UNIVERSITY OF
LIVERPOOL

Weight loss in obese patients with asthma: mechanical or immunological mechanisms?

Thesis submitted in accordance with the requirements of the University of Liverpool for the degree of Doctor in Philosophy by

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November 2012.

I declare that this thesis entitled:

"**Weight loss in obese patients with asthma: mechanical or immunological mechanisms?**"

is entirely my own work.

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Acknowledgements

I would like to thank my supervisors Prof Calverley and Prof Wilding for all their encouragement and support. I would also like to thank Jacquie Currie for her incredible help with logistics and all of the team in University Hospital Aintree lung function department especially Dr Sandy Jack for their support and companionship.

Thanks to Jenny Hawkes for all her help within the lab at the clinical sciences building in University Hospital Aintree in sourcing my lab materials and the team in the pathology department for allowing me to use their facilities to prepare my slides. Thanks also to all the dieticians involved.

I would like to thank staff at the Institute for Lung Health Leicester and Montreal General Hospital for their help training me to perform induced sputum, its processing and performing the differential cell counts.

Thanks go to my research and clinical colleagues at University Hospital Aintree. There are many individuals who I do not have space to mention but know who they are to whom I am extremely grateful for their support to get this done when I have felt like quitting.

Lastly and most importantly I would like to thank my wife Jo for her ongoing support and understanding when I have needed time for this work. Also my two sons Ben and Sam who were both born during the thesis writing and have had to put up with their Dad sitting in front of a computer during their holidays.

Abstract

Background: Asthma is characterised by symptoms and variable airflow obstruction as a result of airway inflammation. Obesity is defined as the accumulation of excessive body fat over daily metabolic demands resulting in a body mass index (BMI) over 30Kg/m^2. Both are increasing in prevalence and have been epidemiologically linked. This has led to suggestions that asthma severity may be influenced by adverse metabolic effects of excess adipose tissue or alterations to the mechanics of breathing in obesity. Whether the asthma severity can be improved by medical weight loss is less clear. In this thesis I aim to explore the hypothesis that "*Medical weight loss improves asthma severity in obese asthmatics.*"

Methods: Obese subjects with a prior physician diagnosis of asthma on inhaled medication were randomised to either a medical weight loss (dietician) or a control group. Measures of generic and disease specific health related quality of life, airway inflammation and bronchial responsiveness were measured at baseline, 3 and 6 months using Short Form-36, St George's Respiratory Questionnaire and the Impact of Weight on Quality of Life – Lite questionnaires plus bronchial responsiveness to methacholine avoiding deep inspiratory manoeuvres, exhaled nitric oxide and induced sputum differential cell counts.

Results: 397 subjects were screened for obesity and asthma. Of 91 subjects tested for bronchial responsiveness 36.3% did not demonstrate bronchial responsiveness and were excluded. There was no significant difference in disease specific Health Related Quality of Life between those with and without significant bronchial responsiveness. There was a significant correlation between HRQoL and BMI but no relationship with other measures of asthma severity. 51 patients with obesity and asthma with bronchial hyperresponsiveness were randomised into the dietician group (26) and control group (25). Both groups achieved weight loss, reaching significance in the dietician arm at 3 and 6 months with no significant difference between groups. Similar proportions of patients achieved clinically significant weight loss (≥5% baseline) in both groups. HRQoL scores improved at 3 and 6 months with no significant differences between groups and no correlation with BMI or % weight lost. There was no significant difference between groups for induced sputum differential cell counts, exhaled nitric oxide or bronchial responsiveness. There was also no correlation between change in weight and these variables.

Conclusion: In a population of obese asthmatics on medication there was a significant effect on health related quality of life influenced by BMI rather than asthma severity. Moderate weight loss was achieved with medical intervention, but there were no clear relationships between BMI, markers of airway inflammation or airway responsiveness and weight loss did not improve measures of asthma severity. The effect of obesity on asthma is complicated and further studies are required to investigate the interaction between lung volumes, symptoms and inflammation.

Publications and presentations

Publications:

Scott S, Currie J, Albert P, Calverley P, Wilding JP. Risk of misdiagnosis, health related quality of life and BMI in patients who are overweight with doctor diagnosed asthma. *Chest.* 2012 Mar;141(3):616-24

Oral presentations:

Asthma and obesity. Mersey and Northwest Deanery SpR meeting.November 2006

Symptoms are poor indicators for the presence of asthma in obese adults measured by SGRQ and SF36. To the American Thoracic Society International Conference 2006.
Am J Respir Crit Care Med 2006;3:A527

Benefits of weight loss in obese patients with asthma: mechanical or immunological mechanisms? University of Liverpool postgraduate student seminars 2007.

Obesity and anaesthesia. 18th Anaesthesia, Intensive Care Medicine and Pain management update. Belle Plagne. 2007

Asthma & Obesity: Second Midlands and North England Asthma Meeting. Sheffield 2009

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Poster presentations

Stephen Scott, Paul Albert, Jacqui Currie, Pam Parry, Peter Calverley, John Wilding. A prospective randomised control trial of medical weight loss in obese asthmatics
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Abbreviations

AHR – Airway hyperresponsiveness

ANOVA – Analysis of variance

ATS – American Thoracic Society

AQLQ – Asthma Quality of Life Questionnaire

BAL – Bronchoalveolar lavage

BDP – Beclomethasome dipropionate

BMI – Body Mass Index

BR – Bronchial responsiveness

BRI – Bronchial response index

C_{air} –Airway concentration

C_{alv} – Alveolar concentration

Ccw – Compliance of the chest wall

$CD4^+$ - Cluster of differentiation 4

C_E – Expiratory concentration

cGMP – Cyclic guanosine monophosphate

C_I – Inspiratory concentration

CO – Carbon monoxide

CO2 – Carbon dioxide

CO2RT – Carbon dioxide recovery time after work

COPD – Chronic obstructive pulmonary disease

CRP – C reactive protein

CT – Computed tomography

DLCO – Diffusing capacity of the lung for carbon monoxide

DLCO/VA – Transfer coefficient of the lung

D-PBS – Dulbecco's phosphate buffered saline

DRS – Dose response slope

DSPC – Disaturated phosphatidylcholine

DTT – Dithiotreitol

ECO2 – Carbon dioxide output of work

ECP – Eosinophil cationic protein

EELV – End expiratory lung volume

EILV – End inspiratory lung volume

eNOS – Endothelail nitric oxide synthase

EO2 – Oxygen cost of work

ERS – European respiratory society

ERV – Expiratory reserve volume

EV – Ventilatory cost of work

FBC – Full blood count

FEF_{25-75} – Forced expiratory flow between 25 to 75% of forced vital capacity

FcεRII – Low affinity receptor for IgE

FeNO – Fraction of exhaled nitric oxide

$FeNO_{50}$ - Fraction of exhaled nitric oxide at 50ml/s flow rate

FEV_1 – Forced expiratory volume in 1 second

FFMI – Fat free mass index

FOT – Forced oscillometry technique

FRC – Functional residual capacity

FVC – Forced vital capacity

Gaw – Airway conductance

GINA – Global initiative for asthma

GOAL – Gaining optimal asthma control

GP – General practitioner

HRQoL – Health related quality of life

IC –Inspiratory capacity

IFNγ – Interferon γ

IgE – Immunoglobulin E

IL* – Interleukin* (where * is a number for example IL4)

iNOS – Inducible nitric oxide synthase

IQR – Interquartile range

IRV – Inspiratory reserve volume

IWQOL-Lite – Impact of weight on quality of life questionnaire-Lite

ISRCTN – International standard randomised controlled trial number

$J_{t:g,air}$ – Total flux of nitric oxide from tissue to air

$J_{t:g,alv}$ – Total flux of nitric oxide from alveolar tissue

KC – Kupffer cells

Kg – Kilogram

L – Lung

LCD – Low calorie diet

M – Metre

MBP – Major basic protein

MCP – Macrophage chemotactic protein

MCS – Mental health component summary (SF36)

MIP-2 – Macrophage inflammatory protein

MMD – Mean mass diameter

MMEF – Maximal mid expiratory flow

MMP – Matrix mettaloproteinase

MRC – Medical research council

MRI – Magnetic resonance imaging

mRNA – Messenger ribonucleic acid

MVV – Maximal voluntary ventilation

NAASO – North American association for the study of obesity

NADPH – Nicotinamide adenine dinucleotide phosphate

NICE – National institute of clinical excellence

nM – Nanomolar

nNOS – Neuronal nitric oxide synthase

NO – Nitric oxide

NO^- - Nitroxyl

$NO2^-$ - Nitrite

NOS – Nitric oxide synthase

eNO – Exhaled nitric oxide

cNOS – Constitutive nitric oxide synthase

eNOS – Endothelial nitric oxide synthase

iNOS – Indicible nitric oxide synthase

O2 – Oxygen

OR – Odds ratio

PAF- Platelet activating factor

PC_{20} – Provocative concentration of a substance to produce a 20% fall in FEV1

PC_{45} - Provocative concentration of a substance to produce a 45% fall in sGaw

PCS – Physical health component summary (SF36)

Pcw – Pressure chest wall

PD_{20} – Provocating dose to induce a fall in FEV_1 of 20%

PEF – Peak expiratory flow

PEFR – Peak expiratory flow rate

PEmax – Maximum expiratory pressure

PImax – Maximum inspiratory pressure

P_L – Pressure lung

PmPeak – Peak pressure at the mouth

PMR – Partial meal replacement

PO2 – Partial pressure of oxygen

PPB- Parts per billion

Prs – Pressure respiratory system

RANTES – Regulation on activation, normal T expressed and secreted

Raw – Airway resistance

RCD – Reduced calorie diet

rs – Respiratory system

RV – Residual volume

SABA – Short acting beta agonist

SD – Standard deviation

SF12 – Short form 12

SF36 – Short form 36

sGaw – Specific airway conductance

SGRQ – St George's respiratory questionnaire

SMART – Symbicort maintenance and reliever therapy

SPSS – Statistical package for the social sciences

SST – Serum separating tube

T - Time

TGF-β – Transforming growth factor beta

Th – T helper cell

TLC – Total lung capacity

TLCO – Transfer factor for carbon monoxide

TNFα – Tumour necrosis factor alpha

TOOLS – Task force on developing obesity outcomes and learning tools

TSH – Thyroid stimulating hormone

TV – Tidal volume

U & E – Urea and electrolytes

V - Volume

VA – Alveolar volume

V_{air} – Airway volume

V_{alv} – Alveolar volume

VC – Vital capacity

V_E – Ventilation expiratory flow

VE/O2 – Ventilation equivalent for O2

V_I – Ventilation inspiratory flow

VLCD – Very low calorie diet

VO2 – Oxygen uptake

VO2max – Maximal oxygen consumption

V_r – Volume at rest

VTh – Ventilatory threshold

Chapter 1. Introduction

1.1 Overview

Obesity is a risk factor for the development of asthma[1]. Possible mechanisms include the effects of obesity on the mechanical aspects of respiration, symptoms and also the effect of an induced systemic inflammatory state associated with obesity which may influence the local respiratory tract inflammatory processes leading to airway narrowing through bronchial responsiveness[2].

Work is needed to understand these processes in more detail, primarily by studying changes in airway responsiveness, inflammatory markers and symptoms that occur with weight change.

Many studies have been cross sectional and have relied on subjective measures to obtain a diagnosis of asthma[3]. Furthermore, longitudinal weight loss studies have generally been based on relatively small numbers of patients undergoing surgical weight loss procedures without objectively diagnosing asthma[4-7].

This research proposes to explore the effect of obesity on symptoms and quality of life, airway responsiveness and non invasive markers of airway inflammation in subjects with objectively proven asthma. It also explores how these variables relate to medically achieved weight loss comparing an intervention group with a control.

1.1.1 Hypotheses:

1. **Impairment in health related quality of life (HRQoL) in obese asthmatics is better explained by the degree of obesity rather than measures of asthma severity such as airway hyper-responsiveness.**
2. **A higher BMI leads to increases in asthma severity measured by airway responsiveness which improves with weight loss.**
3. **Change in BMI in obese asthmatics will be associated with changes in HRQoL.**
4. **Obese asthmatics are more likely to lose weight when receiving a specific weight loss programme than asthmatics offered standard weight loss advice.**
5. **Change in BMI in obese asthmatics will be associated with changes in measures of airway inflammation.**
6. **Change in BMI in obese asthmatics will be associated with changes in airway responsiveness and specific airway conductance.**

1.2 Asthma

The Health Survey for England gave an overall prevalence of a combined variable of recent wheeze, asthma diagnosis ever and treatment for asthma of 8.1% [8] and the global prevalence of asthma ranges from 1% to 18% of the population in different countries [9, 10] (GINA 2006).

Asthma is a disease of the airways and diagnosis is clinical.[11] Although there is no agreed definition of asthma, the International Consensus Report describes asthma as "*a chronic inflammatory disorder of the airways... in susceptible individuals inflammatory symptoms are usually associated with widespread but variable airflow obstruction and an increase in airway response to a variety of stimuli. Obstruction is often reversible, either spontaneously or with treatment*"[12].

The Global Initiative for Asthma uses a descriptive definition of asthma as "*Asthma is a chronic inflammatory disorder of the airways in which many cells and cellular elements play a role. The chronic inflammation is associated with airway hyper-responsiveness that leads to recurrent episodes of wheezing, breathlessness, chest tightness, and coughing particularly at night or in the early morning. These episodes are usually associated with widespread, but variable airflow obstruction within the lung that is often reversible either spontaneously or with treatment.*" This lack of a working definition for asthma presents problems for the study of asthma[13] and the identification of asthma includes a variety of methods.

The fundamental problem in asthma is narrowing of airways caused by hyper-responsiveness of airway smooth muscle, airway wall thickening and mucous hypersecretion which can lead to recognizable symptoms and signs.

The driving factor for these changes is airway inflammation usually driven by an allergic type response mediated by IgE, mast cells and eosinophils[14]. Various clinical tests can detect these factors.

Asthma shares many symptoms in common with other diseases of the respiratory system but also in other systems such as cardiovascular disease. Features of an airway disorder such as cough, wheeze and breathlessness should be corroborated where possible with measurement of airflow limitation[15].

As asthma is a multifactorial condition then we need multiple measures to monitor its severity. Asthma severity can be monitored by symptoms, tests of bronchial responsiveness and measures of airway inflammation. To understand these processes it is necessary to explain the pathology of the condition.

1.2.1 Symptoms.

Symptoms of asthma are common amongst a variety of diseases but the character of symptoms can indicate a diagnosis of asthma. These include: wheeze, shortness of breath, chest tightness and cough. The characteristics that suggest asthma as a diagnosis being: variability, intermittent, worse at night and provocation by triggers or exercise[15].

Other factors to consider for a diagnosis of asthma include a personal or family history of asthma or other atopic conditions, deterioration after exposure to triggers and worsening of symptoms after taking aspirin / non-steroidal anti-inflammatory medication or the use of β-blockers.

1.2.2 Signs.

As asthma is an episodic disease, signs may be absent for most of the time. However, during exacerbations, patients may have wheeze which is usually diffuse, polyphonic, bilateral and particularly expiratory. Lung function will be reduced with an obstructed pattern on spirometry and a reduced peak expiratory flow[12].

1.2.3 Pathology.

There is widespread acknowledgement that asthma is caused by a chronic inflammatory response in the airways[13] and this leads to the pathological and clinical features of asthma. The syndrome of asthma arises from a number of poorly understood inducing stimuli, such as allergens and chemicals, in a group of patients who are in some way genetically predisposed[16]. The patient with asthma is 'primed' or at risk of severe bronchospasm if exposed to trigger factors and may show markedly heightened responses to direct bronchoconstrictor agents[17].

Histological specimens from lungs of asthmatics show shortening of the airway musculature and inflammatory oedema of the whole airway, particularly the submucosal layer[18]. There is thickening of the epithelial basement membrane and damage to the bronchial epithelial lining with desquamation that exposes the epithelial basement membrane. Excessive mucus production occurs due to hypertrophy and hyperplasia of submucus glands and goblet cells. The muscularis layer shows smooth muscle cell hypertrophy and hyperplasia and there is microvascular dilatation in the adventitial layer. All layers of the airway reveal intense infiltration from

inflammatory and immunological cells in the form of granulocytes, especially eosinophils with evidence of degranulation and disgorgement of highly histotoxic products such as major basic protein (MBP). There are also infiltrations of chronic inflammatory mononuclear cells, including T lymphocytes, particularly CD4+ cells[19].

1.2.4 Asthma and inflammation.

Asthma is characterised by a specific pattern of inflammation that is largely driven via immunoglobulin (Ig)E-dependent mechanisms[20]. The airway wall is oedematous and infiltrated with inflammatory cells, which are predominantly eosinophils, lymphocytes, activated mast cells and T-lymphocytes. There is vasodilatation, plasma exudation, oedema and sensitisation with activation of sensory nerves.

Although most asthmatics are atopic, some have normal total and specific IgE and negative skin tests. This "intrinsic" asthma is usually late in onset and more severe[21]. It has a similar pathophysiology to allergic asthma with evidence of local IgE production, possibly directed at bacterial or viral antigens[22]. The inflammation leads to increased symptoms directly by causing cough and chest tightness by activation of airway sensory nerve endings and also indirectly by increased airway hyper-responsiveness. This inflammation persists over many years and can cause a chronic inflammatory state.

More recently different inflammatory phenotypes and endotypes[23] of asthma have been identified by cluster analysis of a heterogenous population with different asthma characteristics including inflammatory profiles[24, 25].

1.2.5 Inflammatory cells.

The precise role of all inflammatory cells is unknown and no single cell can account for the complex pathophysiology of allergic disease, but some cells predominate[26]. **Mast cells** in airway smooth muscle[27] secrete cytokines, such as interleukin (IL)-4 and tumour necrosis factor (TNF)-α[28]. **Macrophages** derived from blood monocytes activated by low affinity IgE receptors (FcεRII)[29, 30] produce many different products, including a large variety of cytokines and have an impaired anti-inflammatory role in asthma[31-34]. **Dendritic Cells** are specialised macrophage-like cells that promote differentiation of T-helper (Th)2 cells and eosinophilia important in the allergic response[35-37].

Eosinophils are characteristic of allergic inflammation and there is a correlation between eosinophil counts in peripheral blood or bronchial lavage and airway hyper-responsiveness[38]. Several mediators are involved in the migration of eosinophils from the circulation to the airway and prolong survival by avoiding apoptosis. **Neutrophils** are a more prominent cell type in airways and induced sputum of patients with more severe asthma[39-41] possibly due to rapid kinetics of neutrophil recruitment or steroid use[42-44], however it is possible that neutrophils are actively recruited in severe asthma, with increased levels of IL-8 due to increased levels of oxidative stress[40, 45]. The role of neutrophils is unknown but it is possible that they may be associated with reduced responsiveness to corticosteroids.

T-Lymphocytes coordinate the inflammatory response by release of specific patterns of cytokines, resulting in recruitment and survival of eosinophils and in the maintenance of mast cells in the airways[46]. Other cells

are present such as **B-Lymphocytes** that secrete IgE[47] and **Basophils** whose role is uncertain[48].

Lastly, **platelets** are activated and aggregated by Th2-mediated inflammation[49], whilst **structural cells** may also be an important source of inflammatory mediators[50-52].

1.2.6 Inflammatory mediators.

Various mediators are involved in asthma which produce the effects of airway smooth muscle contraction, increased microvascular leakage, increased airway mucous secretion and the attraction of inflammatory cells including lipid mediators such as cysteinyl-leukotrienes, PAF and prostaglandins which are potent constrictors of human airways and also have weak inflammatory effects[53-55]. Cytokines such as IL-4, IL-5, IL-12, IL-13 and IL-18 released from inflammatory cells are important in chronic inflammation and play a critical role in orchestrating the type of inflammatory response[56, 57]. Chemokines including eotaxins, RANTES and MCP-4 are involved in the recruitment of inflammatory cells in asthma[58] acting in sequence in determining the final inflammatory response and increasing airway hyper-responsiveness[59-61]. Reactive oxygen species causing oxidative stress result in increased concentrations of 8-isoprostane (a product of oxidised arachidonic acid) in exhaled breath condensates[45] and increased ethane (a product of oxidative lipid peroxidation)[62]. Endothelins induce airway smooth muscle cell proliferation and promote a profibrotic phenotype and may therefore play a role in the chronic inflammation in asthma[63-66]. Finally nitric oxide is produced

by NO synthases in several cells and this will be explored in more detail below[67, 68].

1.3 Markers of asthma severity

Measurement of inflammation in asthma.

I shall concentrate on two of the least invasive ways to measure inflammation in asthma as they have been employed in the methods. These are measurement of exhaled nitric oxide and the use of induced sputum.

1.3.1 Biology of Nitric Oxide

Nitric oxide (NO) is formed by the oxidation of the terminal guanidinium nitrogen on the amino acid L-arginine via an oxygen and nicotinamide adenine dinucleotide phosphate (NADPH) dependent mechanism producing L-citruline and nitroxyl (NO^-)[69] catalysed by the enzyme nitric oxide synthase (NOS)[70]. NOS is present in three isoforms, two constitutive and one inducible: 1.) constitutive neuronal NOS (NOS I or nNOS); 2.) inducible NOS (NOS II or iNOS); and 3.) constitutive endothelial NOS (NOS III or eNOS). All forms are expressed in the airways[71-75]. The constitutive form of NOS is released within seconds by a Ca^{2+} and calmodulin-dependent mechanism producing small amounts of NO in the range of fentomolar or picomolar concentrations after receptor stimulation by selective agonists. The inducible form is produced over a longer period in larger amounts (nM) after induction by proinflammatory cytokines at a pretranslational level. This may be many hours after stimulus and may continue for hours or days. Cytokines such as tumour necrosis factor-α (TNF-α), interferon-γ (IFN-γ), and interleukin (IL)-1β can stimulate

expression of iNOS[76] as well as exogenous factors such as bacterial toxins, virus infection, allergens, environmental pollutants, hypoxia, tumours[77-79] which can be reduced by corticosteroids[80].

The bioactivity of NO is divided into NO mediated/cGMP dependent such as smooth muscle relaxation and cGMP independent such as virus killing. The high level of NO released by iNOS has an effect as an immune effector molecule in killing tumour cells[81], in halting viral replication[82], and in eliminating various pathogens. NO may also inhibit pathogen virulence and replication by *S*-nitrosylation of cysteine proteases[83]. The release of NO activates soluble guanylyl cyclase and causes an increase in intracellular cGMP[84] and endogenously formed NO produces most of its effects by this mechanism. NO can also activate or inhibit other enzymes and NO itself can inhibit NOS activity directly or as a result of inhibition of the induction of iNOS[85].

NO is a highly reactive molecule and small amounts produced by constitutive enzymes are removed safely by reaction with haemoglobin, however when produced in larger amounts reactions of NO with other free radicals can lead to the production of toxic reactive nitrogen species e.g. nitrogen dioxide, peroxynitrite and dinitrogen trioxide which can produce toxic effects by potent oxidative actions[86, 87].

1.3.2 Exhaled Nitric Oxide and measurement

Nitric oxide (NO) as a marker of inflammation, can be measured in the exhaled breath in ppb by chemiluminescence analysers[88] and has been found to be increased in asthmatics compared to non-asthmatics[89]. It has also been shown to be useful in monitoring asthma to guide treatment[90].

The chemiluminescence reaction is based on the findings that ozone reacting with NO yields excited NO_2 which emits infrared light, which is directly proportional to the original NO levels, and the light (photons) can be counted by a photomultiplier tube[91]. NO-free inspired air must be used when exhaled NO measurements are performed to avoid possible effects of outdoor air pollution and NO in ambient air. Exhaled NO measurements can be affected by a number of factors and the avoidance of many of these confounding factors should be attempted prior to measurement[92].

The measurement of exhaled nitric oxide (eNO) is dependent on expiratory flow[93] and thus measurements must be standardised as suggested by international guidelines for the measurement of NO in adults and children[94-96]. The levels of NO in exhaled air are dependent on 1.) production of NO by cells in the airways of lung parenchyma, 2.) diffusion of NO into the capillary circulation, and 3.) alveolar ventilation and bronchial airflow.

NO measured from the mouth is from a combination of sources: the lower respiratory tract, nasal[97], NO from salivary NO_2^- and gastric regurgitation[98, 99]. Nasal NO can potentially contaminate gasphase NO during exhalation manoeuvres[96, 100] and therefore expiratory resistance to close the soft palate with raised mouth pressure is used during exhaled NO

measurements in spontaneously breathing subjects[94, 96]. To measure airway NO the plateau phase is taken after excluding the dead space [92].

It has been shown that NO production and expiratory NO can be predicted by a two-compartment model of the lung (Fig 1.), consisting of a nonexpansible compartment of the conducting airways and an expansible compartment of respiratory bronchioles and alveoli[101]. The model shows that both compartments contribute to exhaled NO and the relative contributions of airways and parenchyma can be separated by analysis of the relationship between exhaled NO output (nl/s) against expiratory flow rate (ml/s).

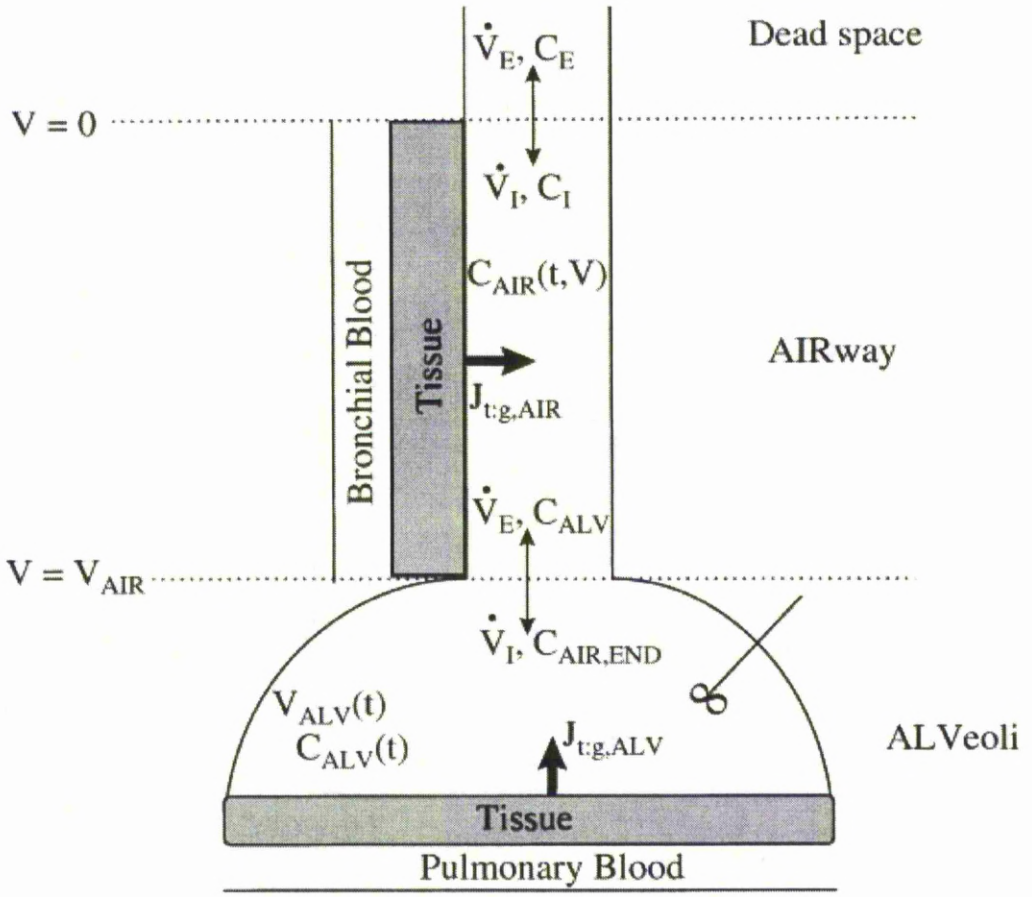


Fig 1. legend, Schematic of 2-compartment model for nitric oxide (NO) pulmonary exchange. First compartment represents relatively nonexpansile conducting airways; second compartment represents expansile alveoli. Each compartment is adjacent to a layer of tissue that is capable of producing and consuming NO. Exterior to tissue is a layer of blood that represents bronchial or pulmonary circulation and serves as an infinite sink for NO. V˙ E and V˙ I, expiratory and inspiratory flow, respectively; CE and CI, expiratory and inspiratory concentration, respectively; Cair and Calv, airway and alveolar concentration, respectively; Vair and Valv, airway and alveolar volume, respectively; *J*t:g,air and *J*t:g,alv, total flux of NO from tissue to air and from alveolar tissue, respectively; *t*, time; V, volume.

Fig. 1. Two compartment model of nitric oxide production in the airways[101] (Reproduced from: Tsoukias NM, George SC. A two-compartment model of pulmonary nitric oxide exchange dynamics. J Appl Physiol 1998;85(2):653-66.) Permission not required.

1.3.3 Exhaled NO and asthma

Exhaled NO has been shown to be raised in atopic asthma when compared to controls[89], and the source of NO is mostly generated in the lower airways[102] mainly by iNOS in airway epithelial and inflammatory cells[72]. There is a strong association between elevated levels of eNO and skin prick test scores, total IgE[103], and blood eosinophilia[104] in mild asthma. It has also been shown to be an indicator of asthma control[105] and asthma severity[106]. Thus eNO has been used to monitor asthma exacerbations[107] and the effect of anti-inflammatory therapy[106]. Corticosteroids may influence the levels of eNO by the reduction in asthmatic inflammation and also by direct inhibitory effects on iNOS itself. Oral and inhaled corticosteroids have been shown to result in a rapid and dose dependent reduction in exhaled NO[108].

NO levels may increase before any significant changes in other parameters, such as lung function and sputum eosinophils, and may therefore serve as an early warning of loss of control.

Exhaled NO in asthmatics is correlated with airway hyper-responsiveness to methacholine[109], as well as peak flow variability[110] and is also associated with eosinophilic inflammation as determined in blood[104], urine[111], bronchoalveolar lavage[110], and sputum[112].

Exhaled nitric oxide has also been shown to be increased by other chest diseases such as mild-moderate COPD, rhinitis, bronchiectasis, active pulmonary sarcoidosis, active fibrosing alveolitis, acute lung allograft rejection and acute viral lung infection. Exhaled nitric oxide is also affected by active smoking and levels are decreased in smokers compared with non-smokers.

To conclude; eNO is a novel non-invasive biomarker reflecting airway eosinophilic inflammation in asthma.

1.3.4 Induced sputum differential cell counts and asthma.

Inflammation of the airways can be assessed through biopsies obtained via bronchoscopy, this is however an invasive technique, involving a procedure that is uncomfortable to the patient and also carries a small risk of complications. Assessing the induced sputum of patients is a less invasive method for obtaining information about the underlying airway inflammation in the airways of asthmatics[113]. The inflammation in asthma is mainly eosinophilic[114] which is steroid responsive[115, 116]. Other types of inflammation may be present such as neutrophilic[24] which is less likely to respond to steroid treatment and different phenotypes of asthma have been identified based on symptoms and predominant cell types in induced sputum[25]. Pin et al[117] showed raised numbers of eosinophils and metachromatic cells in the sputum fraction from asthmatics compared with healthy subjects using a protocol based technique to induce sputum. This has also been shown to be reproducible and valid[114]. The technique has been refined to improve cell viability, reduce squamous contamination and provide reproducible differential cell counts and has also been shown to be safe, even in the presence of moderate to severe exacerbations[118]. Induced sputum cell counting is therefore established as a valuable tool in investigating airways disease.

Guidelines on methodology have been developed to obtain sputum by induction with hypertonic saline and standardise its examination[119]. A standardised method was shown to result in successful sputum induction in

76% of normal and asthmatic subjects who cannot produce sputum spontaneously[117] and if performed carefully with salbutamol premedication and FEV_1 monitoring is relatively safe even in those with airways disease[113].

Once sputum is obtained it must be processed to obtain cells for a cell count and supernatant for measurement of fluid phase components. Dithiotreitol (DTT) is used in the process to improve cell dispersion[120, 121]. This is a sulphydryl reagent that produces mucolysis by opening disulphide bonds which crosslink glycoprotein fibres and maintain sputum in its gel form[121]. Although this makes cell differentiation easier and quicker, it must be noted that it may affect the measurement of certain fluid phase components in the supernatant[115, 122-124].

There is some discussion surrounding the selection of sputum from the whole expectorate in that some of the mediators may be lost when saliva is excluded, however the quality of cytospins are better and significant dilution of mediators may happen in saliva.

Normal ranges for sputum cell counts have been published[113] (table 1.) and it has been shown that in asthmatics, eosinophils and metachromatic cells are increased[114], plus there is also a slight increase in the numbers of neutrophils. These sputum cell counts are highly repeatable with within subject repeatability of sputum eosinophil counts in subjects with asthma being such that 95% of repeated measures lie within the twofold range of the original measurement.

Cell	Normal range Median	Interquartile range
Total cell count ($x10^6$/ml)	3.1	4.0
Eosinophils (%)	0.5	1.1
Neutrophils (%)	24.1	26.8
Macrophages (%)	62.9	30.2
Lymphocytes (%)	1.3	1.6

Table 1. Normal ranges for sputum total cell count and differential inflammatory cell counts derived from 10 normal subjects[113]

There is a weak relationship between the severity of asthma as defined by lung function, airway responsiveness or symptoms and the sputum eosinophil count[125] and studies have shown that induced sputum can be used to monitor asthma control and guide treatment[126].

