work, as discussed in Chapter 7, as breaking this catch 22 scenario is key to the successful design and implementation of a fully powered study, and in the longer term, the wide spread adoption of exercise as a disease modifying intervention. Exercise interventions combined with psychological input may be useful in facilitating adoption of exercise, and this will be considered in the design of a follow on study.

Chapter 7 Discussion and further work

7.1 Introduction

Asthma morbidity and mortality remain relatively static despite the introduction of new biological treatment options over the last 10 years (148), with many patients either not demonstrating the appropriate biological marker for these therapies or remaining sub-optimally controlled on these treatments (149, 150). Exercise training in health has demonstrated immunomodulatory properties, and murine (277, 287, 291) and human (5, 279) studies suggest a role for exercise intervention in the modulation of asthma associated inflammation.

The results presented in this thesis extend the support for exercise training as a clinically relevant and disease modifying treatment for asthma. If further qualification of efficacy can be demonstrated in fully powered and adequately controlled study, then investigation into the cost effectiveness of exercise intervention as a treatment in comparison to standard management would be useful. The hypothesis proposed in the introduction is supported by results presented in this thesis, in that SRETP for 12 weeks appears to result in a significant improvement in asthma symptoms, as assessed by the Asthma Control Questionnaire, and in asthma related quality of life, as assessed by the Asthma Quality of Life Questionnaire. There is further support for the hypothesis in the demonstration of reduction of systemic inflammatory markers following SRETP, with support for the hypothesis demonstrated in the laboratory work that shows decreased inflammatory burden and an increase in antioxidant capacity with exercise training. A control group would have facilitated clearer understanding as to whether these changes were due to the intervention. This chapter will first address potential limitations of the study design, and with these caveats in mind, discuss the implications of these findings and outline plans for further work.

7.2 Limitations to the study

There are a number of limitations to the work presented in this thesis. The exploratory design of this study and low patient numbers have resulted in insufficient power to detect significant changes in clinical and immunological outcome measures, and therefore

further work is required to determine the significance of observed changes. Those enrolled, by definition, due to their interest in taking part, are also likely to be a motivated group. Potential barriers to recruitment and adherence to the exercise training include a hospital centred intervention and the time constraints surrounding this, which was necessary at this stage to confirm safety, efficacy and compliance. However, moving forwards to planning future work, and particularly in the context of the COVID 19 pandemic, there needs to be a shift towards out of hospital testing with visits only for sampling. A precedence and feasibility for translation to an out of hospital intervention has already been demonstrated with the transition of the Wesfit Study to the Safefit training model. In the Safefit Study, the exercise training intervention is less metabolically controlled, and adaptation of this would need to be made to ensure a SRETP equivalent exercise intervention could be replicated at home.

With any intervention study in asthma, there is the question of whether adherence to inhaled corticosteroids have improved, and caused the improvements in symptoms and inflammatory parameters demonstrated in this cohort, as opposed to the exercise intervention resulting in these changes. It is of particular relevance in the context of known poor compliance in asthma patients. One recent study demonstrated the mean proportion of days covered by prescription collection over a 12-month period was only 19% (419). Therefore, excluding confounding by increased adherence is relevant to demonstrating the efficacy of a new intervention in asthma.To mitigate this, patients were asked at the beginning and end of the intervention whether adherence patterns have changed over the course of the study, and there were no suggestions from these conversations that it had. Although some participants were non-compliant at enrolment, they remained, on questioning at the beginning and end of the study, non-compliant throughout the study period. Assessment of compliance within the context of an immunomodulatory intervention that may mediate its effects through modification of glucocorticoid related pathways is complex. A cortisol assay, for example, may be affected by the exercise training as much as any changes in adherence. There are some important points to be noted in support of the exercise intervention rather than increased adherence resulting in clinical improvements demonstrated. One of the patients who demonstrated an improvement in symptoms, a reduction in peripheral blood eosinophil count and gain of 490 ml in pre-bronchodilator spirometry by the end of the intervention, was not on inhaled corticosteroids during the study, so changes in this

case cannot be explained by increased compliance with medications. Additionally, any improvement in inhaled corticosteroid compliance should be accompanied by a marked change in FeNO, with FeNO suppression after directly observed inhaled corticosteroid treatment used as a measure of identification of non-adherence in difficult asthma (420). FeNO levels in this cohort did not significantly differ in spite of the improvements in clinical symptoms and systemic markers of inflammation, although this could be due to underpowering. Inclusion of a prescription pick up check as an indirect measure of adherence should be considered for future work, as prescription pick up has been demonstrated to be a reliable method of assessing medication adherence(421).

A control group of asthma patients would have been useful to demonstrate whether any changes were a result of the exercise intervention and not a result of confounding factors such as potential increases in compliance or improved wellbeing known to be associated with study participation(422). A control group of healthy individuals undertaking the same exercise intervention would have been useful in terms of clarifying whether the redox responses seen were specific to asthma or reflective of generalised redox responses to this particular exercise intervention. It has been demonstrated previously that both continuous and high intensity intermittent exercise are able to increase total antioxidant capacity in healthy individuals (423), and it may be that we are seeing changes in redox capacity that do not have disease specific implications in asthma.