There has been conflicting evidence of correlation between functional airway parameters and sputum inflammatory cells and markers[127] although asthmatics with higher baseline sputum eosinophilia are more likely to exacerbate and a change in sputum eosinophilia correlates well with reduction in airway function heralding an exacerbation rather than symptom scores[128].

In chronic stable asthma compared to healthy subjects there are raised numbers of $CD4^+$ T lymphocytes expressing surface activation markers, reduced natural killer cells and raised B lymphocyte counts that correlate with sputum eosinophil counts. There are increased levels of secretory immunoglobulin IgA. Increased levels of cysteinyl-leukotrienes relate to asthma severity and a number of proinflammatory cytokines (IL-1b, IL-5, IL-6, TNFα, RANTES and IL-8) are raised with the reduction in levels of the anti-inflammatory cytokine IL-10. Levels of matrix metalloproteinases (MMPs) have also been shown to be raised [129].

During asthma exacerbations high numbers of neutrophils have been found[130], as well as high eosinophil counts that fall with treatment[116]. Sputum eosinophil counts and levels of ECP are related to the severity of asthma[131] and this has been shown for neutrophil counts also[40]. Sputum eosinophil counts have also been shown to be significantly inversely associated with methacholine and adenosine bronchial responsiveness[132, 133]. The role of eosinophils in the sputum of asthmatics has some doubt following the demonstration that sputum eosinophilia can be reduced by anti-IL-5 antibodies without reducing airway hyperresponsiveness[134]. There has been noted a group of asthmatics with a neutrophil predominant sputum differential cell count with evidence of symptoms, variable airflow obstruction with a sputum eosinophil cell count <1.9% described as non eosinophilic asthma. These patients are less likely to respond to inhaled corticosteroids and highlight that the type of inflammatory response in symptomatic patients may be heterogenous and more complex than previously thought[24].

Treatment strategies based on titrating treatment to induced sputum eosinophil counts have shown improvements in exacerbation rate and this further suggests that these measures are useful in monitoring asthma severity although there is little evidence that this is reflected in other traditional measures of asthma control such as FEV1, symptoms, AHR and SABA use[126].

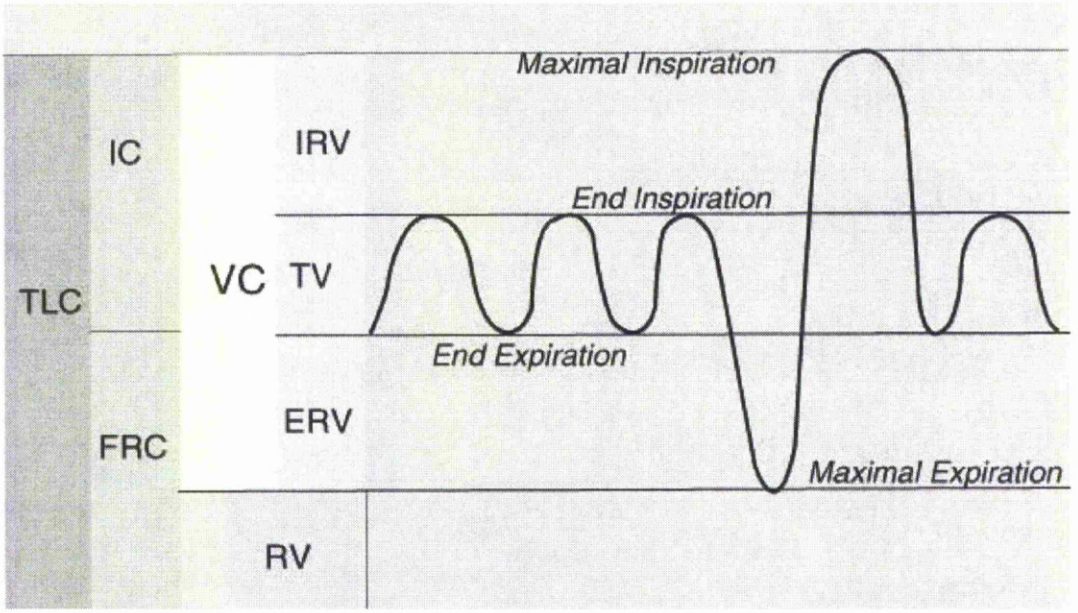
In summary sputum eosinophil counts may be useful in the diagnosis of asthma, predicting the response to corticosteroids, monitoring treatment and predicting exacerbations of disease[129]. They are therefore a useful

measure of asthma control as a marker of inflammation of the airways and a useful tool in assessing airway inflammation in research studies.

1.4 Lung function and airway obstruction.

Respiratory physiology can be measured in a number of ways to measure airflow, lung volumes and bronchial responsiveness. These can be affected variably in asthma and can range from normal to abnormal depending on whether the disease is controlled[14].

Static lung volumes are shown below in fig (2).



TLC – Total Lung Capacity, IC – Inspiratory capacity, FRC – Functional Residual Capacity, VC – Vital Capacity, IRV – Inspiratory Reserve Volume, TV – Tidal Volume, ERV – Expiratory Reserve Volume, RV – Residual Volume.

Fig. 2. Static lung volumes

In asthma these can be normal, however in those with airway narrowing due to airway smooth muscle activation, increased airflow obstruction and gas trapping there can be increases in residual volume (RV),

expiratory reserve volume (ERV) and total lung capacity (TLC)[135]. This can be measured using a spirometer into which the subject exhales from TLC until RV. This can measure the volume of air expelled over time resulting in the vital capacity (VC). Using body plethysmography in which a subject is enclosed in a sealed box and pressures measured at the mouth and outside the body, the TLC and FRC can be derived using Boyles law. From this all other measures including inspiratory reserve volume (IRV), expiratory reserve volume (ERV) and residual volume (RV) can be derived[136].

The characteristic feature of asthma is airway obstruction due to smooth muscle contraction, hypertrophy, remodelling, airway oedema and mucous plugging[11]. This can be measured by tests of airflow such as peak expiratory flow rate which is reduced in asthma and spirometry which measures volume exhaled over time[137]. The forced expiratory volume in 1 second (FEV_1) is reduced in uncontrolled asthma which can reverse with bronchodilators and the ratio with forced vital capacity (FVC) or FEV_1/FVC ratio is reduced as well as the flow at mid expiration or FEF_{25-75} or MMEF[138]. The peak expiratory flow rate can be measured serially over days by the patient and the variability of day to day peak flows can be used as a measure of asthma control with increased variability indicating worse control.

These ventilatory function tests reflect changes in airway resistance (Raw) and its reciprocal, airway conductance (Gaw) which can also be measured using body plethysmography[139] by measuring the pressure at the mouth and air flow. These in themselves can be affected by lung volumes and therefore it is important that measures are carried out to agreed and standardised protocols[17]. Airway resistance can be increased at lower lung

volumes due to a reduced airway diameter and vice versa, however specific airway conductance (sGaw) is expressed as conductance per unit of lung volume and therefore takes this into account[140, 141].

It is important that tests of expiratory flow rates are performed with a deep breath to total lung capacity and maximal effort is used by the subject. Measures of airway resistance are measured dependent on lung volume and are measured indirectly using body plethysmography at the same time as measuring lung volume to derive sGaw. In asthma, Raw is increased and its reciprocal Gaw and sGaw are decreased accordingly.

As asthma is characterised by variable airflow obstruction it is important to note that the changes in airway physiology described can vary from obstructed to normal and therefore it is important to check for variability in these measures. If airflow obstruction is noted then the measurements, if repeated after the application of a bronchodilating agent will return to normal. Alternatively if the measures are normal then a bronchoconstrictor challenge test can be performed as described in the next section[17].

It is important to note however, that a process of airway remodelling can take place in asthma in which the airway wall is thickened, smooth muscle is increased, mucous glands are increased, surface tension increases with increased inflammatory exudate and thickening of the reticular basement membrane occurs. These changes can become irreversible leading to fixed airflow obstruction that resembles chronic obstructive pulmonary disease that some describe as a chronic obstructive pulmonary disease-asthma overlap[19].

Lastly, transfer factor of the lung for carbon monoxide (TLCO) is a measure for the ease of molecules of carbon monoxide molecules to cross

from alveolar gas to the circulation. This is assessed using a single breath technique where a subject exhales to RV then inspires a test gas including carbon monoxide (CO) to TLC and holds their breath before exhaling into a sample bag. The change in CO can be used to calculate the TLCO adjusted for haemoglobin. The TLCO in asthma can be normal or increased due to hyperaemic airways and increased perfusion of the apices of the lungs due to increased pulmonary arterial pressure[142].

1.4.1 Airway responsiveness

Airway obstruction may be absent in well treated or mild asthmatics, however these patients may demonstrate increased smooth muscle tone or increased bronchial reactivity. Asthmatic airways become sensitive and stimuli can result in smooth muscle contraction and airway narrowing. This is the basis of bronchial challenge testing and the severity of responsiveness to challenge can be a marker for asthma and asthma severity or control[14].

Reversible airway obstruction as a result of hyper-responsiveness of bronchial wall smooth muscle is therefore characteristic of asthma and bronchial hyperesponsiveness is responsible for recurrent episodes of wheezing, breathlessness, chest tightedness, and coughing. This can be measured using bronchial provocation challenge testing, non-selectively, either directly or indirectly by exposing the airways to various stimuli[14]. Indirect challenges involve the use of chemical stimuli to initiate one or more of the intermediate steps leading to bronchoconstriction and direct challenges involve the use of substances such as muscarinic agonists (e.g. methacholine) to directly stimulate receptors on airway smooth muscle.

Challenge testing can be used to assist with making a diagnosis and to assess asthma control or severity, however airway hyper-responsiveness (AHR) to methacholine is not synonymous with asthma and its severity is not synonymous with asthma severity. Despite this the measurement of bronchial hyper-responsiveness to methacholine is accepted as a way of assessing asthma severity in clinical trials and a way of tracking change with intervention[143].

There is a correlation between asthma severity and the severity of AHR[144, 145] which improves with anti-inflammatory therapeutic strategies such as inhaled steroids[146]. There is a modest correlation between the severity of direct AHR and airway inflammation with mainly eosinophils or metachromatic cells[147]. There is also an increased response to direct stimuli with nonasthmatic airflow obstruction closely related to the severity of chronic bronchial obstruction felt to represent a geometric issue with regard to airway diameter[148].

There is felt to be two components to AHR, a variable and fixed component with the variable component being able to change with improvement in airway inflammation and the fixed component being related to structural and functional changes in the airway termed airway remodelling[149]. Although AHR is felt to be related to eosinophilic airway inflammation, some studies have dissociated the relationship between eosinophils and AHR. Studies using Mepolizumab an anti-interleukin-5 agent found that patients that had a reduction in eosinophils continued to have AHR and symptoms[134].

Historically, for diagnostic purposes asthma challenge tests target a significant change in FEV_1 with a 20% fall in FEV_1 being considered a positive

test and an arbitrary cut off to exclude significant bronchial responsiveness for most research studies set at 8mg/ml using increasing doses of methacholine.

Standardised methods have been developed to perform methacholine challenge tests[150]. a doubling concentration of methacholine is administered with assessment of the FEV_1. The dose of methacholine calculated to induce a 20% drop in FEV_1 is used to define bronchial responsiveness and is termed PC_{20}. Alternatively airway constriction can be measured using body plethysmography which can avoid deep inhalations to measure increase in airway resistance or its reciprocal, specific airway conductance (sGaw) and the cut off of a 45% drop in sGaw is used to produce PC_{45} which equates to PC_{20}. Two standardised methods are also described to administer methacholine, one requires deep inhalations and the other a tidal breathing method.

The speed or intensity of response to a bronchoconstricting agent has been shown to have a better relationship with HRQoL related to severity of asthma[151]. The slope of the dose-response curve has been shown to be more useful in identifying patients with asthma[152] and in maintaining a better relationship with the degree of oxidative stress of patients[153]. This stems from the observation that a plateau is reached in the dose response curve of non asthmatic individuals that is not present in asthmatics. As this cannot be measured safely in asthmatic subjects the slope of the dose response can be used instead. Therefore bronchial responsiveness of asthmatics can be expressed in terms of the provocative concentration to cause a 20% drop in FEV1 from baseline and also the strength of the response to a provocative stimuli.

It is important to note the caveat that increased bronchial responsiveness may not indicate "asthma" as bronchial hyper-responsiveness can be induced in normal subjects by restricting chest expansion and therefore eliminating the bronchoprotective effect of smooth muscle stretch in airways[154].

1.4.2 Airway remodelling and chronic obstructive pulmonary disease / asthma

As described briefly above, asthma traditionally is thought of as being a reversible airway obstruction characterised by eosinophilic airway inflammation. However it has also been noted that in some cases asthma may be present with fixed airflow obstruction secondary to airway remodelling as a result of chronic airway inflammation. This results in narrowing due to increased airway wall thickness. This can be due to increased smooth muscle thickness, increased inflammatory infiltrate causing increased airway stiffness, epithelial goblet cell hyperplasia and metaplasia. Thickening of the lamina reticularis occurs and proliferation of airway blood vessels and nerves. These changes are less responsive to corticosteroids and it can be difficult to determine the difference between asthma with airway remodelling and chronic obstructive pulmonary disease which has a different aetiology[155]. The two diseases may be differentiated by examination of histology from bronchial biopsy specimens and by determining a typical history such as a significant past history of smoking.

1.5 Symptoms and Health related quality of life in asthma.

Health related quality of life (HRQoL) is used to refer to the "physical, psychological, and social domains of health, seen as distinct areas that are influenced by a person's experiences, beliefs, expectations, and perceptions"[156]. HRQoL reflects an individual's subjective evaluation and reaction to health or illness[157] rather than a medical professionals evaluation. Tools such as the Short Form 36 (SF 36)[158] have been developed to measure HRQoL in subjects and therefore to try to quantify this for research purposes. HRQoL is recognised to be multidimensional and tools generally measure the functional ability, physical, emotional and social wellbeing of individuals[159, 160]. As HRQoL is generally poorly related to functional measures of asthma control such as lung function and markers of inflammation they are useful complimentary sources of information in research to evaluate the patient's own perception of asthma on their quality of life.

Asthma is known to affect the HRQoL of patients and increased severity of asthma can have a greater impact on this measure, although the reciprocal relationship can occur with those with a worse quality of life having worse asthma control[161]. Measuring HRQoL in asthma is also difficult as it can be affected by other factors that may be important such as comorbidity[162], and social circumstance: for this reason more specific tools to measure quality of life such as the St Georges Respiratory Questionnaire and Juniper Asthma Quality of Life Questionnaire have been developed to have greater sensitivity to detect changes related to a change in respiratory status[163, 164].

1.5.1 The Short Form 36 (SF 36)

Ware, the developer of the SF 36 emphasised that health has dimensionality – physical health, mental health, everyday functioning in social and role activities, and general perceptions of well-being- and can range from the negative states of disease to more positive states of well being[159].

The Short Form-36[160, 165] is referred to as a generic measure of quality of life which represents eight of the most important health concepts included in the Medical Outcomes Study which was a large scale test of the feasibility of self-administered patient questionnaires and generic health scales for those with chronic conditions, including the elderly[158]. It is self administered, includes one multi-item scale measuring each of eight health concepts and the scores for the SF-36 are also represented as summary scores for physical health and mental health. Further explanation of the SF-36 questionnaire is described in chapter 2.

1.5.2 The St George's Respiratory Questionnaire

The SGRQ is designed to measure health impairment in patients with asthma and Chronic Obstructive Pulmonary Disease and therefore has been designed to explore those aspects of HRQoL that were identified to be specific to respiratory disease rather than generic[163]. It is therefore more responsive to changes in relation to a change in respiratory disease. There are other questionnaires such as the Juniper Asthma Quality of Life Questionnaire (AQLQ)[164] that are specific for asthma, however in a study or comparison between the two[166], in overall terms, not one of these instruments behaved better than the other and therefore the SGRQ has been used in this

study. Cough and wheeze have been shown to correlate with SGRQ symptom score and 6-min walking distance and MRC dyspnoea grade correlates with activity score. There are also significant correlations with FEV1, FVC, dyspnoea, anxiety and depression.

It is in two parts which includes 16 questions. Part 1 consisting of questions 1 to 8 produces the symptoms score, and part 2, consisting of questions 9 to 16, the activity and impact scores. A total score is also produced.

1.6 Obesity and its measurement

The prevalence of obesity is increasing worldwide [167] and this has many consequences for healthcare and the health of individuals. Obesity is associated with many comorbidities [168] including cardiovascular disease, diabetes, obstructive sleep apnoea and gastroesophageal reflux disease. Recently there has been interest in its possible relationship with asthma [1].

Obesity can be defined as an accumulation of excessive body fat, well over the daily metabolic requirements of facultative energy storage in the form of triglycerides. It can be assessed in a number of ways [169] which measure different aspects of obesity such as total or regional adiposity. Weight per se is a poor measure of obesity as this measures adipose tissue as well as muscle and other lean tissue as a whole. Body mass index (BMI) is a more useful measure as it takes height into account. BMI is calculated by dividing weight (Kg) by the height (m) squared. A BMI >30kg/m2 is accepted as obese. BMI <18.5 underweight, 18.5-24.9 healthy, 25-29.9 overweight and >40 morbidly obese.

Waist circumference is at least as good an indicator of body fat as BMI and is the best predictor of visceral fat. Waist-hip ratio was introduced on the assumption that it would predict fat distribution better than waist circumference alone. Subsequent research, however, showed that it did not. It is also noted that due to a greater measurement error for waist circumference, body weight is the best measure to use for monitoring change[170].

Other methods of measurement include densitometry employing the principle of water displacement, imaging with CT, or MRI which give the best assessment of visceral fat although these are less easy to perform. Bioimpedence is another method that crudely estimates total body water as a component of lean mass and therefore an estimate of fat mass can be obtained however there is no evidence that bioimpedence analysers are better than waist circumference in measuring body fat in adults.

In this study we have employed a combination of these methods to assess obesity in our population.

As shown later, adipose tissue is now thought to be metabolically active and obesity is now thought of as a low grade inflammatory state.

1.7 Obesity and the lung

1.7.1 Historical aspects

Hutchinson[171] was the first to describe the effect of weight on pulmonary function in 1846 when describing the use of his spirometer as he was able to accurately record lung volumes with a reproducible technique for the first time. He noted that with increasing weight, vital capacity increased, remained stable and then reduced. He postulated that this was likely to be due to 'the mere circumstance of fat preventing the mobility of the thoracic boundaries'. Realizing the importance of this relationship he stated that 'the examination of corpulent persons must not be compared with those not corpulent, though in all other aspects the same.'

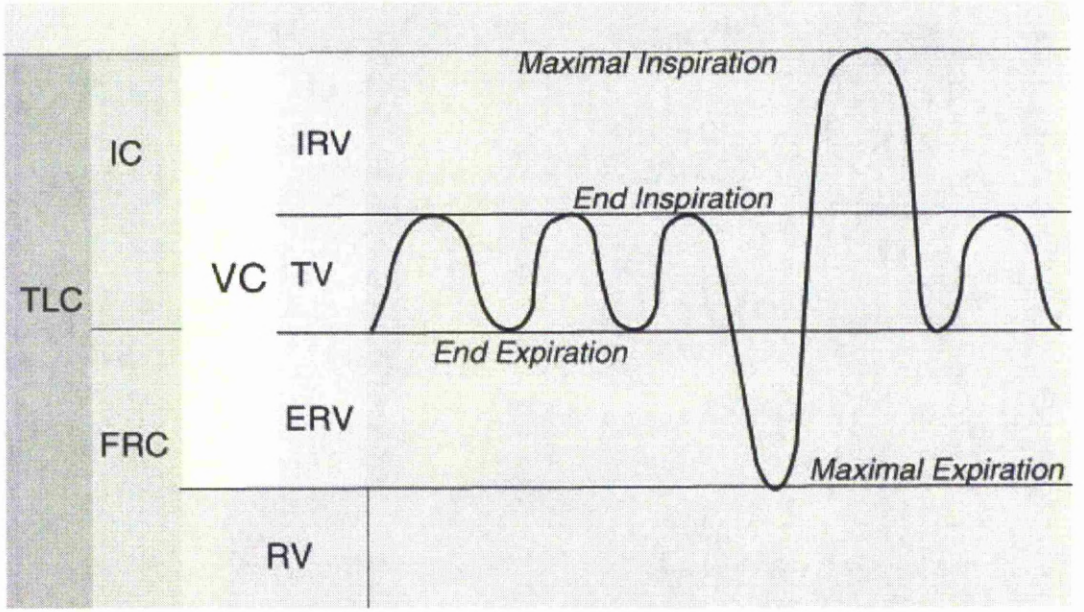
Much early interest in the 1950's of a condition of obesity hypoventilation, later to be given the term 'Pickwickian Syndrome' stemmed from a paper by Kerr and Lagen in 1936[172]. They 'refer especially to a type of obesity which appears to be exogenous in origin, arising in persons whose dietary habits lead to a caloric intake beyond their daily requirements'. They describe reduction of tidal volume, and vital capacity due to changes in posture plus kyphosis and coin the term orthostatic dyspnoea, going on to describe the likely consequences of this in the form of polycythaemia and cyanosis. The first actual use of the term 'Pickwickian Syndrome' was by Bickelmann et al[173] in 1956 who also acknowledge the 'first modern and scientific description' of this syndrome by Seiker et al[174] in 1954 who described four patients with similar symptoms and signs related to obesity. Seiker reported a 20% decrease in total lung volume and a 50% decrease in expiratory reserve which decreased on recumbency to 150cc or 17% of the

average normal value. They postulated that extreme obesity markedly reduces the functional residual capacity and also stated that weight reduction corrected the abnormalities. Bickelmann's paper goes on to describe extensive pulmonary function tests on their patient both before and after weight reduction. These tests revealed low total vital capacity, expiratory reserve volume, residual volume, functional residual capacity and total lung volume which all increased with weight reduction with an increase in maximum breathing capacity.

Since then we have further understanding of the effects of weight on the lung.

1.7.2 Current understanding: Lung volumes.

As noted above compartmentalisation of gas within the lung can be described as per fig 3. below.



TLC – Total Lung Capacity, IC – Inspiratory capacity, FRC – Functional Residual Capacity, VC – Vital Capacity, IRV – Inspiratory Reserve Volume, TV – Tidal Volume, ERV – Expiratory Reserve Volume, RV – Residual Volume.

Fig 3. Static lung volumes

Obesity can affect these volumes to varying amounts[175] and this can depend on the severity of obesity generally measured as BMI.

As already described, the effect of obesity is a general reduction of all lung volumes in these individuals. More recently however the extent to which these volumes are affected has been investigated in more detail: Jones and Nzekwu[176] found a significant inverse linear relationship between BMI for VC, TLC and RV although TLC and RV are not affected until the BMI becomes very large. In contrast FRC and ERV are affected more dramatically at lower BMI and exponentially when BMI becomes greater than 40 kg/m^2.

As lung volumes diminish, so the small airways also diminish in size thus as ERV reduces with increasing BMI, lung volumes come close to RV bringing individuals close to closing volume[177] , at which point the volume of the lung is so small that airways close and gas trapping occurs so that no more gas volume can be expelled[140, 178].

Another important observation is that obese individuals tend to breathe at low tidal volumes with higher respiratory rates when compared to those with normal BMI[179].

When investigating spirometric values there is conflicting information with some studies reporting preserved FEV1/FVC ratios with reduction in both components[180] and others have reported reduced FEV1/FVC ratios[181].

The distribution of fat has been shown to be important in its effect on lung volumes. FVC, FEV1 and TLC have been shown to be affected by subscapular skinfold thickness[182, 183] after removing the effects of BMI and FVC has also been shown have a negative association with fat% and is more affected by central adiposity when measured as waist/hip ratio[184]. Cotes et al[185] found that fat% and fat free mass index had different contributions to lung volumes with fat % contributing to RV and ERV, and hence to FRC, VC and TLC with a negative sign. FFMI contributed negatively to ERV and hence FRC but made a positive contribution to IC, TLC, FEV1 and FVC. More recently abdominal height has been shown to affect pulmonary function[186].

Another contributor to effects on lung function and volumes is body position which can have a greater effect in the obese individual due to the increased pressure from abdominal contents and redistribution of blood cephalically[187, 188] upon becoming supine. FVC reduces on sitting in obese

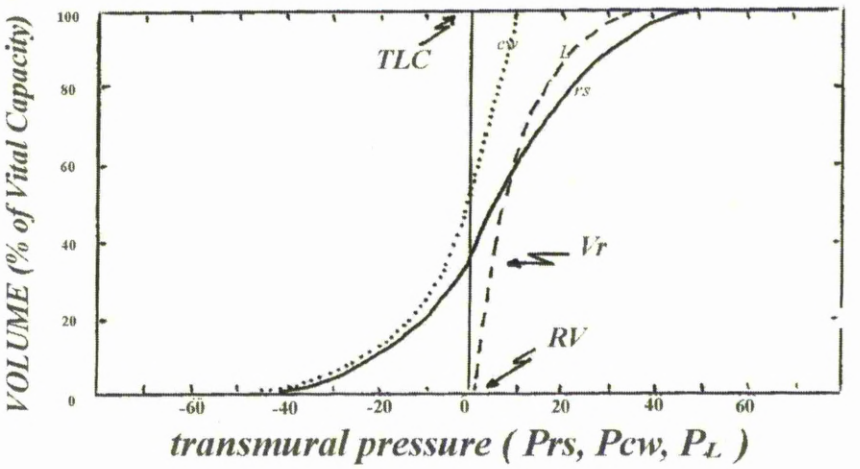
subjects which is not shown in normal subjects[189] and also on becoming supine along with TLC and VC. One group however has shown an increase in FRC on assuming a supine position from sitting, possibly due to increased fat in the abdomen pushing up the diaphragm[190].

These changes in lung volumes are thought to be a combination of changes in abdominal pressure, changes in lung compliance, increased blood volume and increased fat content of the thoracic cavity some of which will be covered later.

There have also been suggestions that gender[186] may have an influence on changes in lung volumes, possibly due to changes in fat distribution, however this is not clear.

1.7.3 Compliance.

The following figure (Fig 4.) shows an example of the volume pressure or compliance curve of the respiratory system[191]. Obesity is thought to affect this system in many ways.



TLC – Total lung capacity, Vr – Volume at rest, RV – Residual Volume, cw – chest wall, L – Lung, rs – Respiratory system, Prs – Pressure respiratory system, Pcw – Pressure chest wall, P_L – Pressure lung.
Fig 4. Pressure Volume curve of the respiratory system[191]

Usually the point of FRC lies on the straight part of the compliance curve so that a small change in pressure results in a relatively large change in volume. As has been described already, however the lung volumes in obesity are lower with a lower FRC as BMI increases. As a result of this the FRC lies on a point of the compliance curve below the straight portion of the curve i.e. below the inflection point[191]. This flatter part of the curve results in less volume change of the lung in proportion to a change in pressure.

Total respiratory compliance is therefore related to vital capacity and Pelosi[192-195] showed that a lower respiratory system compliance is caused by a decreased lung and chest wall compliance with predominance from the lung

component that may be due to increases in both elastic and resistive work[192-195]. Chest wall elastance is significantly correlated with decreased end-expiratory lung volume as a result of increased BMI due to changes in the elastic properties of the lung, vascular engorgement[188], changes in the surface lining i.e. changes in surfactant and collapse of alveoli[196]. Chest wall compliance may also be reduced due to increased adiposity around the ribs, diaphragm and abdomen or limited movement of ribs due to thoracic kyphosis or lumbar lordosis from excessive abdominal fat content[192].

1.7.4 Resistance

Airway resistance is dependant on airway calibre, which is reduced at lower lung volumes[197]. A strong association between BMI and lung volume and airway calibre has been shown and therefore in obesity as lung volume decreases there is increased airway resistance[198, 199]. This increased airway resistance has been shown by plethysmography and forced oscillation technique (FOT)[198]. There is evidence however that changes in airway resistance in obesity cannot be explained by changes in lung volumes alone as airways have been found to be narrower than expected on the basis of lung volume and other factors are involved[199].

Reductions in lung volumes with reduced airway diameter and reduction in smooth muscle stretch over time can lead to alterations in smooth muscle function with a change from rapidly cycling actin-myosin cross-bridges to slow cycling latch bridges leading to airway narrowing by smooth muscle[200].

Airway resistance also increases as obese subjects move to a supine position from sitting due to vascular engorgement as blood moves cephalically

and the diaphragm moves up with areas of atelectasis and alveolar collapse contributing to increased airway resistance[188].

The involvement of the upper airways such as in obstructive sleep apnoea may have an effect on airway resistance as the effort of breathing in through a narrowed upper airway can cause airway collapse[201].

The increase in total respiratory resistance in obesity has been shown to be almost entirely due to increased pulmonary resistance with maximum resistance of the lung up to three times higher in obese subjects than normals as a result of increased airway resistance and an increase in additional resistance of the lung caused by stress relaxation or time constant inequalities within the respiratory system tissues[192].

Maximum resistance of the chest wall has been shown to be higher but not significantly so in the obese group with no correlation between chest wall resistance and BMI[194].

Relative contributions of the lung and chest wall to the maximum resistance of the respiratory system were similar between normal and obese subjects which suggests that the increase is probably due to decreased lung volumes rather than airway narrowing.

Therefore in summary, increased resistance in the lung is due to a number of factors including reduced airway diameter secondary to reduction in lung volumes, loss of deep inspiration effects on smooth muscle, collapse of small airways, vascular engorgement and the effects of the upper airways.

1.7.5 Ventilation – Perfusion

Usually, the ventilation and perfusion of areas of the lung are well matched, however in obesity this may be altered[202].

As already stated, there is a tendency to small airway closure and gas trapping due to the low lung volumes associated with obesity. This can lead to areas of atelectasis and underventilated areas of lung. This coupled with the increased blood volume and vascular engorgement associated with obesity can lead to ventilation – perfusion mismatch. The distribution of perfusion has been shown to be more uniform from top to bottom of the lung in obesity than in normal subjects, possibly due to increased perfusion pressure and Holley et al[203] showed that ventilation to lower zones can become seriously impaired when breathing at low lung volumes in some obese subjects with this being more closely related to ERV than to the degree of obesity.

It has been demonstrated that DLCO can be reduced in obesity[204] although some have reported normal values[181]. There have also been reports of raised DLCO/VA[205] thought to be due to vascular engorgement of ventilated areas.

1.7.6 Exercise and work of breathing

A combination in the change of airway mechanics and oxygen demands can affect exercise and the work of breathing in obese individuals. At a constant respiratory rate, the work of pulmonary ventilation during a single breath increases with tidal volume and respiratory rate[206]. Obese subjects breathe at lower lung volumes and have a reduced FRC which may partly explain increased respiratory work involved in these subjects. Obese

subjects have a higher energy cost of breathing shown by a greater drop in VO2 from spontaneous breathing and after intubation, ventilation and paralysis[207]. Increases in respiratory resistance can also increase respiratory work and may account for increased VO2.

The increase in total respiratory work in obese subjects mainly consists of work done upon the lung, however in the case of obesity hypoventilation this increase in work is spread between the thorax and the lungs[208]. Excess tissue in the obese patient is metabolically active leading to increased oxygen demand at rest and an increased resting metabolic rate. Also the sheer fact of carrying increased body weight increases metabolic demands generally[209].

When examining O2 uptake and CO2 production during exercise, obese subjects maintain a consistently higher level than normal subjects with an increase linearly with work intensity[210]. As exercise increases, O2 consumption, pulmonary ventilation, and breathing frequency increase: this occurs more rapidly in the obese compared to non obese. Minute ventilation increases faster with increased work intensity and this leads to a significantly greater ventilation equivalent for O2 (VE/O2). The maximum amount of oxygen available per kilogram of body weight decreases as obesity increases[210].

There is a significantly lower VO2max (ml/kg/min) in obesity, however as a percent predicted VO2 max based on ideal body weight, cardiorespiratory functional capacity is similar to the non obese[204].

In obese individuals there is a fall in end expiratory lung volume (EELV) until ventilatory threshold (VTh) due to recruitment of expiratory muscles increasing workload then a rise back to resting levels at peak exercise. End

inspiratory lung volume (EILV) shows similar patterns to EELV during exercise and obese subjects approach TLC during maximal exercise producing large increases in the oxygen cost of breathing[204].

Some obese subjects experience expiratory flow limitation during exercise not present during rest which can limit exercise capacity shown by increases in thoracic pressure at rest, VTh, and peak exercise produced by a likely combination of reduced lung volumes, expiratory flow limitation and expiratory resistive work[204].

In summary a combination of factors are involved to increase respiratory work in obese subjects: greater oxygen demands from metabolically active tissues and moving a greater mass, increased work required to move against higher respiratory resistance and a less compliant respiratory system and increased ventilation from faster and lower tidal volumes. Finally limitation of the ability to reduce EELV and increase EILV against flow limitation and a lower TLC all increase work of breathing in the obese.

1.7.7 Bronchial responsiveness

One of the hallmarks of asthma is the presence of bronchial responsiveness or the responsiveness of the airway smooth muscle to contract in the face of a stimulus. This leads to reversible airway obstruction and wheeze.

It has been shown that breathing at low lung volumes and avoiding deep inspiration can lead to increased airway responsiveness[154] and

theoretically this should occur in obesity as these subjects breathe at low lung volumes with small but rapid TV.

Litonjua[211] was the first to investigate the risk of onset of airway hyper-responsiveness measured by methacholine challenge in relation to BMI. They showed a U shaped distribution with those with the lowest and highest quintiles for BMI being more at risk of developing AHR. They also showed that there was a relationship with the rate of increase per year in BMI and the onset of AHR. Increased airway responsiveness with increased BMI has also been found by others also[212].

There is also evidence to the contrary - Schacter et al[213] investigated obese subjects with questionnaire analysis for the presence of wheeze, asthma diagnosis and medication and performed methacholine challenge testing. They found obesity to be a risk factor for recent asthma, wheeze and medication use as measured by questionnaires but did not find it to be a risk factor for airway hyper-responsiveness. Other investigators have found a similar relationship[180, 214, 215].

1.7.8 Effects of weight change

It has been shown that weight gain is an important predictor of decline in FEV1 and FVC in men and women with some suggestion that this relationship is stronger in males[216]. There are few studies which investigate other respiratory volumes over time to describe other changes with weight gain.

Studies involving weight loss have shown improvement of lung volumes after weight loss with increase in FEV1, FVC, ERV, FRC and TLC

[217, 218]. There was no change in DLCO or RV [219]. Resistance reduced as did ventilation due to a reduction in tidal volume. In subjects undergoing surgical weight loss the above were noted plus there were also increases in respiratory muscle strength and endurance as shown by increases in PImax, PEmax and PmPeak/ PImax ratio [217].

Subjects who previously showed closing volume above FRC reversed this ratio after weight loss [219]. Any hypoxaemia prior to weight loss tends to improve and carbon dioxide tension tends to fall. This has been shown not to correlate with the amount of weight loss and thus the change in closing volume and recruitment of areas of the lung correcting the ventilation perfusion mismatch may be important [218].

Resting O2 uptake (VO2), O2 cost of work (EO2), resting ventilation and ventilatory cost of work (EV) have all been shown to decrease with weight loss. CO2 recovery time after work (CO2RT) decreased and CO2 output of work (ECO2) rose slightly. The reduction in EV and ECO2 appears to be due to a reduction in the O2 cost of breathing [209].

The effect of weight loss on lean body mass and muscle structure and function is complex [220], with weight loss reducing energy stores and reducing muscle bulk, however the reduction in surrounding adipose tissue improving convection of heat, increased capillary density and shorter diffusing distance with improved glucose tolerance can improve the efficiency of muscle work with the appropriate consequences for the respiratory system. Muscle endurance has also been shown to improve following weight loss due to changes in substrate utilisation by muscles.

1.8 Biochemical changes and inflammation in obesity.

As previously discussed in relation to asthma, many inflammatory mediators are involved within the respiratory system including the balance between Th1 and Th2 pathways and their associated inflammatory pathways. Studies in humans and animals have identified important biological mediators which may have an influence on airway inflammation in obesity and adipose tissue appears to exhibit a significant overlap in function with T lymphocytes and macrophages.

Adipose tissue produces and releases a number of cytokines and hormone like proteins such as leptin, IL-6, TNF-α, IL-8, plasminogen activator inhibitor-1, TGF-β1, CRP and adiponectin [221, 222], all of which may be of importance for the association between obesity and health complications leading to a systemic proinflammatory state.

Protein levels of cytokines such as IL-6 and TNF-α are found to be elevated in plasma as well as in the adipose tissue of obese subjects, and weight loss is associated with changes in these adipose tissue-derived cytokines. The importance of this source of inflammatory cytokines has been shown by investigating the arterio-venous difference in IL-6 over the abdominal subcutaneous adipose tissue depot in the basal situation [223]. IL-6 was found to be released in the circulation in a sufficient concentration to elicit endocrine effects.

Leptin, a protein coded by the obese (*ob*) gene is involved in some pathophysiological aspects and is a central mediator of inflammation in obesity. It shares structural homology with long-chained helical cytokines, such as IL-6, and has been shown to recruit and activate monocytes and

macrophages, and promote angiogenesis [224]. Serum leptin concentration is increased in obesity and is strongly correlated with total body fat mass [225] and there are indications of a resistance to the effect of leptin in obesity [226].

Leptin is also important for normal lung development, serving as a critical mediator of the differentiation of lipofibroblasts to normal fibroblasts and of pulmonary surfactant phospholipid synthesis. Obese mice that are genetically leptin deficient (*ob/ob*) demonstrate profound pulmonary hypoplasia. Genetically obese *ob/ob* and *db/db* mice, as well as Zucker fatty rats, have mutations in the leptin or leptin receptor gene, but no equivalent mutations have been detected in the majority of humans with obesity [227].

The adult lung displays particularly high levels of putative functional leptin receptor as well as its splice variants. Bergen et al [228] demonstrated that the lung as a whole and fetal type II cells in particular express functional leptin receptors and respond to leptin stimulation by increasing precursor incorporation into DSPC, a specific marker for pulmonary surfactant, suggesting synthesis of this phospholipid is increased and that leptin may have a role in pulmonary maturation.

Exogenous leptin has been shown to modulate allergic airway responses in mice, independent of obesity [229]. Increased leptin levels in mice have been shown to increase airway hyper-responsiveness and increases serum IgE after inhaled ovalbumin challenge. This is not seen with inhaled phosphate buffered saline although BAL cell counts or lung tissue cytokine mRNA expression is not affected. Nonallergic immune function can also be affected as exogenous leptin can enhance bacterial clearance, killing and leukotriene synthesis in a murine pneumococcal pneumonia model [230].

Overfeeding wild-type lean mice can lead to increased leptin levels and these show increased antigen-induced T-cell responses, increased mitogen increased splenocyte IFN-γ production, and increased number of tracheal mast cells compared with lean control animals, although ovalbumin-specific immunoglobulin levels were paradoxically reduced in obese mice versus lean control mice [231]. These studies suggest that leptin appears to have an important immunomodulatory role that is relevant to airway function and immune response, independent of body mass.

Ob/ob mice which are leptin deficient also show increased AHR with increased BAL levels of CC chemokine eotaxin after exposure to ozone and a predominant Th1 inflammatory phenotypic response with elevated levels of IL-6 and the neutrophil chemoattractants macrophage inflammatory protein 2 (MIP-2) and KC compared to normal mice [232]. Exogenous leptin may alter airway immune response differently between obese and lean animals, dependent on factors such as endogenous leptin concentrations, receptor number or affinity, or other concurrent modifications of inflammatory pathways.

Increased levels of eotaxin expression have been found in obese mice and also in humans which was reduced with weight loss by caloric restriction or bariatric surgery [233]. The source of the eotaxin is at least in part related to adipose tissue.

Oestrogen may also have an influence on airway inflammation and may explain the possible gender influence on asthma and obesity.

Guler et al [225] showed a significant but weak correlation between log IgE and log leptin levels among asthmatic children and Sood et al [234] showed

that serum leptin concentrations were associated with current asthma in adults. Lastly Sin and Man [235] showed a strong inverse relationship between FEV1 and serum leptin. Thus indicators suggest that biomarkers of obesity may be important in respiratory disease.

Also of note is a decreased level of the adipokine adiponectin in obesity which has been shown to have anti-inflammatory properties in the airways.

1.8.1 Induced sputum and obesity

There is little information on sputum cell counts in relation to BMI available. A retrospective review of a large database (727 subjects) by Todd et al showed that BMI did not correlate with any cell count including total and differential counts: total, neutrophils, eosinophils, lymphocytes and macrophages. In asthmatics there were higher numbers of sputum eosinophils however again there was no correlation between BMI and counts[236]. Thus there was no correlation between any measure of cellular inflammation in the airway and BMI.

Basyigit et al[237] found no correlation between sputum levels of TNF-alpha and BMI also and in a study by Salerno et al[238] in obstructive sleep apnoea, there was no significant correlation between BMI and any cellular components of induced sputum.

1.8.2 Exhaled NO and obesity

We know little about the effects of obesity on exhaled nitric oxide and few studies so far have presented results regarding the possible relationship

between body mass index and exhaled nitric oxide. Obesity has been shown to be an inflammatory state[222] which may be reflected in increased biomarkers of inflammation such as exhaled NO. It has been suggested that levels of exhaled nitric oxide increase with increasing BMI[239], however in asthmatics this relationship does not occur, possibly due to the masking effect of asthmatic inflammation[240]. There are suggestions however, that as BMI increases further into the obese range i.e. above 30kg/m^2 the levels of eNO become reduced likely due to changes in airway physiology and levels of eNO have been shown to increase following surgically induced weight loss[4].

The release of proinflammatory cytokines from adipose tissue such as IL-6 has the potential to modulate the T-helper 2 immunity which is present in asthma and as a result may be associated with an increase in exhaled nitric oxide. This is expanded in the next section[241].

1.8.3 Possible link between systemic and local inflammation in obesity

As noted above adipose tissue releases a number of substances that can lead to a systemic inflammatory state such as IL-6, TNF-α, IL-8, PAI-1, TGF-β1, CRP, leptin and adiponectin. This increased systemic inflammation could lead to an increase in local airway inflammation through interaction with $CD4^+$ lymphoctes which can produce cytokines that lead to airway cellular inflammation and are important in asthma where they have been shown to produce Th2 cytokines[242] that can lead to an increase in IgE. However some have noted an increase in neutrophils rather than eosinophils in obesity suggesting that there may be an increase in Th1 inflammation driven by IFN-γ and leptin, this is also shown by a reduction in exhaled nitric oxide – a marker

of eosinophilic airway inflammation - as BMI increases. The link between systemic and airway inflammation is little understood and work is required to explore this further. It is known that the asthma syndrome can consist of a number of inflammatory phenotypes, therefore either of these mechanisms are plausible to explain a possible link between the systemic inflammatory state leading to the local inflammation present in asthma. Studies so far however have not been able to show a definite association between airway and systemic inflammation in asthmatic obese subjects although this has been shown in obese non-asthmatic individuals[2, 241].

1.9 Quality of life and obesity

As previously stated above, health related quality of life is used to refer to the "physical, psychological, and social domains of health, seen as distinct areas that are influenced by a person's experiences, beliefs, expectations, and perceptions"[156], HRQoL reflects an individual's subjective evaluation and reaction to health or illness rather than a medical professionals' evaluation[157]. Obesity has been shown to worsen HRQoL in many dimensions as measured by generic questionnaires such as the SF-36 and HRQoL improves with weight loss[243]. As for asthma, disease specific HRQoL questionnaires have been developed to be more sensitive to changes related to changes in weight. One such questionnaire is the Impact of Weight on Quality of Life-Lite questionnaire[244].

1.9.1 Impact of Weight on Quality Of Life-Lite (IWQOL-Lite)

The IWQOL-Lite is the short form of the IWQOL which was the first instrument specifically developed to assess the effects of the obese condition on the quality of life of persons who are seeking treatment for this condition[244]. It was designed around issues expressed by patients attending an intensive treatment programme for obesity at the Duke University Diet and Fitness Centre. Patient expressed dissatisfaction with various aspects of their lives due to obesity which covered health and physical functioning, social/interpersonal life, work, mobility, self-esteem, sexual life, activities of daily living, and comfort with food. The Task Force on Developing Obesity Outcomes and Learning Tools (TOOLS) was convened by the North American Association for the Study of Obesity and this was charged with choosing outcome measures to be used by clinicians and researchers. They recommended the use of the IWQOL-Lite in clinical practice and in research studies on obesity[245].

The IWQOL-Lite is a 31 item questionnaire that begin with the phrase, "because of my weight...". They are separated into 5 domains: Physical esteem (11 items), self-esteem (7 items), sexual life (4 items), public distress (5 items) and work (4 items). There is also a total score. Each item has 5 response options as follows: (1="never true", 2="rarely true", 3="sometimes true", 4="usually true", and 5="always true.")

1.9.2 SGRQ and obesity in asthma

There is little data on the influence of BMI in asthma on the respiratory specific health related quality of life measured by the SGRQ. Two studies

have shown improvement in SGRQ with weight loss[2, 246] although the change in SGRQ scores did not correlate with amount of weight lost.

1.10 Asthma and obesity

There are many ways that obesity may affect the respiratory system as I have already demonstrated through the effects on respiratory mechanics, the influence of adipose tissue on inflammation and there are also suggestions of a shared genetic basis for susceptibility to both asthma and obesity[247]. Studies have related increasing rates of obesity with increasing incidence of asthma and linked the two. It is difficult to ascertain whether there is a true influence of obesity on asthma as the definition of asthma may vary between studies. Some rely on the subjects self reporting of symptoms [248] or a physicians diagnosis of asthma and it has been shown that up to a third of patients with a diagnosis of asthma may not have the disease[249]. Also some studies rely on self reported height and weight to obtain BMI rather than those directly measured and it has been shown that subjects can underestimate weight and overestimate height[250] although other studies that use measured height and weight still show a significant association with BMI and asthma.

Nevertheless cross sectional studies involving large numbers of subjects have demonstrated an increased prevalence of asthma in obesity [251-253]. And the reported odds ratios for incident asthma in obese or extremely obese compared with normal weight individuals range from 1.0 to 3.5 [254].

As with most cross sectional studies, direction or causality may be difficult to establish as asthma may increase the risk of becoming obese due to the use of corticosteroids and reduced activity. Also obesity and asthma

may also be independently associated with other unmeasured confounding conditions, such as obstructive sleep apnoea or gastroesophageal reflux disease [255, 256].

Some prospective studies have found an increased risk of developing asthma with increasing BMI and most have shown a steady dose-response relationship with incident asthma and increasing BMI, and the majority also demonstrate the effect to be stronger in women than men although this is controversial [3]. Many of these longitudinal studies also control for diet and physical activity, strengthening the conclusion that it is obesity itself, and not a lack of exercise or dietary factors, that is associated with asthma[257]. Again studies have varied in whether height and weight is self reported or measured but in either case the majority of prospective studies have reported that obesity is a risk factor for the development of a new diagnosis of asthma. The odds ratio is between 1.1 and 3.0 comparing lowest and highest BMI categories[3].

Paediatric studies show heterogeneity in the strength and direction of the relationship between asthma and obesity[258]. With differences reporting links between asthma and obesity between boys and girls varying between studies, this may be accounted for by differences in the way obesity is measured.

A recent meta-analysis [254] of seven studies in adult subjects with a primary outcome of incident asthma using BMI as a measure of overweight with at least one year follow up found that compared with normal weight, overweight and obesity conferred increased odds of incident asthma, with an odds ratio of 1.51. A dose response effect of elevated BMI on asthma

incidence was observed. Comparing normal weight with overweight the OR was 1.38 and normal weight with obese OR was 1.92. There was no significant difference between men and women. Not all studies agree with the direction of causality and one study has shown that obesity was not a risk factor for subsequent asthma but asthma was a risk factor for subsequent obesity[259].

As mentioned above, it is difficult to be clear whether the relationship between asthma and obesity exists in many of these studies as many rely on a diagnosis made from symptoms or physician reported diagnosis. However, the effects of obesity on the respiratory system can lead to symptoms of breathlessness which may be mistaken for asthma[260]. Some have shown that there is an increased risk of wheeze and breathlessness but not bronchial responsiveness [213] which may suggest that not all subjects that respond positively to questions of symptoms may have asthma and we must treat these studies with caution.

1.10.1 Weight loss studies

Weight loss secondary to bariatric surgery has been shown to improve the clinical status of many morbidly obese patients with asthma, with resolution of the condition and improvements in number of attacks, medication use, hospitalisation and severity score for up to 90% of patients [3]. Also data from the Swedish Obese Subjects Intervention Study found reductions in the cost of medications to treat asthma in a surgically treated group but not in a conventionally treated group of obese patients [261]. Some of these early studies are limited to lack of control groups and a lack of testing of pulmonary

function or bronchial reactivity. More recent studies however with control groups have shown improvements in pulmonary function and asthma control with reductions in exacerbations plus rescue medication use but not exhaled nitric oxide suggesting that improvement in status is likely due to improvement in lung mechanics rather than inflammation[241]. This has been shown more recently also with weight loss after surgery failing to show improvements in inflammatory markers such as eosinophils in bronchoalveolar lavage fluid although there was an improvement in bronchial responsiveness[2]. However it is important to note bariatric surgery is usually carried out on the very obese (BMI>40Kg/m^2) and may not be applicable to the general population.

Weight loss studies secondary to dietary intervention or dietary intervention plus behavioural change have also been carried out. Improvements in FEV1, FVC, dyspnoea, use of rescue medication, number of exacerbations, and health status have been shown with a weight loss of 14.5% in 8 weeks in a pivotal clinical trial in which Stenius-Aarniala et al[246] recruited 38 obese subjects into an open, randomised parallel group study. This included an eight week, very low-energy diet plus a control group. The same group showed improvement in day to day PEF variability, morning PEF and FEV1, mid-expiratory flow, airway resistance (Raw) and FRC with mean weight loss of 13.7Kg in 14 obese patients with asthma. Another study with a weight loss of 14% showed improvements in day to day peak flow variation, FEV1, FVC, ratio of forced midexpiratory flow rate to FVC, FRC, ERV and resting minute ventilation[262]. One study has shown improvements in FEV1, FVC and total lung capacity, but no significant change in bronchial reactivity [213].

1.11 Current study

The studies described in this thesis were designed to examine the effects of weight loss on asthma severity in obese individuals with a previous diagnosis of asthma, on treatment. This is a case control study examining various mechanical aspects of the respiratory system, mainly focussing on the effects of airway calibre and bronchial reactivity, inflammatory markers of asthma within the respiratory system and symptoms.

Chapter 2 Methods and study design

This study was an open, randomised, parallel group study investigating the effects of weight loss using a meal replacement strategy combined with a behavioural program to encourage long term weight maintenance, versus simple dietary advice on symptoms, respiratory function and immunological markers of asthma severity. This was a mechanistic study and therefore duration was six months to allow maximal weight loss in the intensive intervention group and to minimise the chance of dropouts.

Ethical approval was obtained from Sefton Local Ethics Committee (04/Q1508/51) and the trial was registered with the International Standard Randomised Controlled trial Number register (ISRCTN 54432221). All subjects gave written informed consent.

2.1 Selection of subjects

This study was powered to investigate weight loss in patients based on the study by Stenius-Aarniala *et al* who investigated the effect of weight loss on asthmatics and achieved a 14.5% weight loss with dietary intervention compared to 0.3% in a control group[246]. It was designed to recruit eighty subjects with half randomised to intensive treatment and the others to simple dietary advice. Allowing for a dropout rate of 5 patients per group, 25 subjects per group were required to demonstrate significant changes in body weight, assuming a 14Kg change in weight with a SD of 15Kg; 30 subjects per group should be sufficient to show a change in PEFR (based on previously published studies at the time of the study inception showing clear differences in body weight and measures of asthma severity with 19 subjects per group.

This suggested that this sample size should be adequate for the primary outcome of improvement in bronchial responsiveness).

2.2 Pre-screening subjects for inclusion

I wished to include subjects with asthma and a Body Mass Index ≥ 30 Kg/m^2. Subjects were recruited from clinics at University Hospital Aintree and also by poster and newspaper advertisements in the local press. Subjects were asked to contact the department by telephone if they had been given a diagnosis of asthma by a physician, were taking medication and were overweight. When the subjects contacted the department they were screened for inclusion and exclusion criteria outlined below and if appropriate were invited to the department for a screening visit to investigate suitability for the study.

For the purposes of the study asthma was excluded if the subject did not achieve a 20% drop in baseline FEV_1 with doubling doses of methacholine up to 32mg/ml using the 5 breath dosimeter method or did not show reversibility in FEV_1 to nebulised salbutamol of ≥ 15% of baseline.

Other inclusion and exclusion criteria are as follows:

Inclusion criteria:

- Obesity (body mass index ≥ 30 Kg/m^2)
- Age 18-65 years. Male or Female
- Asthma requiring treatment with at least an inhaled corticosteroid and an inhaled β-agonist

Exclusion criteria:

- Subjects on long-term oral corticosteroid therapy (previous use for acute exacerbations, but not within three months of study entry was permitted)
- Diabetes mellitus
- Pregnancy or breastfeeding
- History of major eating disorder (anorexia or bulimia nervosa)
- History of food allergy or allergy to constituents of Slimfast
- Major psychiatric disease (including current use of antidepressants)
- Current smokers
- Uncontrolled thyroid disease (patients on stable thyroxine replacement could be included)
- History of severe cardiac, hepatic or renal disease, malignancy, or any other condition that might, in the opinion of the investigators preclude completion of the study.

2.3 Groups - Randomisation

Groups were randomised following screening at the baseline visit by opening sequential envelopes stratified by gender containing a pre-prepared envelope containing a card with group A (dietician) or group B (control) printed on it. Randomisation was prepared in a 1:1 ratio by the statistics department at the University of Liverpool based 80 subjects stratified to ensure equal numbers of male and female patients in each group. The investigators were blinded to the process of envelope preparation.

2.4 Intervention

2.4.1 Dietician (meal replacement) group

The study investigators were not involved in the intervention at any point to ensure that they could not influence the study and therefore reduce the risk of bias. This group was assessed by a study dietician, their energy needs calculated and were given advice on diet using meal replacements; they were provided with sufficient Slimfast™ meals (drinks/bars. Slim.Fast; Slim.Fast Foods Company, West Palm Beach, FL) to provide up to 3.35 MJ (800 kcals) per day. An eating plan, incorporating meal replacement products with an energy deficit of at least 2.09MJ (500 kcal) per day was negotiated with the subject. Subjects were allowed to select a meal of their choice for the evening meal, up to a total of 5.02-6.28MJ (1200-1500 kcal) per day. Subjects also entered a programme of dietary management designed to encourage long term behavioural change. This was developed for use in Aintree Hospital weight management unit based on regular (two weekly) visits to a dietician for three months, and monthly visits thereafter. Areas covered include dietary history, nutritional education, advice about physical activity, identifying the stage of change and dealing with barriers to change, motivating change, and coping strategies to deal with challenging or difficult situations.

Following the first three months subjects were allowed to introduce a second meal of their choice but continued with at least one meal replacement for the duration of the study

The following protocol was used by the dietician:

Assessment Appointment

- ❑ Measure of weight and calculation of energy requirements
- ❑ Weight and dieting history
- ❑ Reasons for wanting to lose weight
- ❑ Social information
- ❑ Current activity levels
- ❑ Current dietary patterns (typical day)
- ❑ If motivation seems low then assess importance and confidence and consider using ambivalence grid

Information exchange –(information usually given at this appointment):

- ❑ Rate of weight loss and 10 % target
- ❑ How to take meal replacements
- ❑ Daily eating plan
- ❑ Food portion guide
- ❑ Additional Meals
- ❑ Snacks and drinks
- ❑ Food diary
- ❑ Slimfast® preference chart

Discuss Slimfast® choices and give samples (enough to last 2 weeks) of ones subject thinks they may like. Ask them to complete preference chart and bring to next appointment.

Follow up appointments

Review every 2 weeks for 3 months then monthly for 3 months thereafter.

- ❑ Weight Check
- ❑ Provision of sufficient slimfast® for the next 2 weeks - & note what given
- ❑ Review of progress including whether taking slimfast® as advised & if following eating plan
- ❑ Asking re hunger and cravings & any lapses
- ❑ Review of food diaries
- ❑ Review of any lifestyle goals set at previous appointments
- ❑ Negotiating goals for lifestyle change
- ❑ Information exchange (can use agenda setting chart)
- ❑ If motivation seems low consider assessing motivation and confidence and / or using ambivalence grid
- ❑ Other advice/ support as appropriate

2.4.2 Control group

Those subjects randomised to receive conventional advice were given a standard leaflet on healthy eating (British Heart Foundation – Healthy Eating), and advised that weight loss might help their asthma; they were not given further advice on weight loss for the duration of the study.

2.5 Asthma management

No intervention was made to the subjects' asthma treatment throughout the study and treatment continued according to the British Thoracic Society Guidelines[15]. Subjects were educated regarding correct inhaler technique and given advice regarding adjustment of treatment, and when to seek advice from the physician or emergency department if required at the initial visit. Subjects were asked to complete a 2-week asthma diary card recording morning and evening PEFR as the best of three consecutive measurements using a mini-Wright peak flow meter for two weeks prior to each visit. Asthma symptoms and rescue medication were also recorded.

2.6 Procedures and rationale

2.6.1 Measuring height, weight and % fat mass

Height and weight were measured in meters with the patient in stockinged feet using a calibrated wall mounted stadiometer and in kilograms using calibrated weighing scales respectively. Body mass index (BMI) was calculated with the following equation: weight in Kg / height in meters^2 = BMI (Kg/m^2).

%Fat mass was measured using a Quadscan body composition & fluid measuring device (Bodystat®). Subjects were asked not to eat or drink from the night prior to their visit, had no alcohol or caffeine consumption 24hours prior to their visit and refrained from exercise 12 hours prior to the visit. Patient age, height, weight and sex were entered into the device and the subject was asked to lie in a comfortable, relaxed position with the arms and legs spread slightly so that no parts of the body were touching one another.

Self-adhesive disposable electrodes were attached to the right hand and right foot in order to avoid battery current passing through the side of the body where the heart is situated. Red (injecting) leads were connected to electrodes placed just behind the finger and the toe and black (measuring) leads were connected to electrodes placed on the right wrist and right ankle. Once the subject was lying in the supine position, 4-5 minutes elapsed before commencing a measurement to ensure that fluid levels have stabilised in the body.

Results of Fat% and Lean% of total body weight were recorded from the device which is calculated from a regression equation programmed by the manufacturer. Fat and lean mass are determined due to the two compartments having a different impedence or ability to conduct electricity with the body's lean component having less impedence than the fat component.

These anthropometric values were recorded at each subject visit.

2.6.2 Questionnaires, patient peak flow diaries and symptom diaries

Patient health related quality of life was assessed with the following questionnaires: Generic quality of life with the Short Form – 36 (SF-36), Respiratory specific quality of life with the St George's Respiratory Questionnaire (SGRQ) and Weight related quality of life with the Impact of Weight on Quality of Life – Lite (IWQOL – Lite) copies of these are included in Appendix A. At each visit patients were asked to self complete the questionnaires following anthropometric measurements but before any other intervention. Subjects were allowed to ask questions of the investigators if

they were unsure of how to answer any questions but the investigators took care not to influence the response.

The questionnaires were issued at all visits and results calculated as per methods recognised by their respective developers.

2.6.3 Quality of Life Questionnaires

2.6.3.1 The Short Form 36 (SF 36) (UK version)

The United States Task Force on Developing Obesity Outcomes and Learning Standards (TOOLS) recommends utilizing the SF-36 as the generic measure of choice in obesity research (Anne Wolf, NAASO meeting 2000) (Kolotkin 2001obese rev) because it is comprehensive, brief, consistent with current guidelines and psychometrically sound. It is not age, disease, or treatment specific and assesses health-related quality of life outcomes known to be mostly affected by disease and treatment.

Ware, the developer of the SF 36 emphasised that health has dimensionality – physical health, mental health, everyday functioning in social and role activities, and general perceptions of well-being and can range from the negative states of disease to more positive states of well being.

The Short Form-36 is referred to as a generic measure of quality of life which represents eight of the most important health concepts included in the Medical Outcomes Study which was a large scale test of the feasibility of self-administered patient questionnaires and generic health scales for those with chronic conditions, including the elderly. It includes one multi-item scale measuring each of eight health concepts: (1) physical functioning, (2) role limitations due to physical health problems, (3) bodily pain, (4) general health,

(5) vitality (energy/fatigue), (6) social functioning, (7) role limitations due to emotional problems, and (8) mental health (psychological distress and psychological well being). Information about these health status scales is summarised in table 2.

Concepts	No. of items	No. of levels	Meaning of scores: Low	Meaning of scores: High
Physical functioning	10	21	Limited a lot in performing all physical activities including bathing or dressing due to health	Performs all types of physical activities including the most vigorous without limitations due to health
Role-Physical	4	5	Problems with work or other daily activities as a result of physical health	No problems with work or other daily activities as a result of physical health
Bodily pain	2	11	Very severe and extremely limiting pain	No pain or limitations due to pain
General Health	5	21	Evaluates personal health as poor and believes it is likely to get worse	Evaluates personal health as excellent
Vitality	4	21	Feels tired and worn out all of the time	Feels full of pep and energy all of the time
Social Functioning	2	9	Extreme and frequent interference with normal social activities due to physical or emotional problems	Performs normal social activities without interference due to physical or emotional problems
Role-Emotional	3	4	Problems with work or other daily activities as a result of emotional problems	No problems with work or other daily activities as a result of emotional problems
Mental Health	5	26	Feelings of nervousness and depression all of the time	Feels peaceful, happy, and calm all of the time
Reported Health Transition	1	5	Believes general health is much better now than one year ago	Believes general health is much worse than one year ago

Table 2. Health scales and explanation of domains for the Short Form 36 HRQoL questionnaire

The questionnaire has proven useful in surveys of general and specific populations, in comparing the relative burden of disease, and in differentiating the health benefits produced by a wide range of instruments.

The questionnaire is self administered and subjects were excluded if they were unable to read the questionnaire. It was administered before the subject was asked about other health questions and concurrent illnesses so that any discussion of health problems did not influence the subject's

answers. The subject was instructed to read the instructions on top of the first page and choose the response that best represented how they felt. The questionnaire was answered by the subject themselves and spouses, or other family members, or visitors, were asked not to assist in completing the questionnaire. Once the questionnaire was completed the investigators checked for any missing answers and checked with the subject if they missed the question by accident and to complete the missing answer or asked if there was any other reason for not completing the questionnaire.

The SF-36 items and scales are scored so that a higher score indicates a better health state. After data entry, items and scales were scored in three steps:

(1) item recording, for the 10 items that require recoding;

(2) Computing scale scores by summing across items in the same scale (raw scale scores);and

(3) Transforming raw scale scores to a 0-100 scale (transformed scale scores).

Scoring was performed using an excel spreadsheet programmed with the scoring algorithm outlined in the SF-36 Health Survey Manual & Interpretation Guide.

Norms for the general U.S. population are shown in table 3.

	Physical functioning	Role-physical	Bodily Pain	General Health	Vitality	Social-functioning	Role-Emotional	Mental Health
Total	84.15 (23.28)	80.96 (34)	75.15 (23.69)	71.95 (20.34)	60.86 (20.96)	83.28 (22.69)	81.26 (33.04)	74.74 (18.05)
Males	87.18 (21.29)	86.61 (30.88)	76.88 (22.97)	73.48 (20.02)	63.59 (20.04)	85.23 (21.28)	83.28 (31.31)	76.37 (17.16)
Females	81.47 (24.6)	77.77 (36.20)	73.59 (24.25)	70.61 (21.50)	58.43 (21.47)	81.54 (23.74)	79.47 (34.43)	73.25 (18.68)

Table 3. Normative scores for the SF36 HRQoL questionnaire for the general population (US) means (sd) given

The scores for the SF-36 can also be represented as summary scores for physical health and mental health. The eight SF-36 scales define distinct physical and mental health clusters. It is known that 80 to 85 percent of the reliable variance in the eight SF-36 scales is accounted for by physical and mental components of health and this suggests that psychometrically-based summary measures can reduce the number of statistical comparisons required in analyzing SF-36 data from eight to two without substantial loss of information. Therefore the mental component summary (MCS) and physical component summary (PCS) component measures were constructed using principle components analysis factor analytical method.

2.6.3.2 The St George's Respiratory Questionnaire (SGRQ)

The SGRQ is a respiratory specific Health Related Quality of Life questionnaire designed to measure health impairment in patients with asthma and Chronic Obstructive Pulmonary Disease. There are other questionnaires such as the Juniper Asthma Quality of Life Questionnaire (AQLQ) that are specific for asthma, however in a study or comparison between the two, in

overall terms, not one of these instruments behaved better than the other[166] and therefore the SGRQ is fit for our purpose. It is in two parts which includes 16 questions. Part 1 consisting of questions 1 to 8 produces the symptoms score, and part 2, consisting of questions 9 to 16, the activity and impact scores. A total score is also produced.

The first part covers the subject's recollection of symptoms over a preceding period of 1 month to asses the subject's perception of their recent respiratory problems and frequency of respiratory symptoms. The 1 month version was used due to the time frame of the study and it is noted that this is not designed to be a precise epidemiological tool. The second part addresses the subject's current state (i.e. how they are these days). The activity score just measures disturbances to the subject's daily physical activity. The impacts score covers a wide range of disturbances of psycho-social function. Validation studies showed that this component in part relates to respiratory symptoms, but it also correlates quite strongly with exercise performance (6-minute walking test), breathlessness in daily life (MRC breathlessness score) and disturbances of mood (anxiety and depression). The impacts score is, therefore, the broadest component of the questionnaires, covering the whole range of disturbances that respiratory subjects experience in their lives.

The questionnaire is designed for supervised self administration and was completed in a quiet area, free from distraction with the patient sitting at a desk. The investigator explained to the subject why they were completing it, and how important it was to understand how their illness affects them and their daily life. They were asked to complete the questionnaire as honestly as possible and that there were no right or wrong answers, simply the answer

that they felt best applied to them. They were advised to answer every question and someone was close at hand to answer any queries about how to complete the questionnaire.