7.3 Exercise training in symptomatic asthma

The current study demonstrates that interval training in the context of symptomatic asthma, defined by an enrolment ACQ6 score of ≥1.5, performed for 3 x thirty-minute sessions for a total of twelve weeks, seems to result in significant improvements in asthma symptom control and asthma related quality of life, a reduction in peripheral blood cell counts and improved pre-bronchodilator spirometry. Inclusion of a control group would allow greater confidence that this improvement is related to the exercise intervention rather than to confounding factors. The cohort has co-morbidities similar to a difficult asthma group (330), and reflects a spectrum of disease severity as evidenced in the variation in level of asthma medications from SABA only through to biological treatment. The first finding of note from this study is that CPET appears to be safe and well tolerated in a group of patients in whom caution has been employed when utilising

CPET for fear of inducing exacerbation. A caveat to this interpretation is that none of the participants reported exercise induced bronchospasm. Consideration needs to be made as to whether patients with exercise induced symptoms should be screened for exercise induced bronchospasm as part of their baseline CPET. CPET has demonstrated good negative agreement agreement with the gold standard eucapnic voluntary hyperventilation tests for indentification of exercise induced bronchospasm (424). All participants were able to achieve peak exercise without need for rescue medication at all time points. Only one patient was excluded following their CPET, as a result of identification of underlying pre-existing cardiac arrythmias. This finding alone is of clinical revelance. Many patients with difficult and severe asthma present with symptoms that are not consistent with their traditional investigations, which may be due to exercise induced bronchospasm or underlying unidentified cardiac disease. For these patients, CPET would be very useful in identification of the pathology that is driving their symptom burden, as demonsrated by McNicholl et al. Furthermore, interval exercise training appears to be safe and well tolerated within this group, with no adverse events resulting from exercise intervention despite the sub optimal symptom levels within the group. This is reassuring to both patients and physicians, and contradicts the perception that patients with symptomatic asthma are more likely to suffer from exercise induced symptoms (425). Assessment of asthma control using the ACQ remains a subjective assessment of asthma control, and methacholine challenge testing would provide a more objective assessment of asthma control for future work.

The addition of strength training exercises increases requirements in terms of participant time, thereby increasing the participant burden of exercise without sufficient evidence of efficacy. There are not sufficient data to conclude whether there were additional benefits from resistance training in this study. However, if clinical benefit is confirmed in a fully powered study with an interval training intervention alone, then interval training alone may be preferable to patients. Benefit from aerobic training alone demonstrated in COPD, where endurance training at moderate intensity induced the same upregulation of muscle antioxidant capacity as resistance training (426). Finally, the intensity of the current resistance training programme may not have been great enough to induce additional anti-inflammatory benefit. It has been demonstrated that resistance exercise induce lymphocyte apoptosis in an intensity dependant manner, suggesting varying levels of immunomodulation with different intensities of resistance intervention (427). with

comparison of a 60% of 1RM test and a 75% 1RM test. In this study, the 60% group did not demonstrate changes in IL-6 and cortisol levels post intervention but changes were seen in the 75% group. The mechanism behind this appears to be glucocorticoid receptor driven, which demonstrates similarities in the proposed mechanisms for the effect of exercise on inflammation in asthma (427). Perhaps a higher intensity resistance training programme is necessary for additional anti-inflammatory benefit in asthma patients than the 60% 1RM intervention tested in this study. However, given the significant benefits conveyed by interval training alone that are described in this thesis, and the time constraints that are likely to factor in uptake of exercise programmes in this cohort, an amendment was submitted to limit the exercise intervention to interval training with optional resistance training.

7.3.1 The effect of SRETP on physical parameters

The stability of body mass index with this exercise intervention is relevant in the context of this study. Adipose tissue is known to behave as a complex endocrine organ that produces cytokines that can exacerbate the inflammatory pathways involved in asthma(67). Weight loss is an important potential confounder in studies exploring the utility of exercise as an anti-inflammatory treatment for asthma. There were also no change in body fat percentage and free fat mass in the cohort described herein, further supporting that the reduction in inflammation can be considered independent to reduction in the inflammatory drive conferred by adipose tissue. The study of an exercise intervention in obese asthma patients demonstrated the largest number of improvements in inflammatory parameters in published work to date, and it is important to note that significant weight loss was a potential confounder of these results (5).

The results presented in this thesis demonstrate improvements in exercise capacity and physical fitness as assessed by CPET following the intervention. The improvement in physical fitness as assessed by AT and a trend to improvement in $\dot{V}O_2$ peak are reflected in results from cancer studies employing the same training protocol (312). Similarly, the significant improvement in the cancer patients was demonstrated by 6 weeks, with demonstration of improvement in AT and peak at 3 and 6 weeks in the group presented in this thesis.

There are a number of considerations that need noting when comparing across the two groups. An obvious difference when drawing comparisons is that the underlying diagnoses of the two groups are very different in terms of baseline physiology. The difference in underlying diagnoses are particularly relevant in the context of concurrent chemotherapy. However, in line with the hypothesis, both a chemotherapeutic insult and the chronic inflammation of asthma would reduce redox buffering capacity or resilience. In cancer, both the cancer cells themselves generate high levels of oxidative stress, in addition to many standard chemotherapeutic agents demonstrating cytotoxic properties as a result of their propensity to induce rapid and high increases in reactive oxygen species(428). In asthma, the inflammatory cells recruited to the asthmatic airways are responsible for increased levels of reactive oxygen and nitrogen species (100), with resultant reduced redox buffering capacity in both asthma and cancer (99, 429, 430).

Another consideration to note is that the sample size may explain the lack of significance in change in $\dot{V}O_2$ peak . This point regarding sample size is supported by the change in absolute numbers, which, whilst not demonstrating significance statistically, do show an increase in the median between baseline and week 6 that is greater than the MCID of 2ml/kg/minute.