The subjects completed the questionnaire themselves, but someone was available to give advice if required. It is designed to elicit the subject's opinion of his/her health, not someone else's opinion of it, so family, friends or members of staff did not influence the subject's responses. The questionnaire was checked to ensure that there were no missing responses and if found the subject was asked to complete them.

A copy of the SGRQ used in this study is included in appendix A. Scores obtained from subjects are weighted and calculated as per the scoring algorithm suggested by the originators. SGRQ scores in healthy subjects with no history of respiratory disease quoted in the SGRQ manual are shown in table 4: means (95% confidence intervals).

N	Age-years	FEV as % predicted	Symptoms Score	Activity Score	Impacts Score	Total Score
74	46 Range 17-80	95 (91-99)	12 (9-15)	9 (7-12)	2 (1-3)	6 (5-7)

Table 4. Normal scores the domains of the SGRQ in healthy subjects quoted in the SGRQ manual

Scoring of the questionnaires was performed using a computer programme designed by a member of the research team at University Hospital Aintree based on the scoring algorithm designed and described in the St George's Respiratory Questionnaire Manual. Lower scores indicate better quality of life and a meaningful change in SGRQ score is a change of 4.

2.6.3.3 Impact of Weight on Quality of Life-Lite (IWQOL-Lite)

The IWQOL-Lite is the short form of the IWQOL which was the first instrument specifically developed to assess the effects of the obese condition on the quality of life of persons who are seeking treatment for this condition. It was designed around issues expressed by patients attending an intensive treatment programme for obesity at the Duke University Diet and Fitness Centre. Patients expressed dissatisfaction with various aspects of their lives due to obesity which covered health and physical functioning, social/interpersonal life, work, mobility, self-esteem, sexual life, activities of daily living, and comfort with food. The Task Force on Developing Obesity Outcomes and Learning Tools (TOOLS) was convened by the North American Association for the Study of Obesity and this was charged with choosing outcome measures to be used by clinicians and researchers. They recommended the use of the IWQOL-Lite in clinical practice and in research studies on obesity[245].

The IWQOL-Lite is a 31 item questionnaire that begins with the phrase, "because of my weight...". They are separated into 5 domains: Physical esteem (11 items), self-esteem (7 items), sexual life (4 items), public distress (5 items) and work (4 items). There is also a total score. Each item has 5 response options as follows: (1="never true", 2="rarely true", 3="sometimes true", 4="usually true", and 5="always true.")

The questionnaire was administered to the subjects to complete by themselves and due to the sensitive nature of some of the items respondents were allowed to leave a few items blank. This does not affect the scoring and it is acceptable for people to omit items, unless it is *careless* omission of

items. This was checked in the appropriate manner by the researcher with the subject.

A meaningful change in IWQOL-Lite total score is determined using an algorithm described by Crosby and Colleagues. Based on this algorithm, subjects' IWQOL-Lite scores are considered to have shown meaningful improvement from baseline to one year if they increased between 7 and 12 points, depending on baseline severity in comparison to the normative mean. Normative means for the IWQOL-Lite have been derived from a sample of 534 non-obese individuals who were not enrolled in any weight loss treatment programme shown in table 5.

The scoring system for the IWQOL-Lite questionnaire is described by the originators. The scores range from 0 (worst quality of life) to 100 (best quality of life).

IWQOL scale	Sex	BMI 18-24.9	BMI 25-29.9	BMI 30-34.9	BMI 35-39.9	BMI 40+
Physical Function	Females	94.8 (7.3)	82.0 (15.0)	71.8 (19.1)	61.9 (22.0)	43.9 (24.6)
	Males	93.8 (11.0)	88.8 (11.0)	78.1 (17.4)	67.6 (20.6)	46.2 (25.7)
	Total	94.5 (8.5)	84.4 (14.1)	73.6 (18.8)	63.4 (21.8)	44.5 (24.9)
Self-Esteem	Females	85.5 (20.1)	65.0 (26.0)	55.4 (26.4)	49.3 (26.7)	40.1 (27.2)
	Males	95.0 (12.6)	87.9 (16.1)	77.4 (20.7)	68.3 (24.0)	53.1 (27.4)
	Total	88.2 (18.8)	73.4 (25.4)	61.8 (26.8)	54.4 (27.3)	43.1 (27.8)
Sexual Life	Females	94.5 (14.4)	78.6 (24.8)	71.3 (27.3)	67.3 (28.6)	57.6 (32.6)
	Males	97.7 (10.9)	94.3 (13.0)	86.3 (19.3)	80.5 (22.9)	66.1 (29.6)
	Total	95.4 (13.5)	84.5 (22.5)	75.7 (26.1)	70.9 (27.8)	59.6 (32.1)
Public Distress	Females	97.8 (8.1)	94.9 (10.2)	89.3 (15.0)	78.2 (21.2)	51.9 (27.9)
	Males	97.3 (10.9)	97.3 (6.8)	93.2 (11.1)	84.5 (17.5)	55.7 (27.4)
	Total	97.7 (9.0)	95.8 (9.2)	90.4 (14.1)	79.9 (20.4)	52.8 (27.8)
Work	Females	97.6 (8.3)	89.1 (16.3)	84.2 (18.8)	77.6 (22.4)	63.7 (28.4)
	Males	96.7 (9.9)	93.3 (12.6)	88.5 (15.1)	83.4 (18.5)	67.7 (26.3)
	Total	97.4 (8.7)	90.7 (15.2)	85.4 (17.9)	79.1 (21.6)	64.6 (28.0)
IWQOL-Lite Total	Females	93.5 (8.8)	80.7 (13.8)	72.5 (16.6)	64.4 (19.1)	48.5 (22.3)
	Males	95.5 (10.0)	91.3 (9.1)	82.8 (13.4)	74.2 (16.4)	54.6 (22.1)
	Total	94.0 (9.2)	84.6 (13.3)	75.4 (16.5)	67.0 (18.9)	49.9 (22.4)

Table 5. Means and standard deviations of normative scores for IWQOL-Lite Scores by BMI and Gender. (Manual for the impact of weight on quality of life IWQOL and IWQOL-lite measure)

Scoring of the IWQOL-Lite was done using an excel spreadsheet designed on the scoring system documented in the manual for the impact of weight on quality of life (IWQOL and IWQOL-Lite) measure. Lower scores indicate better quality of life and a meaningful change in IWQOL-Lite scores are determined using an algorithm described by Cosby and colleagues suggesting a meaningful improvement from baseline to one year if they increased between 7 and 12 points, depending upon baseline severity in comparison to the normative mean.

2.6.4 Peak flow and symptoms diaries

The subject was instructed in the use of a peak flow meter and given a mini-Wright™ EN1326 standard peak flow meter (Clement Clarke International Ltd) to take home along with a peak flow diary which included questions on daily symptoms. Subjects were instructed to complete this with twice daily peak flow recording prior to medication for two weeks prior to their subsequent visit. Diaries were collected at baseline, 3 months and 6months and a new diary provided at 3 months and 6 months.

2.6.5 Skin prick Testing

Atopic status was checked by skin prick testing with a battery of commercially available common aeroallergens (including the following: saline control, histamine control (Histamine hydrochloride 1.0 mg/ml), cat, dog, house dust mite, tree and grass. A positive result being defined as at least one response with a wheal diameter ≥3mm or larger (Dreborg S 1989) than a positive control response after 15 minutes. The test was carried out by an experienced investigator on the volar aspect of the forearm with a calibrated lancet (1mm) held vertically. The reactions were read after 15 minutes and the wheal size was measured in two perpendicular directions including the longest diameter with the mean recorded as the response. Subjects with at least 1 positive result were regarded as atopic.

2.6.6 Exhaled nitric oxide

Exhaled nitric oxide was measured using a NIOX chemiluminescence online analyser (Aerocrine, Solna, Sweden) in line with ATS / ERS

recommendations for standardised procedures for the online measurement of exhaled lower respiratory nitric oxide[94]. Subjects were asked not to eat for 12 hours prior to their visit and avoid caffeinated drinks.

The analyser was calibrated according to the manufacturer's instructions every 14 days. The analyser has a visual biofeedback mechanism to ensure correct technique from the subject and the subject was instructed in the correct technique beforehand. Any incorrect measurements are rejected automatically by the analyser software to avoid error due to poor expiratory flows etc.

Once seated comfortably the subject was asked to inhale through the machine mouthpiece / filter for 2 to 3 seconds to total lung capacity which provides nitric oxide free air by passing this air through a scrubber. The subject then exhaled immediately, because breath holding may affect FeNO. TLC is recommended because this is the most constant point in the respiratory cycle and patients accustomed to spirometry are familiar with inhaling to this volume.

To exclude nasal NO the subject exhaled against an expiratory resistance by maintaining a positive mouthpiece pressure initially at a flow rate of 50 ml/s for 10 seconds by using the appropriate manufacturers flow control supplied with the analyser and the software set to the appropriate rate. The screen of the analyser provides visual feedback to the subject to help maintain a constant flow rate and pressure for the recommended duration of time. If the flow rate or pressure does not meet the manufacturers tolerances (+/- 10%) then the measurement is not accepted. The plateau nitric oxide level (ppb) was recorded.

To calculate flow independent parameters of exhaled nitric oxide concentrations i.e. airway wall NO flux and alveolar nitric oxide concentrations, the procedure was repeated using 10ml/s, 30ml/s, 100ml/s and 200 ml/s flow rates for 20, 10, 6 and 6 seconds respectively by using different resistors supplied by the manufacturer to alter mouth pressure and altering software settings as per manufacturers instructions. These settings ensure that the total volume of air exhaled at each flow rate accounts for the exclusion of dead space. The manufacturer's information about the NIOX analyser states that the accuracy of the FeNO measurement is ± 2.5 ppb of measured value <50 ppb, and ± 5% of measured value >50 ppb, and the linearity is <2.5 ppb integral linearity.

Three acceptable readings were recorded at each of the five flow rates in each sitting. Recalibration was not required after each change of resistors.

Exhaled nitric oxide levels (ppb) were obtained at screening using an expiratory flow rate of 50ml/s. At subsequent visits exhaled Nitric Oxide was measured at 10ml/s, 30ml/s, 50ml/s, 100ml/s, and 200ml/s to determine flow-independent parameters based on the two compartment model of Tsoukias and George[101]. Alveolar NO concentration was determined as the slope of the regression line of the 100ml and 200ml flow rates after inspection of the trends. Bronchial NO flux was determined as the intercept of this regression line[263].

2.6.7 Bronchial challenge testing / reversibility testing for screening visit

Before their visit, subjects withheld medication as per ATS guidelines for challenge testing[17]. Subjects were also asked to refrain from taking caffeinated drinks for this period.

Historically, for diagnostic purposes asthma challenge tests target a significant change in FEV_1 with a 20% fall in FEV_1 being considered a positive test and an arbitrary cut off to exclude significant bronchial responsiveness for most research studies set at 8mg/ml using increasing doses of methacholine.

Standardised methods have been developed to perform methacholine challenge tests (ATS guidelines)[17]. A doubling concentration of methacholine is administered with assessment of the FEV_1. The dose of methacholine calculated to induce a 20% drop in FEV_1 is used to define bronchial responsiveness and is termed PC_{20}.

Methacholine inhalation testing was performed using the five breath dosimeter method as per ATS guidelines[17]. Airway responsiveness to methacholine was expressed as the provocative concentration of methacholine inducing a 20% fall in FEV1 (PC_{20}). Bronchial hyper-responsiveness was defined by $\geq$ 20% drop in FEV1 with $\leq$ 8mg/ml methacholine

FEV_1 was determined using a spirometer appropriately calibrated beforehand with the subject seated and breathing into the mouthpiece with a nose clip in place following a deep inspiration to total lung volume. The subject was asked to exhale as hard as they could to residual volume for at least 6 seconds. The total volume expired was recorded as the Forced Vital

Capacity (FVC) and the volume of air expired in the first second recorded as the FEV_1.

The following method was used and adapted from the ATS guidelines.

1. Prepare the following 10 doubling concentrations of methacholine in sterile vials, place them in a holder, and store them in a refrigerator:

   Diluent: 0.03, 0.06, 0.125, 0.25, 0.5, 1, 2, 4, 8, 16, 32 mg/ml

2. Remove the vials from the refrigerator 30 min before testing, so that the mixture warms to room temperature before use. Insert 3 ml of the first concentration into the nebuliser, using a sterile syringe.
3. Perform baseline spirometry and calculate a target FEV_1 that indicates a 20% fall in FEV_1 (baseline FEV_1 x 0.80)
4. Ask the subject to hold the nebuliser upright with the mouthpiece in his / her mouth. Watch the subject during the breathing manoeuvres to ensure that the inhalation and breathhold are correct and that the nebuliser is not tipped. The subject should wear a nose clip while inhaling from the nebuliser.
5. At end exhalation during tidal breathing (FRC), instruct the subject to inhale slowly and deeply from the nebuliser. The nebuliser is automatically triggered soon after inhalation. The subject is encouraged to continue to inhale slowly (about 5s to complete the inhalation) and to hold the breath (at total lung capacity) for another 5s.
6. Repeat the previous step for a total of five inspiratory capacity inhalations. Take no more than a total of 2 min to perform these five inhalations.

7. Measure the FEV_1 at about 30 and 90s after the fifth inhalation from the nebuliser. Obtain an acceptable quality FEV_1 at each time point. This may require repeated attempts. Perform no more than three or four manoeuvres after each dose. It should not take more than 3 min to perform these manoeuvres. To keep the cumulative effect of methacholine relatively constant, the time interval between the commencement of two subsequent concentrations should be kept to 5 min.
8. At each dose report the highest FEV_1 from acceptable manoeuvres.
9. If the FEV_1 falls less than 20% replace the nebuliser reservoir and move on to the next concentration.
10. If the FEV_1 falls more than 20% from baseline (or the highest concentration has been given) give no further methacholine, note signs and symptoms, administer inhaled salbutamol, wait 10 min and repeat the spirometry.

2.6.8 Reversibility testing

Prior to testing subjects were asked to withhold inhaled medication for 12 hours prior to testing in all cases. Patients were also asked to refrain from taking caffeinated drinks also for this period.

Subjects unable to undergo methacholine testing due to an FEV1 ≤50% predicted underwent spirometry with bronchodilator response to nebulised salbutamol.

FEV_1 was performed at baseline. 5mg of nebulised salbutamol was given to the subject and the FEV_1 was repeated after 15-20 minutes.

Bronchial hyper-responsiveness was defined by an increase ≥ 15% and 200ml in FEV1 from baseline following nebulised salbutamol.

2.6.9 Bronchial challenge testing for study visits

Before their visit, subjects withheld medication as per ATS guidelines for challenge testing[17]. Subjects were also asked to refrain from taking caffeinated drinks for this period.

Bronchial challenge testing was carried out using a tidal breathing method to avoid the bronchoprotective effect of deep inspiratory manoeuvres which may affect the bronchial responsiveness in obese subjects. Methacholine was used and we followed the American Thoracic Society Guidelines for Methacholine and Exercise Challenge Testing – 1999. Bronchial obstruction was measured using body plethysmography, again to avoid deep inspiratory manoeuvres a change in specific airway conductance (sGaw) of ≥45 % was used to terminate the test and calculate PC_{45}.

All procedures were carried out by the author following instruction by laboratory staff at University Hospital Aintree pulmonary function unit.

Prior to performing the challenge test the equipment was assessed to ensure that it was able to deliver an aerosol with a particle mass median diameter (MMD) between 1.0 and 3.6 μm. We used a Respironics disposable sidestream nebuliser (Phillips) which is able to produce these requirements according to manufacturer's information. The nebuliser output was checked with the following method to ensure an output within 10% of 0.13 ml/min:

2.6.9.1 Nebuliser calibration method.

1. Put 3 ml of room temperature saline into the nebuliser.
2. Weight the nebuliser, using a balance accurate to 1.0 mg (preweight).
3. Adjust the flow meter to 7.0 L/min and nebulise for exactly 2 min.
4. Reweigh the nebuliser (postweight). Empty the nebuliser.
5. Repeat steps 1-4 three times for each of the following air flows: 4.0, 5.0, and 6.0 L/min
6. Calculate and plot the average nebuliser output at each airflow.
    - The nebuliser output in millilitres per minute, assuming 1 ml of saline equals 1,000 mg, is calculated as

      Output (ml/min) =[(preweight (mg) – postweight (mg))/time (min)]/1000.
    - By interpolation, determine the airflow that will generate an output of 0.26ml over 2 min (0.13 ml/min). Record the airflow for the nebuliser and the date of the calibration check.
7. Subsequent checks of nebuliser output need only test the nebuliser output at the flow that generates the correct output. If the output is within specification (0.13 ml/min, ± 10%) testing at other flows is not necessary.

2.6.9.2 Two-minute tidal breathing dosing protocol for methacholine administration.

The following method was used and adapted from the ATS guidelines.

1. Prepare the following 10 doubling concentrations of methacholine in sterile vials, place them in a holder, and store them in a refrigerator:

   Diluent: 0.03, 0.06, 0.125, 0.25, 0.5, 1, 2, 4, 8, 16, 32 mg/ml

2. Remove the vials from the refrigerator 30 min before testing, so that the mixture warms to room temperature before use. Insert 3 ml of the first concentration into the nebuliser, using a sterile syringe.

3. Perform baseline plethysmography and calculate a target sGaw that indicates a 45% fall in sGaw (baseline sGaw x 0.55)

4. A nebuliser and mask was used. Medical air was used to drive the nebuliser at a flow rate of 6 L/min to give the correct nebuliser output as described above.

5. Instruct the patient to relax and breathe quietly (tidal breathing) for 2 min. Set the timer for 2 minutes.

6. Ask the patient to hold the nebuliser upright start the timer and begin nebulisation.

7. Watch the patient to ensure that he / she is breathing comfortably and quietly, and not tipping the nebuliser. After exactly 2 min, turn off the air and take the nebuliser from the patient.

8. Measure the sGaw 90 seconds after the nebulisation is completed. Obtain an acceptable quality sGaw. This may require repeated attempts. It should take no more than 3 min to perform these manoeuvres. To keep the cumulative effect of methacholine relatively constant, the time interval

between the commencements of two subsequent concentrations should be kept to 5 min.

9. At each dose, report the highest sGaw from the acceptable manoeuvres.

10. If the sGaw falls less than 45%, empty the nebuliser, add 3ml of the next highest concentration and repeat steps 5-8 above.

11. If the sGaw falls more than 45% from baseline (or the highest concentration has been given), give no further methacholine, note signs and symptoms, administer 5mg nebulised salbutamol, wait 10 min, and check spirometry.

2.6.9.3 Measuring sGaw: Body plethysmography

Prior to the procedure the equipment was calibrated as per manufacturer's instructions. A Medgraphics™ Elite Plethysmograph was used which is capable of accommodating patients up to 180 Kg. Data was interpreted using Breeze Suite software. As we wished to avoid deep inspiratory manoeuvres so as to avoid the bronchoprotective effect on bronchial reactivity, we measured airway resistance only but not full lung volumes of the subjects.

The subjects were instructed in the correct technique before starting the measurement. Subjects were asked not to take deep breaths and to breathe at tidal volume throughout the procedure.

The subject was asked to enter the body box, a nose clip was applied and the box sealed. Time for equilibration of pressure and temperature was allowed (90 seconds) and then the subject was asked to place their mouth on

the mouthpiece and make a good seal. The patient was asked to breathe normally through a pneumotachograph whilst the operator observed the trace of volume over time on the manufacturer's software to ensure no leak in the system and correct technique. At FRC the mouth shutter was closed and the patient was asked to pant at 1Hz against the closed shutter. In the body box, respiratory efforts against the closed shutter produce changes in alveolar pressure, which are closely similar to changes of pressure at the mouth, and are associated with reciprocal changes in TGV: TGV is decompressed and compressed, causing corresponding changes in box pressure, which are recorded in terms of the change in TGV, denoted as "shift volume".

With the shutter closed the computer displays flow versus shift volume and the slope of this line is used by the software to calculate airway resistance and therefore the reciprocal sGaw (specific airway conductance). Computer displays and manufacturers software were used to accept three reproducible manoeuvres and the average of these three results was obtained for the purpose of the challenge; if a drop of $\geq$ 45% of baseline was met the procedure was discontinued and the subject received nebulised salbutamol as described above otherwise the procedure was repeated after the next nebulisation of methacholine.

Between tests the patient moved outside the body box for the next nebulisation of methacholine.

2.6.9.4 Determination of PC_{45}

The concentration of methacholine required to cause a drop in sGaw of 45% or PC_{45} was calculated using a logarithmic method as follows:

$$PC_{45} = \text{antilog}\ [\ \log C1 + \frac{(\log C2 - \log C1)(45-R1)]}{R2-R1}$$

Where

C1 = second-to-last methacholine concentration (concentration preceding C2)

C2 = final concentration of methacholine (concentration resulting in a 45% or greater fall in sGaw)

R1 = percent fall in sGaw after C1

R2 = percent fall in sGaw after C2

2.6.9.5 Determination of bronchial hyperreactivity: Dose response slope & Bronchial Reactivity Index

To calculate dose response slope and bronchial reactivity index the method described by Burrows et al[264] was used and adapted to PC_{45}. The dose response data were summarised by the expression: percent decline in sGaw / dose, where percent decline sGaw was defined as the decline in sGaw (from the post saline value) after the final methacholine dose administered, and the dose was defined as the final cumulative dose administered. This can be graphically represented as the slope of a line connecting the origin of a dose response curve with the final point of the curve referred to as the dose-response slope.

The slope was calculated by dividing the percent decline in baseline sGaw after the last methacholine challenge by the log of the last methacholine concentration given to account for skewed data. To avoid negative or zero

logarithms in the denominator, all concentrations were expressed as milligrams per decilitre.

The expression used therefore to obtain the dose response slope is as follows:

$$DRS = \frac{\text{percent decline in sGaw}}{Log_{10}C2}$$

C2 = Final concentration of methacholine (mg/dl)

$$\text{Where percent decline in sGaw} = \frac{\text{Baseline sGaw - Final sGaw}}{\text{Baseline sGaw}} * 100$$

Bronchial response index was used to provide a continuous and relatively normally distributed variable for use in statistical analysis[151]:

$$BRI = Log_{10}\ DRS$$

2.6.10 Sputum induction, processing and cell counting

2.6.10.1 Sputum induction

Sputum induction was carried out in a secluded area of the laboratory to minimise embarrassment for the subject with infection control procedures to protect personnel and subjects. A DeVilbiss® large volume ultrasonic nebuliser (DeVilbiss® Ultra-Neb) with an output of approximately 1ml/min was used for the procedure using fresh 6-9ml sterile saline solutions of 3%, 4% and 5% hypertonic saline.

Prior to the procedure the subjects were given a bronchodilator in the form of nebulised salbutamol which had been given following the methacholine challenge test. The subjects then underwent spirometry to obtain a baseline FEV_1 before proceeding. Subjects were instructed prior to the procedure that if they produce sputum felt to arise from the airways to

expectorate into a sterile container. They were instructed to blow their nose and rinse their mouth with water and swallow it prior to their attempt to expectorate to minimise the possibility of contamination from the upper airways and oral cavity. Following this subjects were asked to breathe 3% nebulised hypertonic saline for 7 minutes following which the subject was then asked to cough and spit as previously instructed. Spirometry was then repeated. If the subject stated that they wished to cough at any point during any 7 minute inhalation period the nebuliser was turned off, the specimen obtained and the nebuliser was restarted to complete the inhalation period. If the subject was unsuccessful in producing sputum then the process was repeated with 4% and 5% hypertonic saline solutions. The procedure was discontinued if there was a drop in $FEV_1 \geq 20\%$, the patient was unable to tolerate the procedure or all concentrations of hypertonic saline were completed.

2.6.10.2 Sputum processing & slide preparation

Sputum was processed within two hours of expectoration as per the following protocol kindly supplied by the department of respiratory medicine Glenfield Hospital. Procedures 1-6 below were performed on ice. Centrifuge was set at 4°C.

1. Empty whole sample into a petri dish. Select sputum plugs, using fine forceps, from saliva and transfer onto the petri dish lid (if necessary using inverted microscope). Using blunt forceps gather the sputum plugs into one mass then condense it by moving the entire mass around the lid with small circular motions. The aim is to spread the

saliva across the lid but to keep the sputum in one mass. The selection procedure and condensation / removal of saliva are important in reducing squamous cell contamination.

2. Transfer the concentrated sputum with blunt ended forceps to an empty (pre-weighed) polypropylene centrifuge tube with screw top.
3. Subtract the weight of the empty centrifuge tube from the weight of the centrifuge tube plus selected sputum to obtain the weight of sputum portion to be processed = W
4. Add dithiothreitol (DTT, Sigma, Poole, UK) freshly diluted from a stock solution of 1% (i.e. 200mg in 20ml of water at 4°C for up to 30 days) to 0.1% using Dulbecco's phosphate buffered saline (D-PBS, Sigma, Poole, UK, cat no: D-8662). Use 4x weight / volume (e.g. 4ml DTT per gram of selected sputum).
5. Disperse sputum by repeated gentle aspiration into a plastic Pasteur pipette, vortex for 15 seconds and 15 minutes rocking on a bench spiromix.
6. Add an equal volume of D-PBS (i.e. If 2ml of 0.1% DTT was added to sputum, now add 2ml D-PBS). Vortex for a further 15 seconds, filter through 48 µm nylon gauze (Sefar Ltd) placed in a funnel, pre-wet the gauze with D-PBS and shake off the excess. Filter into a clean 15ml centrifuge tube and note the volume of this cell suspension = X. (This can be done by weighing the tube as in step 3)
7. Assess total cell count viability and level of squamous contamination using a Neubauer haemocytometer and the Trypan blue exclusion method

- Mix 10 µl of cell suspension with 10 µl of Trypan blue (dilution Z=2)
- Flood haemocytometer with the above mixture a perform a cell count within 5 minutes
- Count all cells in 5 or 9 fields of the haemocytometer B (try to count 100 cells). If there are 200 cells per field or more dilute an aliquot of the cell suspension and recount. Cells touching the top and left lines are counted, cells touching the lower and right lines are not. Cells are classified as viable leukocytes, dead leukocytes and squamous (whether viable or not). Calculate the mean number of cells per square and the percentage of viable and squamous cells.

A = Live + Dead Leukocytes (non-squamous cells)

B = Number of fields of Haemocytometer counted

Y = A/B = Mean number of cells in one field of Haemocytometer

$$\% \text{ Squamous cells} = \frac{\text{Squamous cells}}{[\text{Squamous cells} + \text{Viable Leukocytes} + \text{Dead Leukocytes}]} \times 100$$

(This is the only calculation involving squamous cells)

$$\% \text{ Viability} = \frac{\text{Viable leukocytes}}{[\text{Viable} + \text{Dead leukocytes}]} \times 100$$

- Calculate the total number of cells and the total cell count (cells/g sputum)

$$\text{Total number of cells } (x10^6) = \frac{X \times Y \times Z}{100}$$

$$\frac{[(\text{viable} + \text{dead leukocytes})}{5} \times 2 \times \text{volume in ml of filtrate}] / 100$$

Where 5 is the number of fields counted (change this to 9 if p fields were counted) and 2 is the Trypan blue dilution factor

Total cell count (10^6 cells/g sputum) = $\frac{WxYxZ}{Wx100}$ = $\frac{\text{Total number of cells} \times 10^6}{\text{Weight of selected sputum(g)}}$

8. Centrifuge at 2000 rpm (790g) for 10 minutes, brake off

   The purpose of this centrifugation step is to produce a cell and debris free supernatent

9. Carefully remove the supernatant without disturbing the cell pellet and aliquot into labelled cryotubes either in 0.5ml volumes or into 4 equal volumes depending on the amount of supernatant. Supernatent aliquots must be stored at -70°C.

10. Label 4 slides as per study subject codes and label a to d respectively to distinguish between the four slides.

11. Adjust the cell suspension to 0.5 – 0.75 x 10^6 cells / ml

12. To calculate the volume required to give 0.5 – 0.75 x 10^6 cells/ml

    - $\frac{\text{Total number of cells } (10^6)}{0.5 \times 10^6}$ = Vml

      Where V is the final volume the cell suspension must be adjusted to, by adding D-PBS to give a cell concentration of 0.5 x 10^6 cells / ml

    - Always resuspend the cell pellet in 0.5-1.0 ml of D-PBS and aspirate gently to give a single suspension before topping up to the final required volume

13. Use the 50 µl per cytospin to prepare two cytospins (label a and b) and the 75 µl per cytospin to prepare two cytospins (label c and d) at 450

rpm (18.1 g) for 6 minutes using a Shandon III cytogentrifuge (Shandon Southern Instruments, Sewickly, PA, USA).

14. Air-dry the four slides for at least 15 minutes at room temperature and then stain using a modified rapid Giemsa Romanowski stain (Diff-Quik). Slides are dipped into solution A (fixative – Formaldehyde, Methanol & Water) for 10 seconds then into solution B (Blue – Azure dye (Phenothiazin-5-ium, 3,7-bis(dimethylamino)-, chloride)) for 10 seconds followed by solution C (red – xanthene dye (Eosin Y)) for 10 seconds before rinsing in deionized water. The slides are then left to air-dry and coverslips applied.

2.6.10.3 Differential cell count

Differential cell counts were carried out on slides that were found to be adequately prepared to allow the procedure. Slides containing too few cells, spoiled or containing too much squamous contamination were discounted. Cells were then counted using a microscope and a differential count of neutrophils, eosinophils, macrophages, lymphocytes and bronchial cells was performed on at least 300 cells with the aid of a manual differential cell counter.

2.7 Study protocol

Subjects were recruited from clinics at University Hospital Aintree or poster advertisement with a self-reported BMI $\geq$30 kg/m^2, aged 18-65 years, either non-smokers or ex-smokers of >2 years and taking asthma medication. Individuals taking long-term oral corticosteroid therapy, those with other

significant co-morbidities or those reporting an exacerbation within the previous two weeks were excluded. Subjects that fulfilled the entry criteria underwent procedures as per the following protocol:

2.7.1 Screening visit

- Inclusion and exclusion criteria checked
- Check subject has read and understood patient information
- Patient consent obtained
- Weight, Height checked plus body fat % by bioimpedence
- History and physical examination Completion of SGRQ, SF-36 & IWQOL-Lite questionnaires
- Exhaled nitric oxide measurements at 50ml/s flow rate
- Methacholine challenge testing using 5 breath dosimeter method with FEV_1
- Skin prick testing.
- Venesection for full blood count, urea & electrolytes, liver function tests, thyroid function testing and glucose to exclude significant anaemia, hypo/hyperthyroidism, diabetes or other biochemical abnormalities which might adversely affect health status.

2.7.2 Baseline and subsequent visits

(14-28 days from screening visit)

- Weight, Height, collection of PEFR and symptom diaries
- Body fat % by bioimpedence
- Completion of SGRQ, SF-36 & IWQOL-Lite questionnaires

- Exhaled nitric oxide measurements at 10ml/s, 30ml/s, 50ml/s, 100ml/s and 200ml/s flow rates
- Methacholine challenge testing
- Induced sputum using hypertonic saline
- Further PEFR and symptom diaries given
- Randomisation envelope opened

3 months

Repeat of baseline visit as outlined above.

6 months

Repeat of baseline visit as outlined above.

2.7.3 Subjects without bronchial responsiveness at screening

Subjects' general practitioners were informed when the subject volunteered for the study. If subjects did not show bronchial hyper-responsiveness this was explained to them and it was recommended but left to their discretion on whether to inform their general practitioner. If they requested, information was sent with their permission although due to patient confidentiality general practitioners were not informed routinely of the test results. Medication was not withdrawn by the investigator.

Chapter 3: Information gained from the screening visit

Subjects were asked to contact University Hospital Aintree lung function department if they were overweight, had a physician diagnosis of asthma and were taking inhaled medication. Following a pre-screening telephone call subjects were asked to attend the department for a screening visit to check inclusion and exclusion criteria for the study. During the course of this visit various measures were taken and this chapter explains my findings from this visit in which 36.3% did not demonstrate bronchial hyper-responsiveness and were therefore excluded. I wished to explore the differences between those that were and were not excluded to understand why subjects may have been given the diagnosis of asthma without objective measures of bronchial responsiveness. This data has been published in a peer reviewed journal: Scott S, Currie J, Albert P, Calverley P, Wilding JP. Risk of misdiagnosis, health related quality of life and BMI in patients who are overweight with doctor diagnosed asthma. *Chest.* 2012 Mar;141(3):616-24[265]

The prevalence of physician-diagnosed asthma is increasing, in part because of a link between asthma and obesity[266]. Several mechanisms lead to asthma-like symptoms in obese patients[1, 247] including the mechanical effects of increased BMI on lung volumes, increased work of breathing and increased release of adipokines from adipose tissue, although whether these mechanisms are associated with objectively demonstrated bronchial hyper-responsiveness is less certain[213]. As breathlessness is a common symptom of both asthma and obesity there is a risk of diagnostic misclassification of asthma, a view supported by a Canadian study which found a third of subjects with a prior physician diagnosis of asthma had no evidence of asthma judged by symptoms, lung function and bronchial challenge testing[249].

Obesity, like asthma, affects health related quality of life (HRQoL)[267, 268] and increased BMI has been related to increased GP attendance rates[269]. Since HRQoL and asthma control are related[270] it is easy to see how health impairments arising from obesity could be attributed to asthma, further increasing the likelihood of a mis-diagnosis.

I hypothesised that physician diagnosed obese asthmatics are at risk of mis-diagnosis and would have a significantly impaired HRQoL. I also proposed that BMI may correlate more strongly with HRQoL than traditional markers of asthma severity. At the screening visit I collected data about bronchial hyper-responsiveness and health status both generic and disease specific to establish which aspects of their baseline condition related best to their health problems. Additionally the relationship of exhaled nitric oxide (a marker of airways inflammation in asthma[271]) and bronchial responsiveness to HRQoL were secondary outcome measures.

3.1 Methods

The methods and protocol for the screening visit have been outlined in chapter 2 and will be briefly covered here.

3.1.1 Patient Selection

Subjects were recruited from clinics at University Hospital Aintree or poster advertisement with a self-reported BMI ≥30 kg/m^2 , aged 18-65 years, either non-smokers or ex-smokers of >2 years and taking asthma medication. Individuals taking long-term oral corticosteroid therapy, those with other significant co-morbidities or those reporting an exacerbation within the

previous two weeks were excluded. Four subjects were found to have a BMI <30 kg/m^2. In these the BMI was ≥ 28 kg/m^2 and inclusion did not significantly affect the outcome so were included in the intention to recruit analysis.

3.1.2 Questionnaires

Participants completed the St Georges Respiratory Questionnaire (SGRQ)[163], Short Form 36 (SF36)[158] and Impact of Weight on Quality of Life-Lite (IWQOL-Lite)[244] questionnaires validated to assess the effect of respiratory disease, generic factors and weight on quality of life respectively.

3.1.3 Atopy

Atopic status was determined using skin prick testing with a battery of common aeroallergens. A positive result being defined as at least one response with a wheal diameter ≥ 3mm or larger than a control response after 15 min.

3.1.4 Exhaled markers of inflammation

Participants abstained from caffeinated drinks and food for 12 hours before testing. The fraction of exhaled nitric oxide (FeNO) measured in ppb was measured using a chemiluminescence analyser (NIOX®, AEROCRINE, Solna, Sweden) at a flow rate of 50ml/sec as per ERS/ATS guidelines[94]

3.1.5 Bronchial responsiveness

Methacholine inhalation testing was performed using the five breath dosimeter method as per ATS guidelines[17]. Airway responsiveness to

methacholine was expressed as the provocative concentration of methacholine inducing a 20% fall in FEV1 (PC_{20}).

Subjects unable to undergo methacholine testing due to an FEV1 ≤50% predicted underwent spirometry with bronchodilator response to nebulised salbutamol.

Bronchial hyper-responsiveness was defined by ≥ 20% drop in FEV1 with ≤ 8mg/ml methacholine or an increase ≥ 15% and 200ml in FEV1 from baseline following nebulised salbutamol.

3.1.6 Statistical Methods

This was an observational study with sample size determined by numbers of subjects recruited for an interventional trial powered for obese asthmatics with bronchial hyper-responsiveness.

Categorical variables are expressed as percentages of total subjects and compared using Chi squared test. Continuous variables are expressed using mean ± SD and compared using the Students unpaired *t* test if normally distributed. Non-normal distributions determined by Shapiro-Wilk testing are expressed using median and interquartile range. Correlations were performed between normally distributed variables using Pearson correlations two-tailed test and non-normally distributed variables using Spearman's. PC_{20} and FeNO were log transformed to provide normal distributions before correlations calculated with Pearson's. A weak correlation was defined as r = 0.2-0.4, moderate correlation as r = 0.4-0.7 and a strong correlation as r = 0.7-1.0. SPSS version 16 for windows was used for calculation.

Significance was determined if $p<0.05$. Significance of comparisons of multiple variables was adjusted using the Bonferroni correction.

3.2 Results

3.2.1 Subject recruitment

397 subjects underwent telephone screening as outlined in figure 1. 91 subjects were retained in the analysis.

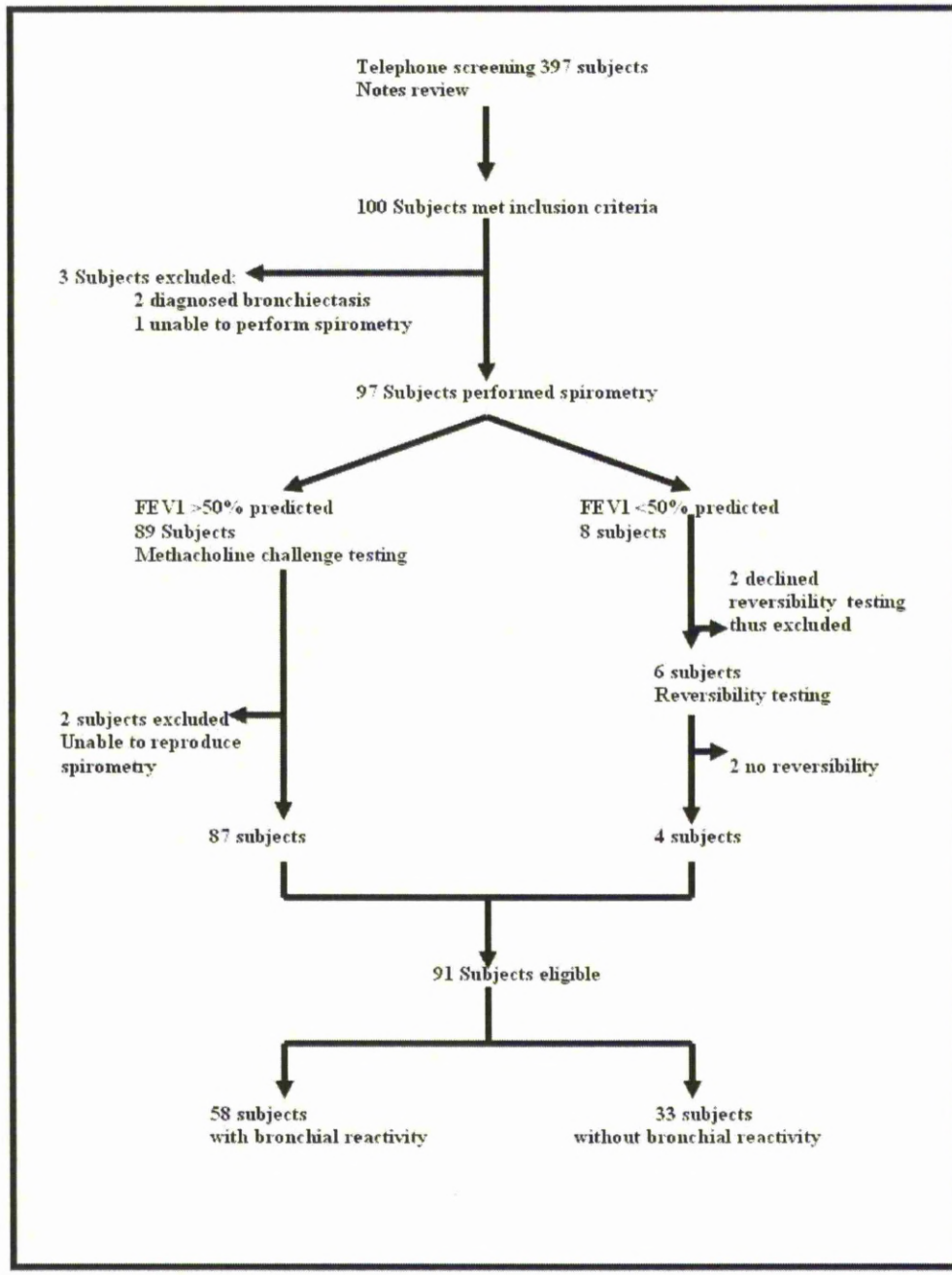


Fig 5. Consort diagram for screening patients

3.2.2 Subject Characteristics: all subjects.

Demographic characteristics, pulmonary function and exhaled nitric oxide of the study participants are summarised in Table 6.

Variables	
Age, yr	49.2 (9.6) yr
Female Gender	60/91 (65.9%)
Ex-Smokers	32/91 (35.2%)
Pack yr (ex smokers)	17.2 (19.3)
BMI Kg/m^2	38 (7) Kg/m^2
Weight Kg	105.6 (22.6) Kg
Subjects with atopy	61/90 (67.8%)
Dose of inhaled steroids µg/d	1273.5 (937.1) µg/d
FEV1 % predicted	85.8 (19.8) %
FVC % predicted	103.1 (17.2) %
FEV1 / FVC	70 (10.6) %
PC_{20} mg/ml	5.087 (6.71) mg/ml
FeNO ppb	25.1 (21.5) ppb

Definition of abbreviations: BMI = Body mass index; FEV1 = forced expiratory volume in 1 second; FVC = forced vital capacity; PC_{20} = concentration in mg/ml methacholine to produce a 20% decrease in FEV1; FeNO = fraction of exhaled nitric oxide at 50ml/s flow rate.
Numbers expressed as mean (sd) or number of cases / number in group (percent)

Table 6. Demographics, medical characteristics, pulmonary function, bronchial responsiveness to methacholine and level of exhaled nitric oxide for all subjects

Subjects were obese with relatively well preserved lung function. Five subjects were taking inhaled steroid medication but did not know their inhaled dose while 4 were not using inhaled steroid. Short acting beta agonists were prescribed in all. 55 (60.4%) used long acting beta agonists. 1 subject refused skin prick testing.

Dose of inhaled steroid (BDP equivalent) weakly related to FEV1 % predicted ($r= -0.29$, $p=0.007$) and FEV1/FVC ($r=-0.26$, $p=0.017$), but not PC_{20}. There was no significant difference in PC_{20} ($p=0.630$), presence of bronchial hyper-responsiveness ($p=0.673$), FEV1 % predicted ($p=0.055$) or FEV1/FVC ratio

(p=0.179) between those taking and those not taking long acting beta agonists.

BMI weakly correlated with PC_{20} (r=0.29, p=0.033) and FeNO (r=-0.32, p=0.025).

3.2.3 Questionnaires

SF36 data were not available in 1 subject due to a completion error. Questionnaire scores for the whole group are shown in Table 7.

SGRQ Domain	Mean (SD)
Symptoms	61.1 (18.4)
Activity	54.7 (22.2)
Impacts	33.2 (17.6)
Total	44.3 (17.0)

SF36 Domain	Mean (SD)
Role physical	53.3 (43.3)
Body pain	62.5 (25.8)
General Health	50.0 (22.3)
Vitality	43.5 (23.3)
Social Functioning	65.3 (26.2)
Role Emotional	61.1 (42.6)
Mental Health	64.4 (19.3)
Mental Health Total	56.9 (21.2)
Physical Health Total	52.0 (22.9)

IWQOL Lite Domain	Mean (SD)
Physical Function	57.2 (25.5)
Self Esteem	49.4 (28.0)
Sexual Life *	68.7 (56.2)
Public Distress *	75.0 (30.0)
Work *	81.2 (37.5)
Total	60.9 (21.6)

*= distribution non-normal

Table 7. Questionnaire scores for all subjects for SGRQ, SF-36 and IWQOL-Lite

Mean (SD) total scores for SGRQ = 44.4 (17.0), SF36 (Mental Health subtotal) = 56.9 (21.2), SF36 (Physical Health subtotal) = 52.0 (22.9) and IWQOL-Lite = 60.9 (21.6) with good correlations between them (p<0.001) Fig 6.

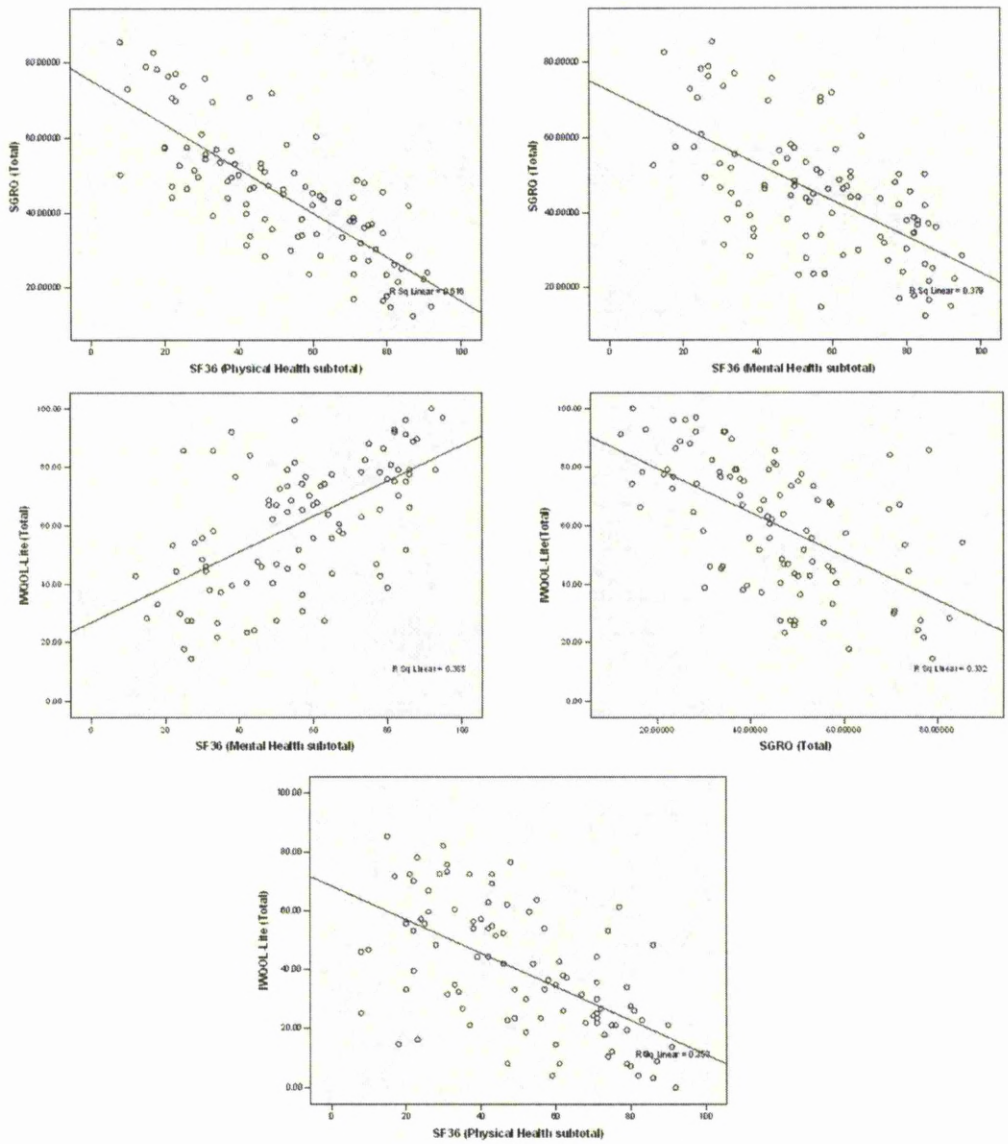


Fig 6. Scatterplots showing correlation of Total scores of SGRQ, IWQOL-Lite and subtotals for mental health and physical health of SF 36

3.2.4 HRQoL, pulmonary function, bronchial responsiveness, BMI and airway inflammation

The influence of pulmonary function, airway responsiveness, BMI and airway inflammation on HRQoL are shown in table 8.

	FEV1 % predicted	FVC% predicted	Log^{10} PC_{20}	BMI	Log^{10} FeNO
SGRQ					
Symptoms	-0.14	-0.07	-0.10	0.09	0.20
Activity	-0.06	-0.24	-0.20	0.42*	-0.15
Impacts	-0.17	-0.09	-0.12	0.24	-0.04
Total	-0.14	-0.16	-0.00	0.33*	-0.05
SF36					
Physical Function	0.01	0.23	-0.10	-0.43*	0.07
Role Physical	-0.13	-0.00	-0.08	-0.26*	0.28*
Body Pain	-0.26*	0.27	-0.00	-0.34*	0.19
General Health	0.19	0.02	0.06	-0.29*	0.21
Vitality	0.03	-0.05	-0.11	-0.29*	0.06
Social Functioning	0.01	-0.06	-0.01	-0.26*	0.13
Role Emotional	0.01	0.01	0.04	-0.30*	0.15
Mental Health	-0.06	0.03	-0.07	-0.22*	0.22*
Physical Health subtotal	0.01	0.13	-0.11	-0.42*	0.25*
Mental Health subtotal	0.04	-0.02	-0.07	-0.35*	0.19
IWQOL-Lite					
Physical Function	0.05	0.14	-0.23	-0.56*	0.30*
Self Esteem	0.07	0.15	0.02	-0.22*	0.14
Sexual Life	0.02	0.14	0.15	-0.19*	0.19
Public Distress	0.12	0.00	0.18	-0.62*	0.28*
Work	0.02	0.7	0.01	-0.39*	0.26
Total	0.07	0.14	-0.11	-0.51*	0.31*

* P<0.05 Bonferroni adjusted

Table 8. Correlations (r values shown) between measures of pulmonary function, airway responsiveness, BMI and airway inflammation

3.2.4.1 Airway inflammation & HRQoL

There were no significant correlations with FeNO and SGRQ domains or SF36 domains following Bonferroni correction. There were statistically significant weak correlations found with FeNO and IWQOL-Lite Physical functioning (r=0.30, p=0.004), Public Distress (r=0.28, p=0.008), and Total (r=0.31, p=0.003) domains.

3.2.4.2 BMI & HRQoL

SGRQ

BMI correlated moderately with the activity domain of the SGRQ ($r=0.42$, $p<0.001$) and weakly with Total SGRQ ($r=0.33$, $p<0.001$) but not symptoms.

SF36

There were moderate negative correlations between BMI, Physical function ($r=-0.43$, $p<0.001$) and Physical Health subtotal ($r = -0.42$, $p<0.001$) and weak negative correlations with Body Pain ($r = -0.34$, $p<0.001$), General Health ($r = -0.30$, $p=0.005$), Role Emotional ($r = -0.30$, $p=0.004$), Mental Health ($r = -0.22$, $p=0.033$)and Mental Health subtotal ($r=-0.35$, $p<0.001$). (Note that a lower score indicates worse HRQoL for SF36).

IWQOL-Lite

There were moderate correlations between BMI, Physical Function ($r=-0.56$, $p<0.001$), Public Distress ($r=-0.62$, $p<0.001$), and Total ($r=-0.51$, $p<0.001$) with a weak correlation between BMI and Work ($r=-0.39$, $p<0.001$).

3.2.4.3 FEV1% predicted, FVC% predicted & HRQoL

There were no significant correlations between any measures of quality of life and FEV1% or FVC% predicted.

3.2.5 Bronchial hyper-responsiveness as an explanatory variable

Subjects with bronchial hyper-responsiveness n=58 (63.7%) were compared to those without n=33 (36.3%) and subject characteristics for each group are summarized in table 9.

	With bronchial hyper-responsiveness n=58	Without bronchial hyper-responsiveness n=33	
Variables			P
Age Years	47.7 (9.7)	52.0 (9.0)	<0.05
Female Gender	38/58 (65.5%)	22/33 (66.7%)	0.911
Ex-Smokers	25/58 (43.1%)	7/33 (21.2%)	<0.05
Pack yr (ex smokers)	17.4 (20.7)	16.1 (14.1)	0.882
BMI Kg/m^2	37.6 (6.5)	38.5 (7.9)	0.560
Weight Kg	105.4 (21.6)	106.0 (22.1)	0.895
Subjects with atopy	45/57 (78.9%)	16/33 (48.5%)	<0.05
Dose of inhaled steroids µg/d (BDP equivalent)	1370.9 (1033.5)	1082.1 (688.0)	0.186
FEV1 % predicted	81.3 (21.3)%	93.7 (13.7)%	<0.05
FVC % predicted	102.2 (19)%	104.8 (13.5)%	0.498
FEV1 / FVC	67 (11.3)%	75.3 (6.3)%	<0.05
FeNO ppb ¥	19.1 (22.8)	15.0 (16.2)	<0.05
Taking SABA	57/58 (98.3%)	32/33 (97%)	0.683
Taking LABA	36/58 (62.1%)	19/33 (57.6%)	0.673

¥ non-normal distribution therefore median ./ IQR quoted
Definition of abbreviations: **BMI = Body mass index; FEV1 = forced expiratory volume in 1 second; FVC = forced vital capacity; FeNO = fraction of exhaled nitric oxide at 50ml/s flow rate. Numbers expressed as mean (sd) or number of cases / number in group (percent)**

Table 9. Demographics, medical characteristics, pulmonary function, bronchial responsiveness to methacholine and level of exhaled nitric oxide between subjects with bronchial hyper-responsiveness, defined as PC_{20} methacholine ≤8mg/ml and those without

Those with bronchial hyper-responsiveness (median PC_{20} 1.64 (IQR 3.48)mg/ml) were younger: 47.6 (9.7) yrs vs 52.0 (9.0) yrs ($p<0.05$), had lower FEV1% predicted: 81.3 (21.3) % vs 93.7 (13.7) % ($p<0.05$), and lower FEV1/FVC: 67 (11.3) % vs 75 (6.3) % ($p<0.05$). There was no significant difference in FVC%. Predicted. FeNO (median (IQR)) was significantly greater: 19.1 (22.8)ppb vs 15 (16.2)ppb ($p=<0.05$) and the percentage with atopy was greater in the bronchial hyper-responsive group 78.9% vs 48.5% ($p<0.05$) as were ex-smokers 43.1% vs 21.2% ($p=<0.05$).

Between groups there was no significant difference in female gender, BMI, dose of inhaled steroids or those taking beta agonists.

There were no significant differences in any domain or total scores for the SGRQ, SF36 subtotals or IWQOL Lite (Table 10) between those with and without bronchial hyper-responsiveness. There were no significant correlations between PC_{20} and any HRQoL domains.

	With bronchial hyper-responsiveness n=58	Without bronchial hyper-responsiveness n=33	
Domain	Mean (SD)	Mean (SD)	P
SGRQ			
Symptoms	63.0 (19.5)	57.7 (16.)	0.194
Activity	53.0 (21.4)	57.7 (23.7)	0.332
Impacts	32.7 (15.8)	34.2 (26.6)	0.710
Total	43.9 (15.7)	45.2 (19.3)	0.721
SF36			
Physical Functioning	51.5 (25.8)	57.2 (23.5)	0.297
Role-Physical	41.7 (42.7)	59.9 (42.7)	0.054
Bodily Pain	55.4 (28.2)	66.5 (23.6)	0.060
General Health	50.5 (24.1)	49.7 (21.3)	0.870
Vitality	45.6 (23.1)	42.3 (23.5)	0.521
Social Functioning	60.5 (27.3)	68.1 (25.4)	0.194
Role-Emotional	56.5 (42.1)	63.8 (43.0)	0.435
Mental Health	63.2 (21.5)	65.0 (18.1)	0.673
Physical Health subtotal	48.9 (24.0)	53.7 (22.2)	0.349
Mental Health subtotal	55.2 (22.8)	57.8 (20.4)	0.585
IWQOL-Lite			
Physical Function	52.7 (27.9)	59.8 (23.8)	0.223
Self Esteem	48.2 (29.5)	50.1 (27.3)	0.773
Sexual Life *	62.5 (65.6)	78.1 (50.0)	0.080
Public Distress *	75.0 (42.5)	77.5 (30.0)	0.304
Work *	75.0 (46.9)	84.4 (37.5)	0.123
Total	56.7 (23.8)	63.3 (20.1)	0.182

* Not normally distributed therefore median and IQR quoted. Mann Whitney U as test of significance

Table 10. Comparison of questionnaire scores for SGRQ, SF-36 and IWQOL-Lite between those with and without bronchial hyper-responsiveness

3.3 Discussion

In a group of obese subjects (mean BMI 38.0 Kg/m^2) with a prior diagnosis of asthma using inhaled medication, 36.3% did not demonstrate bronchial hyper-responsiveness. Although this does not exclude asthma it has a high negative predictive value[17] and suggests a mis-classification of diagnosis supported by lower FeNO[271], higher FEV1/FVC% and less atopy in the unreactive patients.

These patients had significant health impairment despite relatively well preserved lung function, the disease and weight specific quality of life being worse than previous published healthy populations[160, 165, 272, 273]. There was good correlation between total scores of all questionnaires suggesting they were measuring similar outcomes. The variable that correlated strongest with degree of health impairment was BMI rather than other traditional markers of asthma severity i.e. airway responsiveness (PC_{20}), lung function (FEV1 % and FVC% predicted) or airway inflammation (FeNO). There was no significant difference in HRQoL between those with and without bronchial hyper-responsiveness again suggesting less influence than BMI.

This study supports the results of Aaron et al who showed that a third of subjects with a prior physician diagnosis of asthma had no evidence of asthma judged by symptoms, lung function and bronchial challenge testing[249] and extend these observations to a more rigidly pre-specified population where it might be expected that the incidence of hyper-responsiveness in obese patients would be higher[154].

I have shown a consistent negative correlation of increasing BMI with HRQ0L measured by both generic and disease specific instruments. This

effect was much greater than any associations with degree of airway inflammation as assessed by FeNO which might have been expected to track asthma severity[271, 274]. The presence of bronchial hyper-responsiveness itself was not a good discriminator of impaired health status whilst medication use, specifically long-acting bronchodilators in addition to inhaled corticosteroids was neither different in the reactive and non-reactive groups nor predictive of differences in health status. As might be expected reactive individuals tended to have marginally worse lung function, more obstruction and more atopy but none of these factors would be a reliable discriminator.

A reduced quality of life associated with obesity is related to increased attendance rates to primary care[269] where patients have the opportunity to report respiratory symptoms[204, 275, 276] and each visit can potentially lead to mis-classification of asthma diagnosis. Increased physician interaction may explain some of the association of asthma with obesity and care must be taken when interpreting studies of asthma and obesity based on self reporting of asthma diagnosis.

It is likely that the negative correlation of body mass with HRQoL is due to a generic effect[267] as there were correlations across all questionnaires and we did not find a significant correlation between BMI and the symptoms domain of the SGRQ which includes questions on frequency of cough, sputum, breathlessness, wheeze and exacerbations.

This study has some limitations due to its observational nature using data from screening subjects for an interventional study. Subject numbers were not equally matched between groups, but groups were well-matched for age, weight and BMI. Although there were more ex-smokers in the bronchial

hyper-responsiveness group, excluding ex smokers from analysis did not alter outcomes. The study entry criteria precluded the inclusion of patients with normal BMI and so our data are confined to obese patients.

There is no universally accepted definition of asthma[15] and patients can have asthma without demonstrable bronchial hyper-responsiveness. Many studies require the presence of bronchial responsiveness defined as a PC20 calculated by linear interpolation of the log concentration to methacholine to cause a 20% fall in FEV1 of <8 mg/ml or reversibility of FEV1 to inhaled bronchodilators of 15%[17]. I therefore used these criteria towards making a diagnosis of asthma which is supported by the evidence of less airway inflammation, less airway obstruction and less atopy in those that did not show bronchial hyper-responsiveness.

It is possible that the use of inhaled steroids resulted in improvement in bronchial responsiveness[270]. However, there was no difference in mean dose of inhaled steroid between those with and without increased bronchial responsiveness.

The screening protocol was not designed to measure static lung volumes and therefore I was unable to show a relationship between HRQoL and functional residual capacity or expiratory reserve volume which are reduced in obesity[176, 199] possibly linked to bronchial hyper-responsiveness[154]. I did however measure FVC which can give an idea of lung volume and there was no difference in FVC between those with and without bronchial hyper-responsiveness and no correlation between FVC and PC20 or HRQoL.

The SGRQ is not specific for asthma but is validated as a tool for asthma research[277] with a similar ability to discriminate among groups of

patients based on asthma severity and control compared to the asthma quality of life questionnaire[166].

Obesity increases the risk of other comorbidities which may influence HRQoL[278]. I excluded these through screening.

Previous studies of the impact of asthma on HRQoL exist[279] and the effect is multifactorial including disease severity, pulmonary function, symptoms and other measures, little is known about the impact of weight on this complex relationship[268]. There are similar relationships between the effect of BMI on HRQoL[267] and further work is required to explore these complex relationships.

I found a significant number of patients with a potential mis-classification of a diagnosis of asthma in an obese population. The strongest correlations with either generic or disease specific HRQoL were found with BMI. This has some clinical implications. Much of modern asthma management is focussed on symptom reduction either by increasing the intensity of maintenance treatment (GOAL Bateman[280]) or adjusting the daily treatment regime (SMART Rabe[281]). Applying such approaches to patients who remain as symptomatic as my non-reactive obese patients might be harmful. The reactive and non-reactive groups reported similar degrees of symptom intensity and used similar amounts of asthma treatment. Future studies should consider whether therapy can be withdrawn effectively in these obese patients receiving more therapy. Certainly a more robust initial diagnostic approach might save time and money over the long term by identifying patients whose asthma corresponds to more conventional diagnostic criteria.

These data emphasise the complex problems of identifying respiratory disease accurately in obese subjects. Future work is needed to study the impact of weight loss in this patient group and its impact on HRQoL.

Chapter 4: Weight loss in obese asthmatics

4.1 Introduction

Most previous studies investigating possible links between asthma and obesity have relied on cross sectional data[247]. Although this may suggest a possible relationship it is difficult to determine cause and effect. As previously noted, some studies have relied on subjects' self reported history of symptoms of wheeze and a doctor diagnosis of asthma, however, as I have shown in the previous chapter this may not be a reliable indicator for the presence of asthma when objective measures are not included[249]. Other studies investigating a possible relationship between body mass index and asthma have relied on data from large cohort studies to determine body mass index and relate this to the onset of asthma. These studies have suggested a relationship between obesity and asthma and suggest that the risk of developing asthma is greater in individuals with a higher body mass index. Although more robust in terms of determining a temporal relationship between the onset of asthma and the presence of obesity, many of these studies were not designed with this question in mind[3].

Other studies have sought to investigate the relationship between asthma and obesity through interventions inducing weight loss. Some of these studies have involved patients that have undergone bariatric surgery, however the effect of the surgery itself may act as a confounding factor when investigating the effects of weight loss in terms of measuring lung volumes and inflammatory markers. The effect of abdominal surgery may affect diaphragmatic function and wound healing may affect systemic inflammatory markers and therefore introduce possible confounders[282-284].

Non surgical weight loss interventions are available in a number of forms including low or very low calorie diets, meal replacements and pharmacological intervention which should generally be given together with behavioural intervention and support for increased physical activity. (NICE clinical guidelines 43)[285]. Using weight loss techniques that do not involve surgery eliminates the possible confounding factor of the surgery itself, and is applicable to a much wider group of people with obesity. Although there is still the possibility of a change in diet or behavioural intervention affecting asthma outcomes such as symptoms, asthma control and quality of life rather than the effect of weight loss itself, these are less likely to be significant compared to a surgical intervention.

A clinically significant weight loss has been determined by a consensus of obesity experts to be ≥5% of body weight in terms of improving lipid, glucose and blood pressure levels with potential reductions in cardiovascular disease and diabetes risk[286, 287]. We therefore wished to choose a method shown to achieve this target and chose a meal replacement strategy and behavioural intervention. Stenius-Aarniala et al managed to achieve a mean weight reduction of 14.5% using a weight reduction programme including 12 group sessions lasting 14 weeks including 8 weeks taking a very low energy dietary preparation giving a daily energy intake of 1.76MJ[246].

We chose the method outlined in chapter 2 which had previously been used by the clinical research department for endocrinology and diabetes at University Hospital Aintree for weight loss studies, designed to produce an energy deficit of at least 0.5 MJ per day which has been shown to predict a weight loss in itself of 0.45Kg per week[288] and can be enhanced with

behaviour modification[289] and meal replacement strategies[290]. Low calorie diet strategies with partial meal replacement plan, when administered with behaviour modification have been shown to result in a significant amount of weight loss of up to 7% in 3 months and 7-8% at 1 year[290]. It has also been shown to achieve ≥5% weight loss in 72% at 3 months and 74% at 1 year. The meal replacement strategy using meal replacement shakes, soups, hot chocolate and nutrition snack bars (Slim·Fast; Slim·Fast Foods Company, West Palm Beach, FL) has been shown to be effective at producing weight loss of ≥5% and is well tolerated[291] and was therefore used in this study.

I hypothesised that an intervention arm of randomised subjects in a 1:1 fashion would lose clinically significant percentage i.e. ≥5% of their starting weight at 3 and 6 months. I also hypothesised that this would be significantly greater than the control or non-intervention group and this difference could therefore be used to determine differences in asthma control.

4.2 Methods

The methods used during this study have been outlined in the chapter 2 but I will recap briefly here.

4.2.1 Randomisation

Subjects were randomised into one of two groups (an intervention group referred to as the dietician group and control arm) in a 1:1 ratio based on codes generated by the University of Liverpool Statistics Department stratified to ensure equal numbers of males and females in each group The

investigators were blinded to this process. The subjects underwent the following interventions as per their group:

4.2.1.1 Group A – Dietician group

The study investigators were not involved in the intervention at any point to ensure that they could not influence the study and therefore avoid bias. The dietician group entered into a meal replacement strategy low calorie diet by a study dietician following the baseline visit as per the protocol explained previously. Meal replacement products (Slim·Fast; Slim·Fast Foods Company, West Palm Beach, FL) were used

4.2.1.2 Group B – Control arm

At the end of the baseline visit those subjects that were randomised into the control group were given a standard leaflet on weight loss (British Heart Foundation "so you want to lose weight") and were not given any further dietary advice or support during the study.