Also noteworthy when in search of an explanation, is that participants displaying higher levels of inflammatory cytokines have been shown to demonstrate a reduced response to exercise training (343), that would not be expected in the context of immune suppressant chemo and radiotherapy. The authors of this previous study postulated that elevated inflammatory levels may blunt gains in muscle mass by disruption of signalling cascades associated with protein synthesis and degradation during training, thereby reducing potential gains in aerobic capacity. The cohort presented herein may be responding to the exercise training during the intervention period by initially demonstrating a reduction in their inflammatory burden, and secondarily to that will respond with greater improvements in fitness.

7.3.2 Effect of SRETP on symptom burden and quality of life parameters

There are significant and rapid improvements in asthma symptoms as assessed by the asthma control questionnaire following exercise intervention in this cohort. This is reflected in the only other study of interval training in asthma published to date, but only

in the context of dietary and exercise intervention, and not in exercise alone (280). There are also significant reduction in symptom scores as assessed by ACQ in the Freitas study of an exercise and dietary intervention in obese asthmatics; however, as with all outcomes from that study, there is a risk of confounding from the significant reduction in weight with exercise and dietary intervention (5). Of the other exercise intervention studies, one did not demonstrate significant improvement in ACQ score (6), whilst another did not assess asthma control using the asthma control questionnaire, but did demonstrate an increase in symptom free days following exercise training (279).

A potential explanation for variability in improvement in symptom scores is variation in levels of symptoms at baseline. The cohort presented in this thesis are more symptomatic at baseline when assessed by ACQ score (mean ACQ 2.2) than previous cohorts, which had mean ACQ scores ranging from 1.6 (279) to 2.0(5). The rationale behind this potential explanation is the greater the symptom burden at baseline, the greater the potential for improvement. Logically, higher the symptom burden should be reflective of higher the levels of baseline inflammation, with the hypothesis that exercise intervention reduces inflammation and therefore symptoms. However, this assumption is dependent on the extent to which symptom burden is attributable to inflammation. There is demonstration that inflammation can persist in patients with ACQ< 0.75, and that methacholine challenge provided better assessment of inflammation than ACQ score(431), suggesting the relationship between symptom burden and inflammation is more complex. Additionally, there were differences within the exercise interventions in each study, and if exercise is to be regarded as a medication, then it is impossible to compare across different ‘doses’ of exercise.

Another consideration when interpreting improvement in symptoms with exercise training is the effect of seasonality on what is, by definition, a variable disease. It has been demonstrated that patients who train from winter to summer improved their symptoms to a greater extent than those who train from summer to winter (432); however, the cohort presented here was recruited over a 2 year period throughout the year, which should mitigate any impact of seasonal variation on results.

The results from the Asthma Quality of Life Questionnaire (AQLQ) are interesting. There is significant improvement at 6 and 12 weeks, with overall improvement in AQLQ is demonstrated over the duration of the intervention. These results suggest a lag time in

the effect of exercise on improvement in QoL, and may be of relevance when managing patient expectations of response to an exercise intervention; in the same way that medications need to reach a therapeutic level prior to response assessment, exercise needs to be reviewed in the same context.

Looking more specifically at the domain scores, the symptom domain of the AQLQ show a trend to longitudinal improvement that is reflective of the improvement demonstrated in ACQ score. The activity domain does not show a significant improvement over the course of the exercise intervention. The lack of improvement in activity domain is may be reflecting a young cohort who, despite a high asthma symptom burden at baseline, are relatively unlimited in their activities of daily life.

There are significant improvements in the emotional domain, which are not unexpected. Exercise has been consistently demonstrated to improve psychosocial health, and is comparable to antidepressant medications as a first line treatment for mild to moderate depression (433). The link between exercise and psychosocial health is of relevance in asthma, where depression and anxiety are prevalent comorbidities known to effect symptom control(330), and even if exercise exerts some of its positive impact on symptom scores through modification of emotional domains, then that is still of potential wide-ranging benefit in the management of severe and difficult asthma. Additionally, a bidirectional relationship has been demonstrated between inflammation and depression (434), and the improvement in emotion scores demonstrated in this cohort may partially be a result of the anti-inflammatory benefits conveyed by the intervention.

The potentially most relevant significant improvement in asthma quality of life domains following exercise intervention in this cohort is demonstrated in the environmental domain, as this provides support for the hypothesis. It may be that the improvement in quality of life is driven by increased reliance to environmental stressors that is mediated through an increase in tolerance of oxidative stressors following exercise training. It has been suggested that allergen initiated innate and adaptive immune responses result in production of reactive oxygen (ROS) and nitrogen species (RNS) (100) and therefore greater capacity to reduce or buffer these ROS and RNS would be beneficial. An increase in buffering capacity has been demonstrated in this cohort by an increase in nitrite levels and ferric reducing antioxidant capacity of plasma over the course of this intervention, which may equate to a reduction in symptoms experienced by participants on exposure

to these environmental triggers, as assessed by the environmental domain of the AQLQ. This theory has support in the literature (435) and from the associations demonstrated in Chapter 5 of this thesis, but without the inclusion of a control group, it is not possible to conclude that this is a result of the exercise intervention.

7.3.3 Effect of SRETP on clinically assessed parameters of asthma control

The improvements in pre-bronchodilator spirometry (FVC) provide support that the improvements in asthma control and quality of life demonstrated in this thesis are not purely a placebo result from the benefits conferred from research participation or from the overall increase in well-being that increased exercise conveys (354). The improvements in pre-bronchodilator spirometry following the intervention, in combination with the reduction in FEV1 bronchodilator reversibility suggests that exercise training is effective at reducing hyperreactivity of airways that is characteristic of sub optimally controlled asthma. These results are supported by previous studies of exercise training in asthma, which demonstrated reduced bronchial hyperreactivity to methacholine challenge(6). However, the lack of a control group means it is impossible to confirm causation of these changes. The laboratory work discussed in Chapter 5 suggests a role for redox regulation in the mechanistic explanation behind this reduction in bronchial hyperreactivity, assuming the hypothesis of this thesis is correct, with demonstration of an increase in antioxidant capacity following the exercise intervention.

7.3.4 Effect of SRETP on peripheral full blood cell count

The effect of SRETP on peripheral full blood cell count lends further support to the immunomodulatory capacity of SRETP already demonstrated in cancer patients (308). The changes in white cell count in this asthmatic cohort reflect the response to exercise in health, with a reduction with exercise training (364). These results, in combination with the trend for reduction in neutrophil count demonstrated in this cohort is interesting in the context of questioning whether changes in compliance are an explanation for symptom improvement rather than the exercise intervention. Inhaled corticosteroids have been shown to increase white cell count through an increase in absolute neutrophil counts, potentially via reducing neutrophil adhesion to the endothelial surface through a reduction in adhesion molecule expression on neutrophils (370). Conversely, white cell count and neutrophil count following exercise training for weeks or months either

remains static or reduces, as review in (364). The initial rise and subsequent reduction in lymphocyte count is similarly expected with exercise training(364).

Perhaps of most relevance in the context of asthma is the reduction in peripheral blood eosinophil count, and this is the first study in asthma to demonstrate this, although reductions in sputum eosinophil count have been shown by others (279). Anti-eosinophilic treatments in the form of inhaled and systemic corticosteroids, and more recently, anti IL-5 treatments, form the mainstay of treatments for more severe forms of asthma. It is not unreasonable to extrapolate that the reduction in symptom burden may potentially be related to the reduction in eosinophilic inflammation, given the associations presented in Chapter 5, though the lack of control group mean determining causation is impossible.

The lack of demonstrable effect of exercise on total IgE could potentially be explained by atopy-driven increases in total IgE clouding any exercise related reductions in asthma related inflammation in the patient cohort described in this thesis. Mouse models have demonstrated reduction in total and specific IgE in response to exercise training (436). An exercise intervention in asthmatic children reflected this reduction in IgE, but without any significant change in FeNO or blood eosinophil count (278). Given the variability in total IgE in patients with moderate to severe persistent allergic asthma over a year (437), and the missing data, a larger cohort may be necessary to demonstrate significant changes in response to exercise training, with a control group to determine causation.

The lack of significant change in CRP levels in the cohort described herein is not surprising. Levels of CRP within the cohort were low at baseline, and the reduction in CRP following exercise in described in the literature is larger when accompanied by weight loss and a reduction in percentage body fat (438), so less likely to reduce in a cohort where BMI remains static. The ongoing caveat of small numbers and their impact on data interpretation remain.

The number of patients able to produce induced sputum samples within the cohort described in this thesis was low, but not outside of the rates of success seen within this institution in the context of other asthma studies. A larger number of patients may be necessary to demonstrate a change in sputum eosinophil counts expected alongside the reduction in blood eosinophil count, and there are not sufficient data to interpret beyond speculation in this patient group.

FeNO levels did not significantly change with the exercise intervention. The evidence in the literature is mixed, with some(5, 279) but not all (278, 280, 439) showing improvement in FeNO levels with exercise intervention. It may be that allergic rhinoconjunctivits masked any reduction in lower airways FeNO in this study, with some patients within this cohort reporting hayfever symptoms at times of sampling. In one patient in particular, peripheral blood eosinophil count and asthma symptoms improved whilst FeNO increased. This explanation is supported in the literature, with reports of confounding of FeNO correlation with asthma symptoms in the presence of other atopic conditions (440). One study demonstrating independent associations between eczema, allergic rhinitis and atopic status and elevated FeNO levels but not with atopic asthma (441). In another context, the lack of change in FeNO levels is useful. FeNO suppression tests are used as a method to identify non-adherence in difficult to control asthma (375), as discussed in section 7.2. Given FeNO suppression tests are used in identifying non-adherence, it seems reasonable to extrapolate that if the improvements in symptom scores and inflammatory parameters described in this thesis were as a result of improved compliance, then a reduction in FeNO would be anticipated. The lack of this supports the role for the intervention in effecting these changes rather than alterations in compliance, which, as previously discussed, cannot be fully assessed without larger numbers and a control group.

7.3.5 Effect of SRETP on asthma related cytokines

There were changes in levels of a number of asthma related cytokines in plasma but not serum across the course of the intervention. This is in contrast to the most comparable literature of an exercise intervention in obese asthma. Here, there were a number of significant changes in cytokine levels detected within the serum (5). However, levels of cytokines were not assessed in the plasma and it may be that there were also some significant changes in this compartment that were not looked for. The patient cohort, in comparison to the group discussed herein, were obese with significant weight loss following the intervention, and it may be that the reduction in adipose driven inflammation contributed to changes in cytokine levels in the serum where we were not able to demonstrate the same. A number of our serum cytokine readouts were out of range for detection meaning that changes may have been there but in our non-obese cohort were not at a demonstrable level. Interestingly, lower serum cytokine levels are

also demonstrated in the literature, where matched serum and plasma samples were compared (442). Here, they demonstrated that plasma was a more sensitive matrix for the detection of low abundance cytokines due to non-specific background readouts being increased in serum versus plasma. It was recommended in this study that it would seem reasonable to recommend the use of plasma over serum for multiplex assays when possible.