4.2.2 Measures of weight and weight change

At each study visit measures of body mass index (kg/m^2) calculated from weight (Kg) and height (m) were taken using calibrated scales and a stadiometer. Bioimpedence using a Bodystat Quadscan (Tanita) was used to estimate total body fat%.

4.2.3 Statistical analysis

Sample size was determined based on the study by Stenius-Aarniarla[246] to have 80% power to detect a 12% change in morning peak flow

rate. This was felt to reflect a change in asthma severity and therefore would allow comparison of other markers of asthma severity used in this study. This was felt to be 80 patients randomised on a 1:1 basis into an intervention and control group. Allowing for a dropout rate of 5 patients per group it was felt that 25 subjects per group would be required to demonstrate significant changes in body weight and 30 subjects per group to show a change in lung function. A previous study has shown clear differences in body weight and measures of asthma severity with 19 subjects per group and it was felt that this sample size should be adequate for the primary outcome of improvement in bronchial hyper-responsiveness.

Categorical variables are expressed as percentages of total subjects and compared using Chi squared test. Continuous variables are expressed using mean ±SD and compared between groups with the Students unpaired *t* test if normally distributed. Non-normal distributions determined by Shapiro-Wilk testing are expressed using median and interquartile range. Variables compared between visits were compared using paired-samples *t* testing. Correlations were performed between normally distributed variables using Pearson correlations two-tailed test and non-normally distributed variables using using Spearman's.

Significance was determined if $p<0.05$.

4.3 Results

As noted in the previous chapter, from 91 subjects who underwent screening, 58 met the inclusion criteria to be included in the trial and were asked to attend for their first visit (baseline). 7 subjects were lost to follow up

and did not wish to continue. 51 subjects attended the baseline visit and were randomised according to the described protocol stratified by gender. 26 were randomised to the dietician group and 25 to the control group.

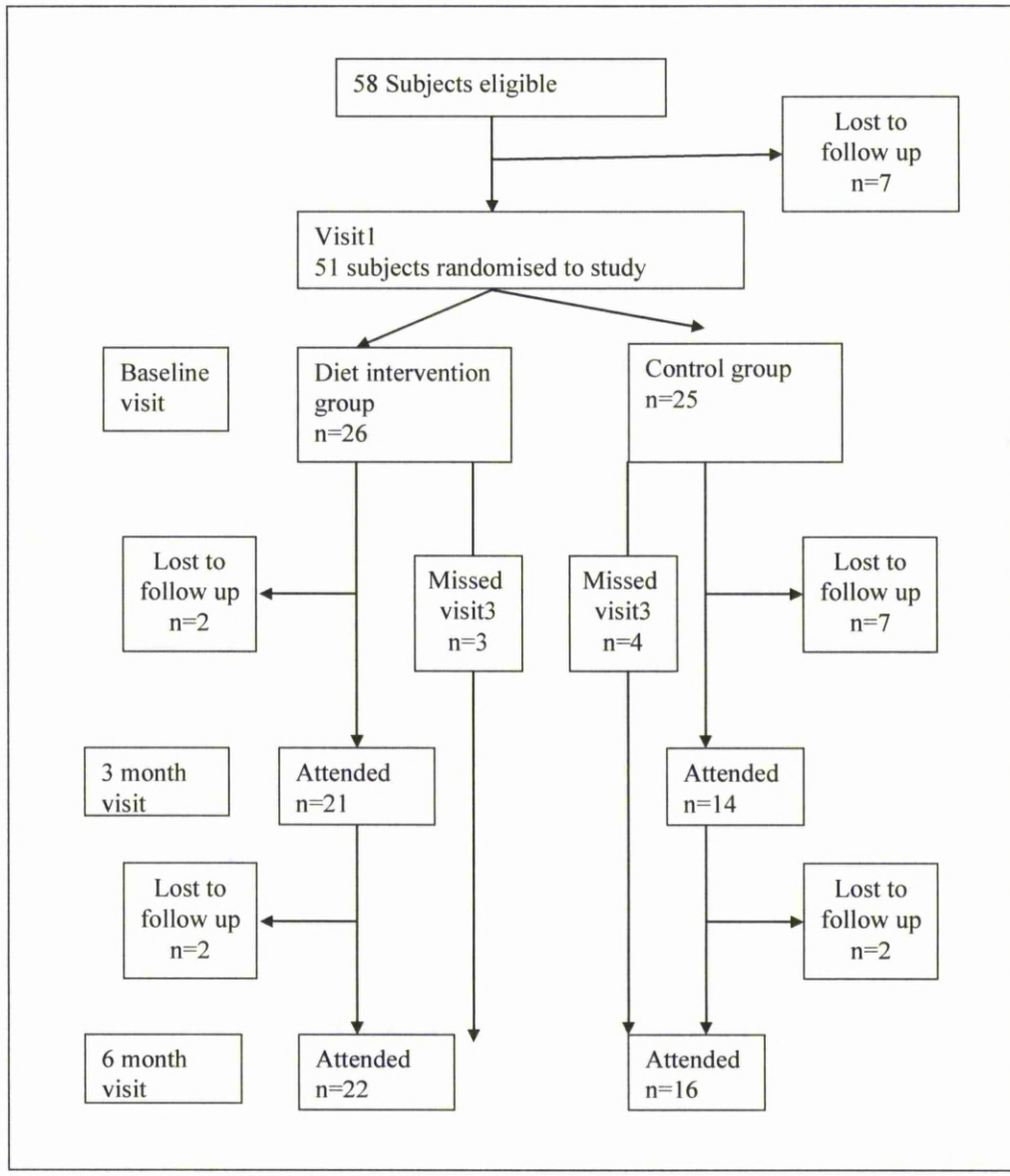


Fig 7. Consort diagram for trial

4.3.1 Subject Characteristics

Demographic characteristics, pulmonary function and exhaled nitric oxide of the study participants are summarised below.

4.3.2 Demographics

	Dietician Group n=25	Control Group n=26	P
Age Years	45.6 (8.9)	49 (10.9)	0.219
Female Gender	16/25 (64%)	16/26 (61.5%)	0.856
Ex-smokers	13/25 (52%)	3/26 (11.5%)	<0.05
Pack yr (ex smokers)	13.2 (8.9)	3.7 (1.2)	<0.05
BMI Kg/M^2	38.2 (5.6)	37.2 (5.5)	0.513
Weight Kg	106.5 (21.5)	107.6 (21.4)	0.850
Fat%	43% (8)	41.8%	0.627
Subjects with atopy	22/25 (88%)	17/26 (65.4%)	0.057
Dose of inhaled steroids µg/d (BDP equivalent)	1287.5 (858.4)	1054.2 (1010.4)	0.393
FEV1% predicted	78.7% (21.7)	88.6% (17)	0.077
FVC% predicted	100.2% (19.6)	105.5% (14.7)	0.279
FEV1/FVC	66.1% (10.8)	70.6% (0%)	0.117
FeNO ppb	28.5 (26.6)	31.7 (25.6)	0.665
Taking SABA	26/26 (100%)	26/26 (100%)	Na*
Taking LABA	15/25 (57.7%)	14/26 (56%)	0.903

*unable to perform chi squared but all pts on SABA therefore no significant difference noted

Definition of abbreviations: BMI = Body mass index; BDP = Beclomethasone dipropionate equivalent; FEV1 = forced expiratory volume in 1 second; FVC = forced vital capacity; FeNO = fraction of exhaled nitric oxide at 50ml/s flow rate; SABA = Short acting beta agonist; LABA = Long acting beta agonist. Numbers expressed as mean (sd) or number of cases / number in group (percent)

Table 11. Demographics, medical characteristics, pulmonary function, level of exhaled nitric oxide and medication for each study group

Subjects were well matched for lung function, exhaled nitric oxide, atopy, age, gender, BMI, weight and medication. There were significantly more ex smokers with a higher pack year history in the dietician group vs the control group. All subjects were using inhaled short acting beta agonist medication. One subject in the dietician group did not know their inhaled

steroid dose and two subjects in the control arm were not taking inhaled steroids. Despite this there was no significant difference in dose of inhaled steroid (BDP equivalent) between groups. There were no significant correlations between steroid dose and FEV1% predicted, FVC% predicted, FEV1/FVC ratio, FeNO or PC20 in either the dietician or control groups:

There was no significant difference in long acting beta agonist use between groups. In the dietician group there was no significant difference in PC20 (p=0.553), FEV1% predicted (p=0.239), FVC% predicted (p=0.302) or FEV1/FVC ratio (p=0.440) between those taking and those not taking long acting beta agonists. This was similar in the control group for PC20 (p=0.734), FEV1% predicted (p=0.284), FVC% predicted (p=0.082), or FEV1/FVC ratio (p=949).

4.3.3 Intention to treat analysis

4.3.3.1 Change in weight between visits

Between groups

	Mean (SD) Kg		
Visit	Dietician group	Control group	P value
Baseline	106.5 (21.5) Kg	107.6 (21.4) Kg	p=0.850
3 months	102.2 (20.9) Kg	107.4 (22) Kg	p=0.485
6 months	104 (18.9) Kg	105.2 (13.2) Kg	p=0.824

Table 12. Weight of subjects at each visit in each group mean (sd) shown

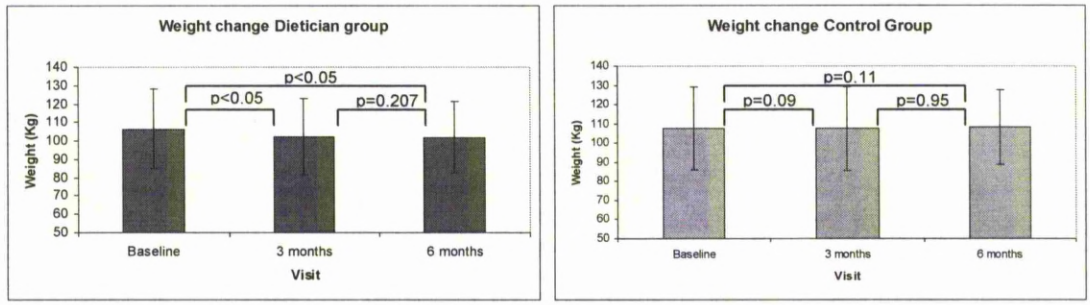


Fig 8. Mean weight of subjects in dietician and control groups comparing differences between baseline to 3 months, 3 months to 6 months and baseline to 6 months

The results of weight loss in Kg show that those in the dietician group achieved significant weight loss between baseline and 3 months and this remained significant when comparing weight at 6months with their original weight although there was no significant weight loss between 3 and 6 months suggesting that most weight loss occurred in the first 3 months of the intervention and was maintained at 6 months. In the control group there was a trend towards weight loss which did not reach significance at either 3 months or 6 months.

The following graph and accompanying data shows weight loss in each group as expressed as percentage of original weight. Numbers of subjects in the table denotes number of subjects that had paired samples (i.e. attended visits at 3 & 6 months, this is different from the total numbers randomised due to drop out or non attendance for the 3 month visit. See consort diagram.)

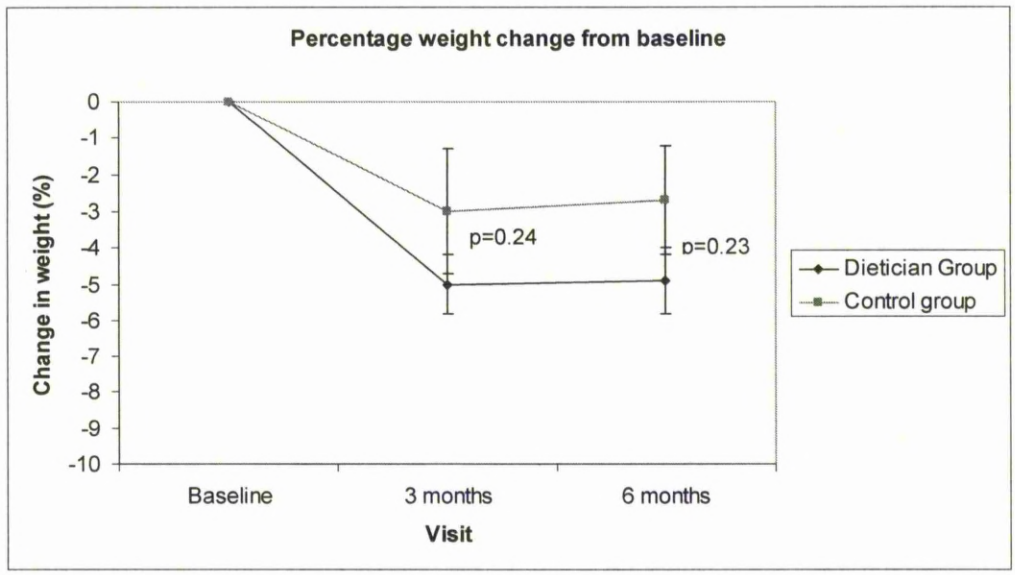


Fig 9. Change in weight as % of baseline at 3 and 6 months for each group. p values indicate differences between groups at each visit

Despite significant weight loss in the dietician group, when percentage weight loss in Kg at 3 and 6 months was compared between groups there was a trend towards increased loss of weight in the dietician group but this did not reach significance. This is also true for BMI and Fat %.

Time from baseline	Dietician group		Control group		
	No of subjects	% weight loss (SD)	No of subjects	% weight loss (SD)	p value
3 months	21	-5 (3.5)%	14	-3 (6.4)%	p=0.24
6 months	22	-4.9 (4.3)%	16	-2.7 (6.6)%	p=0.23

Table 13. Percentage weight loss from baseline for each group. Number of subjects in each group at 3 and 6 months and mean percentage change in weight from baseline. p value indicates comparison between dietician and control groups

4.3.4 Last observation carried forward

Due to the number of patient drop outs or non-attendance we have also investigated the above using last observation carried forward, as this methodology is commonly used in weight loss studies as a means of

assessing response to interventions where dropouts occur and is currently recommended for such studies by regulators such as the US FDA.

4.3.4.1 Change in weight between visits

Between groups

	Mean (SD) Kg		
Visit	Dietician group	Control group	P value
Baseline	106.5 (21.5) Kg	107.6 (21.4) Kg	p=0.850
3 months	102.2 (20.9) Kg	107.4 (22) Kg	p=0.512
6 months	101.9 (19.4) Kg	108.1 (19.3) Kg	p=0.422

Table 14 Weight of subjects at each visit in each group mean (sd) shown

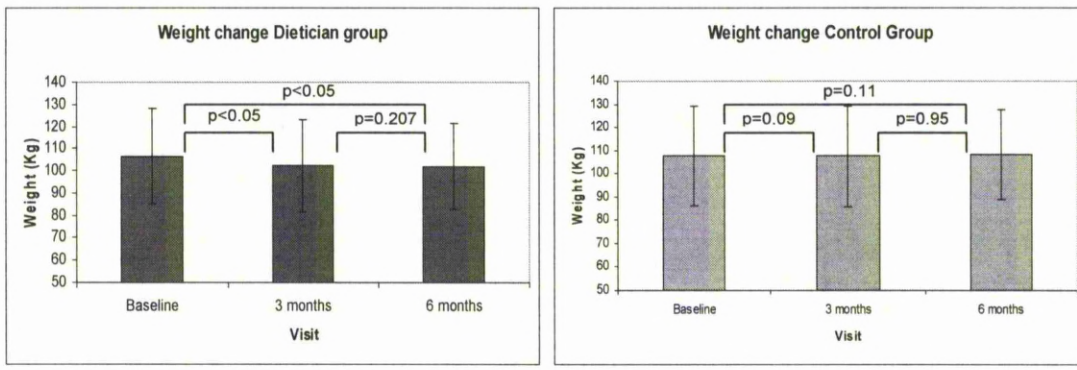


Fig 10. Mean weight of subjects in dietician and control groups comparing differences between baseline to 3 months, 3 months to 6 months and baseline to 6 months

As for the intention to treat analysis there was a significant weight loss in the first three months which is sustained at six months. There was no significant weight loss seen in the control group at either three or six months although there was a trend towards weight loss. When analysing for percentage of weight lost as a percentage of the starting weight there was a trend towards a greater percentage weight lost in the dietician group compared with the control group, however this did not reach significance. The mean percentage weight loss did however reach ≥ 5% in the dietician group

which is thought to be clinically significant in terms of health improvement.

This is also true for BMI and Fat %.

The graph and table is similar to the intention to treat analysis.

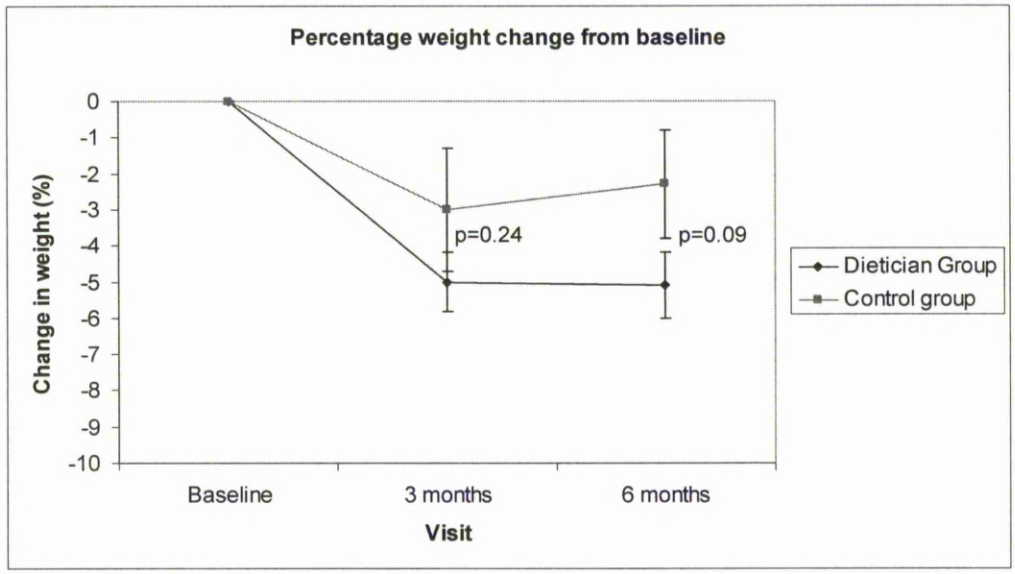


Fig 11. Change in weight as % of baseline at 3 and 6 months for each group. p values indicate differences between groups at each visit

Time from baseline	Dietician group		Control group		
	No of subjects	% weight loss (SD)	No of subjects	% weight loss (SD)	p value
3 months	21	-5 (3.5)%	14	-3 (6.4)%	p=0.24
6 months	24	-5.1 (4.5)%	18	-2.3 (6.3)%	p=0.2

Table 15. Percentage weight loss from baseline for each group. Number of subjects in each group at 3 and 6 months and mean percentage change in weight from baseline. p value indicates comparison between dietician and control groups

4.3.5 Using 5% weight loss as groups

As noted previously clinically significant weight loss is accepted by expert opinion to be ≥ 5%. I therefore examined the groups for any significant differences in the numbers of subjects that achieved this amount of weight loss. The following table shows numbers of subjects in each group at 3 and 6 months that achieved 5% weight loss.

Intention to treat analysis

	Control group n (%)	Dietician group n (%)	P value
3 months	6 (42.9%)	8 (38.1%)	p=0.778
6 months	5 (31.3%)	11 (50%)	p=0.248

Table 16. Number (percentage) of subjects in each group at 3 months and 6 months that lost ≥ 5% of baseline weight. p value represents comparison of means for each group

	Control group n (%)	Dietician group n (%)	P value
3 months	6 (42.9%)	8 (38.1%)	p=0.778
6 months	5 (27.8%)	12 (50%)	p=0.248

Table 17. Number (percentage) of subjects in each group at 3 months and 6 months that lost ≥ 5% of baseline weight. p value represents comparison of means for each group

There was no significant difference between the control and dietician groups for the numbers of subjects achieving 5% weight loss.

4.3.6 Influence of age and gender on weight loss

There was no significant difference in absolute weight loss or percentage weight loss in either the control or dietician groups between male or female subjects. There was no significant correlation with age of subject and absolute amount of weight loss or percentage weight lost.

4.3.7 Fat % as a variable

When using fat % as measured by bioimpedence as a measure of obesity there was no significant differences in outcomes compared to BMI or changes in weight.

4.4 Discussion and Conclusions

I was able to show significant weight loss in the intervention arm of the study at 3 months which was sustained at 6 months. Although there was a trend towards weight loss n the control group this was not significant at any time point. When comparing between groups at 3 and 6 months there was a greater weight loss in the dietician group, however this did not reach significance. This was true in the intention to treat analysis and also the last observation carried forward analysis.

One reason for this is that in both groups there were individual subjects that gained weight as well as lost weight and with both groups achieving an overall mean weight loss the difference between the two was not great enough to achieve significance. There was no difference between the groups for the number of subjects that achieved the clinically significant weight loss of ≥5% although the mean weight loss for the dietician group as a whole did reach this level at 3 and 6 months but was not reached in the control group. One of the reasons for weight loss in both groups may be due to the so called Hawthorne effect in that subjects in a control group of a study experience an effect due to the very fact that they are taking part in the study itself[292].

I can therefore suggest that further analysis of markers of asthma severity can continue using the two groups of subjects although as there were individuals that lost significant amounts in both groups we can include analysis of the group as a whole between those that achieved clinically significant weight loss vs those that did not. Interestingly Fat% measured by bioimpedence did not add any further information and therefore will not be included in further analysis in this thesis.

The choice of weight loss intervention may not have been sufficiently effective: different methods of weight loss include reduced calorie diet, very low calorie diet with meal replacement, pharmacological and weight loss with surgical intervention. I used a partial meal replacement plan and a comprehensive behavioural approach which has previously been reported to induce a 10% to 12% initial weight loss in the first 12 to 16 weeks[293] and had been used in a similar published trial on the effects of weight loss on asthmatic subjects. Most studies have found that patients who completed a comprehensive VLCD program (that includes lifestyle modification) generally lost 15% to 25% of initial weight in 3 to 4 months. A metanalysis of weight management using a meal replacement strategy VLCD vs LCD[293] showed a weight loss between 13.4% and 19.9% at 26 weeks. Another metanalysis of studies of partial meal replacement vs conventional reduced calorie diet showed a 7% weight loss in the PMR group at 3 months and 7-8% at 1 year. There was significant different between the two groups at 3 months (4% vs 7%) and 1 year (3-7% vs 7-8%).

The weight loss seen in this study is similar to what might be expected using a multicomponent approach as recommended by NICE. In the meta-analysis reported in the NICE Obesity Guideline[285] (CG43), mean weight loss at 1 year was 3.82Kg compared to information alone, which is consistent with the weight loss seen here. Cultural differences between our UK study population and those studied by others (in Sweden and the USA) may also explain the difference in response to that previously reported.

It is felt by obesity experts that ≥5% weight loss is clinically significant to improve lipid, glucose and blood pressure levels with potential reductions in

cardiovascular disease[286] and in the Heymsfield metanalysis[290] it was shown that at 3 months, 34 and 72% of RCD and PMR groups lost ≥5% of initial body weight, respectively ($p<0.001$) with 33% vs 74% at 1 year. Again the intervention was designed on a method that was shown to achieve this amount of weight loss.

Partial meal replacement strategies redirect meal/food selections, potentially replacing self selected calorie dense foods with well-defined reduced calorie alternative of known nutritional value. VLCDs replace all meals and represent an extreme in structured diets for weight control. PMRs may function in a similar way while additionally permitting subjects to develop learning skills in portion sizes as well as maintain an acceptable lifestyle. The higher calorie level of PMRs and slower rate of weight loss compared to VLCDs is less likely to promote complications such as cholecystitis and therefore my method was chosen to be safe.

There are some potential limitations to these analyses including the drop out rate. Weight loss studies are known to have high drop out rates of up to 66% in a systematic review of 44 long-term weight loss studies in obese adults[286]. I was able to achieve follow up at six months in 71% of the subjects with a drop out rate of 29% which is comparable to many interventional studies of weight loss also supported in a metanalysis of six VLCD weight loss studies that showed an attrition rate of 22.3% and a metanalysis of partial meal replacement studies[290] with a dropout rate of 19% at 3 months and 47% at 1 year. I have analysed my results with intention to treat analysis which is sometimes criticised as it is felt that this may bias the weight loss arm of the trial as more subjects are likely to continue in the trial if the weight loss is

successful and subjects are more likely to discontinue if they do not lose weight. To overcome this, last observation carried forward analysis is often used and I have also employed this method, however this also has its critics. Despite this the conclusions drawn do not differ between either method of analysis of the data.

4.5 Summary

The weight loss intervention, although not achieving a significant difference between groups at 3 and 6 months, appears to have been effective at achieving significant weight loss at 3 and 6 months. Therefore further analysis of data in this study can use 'between groups' comparisons and also compare between visits at 3 and 6 months. Further analysis can also be carried out between those that achieved clinically significant weight loss of ≥5% and those that did not in both groups for the whole cohort and also using all subjects by weight change.

Chapter 5: Health Related Quality of Life and weight loss

5.1 Introduction

As noted previously in Chapter 3 both weight and asthma can affect health related quality of life (HRQoL) or the "physical, psychological, and social domains of health, seen as distinct areas that are influenced by a person's experiences, beliefs, expectations, and perceptions"[156]. HRQoL reflects an individual's subjective evaluation and reaction to health or illness[294] rather than a medical professional's evaluation and measuring the effects of weight change on HRQoL therefore measures the impact of weight change on aspects of disease that are important to the patient and compliments other objective measures of asthma severity such as bronchial reactivity or measurable airway inflammation. HRQoL is recognised to be multidimensional and tools generally measure the functional ability, physical, emotional and social wellbeing of individuals. I have explored the relationship between weight loss, asthma control and HRQoL with two specific questionnaires and a generic questionnaire, these being the St George's Respiratory Questionnaire (SGRQ)[163] for asthma, the Impact of Weight on Quality of Life-Lite (IWQOL-Lite)[244] questionnaire for weight and the Short-Form 36 (SF36)[160] for generic quality of life. Disease specific questionnaires are more sensitive to change in the particular condition they are designed for and this is why they have been used here alongside the generic questionnaire from which they are derived.

Previous studies have shown that obesity is associated with worse quality of life that improves with weight loss interventions[243]. Studies have also shown the effect of asthma on quality of life which improves with improvement in asthma control[161]. I have already demonstrated how HRQoL

may be related to BMI in obese subjects with a prior diagnosis of asthma with or without bronchial hyper responsiveness and explore this further in this chapter longitudinally with change in weight.

5.1.1 Exploring changes in weight / BMI and health related quality of life

I have explored the different health related quality of life questionnaires and compared changes between the two groups within the study as the primary outcome. Correlations with changes in HRQoL and changes in weight and relationships between change in HRQoL between subjects that achieved clinically significant weight loss (≥ 5% of original weight) and those that did not were secondary outcomes. I have also explored the relationship with bronchial responsiveness, exhaled nitric oxide and the possible effect of gender.

5.2 Methods

The methods, order of investigations and study protocol are outlined in chapter 2 and I will recap the use of the questionnaires here in brief.

All questionnaires were administered to each subject at each visit i.e. baseline, 3 and 6 months. All were self completed as per the designer's recommendations.

On arrival to the department the questionnaires were completed before any procedures were undertaken. The subjects were asked to complete the questionnaires by themselves in a quiet area, free from distraction, although the investigators were on hand to answer any questions if required. They were asked to complete the questionnaires as honestly as possible and were told that there were no right or wrong answers. After completion the

questionnaires were checked to ensure that there were no answers missed in error and the questionnaires were collected.

Questionnaires were scored as per the designer's instructions as described in Chapter 2.

5.2.1 Statistical analysis

Categorical variables are expressed as percentages of total subjects and compared using Chi squared test. Continuous variables are expressed using mean ±SD and compared between groups with the Students unpaired *t* test if normally distributed. Non-normal distributions determined by Shapiro-Wilk testing are expressed using median and interquartile range. Variables compared between visits were compared using paired-samples *t* testing. Correlations were performed between normally distributed variables using Pearson correlations two-tailed test and non-normally distributed variables using Spearman's. Any correlations were checked visually for homoscedasticity to confirm any relationship. Significance for multiple comparisons were adjusted by Bonferroni correction. Significance was determined if $p<0.05$ and alpha level adjusted by Bonferroni for number of observations studied.

5.3 Results

58 subjects completed screening and were randomised into the trial as per the consort diagram Fig 7 (p140). Of 26 subjects in the dietician group, 21 attended at 3 months and 22 attended at 6 months. Of 25 subjects enrolled into the control group 14 attended at 3 months and 16 at 6 months. Due to

errors in completing questionnaires data was missing for 1 subject for SF36 at 3 months and 1 subject at 3 months and 6 months for the IWQOL-Lite.

5.3.1 HRQoL scores for all subjects for all visits

The HRQoL scores for all subjects are shown below for all visits and for each questionnaire. The mean scores for all questionnaires showed worse HRQoL in our population when compared to normative scores previously published (see chapter 2).

SF-36 (mean (sd))

	Baseline (n=51)	3 months (n=34)	6 months (n=38)
Phys Functioning	60.1 (20.9)	64.7 (20.7)*	71.8 (20.5)¥
Role Physical	57.4 (41.9)	75.7 (37.2)*	73.7 (37.2)¥
Bodily Pain	65.5 (25.4)	68.0 (22.4)	70 (24.8)
General Health	51 (21)	51 (21.5)	55.8 (21.4)¥
Vitality	47.2 (21.5)	51.6 (21.5)*	52.9 (20.2)¥
Social functioning	70.3 (23)	76.6 (25.5)*	77.1 (26.1)
Role Emotional	69.3 (38.8)	76.5 (37.2)	73.7 (41.2)
Mental Health	68.8 (15.8)	67.2 (19.7)	67.4 (20.5)
Physical Health summary	56.2 (20.3)	62.2 (18.3)*	64.8 (19.1)¥
Mental Health Summary	61.4 (18)	64.5 (19)*	65.3 (20.9)¥
Total	61.2 (19)	66.4 (18.8*)	66.7 (20.7)¥

* = Significant change ($p<0.05$) between baseline and 3 months
¥ = Significant change ($p<0.05$) between baseline and 6 months
For SF36 higher score = better HRQoL

Table 18. Mean (SD) scores for each domain of the SF36 HRQoL questionnaire at each visit for all subjects

SGRQ (mean (sd))

	Baseline (n=51)	3 months (n=35)	6 months (38)
Symptoms	62.7 (19.5)	58.2 (19.9)*	54.2 (19.3)¥
Activity	50.8 (19.6)	47.2 (21.1)*	41.8 (21.4)¥
Impacts	29.7 (15.4)	25.2 (14.8)*	26.6 (18.7)
Total	41.6 (14.9)	37.4 (15.1)*	35.8 (17.4)¥

* = Significant change ($p<0.05$) between baseline and 3 months
¥ = Significant change ($p<0.05$) between baseline and 6 months
For SGRQ lower score = better HRQoL

Table 19. Mean (SD) scores for each domain of the SGRQ HRQoL questionnaire at each visit for all subjects

IWQOL Lite (mean (sd))

	Baseline (n=51)	3 months (n=34)	6 months (n=37)
Physical Function	65.4 (19.9)	66.7 (19.7)*	69.3 (20.2)¥
Self Esteem	52.7 (30.1)	53.4 (24.9)	57.8 (27.6)¥
Sexual Life	39.1 (28.1)	71.3 (27.1)*	72.1 (29.2)¥
Public Distress	75.4 (25.4)	74.5 (25)	80.8 (23.8)¥
Work	79.2 (20.5)	80.8 (17.6)*	84.3 (20)¥
Total	66 (19.8)	67.5 (18.1)*	70.5 (20.6)¥

* = Significant change ($p<0.05$) between baseline and 3 months
¥ = Significant change ($p<0.05$) between baseline and 6 months
For IWQOL-Lite higher score = better HRQoL

Table 20. Mean (SD) scores for each domain of the IWQOL-Lite HRQoL questionnaire at each visit for all subjects

There was a trend for improvements in all HRQoL total scores in the three questionnaires from baseline to 3 months and baseline to 6 months but no significant change between 3 and 6 months for any domains in any of the questionnaires used.

5.3.2 Comparing HRQoL scores between groups: Dietician group vs Control group

Questionnaire scores for all domains were compared between the dietician and control group at each visit and for each questionnaire. Differences in mean scores were compared between groups for each score

using independent-samples t-test and ANOVA with Bonferroni correction for multiple comparisons.

SF-36

	Dietician group Baseline (n=26)	Control group Baseline (n=25)	Dietician group 3 months (n=20)	Control group 3 months (n=14)	Dietician group 6 months (n=22)	Control group 6 months (n=16)
Phys Functioning	55.2 (20.9)	65.2 (20.1)	63.8 (20.3)	66.1 (21.9)	70.5 (18.4)	73.8 (23.7)
Role Physical	43.3 (41.6)	72 (37.7)	78.8 (34.7)	71.4 (41.4)	76.1 (36.6)	70.3 (39)
Bodily Pain	60.8 (26.1)	70.4 (24.3)	65.2 (23.5)	72.1 (20.9)	68.3 (21.8)	72.3 (28.9)
General Health	46.2 (21)	56 (20.2)	44.3 (24.2)	60.5 (12.3)	51.6 (25.1)	61.6 (13.5)
Vitality	40.2 (21.8)	54.4 (19.1)	50.3 (23)	53.6 (19.9)	51.1 (19.1)	55.3 (21.9)
Social functioning	62.2 (24)	78.7 (18.9)	77.6 (27.1)	75.1 (24)	73.5 (28.3)	82.1 (22.8)
Role Emotional	66.7 (38.9)	72 (39.3)	75 (38.8)	78.6 (36)	69.6 (42.4)	79.2 (40.1)
Mental Health	65.4 (16.5)	72.3 (14.5)	60.8 (20.6)	76.3 (14.6)	63.6 (19.5)	72.5 (21.4)
Physical Health summary	49.1 (19.6)	63.5 (18.7)	60.4 (18.2)	64.8 (18.9)	63.5 (17.2)	66.6 (22)
Mental Health Summary	56.2 (18.6)	66.8 (16.1)	61.5 (20.1)	68.8 (17.3)	61.8 (20.2)	70.2 (21.4)
Total	54.9 (18.3)	67.7 (17.7)	64.5 (18.7)	69.1 (19.2)	63.6 (19.1)	70.9 (22.7)

For SF36 higher score = better HRQoL

Table 21. Mean (sd) scores for each domain for dietician and control groups at each visit for the SF-36 HRQoL questionnaire. n = number of subjects in each group that attended each visit

There were no significant differences (ANOVA) between dietician and control group scores for any domain for the SF36 at any time point following Bonferroni correction for multiple comparisons (adjustment of alpha level: $p<0.00625$).

SGRQ

	Dietician group Baseline (n=26)	Control group Baseline (n=25)	Dietician group 3 months (n=21)	Control group 3 months (n=14)	Dietician group 6 months (n=22)	Control group 6 months (n=16)
Symptoms	66.8 (17.8)	58.5 (20.6)	61.9 (18.6)	52.7 (21.1)	56 (17.4)	51.9 (22)
Activity	55.6 (17.3)	45.8 (20.8)	48.8 (18.4)	44.9 (25.3)	46.3 (20)	35.7 (22.4)
Impacts	32.8 (17)	26.5 (13.2)	28.3 (15.7)	20.5 (12.6)	28.8 (19.2)	23.6 (18.2)
Total	45.4 (14.9)	37.7 (14.1)	40.1 (14.3)	33.3 (15.9	38.6 (17.1)	32 (17.7)

For SGRQ lower score = better HRQoL

Table 22. Mean (sd) scores for each domain for dietician and control groups at each visit for the SGRQ HRQoL questionnaire. n = number of subjects in each group that attended each visit

There were no significant differences (ANOVA) between dietician and control group scores for any domain for the SGRQ at any time point following Bonferroni correction for multiple comparisons (adjustment of alpha level: $p<0.0125$).

IWQOL-Lite

	Dietician group Baseline (n=26)	Control group Baseline (n=25)	Dietician group 3 months (n=20)	Control group 3 months (n=14)	Dietician group 6 months (n=22)	Control group 6 months (n=15)
Physical Function	59.4 (20.4)	71.6 (17.7)	64.8 (18.7)	69.3 (21.4)	68.6 (17.3)	70.5 (24.4)
Self Esteem	46.5 (30.2)	59.1 (29.3)	52 (26.3)	55.4 (23.6)	56.5 (25.4)	59.8 (31.3)
Sexual Life	69 (26.7)	69.3 (30)	73.1 (27.7)	68.8 (27.2)	76.1 (24.2)	66.3 (35.3)
Public Distress	72.3 (24.9)	78.6 (25.9)	74.3 (21.9)	74.6 (29.8)	78.6 (24.2)	84 (23.7)
Work	73.7 (20.1)	85 (19.8)	80 (14.7)	82.1 (21.6)	83.2 (17.1)	85.8 (24.1)
Total	61.2 (19.3)	70.9 (19.4)	66.7 (17.5)	68.6 (19.5)	70.3 (17.4)	70.8 (25.2)

For IWQOL-Lite higher score = better HRQoL

Table 23. Mean (sd) scores for each domain for dietician and control groups at each visit for the IWQOL-Lite HRQoL questionnaire. n = number of subjects in each group that attended each visit

There were no significant differences (ANOVA) between dietician and control group scores for any domain for the IWQOL-Lite at any time point following Bonferroni correction for multiple comparisons (adjustment of alpha level: $p<0.00833$).

5.3.3 HRQoL scores: Change from baseline

Changes in summary or total scores for each questionnaire and for each group at 3 and 6 months from baseline are shown below.

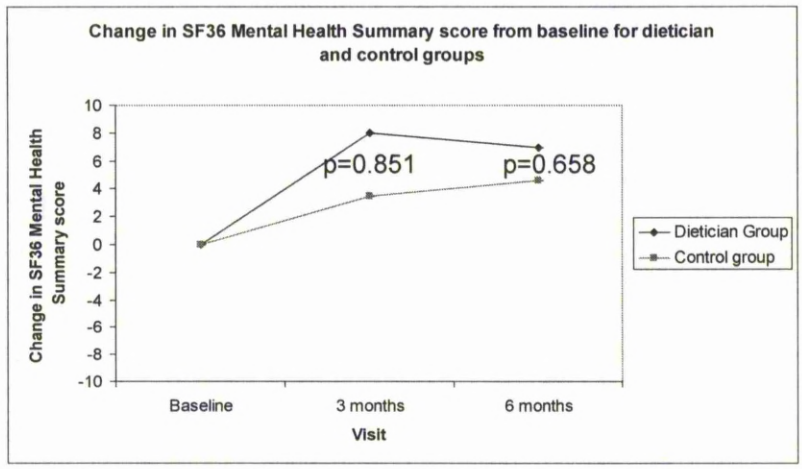
	3 months Dietician group	3 months Control group	6 months Dietician group	6 months Control group
SF36				
Physical Health summary	12.5 (13.3)	-0.2 (14.1)	12.9 (16.8)	3 (11.7)
Mental Health Summary	8 (13)	3.5 (17.5)	6.9 (14.3)	4.6 (16.4)
Total	11.3 (13.2)	1.6 (16.7)	8.2 (19.2)	4.1 (13.9)
SGRQ				
Total	-7.5 (8.7)	-3 (8.2)	-6.1 (9.5)	-4.9 (12)
IWQOL-Lite				
Total	7.3 (8.2)	3.4 (14.6)	8.7 (11.2)	2.3 (11.9)

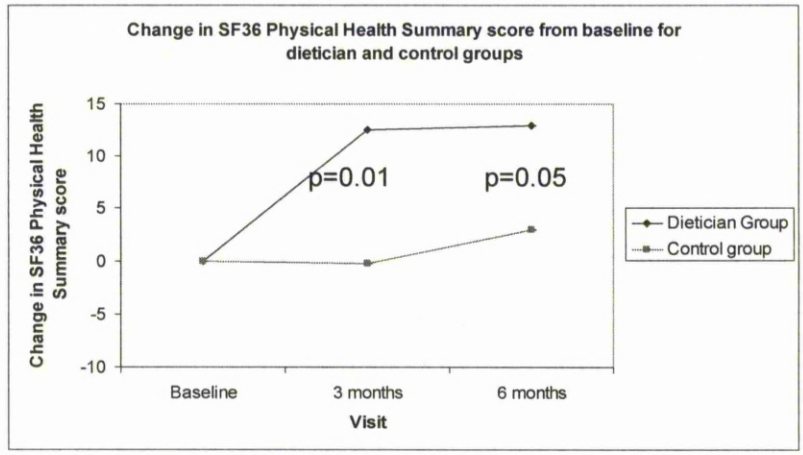
Significant correlation $p<0.05$

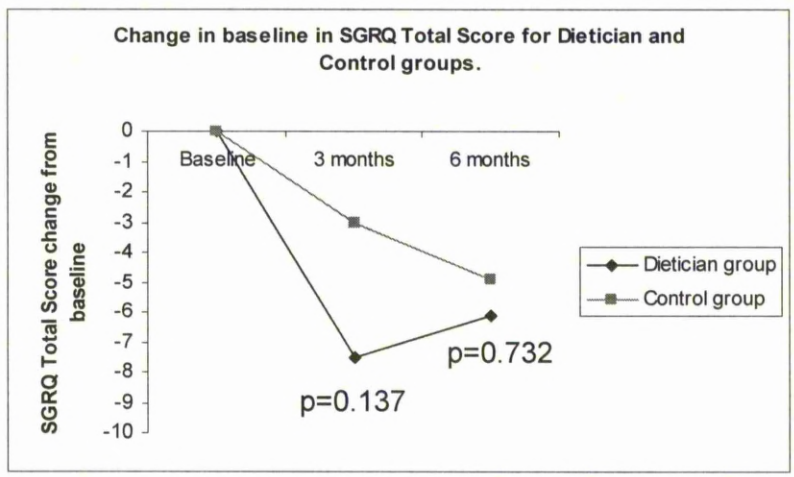
Table 24. Mean (SD) change in scores for each HRQoL questionnaire between baseline to 3 months and baseline to 6 months. Subtotal scores for SF-36 and total scores for SGRQ and IWQOL-Lite shown

Comparing changes in HRQoL scores from baseline there were no significant differences after Bonferroni correction for multiple comparisons between the dietician and control groups for total scores for the SF36, SGRQ or IWQOL-Lite from baseline to 3 or 6 months.

Although there was a trend for an increased improvement in HRQoL scores as can be seen from the graphs below this did not reach significance.


Change in SF36 Mental Health Summary score from baseline for dietician and control groups
Change in SF36 Mental Health Summary score
10
8
6
4
2
0
-2
-4
-6
-8
-10
p=0.851
p=0.658
Baseline
3 months
6 months
Visit
Dietician Group
Control group


Change in SF36 Physical Health Summary score from baseline for dietician and control groups
Change in SF36 Physical Health Summary score
15
10
5
0
-5
-10
p=0.01
p=0.05
Baseline
3 months
6 months
Visit
Dietician Group
Control group


Change in baseline in SGRQ Total Score for Dietician and Control groups.
SGRQ Total Score change from baseline
0
-1
-2
-3
-4
-5
-6
-7
-8
-9
-10
Baseline
3 months
6 months
p=0.732
p=0.137
Dietician group
Control group

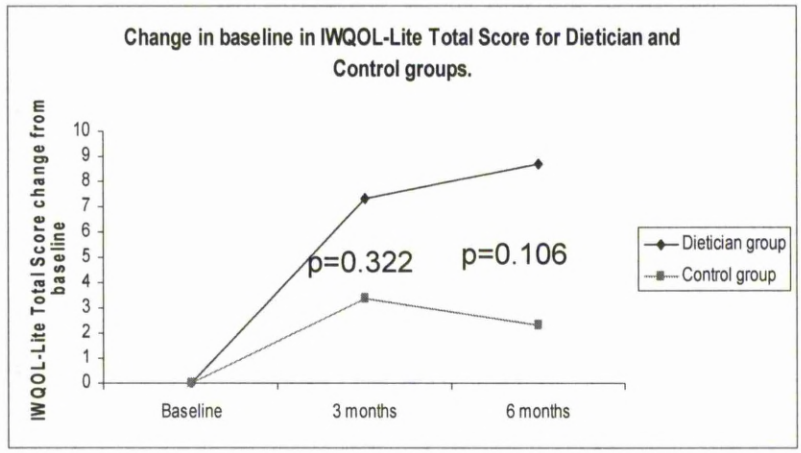


Fig 12. Graphs to show change in change in scores for SF-36 Subtotals, SGRQ and IWQOL-Lite total scores for each group from baseline to 3 months plus 6 months. p values represent comparison of means at 3 and 6 months between study groups

5.3.4 Relationship between weight and HRQoL scores.

5.3.4.1 Correlations between BMI and HRQoL

SF36

There were no significant correlations (appendix B) between BMI and any domain of the SF36 or subtotals after Bonferroni correction for all subjects or either group except the role physical domain at 6 months in the control group (r=-0.693, p=0.003)..

SGRQ

There were no significant correlations after Bonferroni correction (Appendix B) for any visits between BMI and HRQoL domain or total scores for the SGRQ questionnaire for all subjects or either group except the symptoms domain in the control group at 6 months (r=-0.640, p=0.008).

IWQOL-Lite

There were significant correlations after Bonferroni correction with BMI and multiple domains of the IWQOL-Lite questionnaire for all subjects at baseline for physical function (r=-0.437, p=0.001), public distress (r=-0.715, p=0.000) and total score (r=-0.460, p=0.001). This was also seen in the control group at baseline for public distress (r=-0.826, p=0.000) and total scores (r=-0.586, p=0.002). At 3 months there were correlations between BMI and public distress in all subjects (r=-0.704, p=0.000) and the control group (r=-0.752, p=0.003) plus total score for all subjects (r=-0.705, p=0.007) and control group (r=-0.705, p=0.007). Although at 6 months the correlation in all subjects between BMI and public distress (r=-0.581, p=0.000) and total scores (r=-0.471, p=0.003) remained there were no correlations in the control group. In addition there were correlations at 6 months between BMI in all subjects and physical function (r=-0.482, p=0.002) and public distress in the dietician group (r=-0.606, p=0.003).

5.3.4.2 Correlations between change in weight and change in scores

Changes in weight as a percentage of baseline at 3 and 6 months were compared with changes in questionnaire scores at the same time points for all subjects and for each group to investigate the possibility of a relationship between weight change and change in HRQoL. Data is shown in appendix B.

SF36

There was no significant correlation after Bonferroni correction between change in any questionnaire domains for the SF36 with percentage

weight change between baseline to 3 months, 3 months to 6 months or baseline to 6 months.

SGRQ

There was no significant correlation after Bonferroni correction between change in any questionnaire domains for the SGRQ with percentage weight change between baseline to 3 months, 3 months to 6 months or baseline to 6 months.

IWQOL-Lite

There was no significant correlation after Bonferroni correction between change in any questionnaire domains for the IWQOL-Lite with percentage weight change between baseline to 3 months, 3 months to 6 months or baseline to 6 months except at baseline to 3 months for self esteem ($r=-0.511$, $p=0.02$) and total score ($r=-0.551$, $p=0.001$) for the whole group plus self esteem ($r=-0.704$, $p=0.007$) and total score ($r=-0.621$, $p=0.024$) for the control group.

5.3.5 Comparing groups that achieved ≥5% weight loss at 6 months with those that did not

I have explored the relationship between HRQoL and weight change between the study groups. As noted previously there were subjects that lost significant weight in the control group and also those that did not loose weight in the dietician group. Therefore I have also explored possible differences in

change in HRQoL between those that did and did not lose significant weight suggested at ≥5% of original weight by expert opinion.

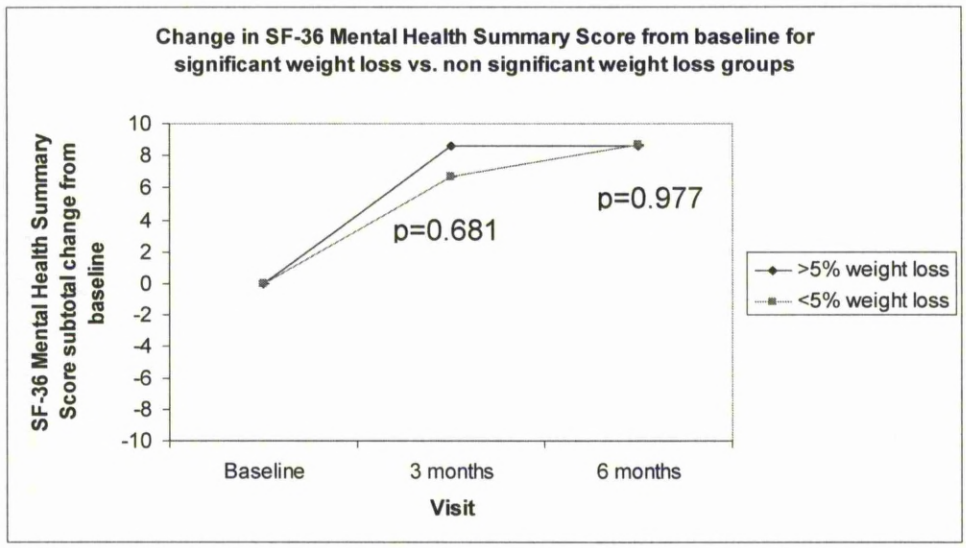
SF36 Scores

There was a trend towards improvement in generic HRQoL measured by SF36 in those that lost ≥5%weight between baseline and 6 months although this was not significant after Bonferroni adjustment for any SF36 domain at 3 or 6 months.

Using paired samples T test change in scores was compared between baseline to 3 months and baseline to 6 months for the group with significant weight loss and those without. Between baseline and 3 months there were no significant differences in scores for any domain in the group that showed no significant weight loss. In the weight loss group there were significant improvements after Bonferroni adjustment in Role Physical (65 vs 91.7), Role Emotional (64.5 vs 91.1), Physical Health summary score (60.7 vs 69.3), Mental Health summary score (62.1 vs 72.1) and Total score (64.1 vs 74.3).

Between baseline and 6 months there were no significant improvements after Bonferroni adjustment in the non weight loss group. In the weight loss group there was significant changes in Physical Functioning (61.9 vs 73.4).

When comparing the overall change in SF36 domain scores from baseline to 3 and 6 months between groups there were no significant differences at 3 or 6 months between those that lost significant weight and those that did not.



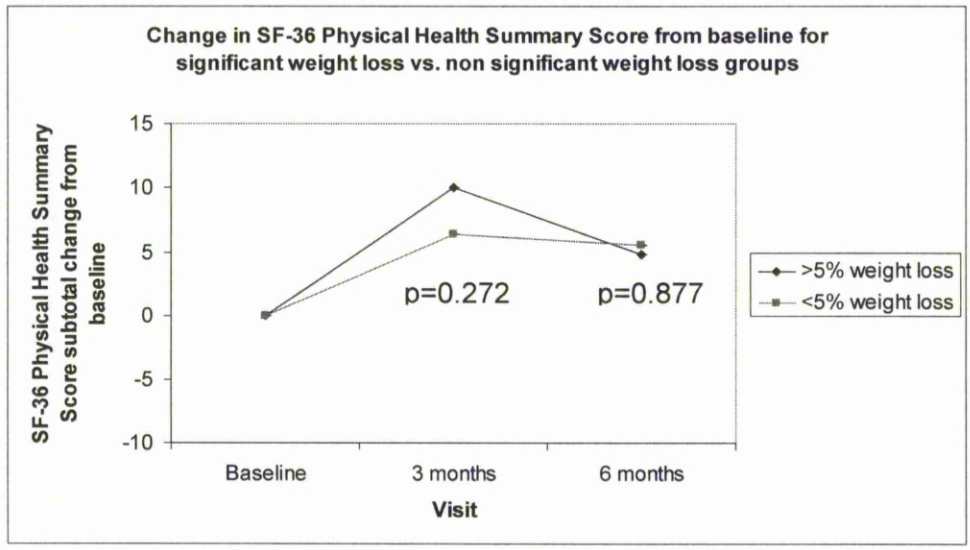


Fig 13. Graphs showing mean change in summary scores of SF-36 for mental health and physical health subtotals for those that lost ≥ 5% of baseline weight from baseline at 3 and 6 months and those that did not. p value represents comparison between groups at 3 and 6 months

SGRQ scores

There was a trend towards improvement in respiratory specific HRQoL measured by SGRQ in those that lost ≥5%weight between baseline and 6 months although this was not significant after Bonferroni adjustment for any domain at 3 or 6 months.

Using paired samples T test change in scores was compared between baseline to 3 months and baseline to 6 months for the group with significant weight loss and those without. Between baseline to 3 months there were no significant improvements after Bonferroni adjustment in quality of life in the non weight loss group, however in the weight loss group there were significant improvements in Activities domain (48.9 vs 39.8), and Total score (38.2 vs 31.3).

Between baseline to 6 months there were significant improvements after Bonferroni correction in scores in the no weight loss group for Activities score (51.6 vs 43.7). This was also true for the weight loss group, Activities score (48.4 vs 39.2).

When comparing the overall change in SGRQ domain scores from baseline to 3 and 6 months between groups there were no significant differences at 3 or 6 months between those that lost significant weight and those that did not.

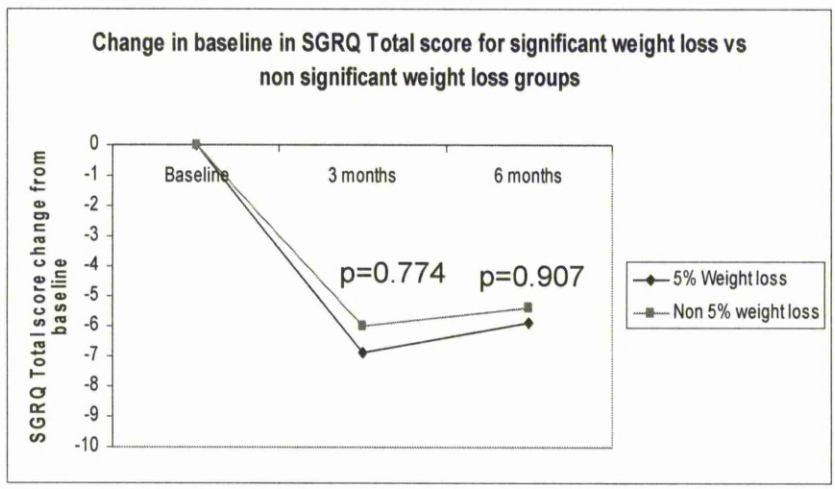


Fig 14. Graph showing mean change in total score of SGRQ for those that lost ≥ 5% of baseline weight from baseline at 3 and 6 months and those that did not. p value represents comparison between groups at 3 and 6 months

IWQOL-Lite

There was a trend towards improvement in weight specific HRQoL measured by IWQOL-Lite in those that lost ≥5%weight between baseline and 6 months although this was not significant after Bonferroni adjustment for any domain at 3 or 6 months.

Using paired samples T test change in scores was compared between baseline to 3 months and baseline to 6 months for the group with significant weight loss and those without. Between baseline to 3 months there were no significant differences after Bonferroni adjustment in scores in the non weight loss group, however in the weight loss group there was significant improvement in scores for Physical function domain (66.1 vs 71.7) and Total score (68.8 vs 74.8).

Between baseline and 6 months there were no significant changes after Bonferroni correction for any domain in the non weight loss group. In the weight loss group there were significant deteriorations in quality of life scores for the Total score (66.8 vs 75.7).

When comparing the overall change in IWQOL-Lite domain scores from baseline to 3 and 6 months between groups there were no significant differences at 3 or 6 months between those that lost significant weight and those that did not.

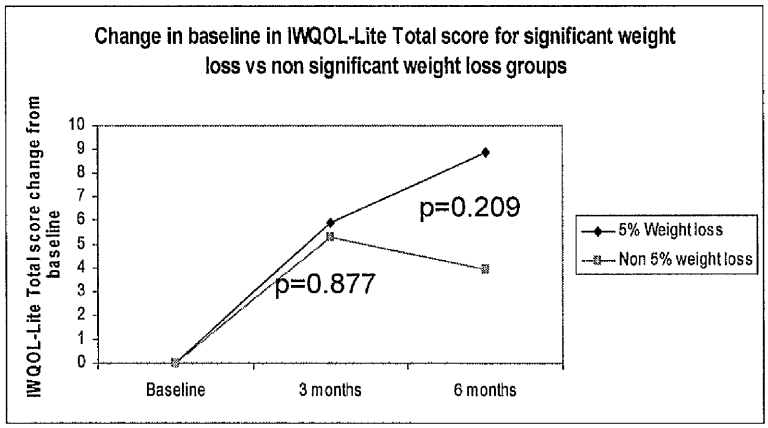


Fig 15. Graph showing mean change in total score of IWQOL-Lite for those that lost ≥ 5% of baseline weight from baseline at 3 and 6 months and those that did not. p value represents comparison between groups at 3 and 6 months

5.3.6 Effect of gender on HRQoL scores

SF36

When comparing males with female subjects there was a consistent trend for HRQoL scores to be higher for males compared to females. These were significant in the following cases. At baseline there were significant differences in questionnaire scores between males and females for the Physical Function score (male 68.4 vs female 55.2).At 3 months, there were significant differences for Physical function (male 75.4 : female 57.3), Role Emotional (male 95.2 : female 63.4), Mental Health summary (male 72.1 : female 59.1) and total score (male 74.2 : female 61). At 6 months, there were significant differences in Physical Function (male 79.1 : female 66), Vitality (male 60.9 : female 46.4), Social Functioning (male 87.6 : female 68.7), Mental Health (male 75.3 : female 61), Mental Health summary (male 72.8 : female 59.3) and Total score (male 72.9 : female 61.6)

SGRQ

When comparing males with female subjects there was a consistent trend for SGRQ scores to be lower for males compared to females. This was significant at baseline for the activities domain (male 40: female 57.2). Again at 3 months there was a significant difference for the activities domain (male 36.6: female 54.3) and at visit 4 the same was true (male 33.6 : female 48.5)

IWQOL-Lite

For the IWQOL-Lite there were consistently lower scores again in the female group compared to males. All were significant at baseline except Public Distress: Physical Function (male 73 : female 60.9), Self Esteem (male 69.9 : female 42.5), Sexual Life (male 83.9 : female 60.4), Work (male 88.8 : female 73.6) and Total (male 76.2 : female 59.9). At 3 months only Sexual life was significant (male 87.5 : 61.3) which remained so at 6 months (male 90.2 : female 58.3) with Total score (male 79 : female 64.1).

5.3.7 Effect of gender on change in questionnaire scores between visits

SF36

There was no significant difference between males and females between each visit for all questionnaire scores for SF36.

There were no significant correlations after Bonferroni adjustment between percentage change in weight between baseline to 3 months, 3 months to 6 months and baseline to 6 months and SF36 questionnaire scores for either males or females.

SGRQ

There was no significant difference after Bonferroni adjustment between males and females between each visit for all questionnaire scores for SGRQ. There were also no significant correlations between percentage change in weight between all visits and SGRQ questionnaire scores for either males or females.

IWQOL-Lite

There was no significant difference after Bonferroni adjustment between males and females between each visit for all questionnaire scores for IWQOL-Lite.

For changes of IWQOL-Lite scores between baseline and 3 months and change in percentage weight there were no significant correlations in males however in females there were significant correlations after Bonferroni adjustment in Self Esteem (r= -0.710, p=0.000) and Total score (r= -0.603, p= 0.005). There were no correlations for males or females between 3 and 6 months or baseline to 6 months.

5.3.8 Comparing dietician and control groups: Effect of gender

SF36

At baseline there were no significant differences after Bonferroni adjustment in questionnaire scores in either the dietician or control groups for all domains of the SF36 between males and females. At 3 months in the dietician group there was no significant difference after Bonferroni adjustment although in the control group there was a significant difference between males

and females for the Vitality domain (male 69.2 : female 41.9). At 6 months there were no significant differences after Bonferroni adjustment in scores between males and females in either group

SGRQ

For baseline, 3 and 6 month visits there was no significant difference after Bonferroni adjustment between males and females in either group for any score for SGRQ.

IWQOL-Lite

In the dietician group there were no significant differences after Bonferroni adjustment in any questionnaire score for all domains at any visit. In the control group there were significant differences at baseline for Self Esteem (male 78.3: female 46.4), Sexual Life (male 88.7: female 56.2), Work (male 98.1 : female 76.2) and Total scores (male 84.2: female 62.1). At 3 months there were significant differences for Sexual Life (male 97.5: female 52.8) in the control group which remained significant at 6 months (male 92.9: female 43).

5.3.9 Relationships between Questionnaire scores and markers of asthma severity

5.3.9.1 Exhaled nitric oxide

There were no significant correlations after Bonferroni adjustment for the SF36 or IWQOL-lite scores at any visit 2 with $FeNO_{50}$, alveolar or bronchial exhaled nitric oxide.

There were no significant correlations after Bonferroni adjustment for SGRQ domains at any visit except for Impacts and $FeNO_{50}$ (r=0.395, p=0.005) and bronchial nitric oxide (r=0.419, p=0.008) at baseline plus Impacts and bronchial nitric oxide (r=0.505, p=0.005) at 3 months.

There were no significant correlations after Bonferroni adjustment between change in questionnaire scores between baseline and 6months and changes in exhaled nitric oxide at 50ml flow rate, alveolar nitric oxide or bronchial wall flux.

5.3.9.2 Bronchial responsiveness and specific airway conductance

There were no significant correlations after Bonferroni adjustment between airway responsiveness or specific airway conductance and questionnaire scores for any visit for SF36, SGRQ or IWQOL-Lite.

There were no significant correlations after Bonferroni adjustment between change in any of the domains for any questionnaire and change in bronchial responsiveness or specific airway conductance between baseline and 6 months

5.3.10 Peak flow and symptoms diaries

The completion and return rate of self completed patient peak flow and symptoms diaries was too low to allow further analysis of these results and are therefore not included.

Additional material for this chapter can be found in appendix B

5.4 Conclusions and discussion

Both obesity and asthma can significantly affect HRQoL in an adverse way[164, 267]. We showed that quality of life score means for all subjects and in each group were worse than published means for the questionnaires SF36[160] and SGRQ[273] and the total scores of all the questionnaires correlated significantly with each other suggesting that they are recording similar effects on health related quality of life. The IWQOL-Lite published normal scores are stratified for BMI, with mean BMI in our subjects being 38 Kg/m^2 our subjects showed scores similar or slightly worse than means published[272] in the IWQOL manual for the BMI range 35-39.9 Kg/m^2.

For all subjects there were significant improvements in generic HRQoL measured by the SF36 physical health summary score, mental health summary score and total scores between baseline and 3 months and also between baseline and 6 months. This was also seen in the specific questionnaires SGRQ total score and the IWQOL-Lite total score. There were no significant differences between the dietician and control groups. Therefore there was no significant effect seen on HRQoL in those that had intervention from a dietician compared to those that did not. Although there was improvement in HRQoL scores at 3 and 6 months, the improvement was not significantly better in either group as the two groups did not diverge significantly despite a trend to greater improvement in scores in the dietician group compared with the control group. Our study may have been underpowered to detect a difference in the two groups as the trend seen did not reach significance. We have also shown previously that not all subjects in the dietician group lost weight and some lost weight in the control group which

may be another reason for the lack of significant differences in HRQoL between these groups. Weight loss achieved was relatively modest in our study of -5% and – 4.9% at 3 and 6 months in the dietician group and -3 and -2.7% in the control group and although there is a linear relationship reported regarding weight loss and improvement in HRQoL, below 10% this may be unreliable[295]. Finally we may have been seeing an improvement in the HRQoL simply as a result of subjects taking part in a study (the so-called Hawthorne effect) rather than the results of interventions or weight loss[292].

There were no significant correlations in the dietician group between BMI and HRQoL scores but there were significant correlations in the control group at 3 and 6 months for the generic and respiratory specific questionnaires, although the total scores showed no significant correlation. There were greater correlations with BMI in the weight specific questionnaire as would be expected which became less clear at 3 and 6 months. There were correlations for total IWQOL-Lite score and BMI for all subjects and the control group at baseline and 3 months but not at 6 months when there was no correlation for total score for any group. The trend again was a possible relationship towards a worse HRQoL with increasing BMI as the correlation coefficients between questionnaire total scores and BMI for each correlation were in the correct direction even if they did not reach significance in most cases. The lack of correlations in the dietician group could be due to the effect of behavioural intervention causing a global improvement in HRQoL which may have masked any effect from BMI itself. However, there were no significant differences in scores between the control and dietician group in those that did not lose clinically significant weight which does not support this

hypothesis as we would expect to see an improvement in HRQoL in the dietician group.

It is known that there is a bidirectional or reciprocal relationship between some aspects of HRQoL such as those associated with psychological well being and weight loss[295]. Therefore it may be that those who had improvements in HRQoL due to the effects of intervention may have achieved greater weight loss because of changes in HRQoL rather than change in weight leading to improvements in HRQoL although some subjects in our study improved HRQoL but did not lose weight.. We are unable to investigate this further as changes coexist during treatment phases of studies. The changes in psychological well-being that take place during weight management programs might independently contribute to their success and causal paths between psychosocial and behavioural / weight changes are most likely closely intertwined. This reciprocal determinism should be explored further in future studies.

Our results therefore suggest that there may be a relationship between HRQoL and BMI. Improvements in HRQoL in these subjects that were seen mostly in the first three months may be related to the period when most weight loss occurred (see chapter 4) which was also over this period. We are unable to comment further on whether this is due to the effect of weight reduction or other improvements in asthma control because there were no relationships between HRQoL scores and FeNO or PC_{20} suggesting that the effect of weight loss is the dominant factor.

Correlations appeared to be stronger for the IWQOL-lite questionnaire vs BMI compared to the other questionnaires. As this is a specific

questionnaire for the impact of weight on quality of life[244] and therefore more sensitive to changes in weight, this would further suggest that the strongest influence on HRQoL in our study is BMI rather than the effect of asthma as there are a lack of significant correlations between BMI and SGRQ which is a specific respiratory based questionnaire[163].