For many years, there has been a quest for the identification of useful biomarkers within asthma, and although a number of biomarkers have been identified further work is required to achieve greater understanding of the clinical utility of these (403). To date, the most widely used clinical biomarker is not a serum or plasma but whole blood eosinophil count, which is used as a prescribing requirement for the rapidly increasing number of anti IL-5 treatments. However, whilst blood eosinophil counts have demonstrated utility for the initial prescribing of anti-IL-5 treatments, they do not convey utility in treatment monitoring (377). Serum periostin has also been identified as potentially useful, but a clear role for the measurement has yet to be determined (403). Therefore, whilst these results are useful in demonstrating an overall reduction in TH2 inflammation following the exercise intervention, the literature remains lacking in evidence to support a clear role for individual serum or plasma markers in monitoring disease.

The specific cytokines identified as having significantly reduced are noteworthy. CCL11/eotaxin is an eosinophil chemoattractant and in combination with the fall in eosinophil count, may be more clinically relevant. Further relevance to our hypothesis is demonstrated with evidence of its modulation via NRF2(387). TNFα, IFNγ and IL-5 also significantly reduced in the serum following the exercise training intervention. However, as the majority of these results were below the lower limit of quantification for the assay and therefore have to be interpreted with caution. In the context of the other results from this study, they provide additional support for he hypothesis with the caveats detailed above.

7.3.6 Effect of SRETP on redox regulation results

SRETP significantly increases the levels of nitrite both pre and post CPET across the course of the intervention. Additionally, amount of increase in nitrite post CPET demonstrates

association with amount of increase in oxygen uptake at AT. There is demonstration of a positive association between plasma nitrite levels and exercise capacity in terms of endurance time in the literature (443). In this work, a positive association between nitrite and exercise capacity was seen at in patients at 80% of maximum workrate and at maximum workrate, without an increase in nitrate. The authors suggest that exercise promotes balance of nitrogenous species to a reduced state. They propose that the reduced nitrogenous species provide a buffer to the oxidative stress induced by exercise, reflective of our hypothesis. There is no published work investigating nitrite or nitrate levels in the context of exercise and asthma. Similarly, there is no comparative asthma work regarding ferric reducing antioxidant capacity of plasma with exercise. In cardiac rehab patients, there has been demonstration of an increase in FRAP with exercise intervention, supporting our findings that exercise improves the overall antioxidant capacity (444).

There appears to be a relationship between improvements in clinical asthma outcomes in our patients following SRETP, through to markers of redox regulation and increases in physical fitness, supporting the mechanistic hypothesis for this thesis. Further, appropriately randomised and controlled work is needed to investigate whether the increases in physical fitness are driving improvements in markers of redox regulation and clinical asthma improvement. I have demonstrated that symptom score at baseline is negatively associated with NRF2 expression, showing that the first link in the mechanistic chain appears to interact with the outcome that has most clinical relevance to patients with asthma. I have also shown that changes in downstream markers of redox regulation correlate through to improvement in quality of life scores. This association is reflective of work in asthma patients, demonstrating a link between redox regulation status and asthma control (110, 445), and reflective of work in non-asthma patients and exercise (260). Finally, I looked at changes in expression of NRF2 and Keap1 in PBMC derived RNA to investigate whether the further downstream results could be attributed to changes in these ‘master antioxidant’ genes. The associations demonstrated here were interesting for a number of reasons, although need to be interpreted in the context that NRF2 and Keap 1 expression did not significantly change over the course of the intervention. In addition, without a control group, it is unclear whether any changes that occurred are a result of the exercise intervention or due to other confounding factors. Accepting these caveats, the first point to note is that the association between NRF2 ratio and

bronchodilator reversibility is not as expected, with a lower NRF2: Keap1 ratio correlating with a greater reduction in bronchodilator reversibility of FVC as a marker of reduced airways hyperreactivity. Similarly, the association between the increase in NRF2 and bronchodilator reversibility of FEV1 is not as expected, with a greater reduction in bronchodilator reversibility as a marker of airways hyperresponsiveness correlating with a reduction in NRF2 over the course of the intervention. It may be that the complex relationship between redox buffering via upregulation of NRF2 is not demonstrable by investigation of NRF2 expression in one compartment, without differentiating between nuclear and cytoplasmic levels, or between peripheral blood and lung levels, and investigation of these compartmental changes would be a focus of further work. We did, however, go on to demonstrate significant longitudinal association between higher week 6 NRF2 levels and reduction (improvement) in ACQ score, suggesting the upregulation of NRF2 precedes improvement in inflammation and symptom scores, which is reflected in mechanistic asthma mouse models of NRF2 upregulation. There are also strong associations between increased levels of NRF2 expression at week 6 and overall reduction in AQLQ total and environmental scores over the duration of the training intervention. Similar, moderate to strong associations were demonstrated between NRF2 levels at week 6 and improvements in lung function and inflammation. To my knowledge, there is no work investigating the role of redox regulation changes in the context of exercise and symptom control in asthma, or the timelines in which these changes occur. There is support for my hypothesis, however, in the demonstration NRF2 expression at both protein and gene level with resolution of inflammatory and oxidative stress in a mouse model investigating responses to diesel exhaust particle exposure (402). Further experimental work is required to determine if the same timeline applies with NRF2 upregulation with exercise in asthma models of inflammation.

7.3.7 Summary

Overall, the results presented herein are reflected in the small number of clinical trials looking at exercise training in the context of asthma (5, 6, 446), with this study demonstrating a larger number of improvements in objective clinical parameters. This work is novel in that it demonstrates improvements in asthma symptoms, lung function and systemic inflammation in one cohort, with significant improvements in downstream markers of redox regulation and strong correlations suggesting an association between

levels of NRF2 expression and asthma symptoms, inflammation and lung function. However, the lack of demonstration of significant change in NRF2 over the course of the intervention and study design limitations mean further work is required to support or refute the hypothesis that is central to this thesis. The only other published work investigating interval training in asthma did not demonstrate any changes in systemic or airways inflammation, and the significant improvements in asthma symptoms (ACQ) were only evident in the combined intervention of diet and exercise, not exercise alone (280).The efficacy and immunomodulatory capacity of this exercise training programme has been demonstrated in other diseases (308) and it may be that interval training is the more optimal exercise training programme for disease modification in inflammatory diseases. This hypothesis, however, requires further assessment.

7.4 Future plans

For the current data set, I plan to analyse stored saliva and exhaled breath as an additional compartment to investigate changes in redox buffering and their role in the mechanism driving exercise induced improvements in asthma related symptoms and inflammation. I also intend to analyse the additional lung function measures of FOT and MBNW as more sensitive measures of lung function to assess whether these improve with exercise intervention, and whether there is any relationship to the redox regulation changes discussed in this thesis. In addition, I will explore whether exercise can improve sensitivity to steroids through a) increases in Treg cells resulting in anti-inflammatory cytokines, and b) through redox related modification of the glucocorticoid receptor.

7.4.1 Future work investigating the role of exercise in modulation the redox regulation system in asthma

A fully powered, randomised controlled study in asthma patients to further investigate the results demonstrated in this thesis is planned. Based on the limitations discussed earlier in this chapter, consideration would need to be made for inclusion of both a sham treated asthma control group and a healthy exercise intervention group to identify whether improvements in asthma control were a result of the exercise intervention, and to determine if any redox regulation responses to the exercise intervention were asthma specific. Power calculations based on the data presented in this thesis suggest numbers of

between 26 and 86 are sufficient to demonstrate a statistically significant change in ACQ6 score (see table 7.1), which should also allow for further mechanistic investigation. Given the subjectivity of the symptom scores, consideration is also being given as to whether a study should also be powered to detect changes in lung function, bronchial hyperreactivity as assessed by methacholine challenge and in vitro measures of inflammation. It would be interesting to power for changes in the redox metabolome, but this is such an experimental area at present and has such inter individual variability, that this is unlikely to be possible. Based on the proof of concept presented in this thesis, I would structure the design of the project similarly in terms of the exercise intervention and sampling process. However, in the context of the COVID 19 pandemic, and also taking into consideration findings presented in Chapter 6 and feedback from PPI representatives (data not presented), consideration for out of hospital, in home training may be necessary. There is precedence for this concept of supervised, out of hospital training within the University of Southampton, with the development of the Safefit trial, which has successfully recruited over 100 participants, with the caveat that the out of hospital training intervention would need to be adapted to provide the same degree of controlled metabolic stress that the SRETP delivers.

Table 7.1 Power calculations based on a primary outcome of improvement in ACQ score, with examples of vaying detection levels, power and drop out rates.

Standard deviation of ACQ in control group	**Difference to detect**	**Power (%) (alpha= 0.05)**	**N (per group)**	**N (Total) allowing for 20% drop-out**
0.62 (Proof of concept study)	***0.5***	***85***	***29***	***74***
	0.5	90	34	86
	0.75	***85***	***14***	***36***
	0.75	90	16	40
	1.0	85	8	20
	1.0	90	10	26

To demonstrate causation between upregulation of NRF2 and reduction in asthma related inflammation and improvement in asthma related clinical outcomes, I would

include additional laboratory work demonstrating the absence of this response to exercise with models that block the NRF2 response.

7.4.2 Future work in COPD

There is suggestion that disruption of the redox regulation system plays a role in the pathogenesis of COPD, and pulmonary rehabilitation has been shown time and again to provide benefit in this context. Animal models suggest a role for NRF2 in the mechanism of this effect (447).Investigation of the mechanism of effect of of exercise in COPD clinical outcomes is planned, with an initial pilot based on the Safefit approach and following an exercise intervention and sampling schedule similar to the approach used for the study presented herein.

7.4.3 Exercise in asthma as part of routine clinical care

The ultimate aim would be for exercise as a disease modifying intervention to be incorporated into routine asthma guidelines, both in primary and secondary or tertiary settings. The scientific rationale for this would be demonstrated in the studies planned for further work, based upon the hypotheses described in this thesis.

7.5 Summary

This preliminary study has shown that interval exercise training in the context of symptomatic asthma is safe and tolerable, and able to improve symptom scores and quality of life measures alongside reductions across a number of inflammatory cell types and improvement in lung function. I have further demonstrated improvement in markers of physical fitness and redox regulation, and demonstrated association between changes from these through to clinical asthma outcomes with exercise intervention. Further, definitive work is planned in asthma and explorative work to assess whether the results presented in this thesis are transferrable to COPD are planned, all with consideration of the context of the global COVID 19 pandemic. This further work aims to better understand the mechanism through which exercise may be anti-inflammatory in symptomatic asthma, and may aid the identification and development of novel drug targets to improve outcomes for patients.

Appendix A Asthma Control Questionnaire

ASTHMA CONTROL QUESTIONNAIRE (ACQ)

ENGLISH VERSION FOR THE UK

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For further information:

Chapter 7

Elizabeth Juniper, MCSP, MSc
Professor

20 Marcuse FieldsBosham,
West Sussex PO18 8NA,
England

Telephone: +44 1243 572124

Fax: +44 1243 573680

E-mail: juniper@qoltech.co.uk
Web: http://www.qoltech.co.uk

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Senior Translator: Pr Elizabeth Juniper

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Please answer questions 1 - 6.

Circle the number of the response that best describes how you have been during the past week.

1. On average, during the past week, how often were you **woken by your asthma** during the night?

0 Never
1 Hardly ever
2 A few times
3 Several times
4 Many times
5 A great many times
6 Unable to sleep because of asthma

2. On average, during the past week, how **bad were your asthma symptoms when you woke up** in the morning?

0 No symptoms
1 Very mild symptoms
2 Mild symptoms
3 Moderate symptoms
4 Quite severe symptoms
5 Severe symptoms
6 Very severe symptoms

3. In general, during the past week, how **limited were you in your activities** because of your asthma?

0 Not limited at all
1 Very slightly limited
2 Slightly limited

3 Moderately limited
4 Very limited
5 Extremely limited
6 Totally limited

4. In general, during the past week, how much **shortness of breath** did you experience because of your asthma?

0 None
1 A very little
2 A little
3 A moderate amount
4 Quite a lot
5 A great deal
6 A very great deal

5.	In general, during the past week, how much of the time did you **wheeze?**	0 Never 1 Hardly any of the time 2 A little of the time 3 A moderate amount of the time 4 A lot of the time 5 Most of the time 6 All the time
6.	On average, during the past week, how many **puffs/inhalations of short-acting bronchodilator** (eg. Ventolin/Bricanyl) have you used each day? *(If you are not sure how to answer this question, please ask for help)*	0 None 1 1 - 2 puffs/inhalations most days 2 3 - 4 puffs/inhalations most days 3 5 - 8 puffs/inhalations most days 4 9 - 12 puffs/inhalations most days 5 13 - 16 puffs/inhalations most days 6 More than 16 puffs/inhalations most days

To be completed by a member of the clinic staff

7. FEV_1pre-bronchodilator: .....

FEV_1predicted: .....

FEV_1%predicted: .....

(Record actual values on the dotted lines and score the FEV_1 %predicted in the next column)

0 > 95% predicted 1 95 - 90%

2 89 - 80%

3 79 - 70%

4 69 - 60%

5 59 - 50%

6 < 50% predicted

Appendix B Asthma Quality of Life Questionnaire

ACTIVITIES

We should like you to think of ways in which asthma limits your life. We are particularly interested in activities that you still do, but which are limited by your asthma. You may be limited because you do these activities less often, or less well, or because they are less enjoyable. These should be activities which you do frequently and which are important in your day-to-day life. These should also be activities that you intend to do regularly throughout the study.

Please think of all the activities which you have done during the last 2 weeks, in which you were limited as a result of your asthma.

HOW **LIMITED** HAVE YOU BEEN DURING THE LAST 2 WEEKS IN THESE ACTIVITIES?

	Totally Limited	Extremely Limited	Very limited	Moderate limitation	Some Limitation	A little Limitation	Not at all Limited	Activity Not Done
	1	2	3	4	5	6	7	
1. _____	☐	☐	☐	☐	☐	☐	☐	☐
2. _____	☐	☐	☐	☐	☐	☐	☐	☐
3. _____	☐	☐	☐	☐	☐	☐	☐	☐
4. _____	☐	☐	☐	☐	☐	☐	☐	☐
5. _____	☐	☐	☐	☐	☐	☐	☐	☐

HOW MUCH **DISCOMFORT OR DISTRESS** HAVE YOU FELT OVER THE LAST 2 WEEKS?

	A Very Great Deal	A Great Deal	A Good Deal	Moderate	Some	Very Little	None
6. How much discomfort or distress have you felt over the last 2 weeks as a result of CHEST TIGHTNESS?	1	2	3	4	5	6	7
	☐	☐	☐	☐	☐	☐	☐

IN GENERAL, **HOW MUCH OF THE TIME** DURING THE LAST 2 WEEKS DID YOU:

	All of the Time	Most of the Time	A Good Bit of the Time	Some of the Time	A Little of the Time	Hardly Any of the Time	None of the Time
7. Feel CONCERNED ABOUT HAVING ASTHMA?	☐	☐	☐	☐	☐	☐	☐
8. Feel SHORT OF BREATH as a result of your asthma?	☐	☐	☐	☐	☐	☐	☐
9. Experience asthma symptoms as a RESULT OF BEING EXPOSED TO CIGARETTE SMOKE?	☐	☐	☐	☐	☐	☐	☐
10. Experience a WHEEZE in your chest?	☐	☐	☐	☐	☐	☐	☐
11. Feel you had to AVOID A SITUATION OR ENVIRONMENT BECAUSE OF CIGARETTE SMOKE?	☐	☐	☐	☐	☐	☐	☐

HOW MUCH **DISCOMFORT OR DISTRESS** HAVE YOU FELT OVER THE LAST 2 WEEKS?

	A Very Great	A Great Deal	A Good Deal	Moderate Amount	Some	Very Little	None
	1	2	3	4	5	6	7
12. How much discomfort or	☐	☐	☐	☐	☐	☐	☐

IN GENERAL, **HOW MUCH OF THE TIME** DURING THE LAST 2 WEEKS DID YOU:

	All of the Time	Most of the Time	A Good Bit of the Time	Some of the Time	A Little of the Time	Hardly Any of the Time	None of the Time
	1	2	3	4	5	6	7
13. Feel FRUSTRATED as a result of your asthma?	☐	☐	☐	☐	☐	☐	☐
14. Experience a feeling of CHEST HEAVINESS?	☐	☐	☐	☐	☐	☐	☐
15. Feel CONCERNED ABOUT THE NEED TO USE MEDICATION for your asthma?	☐	☐	☐	☐	☐	☐	☐
16. Feel the need to CLEAR YOUR THROAT?	☐	☐	☐	☐	☐	☐	☐
17. Experience asthma symptoms as a RESULT OF BEING EXPOSED TO DUST?	☐	☐	☐	☐	☐	☐	☐
18. Experience DIFFICULTY BREATHING OUT as a result of your asthma?	☐	☐	☐	☐	☐	☐	☐
19. Feel you had to AVOIDA SITUATION OR ENVIRONMENT BECAUSE OF DUST?	☐	☐	☐	☐	☐	☐	☐
20. WAKE UP IN THE MORNING WITH ASTHMA SYMPTOMS?	☐	☐	☐	☐	☐	☐	☐

21. Feel AFRAID OF NOT HAVING YOUR ASTHMA MEDICATION AVAILABLE?	☐	☐	☐	☐	☐	☐	☐
22. Feel bothered by HEAVY BREATHING?	☐	☐	☐	☐	☐	☐	☐

IN GENERAL, **HOW MUCH OF THE TIME** DURING THE LAST 2 WEEKS DID YOU:

	All of the Time 1	Most of the Time 2	A Good Bit of the Time 3	Some of the Time 4	A Little of the Time 5	Hardly Any of the Time 6	None of the Time 7
23. Experience asthma symptoms as a RESULT OF THE WEATHER OR AIR POLLUTION OUTSIDE?	☐	☐	☐	☐	☐	☐	☐
24. Were you WOKEN AT NIGHT by your asthma?	☐	☐	☐	☐	☐	☐	☐
25. AVOID OR LIMIT GOING OUTSIDE BECAUSE OF THE WEATHER OR AIR POLLUTION?	☐	☐	☐	☐	☐	☐	☐
26. Experience asthma symptoms as a RESULT OFBEING EXPOSED TO STRONG SMELLS OR PERFUME?	☐	☐	☐	☐	☐	☐	☐
27. Feel AFRAID OF GETTING OUT OF BREATH?	☐	☐	☐	☐	☐	☐	☐
28. Feel you had to AVOID A SITUATION OR ENVIRONMENT BECAUSE OF STRONG SMELLS OR PERFUME?	☐	☐	☐	☐	☐	☐	☐

29. Has your asthma INTERFERED WITH GETTING A GOOD NIGHT'S SLEEP? ☐ ☐ ☐ ☐ ☐ ☐ ☐

30. Have a feeling of FIGHTING FOR AIR? ☐ ☐ ☐ ☐ ☐ ☐ ☐

HOW LIMITED HAVE YOU BEEN DURING THE LAST 2 WEEKS?

	Severely Limited Most Not Done	Very Limited	Moderately Limited Several Not Done	Slight Limited	Very Slightly Limited Very Few Not Done	Hardly Limited At All	Not Limited Have Done All Activities
	1	2	3	4	5	6	7
31. Think of the OVERALL RANGE OF ACTIVITIES that you would have liked to have done during the last 2 weeks. How much has your range of activities been limited by your asthma?	☐	☐	☐	☐	☐	☐	☐

	Severely Limited Most Not Done	Very Limited	Moderately Limited Several Not Done	Slight Limited	Very Slightly Limited Very Few Not Done	Hardly Limited At All	Not Limited Have Done All Activities
	1	2	3	4	5	6	7
32. Overall among ALL THE ACTIVITIES that you have done during the last 2 weeks how limited have you been by your asthma?	☐	☐	☐	☐	☐	☐	☐

Appendix B

DOMAIN CODE:

Symptoms: 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 29, 30

Activity Limitation: 1, 2, 3, 4, 5, 11, 19, 25, 28, 31, 32

Emotional Function: 7, 13, 15, 21, 27

Environmental Stimuli: 9, 17, 23, 26

Appendix C Exercise Therapy Burden Questionnaire

Exercise Therapy Burden Questionnaire

Your physician has asked you to play sports, to exercise on your own or to attend sessions with a physiotherapist in order to treat your condition.

Here are some of the statements we have heard from other patients about difficulties or constraints in following the recommendations or prescriptions they were given about physical exercise in treating their condition. We call "burden" all of these difficulties and constraints that can hinder you in carrying out your exercises.

For each statement, please select a number from 0 to 10 to indicate how some of the difficulties or constraints expressed by these patients also affect you in performing your exercises. The number you choose will express how much the stated proposition is a difficulty or a constraint to your physical exercises.

Please tell us first what is the main prescribed or recommended physical activity you are doing :

☐ *Sports* ☐ *Physiotherapy* ☐ *Home based exercise program*

1. The exercises cause me pain :

Not at all	☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ 0 1 2 3 4 5 6 7 8 9 10	All the time

2. The exercises cause me fatigue :

Not at all	☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ 0 1 2 3 4 5 6 7 8 9 10	All the time

3. I get bored when I exercise (too much repetition, not enough fun) :

Not at all	☐ ☐ ☐ ☐ ☒ ☐ ☐ ☐ ☐ ☐ ☐ 0 1 2 3 4 5 6 7 8 9 10	All the time

4. The exercises to achieve in my program are too difficult :

Not at all	☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ 0 1 2 3 4 5 6 7 8 9 10	All the time

5. I waste too much time exercising :

Not at all	☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ 0 1 2 3 4 5 6 7 8 9 10	All the time

6. Exercising reminds me of my condition :

Not at all	☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ 0 1 2 3 4 5 6 7 8 9 10	All the time

7. I lack support to exercise :

Not at all	☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ 0 1 2 3 4 5 6 7 8 9 10	All the time

8. I lack motivation to exercise :

Not at all	☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ 0 1 2 3 4 5 6 7 8 9 10	All the time

9. The exercises that I am asked to do are not adapted to my physical activity objectives

Not at all	☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ 0 1 2 3 4 5 6 7 8 9 10	All the time

10. I feel that exercising is not efficient in my case :

Not at all	☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ 0 1 2 3 4 5 6 7 8 9 10	All the time

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