Despite these individual effects there was no significant relationship between change in weight and change in questionnaire scores for either specific or generic questionnaires for all subjects or for individual groups. It may be that the questionnaires are not measuring the right thing and as we showed in the screening data in chapter 3, quality of life in asthmatic obese subjects is likely to be more complicated, multi-factorial and therefore difficult to measure. HRQoL can be influenced by many factors and obesity is associated with many comorbidities[162] that we did not measure in this study.

Some studies have suggested a difference in the effects of obesity on asthma between males and females[215]. We explored the effect of gender on HRQoL in our study and found a trend towards a worse quality of life in females compared with males. Although there were no significant differences in change in HRQoL with percentage change in weight, females tended to have greater improvements in some domain scores compared to males. Other studies have found that HRQoL appears to affect females more than males[257].

We showed significant effects on role physical, vitality and social functioning in the generic questionnaire SF36 between baseline to 3 months and baseline to 6 months. Other studies have varied in which domains are affected by weight loss[243] but Kolotkin et al in their review of quality of life and obesity[267] state that quality of life, as measured by the SF-36 improves after

small to moderate amounts of weight loss in physical aspect domains as physical function, vitality and mental health in one study[296], vitality, general health perception and role limitations in another[294] and also physical function and bodily pain in one more[297], more than psychosocial aspects of HRQoL.

Studies on the effect of obesity on HRQoL show that the effects of weight loss generally improve HRQoL. In a ten-year follow up study of the trends in health-related quality of life after surgical and conventional treatment for severe obesity, Karlsson et al[295] showed that measures of HRQoL tracked changes in weight and improvement in quality of life was associated with the magnitude of weight loss. Anxiety however was not a useful measure in the long term although anxiety was reduced in the first four years following weight loss surgery. They showed also that for subjects with <10% weight loss the effects on HRQoL were trivial. As we used a cut off of 5% weight loss this may explain why our results did not reach significance although there was a trend for improvement with weight loss. This study however used different measures of HRQoL to mine and the authors note this as a limitation of the study as they do not measure physical function for example. They also do not state the existence of any co-morbidities which may have contributed to quality of life.

Kolotkin et al[298] studied the effect of weight loss in a group of subjects using pharmacological intervention with the IWQOL-Lite questionnaire. They also found an improvement in quality of life with weight loss which improved in a linear fashion with amount of weight lost although there was considerable variability among different facets of HRQoL in terms of response to weight loss. Among those dimensions measured by the IWQOL-Lite, Physical

Function showed the most improvement with weight loss, followed by Self-Esteem. Again they used 10% weight loss as a clinically meaningful change, below this weight loss they did show improvement in HRQoL however. They also showed as in my study a worse quality of life in females compared to males with an improvement in more domains in females compared to males. They did not use another generic quality of life score and acknowledge this in their discussion.

Interestingly there were improvements seen in the respiratory specific questionnaire SGRQ between visits from 3 months to 6 months when compared to baseline. This could mean that the questionnaire is measuring a global effect on HRQoL which is improving with weight loss or that there are particular aspects of respiratory function or symptoms that have improved with weight loss. There was a lack of correlation between HRQoL scores and bronchial responsiveness or airway inflammation which suggests that improvement in HRQoL is not due to an improvement in asthma severity measured by these markers but is likely due to the reduction in weight itself. Previous studies have shown that associations between HRQoL measures and reference measures of asthma diseases status are generally greater for symptom measures than for lung function[161, 163] i.e. patients symptoms correlated with HRQoL better than FEV1. However I was unable to explore this effect further as a measure of dyspnoea was not part of the protocol and I wished to avoid deep inspiratory manoeuvres. Changes in resting lung volumes, in particular ERV and FRV may improve symptoms with weight loss which in turn may affect HRQoL. Further studies are required measuring full lung volumes to explore this further. In the previous chapter I showed that

those subjects with and without bronchial responsiveness had similar impacts on HRQoL which would support this suggestion that weight has a greater impact on HRQoL than other objective markers of "asthma" such as lung volumes or bronchial responsiveness.

The SF36 questionnaire has been shown to be highly significantly correlated with the severity of asthma assessed by both a validated clinical score and the pulmonary function of the patients[299]. Again I showed significant change from baseline at 3 and 6 months in the group as a whole but did not find significant correlations between markers of asthma severity and SF36 questionnaire scores. This would support the suggestion that the greater impact on quality of life in our subjects was from BMI rather than asthma severity and that improvements in scores were more likely to be due to improvements in weight and the effect of being in a study than any improvement in asthma severity. Again caution must be applied as others have suggested that the SF-36 has only a poor or moderate response involving those with milder asthma[164].

The specific respiratory questionnaire validated in COPD and asthma was the St Georges Respiratory Questionnaire (SGRQ) which has good discriminative capacity and responsiveness for group comparisons although the symptoms domain may show a lack of longitudinal validity and responsiveness if a long recall period version is used[166]. The use of this questionnaire addresses the uncertainty about responsiveness of a generic questionnaire on changes in asthma severity and also in detecting the effect of HRQoL from milder asthmatics. I used a 1 month recall period and hopefully eliminated this problem but we should use caution when using the

symptom domain of the SGRQ to equate to the subjects symptoms in relation to other markers of asthma severity such as specific airway conductance or PC_{20}. Again I found no relationship between severity of asthma and domain scores using the SGRQ questionnaire, confirming the results obtained using the generic questionnaire. Others have found a limited correlation between traditional measures of asthma control such as bronchial obstruction and HRQoL and therefore there may be a clinical-functional dissociation due to a lack of precision in determining HRQoL and asthma symptoms[300]. One study[151] has shown that my interpretation of bronchial responsiveness using the dose threshold of methacholine to trigger bronchoconstriction may not have been the best measure to use and may explain a lack of relationship between HRQoL and PC_{45}. They found that bronchial reactivity index as a measure of the intensity of bronchoconstriction was a better correlator with poorer HRQoL than PC_{20} in patients with stable asthma. The presence of bronchial responsiveness by PD_{20} is associated with poorer HRQoL in moderate to severe asthmatics compared to those with a negative PD_{20} [151, 301]. I excluded those subjects with a negative methacholine challenge and therefore may have excluded a proportion of subjects that may have been defined as having asthma by other criteria which may have explained our results.

The interplay between HRQoL with obesity can affect asthma, the effect on psychological well being can affect asthma quality of life and asthma control[161] and vice versa. Adams showed that psychological distress is more frequent in subjects with asthma compared to those without. However, in those with psychological distress the mental health component summary

score of the SF12 did not differ between asthmatics and non-asthmatics. Therefore, in an obese population that are more likely to have psychological distress than a normal weight group we may lose the discriminatory power of these HRQoL measures in asthmatic patients. This is clearly a complex relationship and one that is difficult to tease apart and we are only beginning to understand.

Along with psychosocial issues, many other conditions can affect HRQoL. I attempted to reduce these to a minimum by excluding those subjects with significant comorbidities, however we could not completely exclude these and were unable to screen for commonly associated conditions with asthma and obesity such as gastroesophageal reflux and sleep apnoea which may influence HRQoL in obese asthmatics. This is another limitation of the study. Asthmatics have been shown to have more comorbidity as reflected in hospitalisations, emergency department visits and ambulatory care than non-asthmatics and comorbidity has been associated with decreased quality of life and poor asthma control[162].

The questionnaires themselves may cause limitations due to the period of recall involved in the questions, I used questionnaires with recall periods that should have been adequate for the length of the study, however the length of the study itself may cause problems as a patient may adopt an avoidant coping strategy or distress caused by asthma may still occur for a period after it has become better controlled[302]. The issue of recall periods may be addressed by the use of symptom diaries rather than questionnaires, however these are more likely to suffer from ceiling and floor effects. I have used symptom diaries also in this study, however, response rate in returning

and completing these diaries was poor. Diaries are however more useful for assessing longitudinal correlations with pulmonary function.

The influence of weight on the relationship between asthma and HRQoL needs to be taken into account in future studies exploring the relationship between clinical aspects of asthma and HRQoL questionnaires Previous studies have used BMI categories amongst others to explore effects on determinants of quality of life. Ford et al[303] used a simplified four item set of health related quality of life questions in a population of asthmatics using a telephone survey and found a U-shaped relation with poor or fair health, increased numbers of physically unhealthy days, mentally unhealthy days and days with activity limitations. I have explored this in a longitudinal study and although we have shown similar findings cross sectionally have failed to show significant improvements with weight loss.

Another limitation to my study which may explain some of the lack of effects of weight loss may come from selection bias. It is known that obese subjects seeking treatment tend to have worse quality of life than those not seeking treatment and we may therefore have selected subjects through recruitment with a worse quality of life than the general obese population[304].

Lastly it is difficult to assess the effect purely of BMI and obesity on HRQoL in this study without the inclusion of a group of subjects with a normal BMI. As this was a weight loss study this was not practical and the study was not designed to include a normal weight group but those that lost weight could be expected to act as a surrogate group with lower BMI to compare with the obese group.

The strengths of this study are the longitudinal nature of the study and the inclusion of a dietician group and a control group. Also the use of generic and specific questionnaires allowed us to compare a generic HRQoL with the SF36 which could possibly compare the HRQoL of our subjects with other diseases and also the more sensitive nature of the specific questionnaires for their respective diseases which would be more sensitive to change than the generic questionnaire. Although we were comparing multiple variables we accounted for this by using correction for multiple variable analysis with Bonferroni correction.

5.5 Summary

In summary my subjects had significantly impaired HRQoL as measured by a generic questionnaire and specific questionnaires for weight and asthma. There was an improvement in questionnaire score at 3 and 6 months into the study compared to the baseline visit. There was a trend towards, but no significant correlation between BMI and HRQoL however there was also a trend towards a relationship between percentage of weight loss and change in HRQoL with a non-significant difference between dietician and control groups. There were no significant relationships between measures of asthma severity i.e. bronchial responsiveness as measured by PC_{45}, airway narrowing measured by specific airway conductance or exhaled nitric oxide. I can therefore tentatively suggest that the greatest impact on HRQoL in obese asthmatics comes from the obesity element of their health impairment rather than severity of asthma and improvements in HRQoL as

weight decreases mainly comes from the direct result of weight reduction rather than any improvement in asthma severity.

The interplay between asthma severity, obesity and HRQoL is complicated and each can influence the other. Further specific studies on HRQoL in this group of patients are therefore required to explore this further.

Chapter 6: Induced sputum differential cell counts and weight loss

6.1 Introduction

Asthma is an inflammatory condition with a type 2 inflammatory response involving mainly eosinophils[20]. It has previously been demonstrated that asthma severity and control can be related to airway eosinophil counts[125, 128] which can be measured non-invasively by collecting sputum induced with hypertonic saline. The methodology has been described in chapter 3. Adipose tissue has been found to produce adipokines[221, 222] which are able to regulate systemic inflammation[305]. Leptin, IL-6, CRP and TNF-α have been shown to be increased in obesity which may modulate Th2 immunity. Conversely IL-10 which inhibits the production of IL-6 and TNF-α is decreased in obesity. It is suggested that the inflammation in asthma may be influenced in obesity by these changes in the production of adipokines in the increased fat mass of the patient and therefore produce a specific phenotype of asthma[306] in obese asthmatics or amplify the usual inflammation related to asthma which could be measured non-invasively by measuring the differential cell count in induced sputum[113].

The results of differential cell counts obtained from induced sputum is one method of determining asthma 'phenotypes' along with patterns of bronchial responsiveness and exacerbations[23]. Haldar et al suggested a model of asthma phenotypes in a population of asthmatics and suggested that obese patients were more likely to have a symptom predominant non-eosinophilic phenotype[25]. Lessard et al compared a group of obese versus non-obese asthmatics to try to determine a specific obese asthma phenotype, part of this involved the use of induced sputum differential cell counts[307]. They found no difference in differential cell counts between obese and non-obese

subjects. In their whole population there was an inverse relationship between eosinophils and waist circumference and a similar trend for BMI. Others have also failed to identify an obese asthma phenotype by investigating the induced sputum differential cell counts in obese and non-obese subjects although not all found the same relationship between eosinophils and BMI[236, 240, 241].

I wished to explore the cell counts of an obese asthmatic population to see whether there was evidence of a relationship between weight or BMI and a specific differential cell phenotype. I also explored the presence of bronchial responsiveness with these cell counts and its relationship to exhaled nitric oxide. Furthermore I wished to explore the effect of a change in weight on these cell counts.

It has been hypothesised that an increase in BMI leads to an increase in airway inflammation due to the increase in the pro-inflammatory substance leptin and a reduction in the anti-inflammatory product adiponectin. The IL-6 like effects of leptin have been linked to an upregulation in TH2 type inflammation and has therefore been linked with the possible relationship between asthma and obesity[305]. I investigated the relationship between individual cell lines from the differential cell count and BMI for the group as a whole, for intervention groups and also in relation
to changes in BMI between visits.

6.2 Methods

Detailed methods are outlined in Chapter 2. Sputum was obtained from subjects at baseline, 3 months and 6 months using hypertonic saline by the investigator with standardised techniques. The investigator received training

from centres established in developing and using the technique. Sputum was processed within two hours of obtaining the sample with a sputum selection method and cytospins prepared and stained with Diff-Quik Giemasa Romanowski stain.

Differential cell counts were obtained manually by the investigator who had previously been trained by established sites performing this technique using 300 cells. Counts were performed twice to assess repeatability and reliability of the procedure.

6.2.1 Statistical analysis

Categorical variables are expressed as percentages of total subjects and compared using Chi squared test. Continuous variables are expressed using mean ±SD and compared between groups with the Students unpaired *t* test if normally distributed. Non-normal distributions determined by Shapiro-Wilk testing are expressed using median and interquartile range. Variables compared between visits were compared using paired-samples *t* testing. Correlations were performed between normally distributed variables using Pearson correlations two-tailed test and non-normally distributed variables using Spearman's. Any correlations were checked visually for homoscedasticity to confirm any relationship. Significance for multiple comparisons were adjusted by Bonferroni correction. Significance was determined if $p<0.05$ and alpha level adjusted by Bonferroni for number of observations studied.

Reproducibility of counting technique was assessed using Bland Altman plots.

6.3 Results

6.3.1 Sputum collection technique, reproducibility, viability etc.

The following data shows the results of the subjects' ability to produce adequate sputum samples during the technique, the proportion processed and numbers of slides produced including those whose quality meant that it was not possible to count the cells required for a result i.e. acceptability

Baseline

Attended: 51

Sputum induction attempted: 51 (100%)

Adequate sputum produced: 36 (71%)

Sputum processed: 36 (71%)

Slides produced: 36 (100%)

Slides unreadable: 8 (22%)

3months

Attended: 35

Sputum induction attempted: 33 (94%)

Adequate sputum produced: 27 (77%)

Sputum processed: 27 (77%)

Slides produced: 25 (93%)

Slides unreadable: 5 (20%)

6 months

Attended: 38

Sputum induction attempted: 36 (95%)

Adequate sputum produced: 26 (72%)

Sputum processed: 26 (72%)

Slides produced: 25 (96%)

Slides unreadable: 5 (20%)

6.3.1.1 Slide quality data

Squamous contamination and cell viability were assessed in each case and good results were obtained for these as outlined below suggesting that good quality slides were obtained from induced sputum samples.

	N	Minimum	Maximum	Mean	SD
Squamous	78	0	50	8.4	10
Viability	78	0.66	100	85.1	20.4

Table 25. Quality data of slides prepared from induced sputum for all samples prepared for squamous contamination and cell viability obtained from haemocytometer sample

6.3.1.2 Reproducibility

The investigator received training in performing the technique in centres with experience in the procedure prior to starting the study. I would like to acknowledge the Institute for Lung Health Leicester and Montreal General Hospital for their generous support in this. To check reproducibility of the results slides were counted more than once and the outcomes of those counts were compared. There were no significant differences between the two counts suggesting that technique was sound. This is also reflected in the

results between visits which show good correlations between visits for differential cell counts for individual subjects.

There were good correlations for cell counts for Neutrophils, Macrophages, and Eosinophils at all visits and also for epithelial cells at baseline and 3 months. Correlations were not significant for lymphocytes and metachromatic cells or epithelial cells at 6 months, however counts were very low which may explain this discrepancy. As phenotype is mainly determined by neutrophilic and eosinophilic inflammation this was felt to be adequate to continue with analysis.

Bland Altman plots for each cell count at each visit are shown in the appendix along with correlations between visits and between two successive differential cell counts for a particular visit.

6.3.2 Differential cell counts for each visit

Differential counts for baseline, 3 month and 6 month visits are represented here for all subjects and also for each group. Results are presented as total cells counted for each cell type from a total of 300 cells rather than as percentages of the total count.

6.3.2.1 All subjects

	Baseline n=28	3 months n=20	6 months n=20
Neutrophils	159 (60)	176 (73)	182 (56)
Macrophages	103 (55)	102 (63)	90 (49)
Eosinophils	24 (37)	15 (26)	22 (25)
Epithelial	12 (11)	5 (6)	4 (4)
Lymphocytes	2 (2)	1 (1)	2 (1)
Metachromatic	0 (0)	1 (1)	0 (0)

Cell counts presented as number of cells counted from a total of 300. Data presented as mean (SD)

Table 26. Mean (SD) total cell counts for each visit from a total of 300 cells counted for each cell line for all subjects

For the whole group of all subjects there were no significant differences between baseline to 3 months, 3 months to 6 months or baseline to 6 months for any cell type in the differential cell count.

6.3.2.2 Comparing dietician and control groups

Differential cell counts for each study group are presented here as per those for all subjects.

	Baseline		3 months		6 months	
Cell type	Dietician group n=15	Control group n=13	Dietician group n=13	Control group n=7	Dietician group n=13	Control group N=7
Neutrophils	172 (49)	144 (69)	184 (73)	161 (75)	187 (65)	174 (35)
Macrophages	97 (46)	111 (65)	90 (58)	122 (71)	88 (59)	93 (28)
Eosinophils	18 (19)	30 (51)	18 (31)	10 (11)	20 (26)	27 (24)
Epithelial	11 (11)	12 (12)	6 (6)	3 (3)	3 (5)	5 (3)
Lymphocytes	2 (2)	3 (2)	3 (1)	2 (1)	2 (1)	2 (1)
Metachromatic	0 (0)	0 (0)	2 (0)	1 (1)	0 (1)	0 (0)

Cell counts presented as number of cells counted from a total of 300. Data presented as mean (SD)

Table 27. Mean (SD) total cell counts for each visit from a total of 300 cells counted for each cell line for dietician and control groups.

When comparing mean cell counts between the dietician and control groups there were no significant differences at any visit. There were also no

significant differences in cell counts in either group between baseline to 3 months, 3 months to 6 months and baseline to 6 months.

6.3.3 Relationship between BMI and differential cell counts

The relationship between individual cell lines from the differential cell count and BMI for the group as a whole, for intervention groups and also in relation to changes in BMI between visits is presented here.

6.3.3.1 All subjects

	Baseline	3 months	6 months
Neutrophils	-0.4 (0.841)	0.206 (0.385)	0.044 (0.852)
Macrophages	0.098 (0.620)	-0.304 (0.193)	-0.130 (0.586)
Eosinophils	-0.081 (0.682)	0.200 (0.399)	0.208 (0.380)
Epithelial	-0.048 (0.807)	-0.100 (0.676)	-0.422 (0.064)
Lymphocytes	0.262 (0.177)	-0.188 (0.428)	0.404 (0.077)
Metachromatic	0.126 (0.523)	-0.446 (0.049)	-0.057 (0.812)

Data presented as r value (p)

Table 28. Correlations coefficients comparing total cell count for each cell line with BMI for all subjects. r (p)

There were no significant correlations with any of the cell counts and BMI for all subjects at any study visit.

6.3.3.2 Comparing dietician and control groups

	Baseline		3 months		6 months	
Cell type	Dietician group	Control group	Dietician group	Control group	Dietician group	Control group
Neutrophils	0.262 (0.345)	-0.339 (0.257)	0.206 (0.385)	0.779 (0.039)	0.075 (0.808)	-0.440 (0.323)
Macrophages	-0.221 (0.428)	0.401 (0.174)	-0.304 (0.193)	-0.849 (0.016)	-0.116 (0.706)	-0.159 (0.734)
Eosinophils	0.002 (0.993)	-0.119 (0.698)	0.200 (0.399)	0.316 (0.490)	0.132 (0.666)	0.828 (0.021)
Epithelial	-0.290 (0.294)	0.240 (0.429)	-0.100 (0.676)	-0.030 (0.949)	-0.432 (0.140)	-0.207 (0.656)
Lymphocytes	0.191 (0.496)	0.420 (0.153)	-0.188 (0.428)	-0.668 (0.101)	0.373 (0.209)	0.558 (0.193)
Metachromatic	-0.004 (0.989)	0.261 (0.390)	-0.446 (0.049)	-0.732 (0.061)	-0.008 (0.980)	-0.421 (0.347)

Data presented as r value (p)

Table 29. Correlations coefficients comparing total cell count for each cell line with BMI for dietician and control groups. r (p)

There were no significant correlations between BMI and any cell type in either the intervention or the control group at any visit.

6.3.4 Correlation between change in differential cell count between visits and change in weight %

On investigating the percentage change in weight between visits and change in cell count there was no significant correlation after Bonferroni correction between percentage change in weight from baseline and changes in differential cell count.

6.3.5 ≥5% weight loss Group vs <5% weight loss group – between group comparisons

As previously discussed, some subjects in the control group lost weight and some subjects in the dietician group gained weight. To explore any

possible relationship with weight loss further I have compared those that lost clinically significant weight, generally accepted as ≥5% with those that did not.

Again there were no significant differences in each type of cell in the differential cell count between those subjects that achieved >5% weight loss and those that did not at any visit. I am therefore unable to find a relationship between BMI and the inflammatory phenotype in obese asthmatic patients. Although there was a possible trend towards an increase in neutrophil count in the dietician group with weight loss this is not reproducible in any of the other analyses.

6.3.6 Exploration of Eosinophilic or Neutrophilic predominant sputum

It is suggested that sputum differential cell counts can be separated in terms of phenotypes into eosinophilic predominant if there is ≥3% eosinophils or neutrophilic predominant if neutrophils ≥60% I therefore investigated whether BMI was significantly different when separated by this definition.

	BMI Kg/m^2			
Visit	≥3% Eosinophils	<3% Eosinophils	≥60% Neutrophils	<60% Neutrophils
Baseline	37.7	39.8	39.1	38.1
3 months	37.5	36.7	38.4	35.4
6 months	36.8	37.8	37.6	36.4

*=p<0.05

Table 30. Mean BMI for each visit when comparing eosinophilic vs. non eosinophilic predominant phenotypes and neutrophilic vs. non neutrophilic phenotypes

There was no significant difference in BMI between eosinophilic vs non eosinophilic or neutrophilic vs non-neutrophilic groups at any visit.

6.3.7 Secondary outcome measures: Relationship of differential cell counts with exhaled nitric oxide and bronchial responsiveness

There were no significant correlations between any of the cell lines in the differential cell count and exhaled nitric oxide using either FeNO at 50 ml flow rate, alveolar nitric oxide or bronchial nitric oxide for all visits.

There were no significant correlations between any of the cell lines in the differential cell count and bronchial responsiveness measured by PC45 for all visits.

6.3.8 Medication and differential cell counts

There were no significant correlations with any cell lines in the differential cell count and inhaled steroid dose.

Additional material for this chapter can be found in appendix C

6.4 Conclusions and discussion

Despite theoretical evidence which would suggest that the possible link between asthma and obesity may be due to an increase in airway inflammation or the development of a specific inflammatory phenotype[232, 234, 305] I have been unable to reproduce this with my own data. I did not find a significant correlation between BMI and differential cell count in these obese asthmatics nor been able to demonstrate a significant change in differential cell count with a change in weight. I am therefore unable to find a relationship between BMI and the inflammatory phenotype in obese asthmatic patients. Although there was a possible trend towards an increase in neutrophil count in the dietician group with weight loss this is not reproducible in any of the

other analyses. This reflects the cross sectional studies by Lessard et a[307]l and Todd et al[236] who found no difference in differential cell counts between obese and non-obese groups. Also more recently Dixon showed no change in differential cell counts with weight loss before and after bariatric surgery for weight loss[2].

I did not find a trend or relationship between BMI and eosinophils in my group which was shown by Lessard. I also did not find a correlation between change in eosinophil count and change in weight or a relationship between eosinophil or neutrophil predominant phenotypes and weight change in either the control or dietician groups. Previous data from Todd et al also supports this. They retrospectively analysed a database of 727 sputum differential cell counts stratified into increasing BMI groups and showed that there was no difference in differential cell counts between groups. They also showed an increase in eosinophils in those with asthma in both the obese and non-obese categories but there were no differences between these groups. Unlike Lessard et al they did not find any correlation between BMI and any measure of cellular airway inflammation which reflects my own data.

Other cross sectional studies that have investigated airway inflammation and obesity include van Veen et a[240]l and Sutherland et al[241]. van Veen showed a lower percentage of sputum eosinophils in an obese group of patients compared with normal BMI and linear regression analysis showed a negative association between BMI and sputum eosinophils. Sutherland showed similar results to Todd et al and showed that asthmatic patients had higher levels of sputum eosinophils than non asthmatics but there were no differences in either group between obese and non-obese

individuals. To summarise this, it appears that asthmatics have higher eosinophils than non asthmatics but BMI does not influence the number of eosinophils in an asthmatic population.

I was able to perform differential cell counting in this group with good quality specimens in most cases and the results were reproducible on repeated differential cell counting. My overall differential cell counts for each visit are similar to other reported studies as outlined in table 31 and most closely mirror the study by Sutherland.

Study	Eosinophils	Neutrophils	Lymphocytes	Macrophages	Metachromatic cells
Todd[236]	0.7%	59.2%	6.7%	0.3%	NA
Lessard*[307]	5.1%	41.5%	1.5%	48.7%	3.2%
Van Veen**[240]	0.5%	54.6%	NA	NA	NA
Sutherland[241]	11.3%	28.9%	1.2%	4.25%	NA
Scott Visit2	8%	53%	0.7%	34%	0%
Scott Visit3	5%	59%	0.3%	34%	0.3%
Scott Visit4	7.3%	61%	0.7%	30%	0%

*Group reported using SABA & ICS

** Do not report values for Lymphocytes, Macrophages or metachromatic cells

Table 31. Comparison of reported mean differential cell counts in published studies of obese subjects and mean differential cell counts at each visit in this study

This is the first study to report longitudinal data of sputum differential cell counts in a group of subjects with non-surgical weight loss and adds to the evidence from cross-sectional and surgical weight loss studies that obesity does not appear to influence the differential cell counts in asthma.

Many of the studies suggesting a link between inflammatory cytokines related to leptin and adiponectin in obesity and asthma involve animal models using leptin deficient mice[247]. Administration of exogenous leptin in leptin-

deficient mice augments airway hyperreactivity following allergen challenge, as well as lung inflammation following ozone exposure[232, 308] and exogenous administration of adiponectin prevents the development of allergen-induced airway hyperreactivity plus also inhibits vascular smooth muscle proliferation but not airway smooth muscle proliferation[309]. Studies in humans are less conclusive and this study reflects this in that although I have tried to control for most factors I still have a heterogenous group of subjects on a variety of medications and different severities of asthma. This may therefore account for my results not showing a predominant cell type, however I would expect to find a signal in the analysis of the longitudinal data from the study but this was not the case.

I was unable to find a relationship with sputum differential cell type or phenotype and other non-invasive markers of airway inflammation i.e. exhaled nitric oxide and also there was no relationship between any sputum cell predominance and bronchial responsiveness in my population.

The relationship between exhaled nitric oxide and BMI is more complex as measurement of nitric oxide is affected by the flow of air through the airways[101] and may reflect areas of gas trapping due to low lung volumes in obesity which may explain the possible negative correlation between exhaled nitric oxide and BMI. This may be one reason why I did not find a correlation between exhaled nitric oxide and differential cell counts. It would be important to include measures of lung volumes and in particular to measure the closing volume of these subjects which I was unable to do due to the nature of the design and is an area for future research.

The effect of BMI on lung volumes may also explain the lack of a correlation between bronchial responsiveness and differential cell counts. A link between sputum eosinophil counts and bronchial responsiveness has been shown previously[310], however it is also known that breathing at low volumes can reduce the bronchoprotective effect of deep inspiration and increase bronchial reactivity[154]. It has also been shown however that in obesity this effect may not occur[311, 312]. There is a possibility that ERV may be important to factor into the relationship between bronchial reactivity and sputum cell counts and again I was unable to measure this in these subjects due to the design of the study to avoid manoeuvres involving deep inspiration. However the lack of a significant correlation between change in BMI and changes in bronchial reactivity, FeNO and differential sputum cell counts would help to take into account the lung volumes and therefore suggest that our conclusion would be correct.

The link between asthma and obesity is complex and is not due to one particular mechanism but likely to be due to a combination of mechanical, psychological and immunological processes. The very fact that asthma can present as many different phenotypes[23] makes it difficult to find a specific phenotype linked with obesity. I propose that rather than a specific phenotype of asthma, obese patients with asthma can reflect the same overall mixture of phenotypes of all asthmatics but may be recognised earlier due to the other effects of obesity which cause them to amplify their symptoms, seek medical help earlier and mimic the symptoms of asthma. I was unable to examine adipokines and other inflammatory markers in the blood but a recent study

has shown that there may still be a link between inflammation and asthma in obesity which does not involve the differential cell type[2].

This study has some weaknesses. Although similar to other published studies using sputum induction for differential cell counts I obtained sputum in 71-77% of patients at each visit and was able to perform differential cell counts on 78-80% of these. Also not all subjects attended every visit. Despite this I was able to analyse significant numbers of subjects similar or larger in number to other published weight loss studies. Another limitation would be the expertise of the observer performing the differential cell counts which were performed by hand. I tried to exclude error by counting slides twice and comparing reproducibility of counts which correlated well, had reasonable bland-Altman plots and there were also good correlations between visits. Another limitation of the study has previously been mentioned in another chapter in that not all subjects in the dietician group lost weight, however when comparing those that lost significant weight with those that did not I found no significant difference. Lastly, my group of subjects were not all taking the same amounts of medication and represented a heterogenous group of subjects in respect to prior smoking status and atopy, although this may have implications on the cross sectional analysis of data the strength of this investigation was its longitudinal nature so any changes in differential cell count in each individual would be related to a change in BMI as long as other variables remained the same. I also found no relationship between the dose of inhaled steroids taken by the subjects and the differential cell counts.

6.5 Summary

My results reflect previously published cross-sectional studies[236, 240, 241] that suggest there is no particular inflammatory 'obese phenotype' as determined by differential sputum cell counts and this is the first reported interventional study using dietary intervention. Asthmatic subjects may have higher sputum eosinophil counts than non asthmatic subjects but there are no differences between obese and non obese asthmatics. Weight loss does not alter the inflammatory profile of the airways measured non-invasively by differential cell counts of induced sputum.

Chapter 7: Exhaled nitric oxide and BMI

7.1 Introduction

The diagnosis of asthma is a clinical one and features that increase the probability of a diagnosis of asthma include symptoms, the presence of variable airflow obstruction and airway inflammation[15].

Airway inflammation is typically described as a type II inflammatory response with an eosinophilic predominance[313], although different phenotypes have been described. Airway inflammation can be monitored non-invasively by measuring exhaled nitric oxide using standardised techniques using a chemiluminescence analyser[94]. Levels of exhaled nitric oxide have been shown to correlate with asthma control or asthma severity[105, 106] and can be used to monitor treatment[90].

Asthma has been associated with obesity and it is not clear whether this relationship is due to the mechanical effects of increased BMI on airway physiology or an inflammatory effect of adipose tissue on airway inflammation[247]. As noted above measuring exhaled nitric oxide is a non-invasive way of measuring this airway inflammation in subjects with asthma. Exhaled nitric oxide has been shown to be increased in obesity[239] in some studies but has also been found to decrease with increasing BMI[240]. The fraction of exhaled nitric oxide measured at the mouth is made up of nitric oxide originating in the alveolus and also in the bronchial wall[101] and this can be affected by airway inflammation, airway physiology with reductions in nitric oxide with bronchoconstriction and the effects of back diffusion of nitric oxide in the airways during gas trapping[314, 315]. Measuring the alveolar and bronchial wall nitric oxide may mitigate the effects of airway narrowing and gas trapping that occurs in obesity and its effects on exhaled levels of nitric oxide.

The aim of this chapter is to investigate the effect of BMI and weight loss on levels of fraction of exhaled nitric oxide through a single flow exhalation rate but also to investigate the effect of BMI and weight loss on the flow independent parameters of alveolar nitric oxide and bronchial wall flux.

7.2 Methods

Details of the measurement of exhaled nitric oxide have been described in Chapter 2 but will be briefly reviewed here.

I monitored exhaled nitric oxide using a NIOX chemiluminescence analyser using a standard technique (ATS/ERS guidelines[316]) after withholding medication, and fasting for 12 hours. Exhaled nitric oxide levels (ppb) were obtained at baseline, 3 and 6 months exhaled Nitric Oxide was measured at 10ml/s, 30ml/s, 50ml/s, 100ml/s, and 200ml/s to determine flow-independent parameters based on the two compartment model of Tsoukias and George[101]. Alveolar NO concentration was determined as the slope of the regression line of the 100ml and 200ml flow rates after inspection of the trends. Bronchial NO flux was determined as the intercept of this regression line[263].

Measurements were taken before any other respiratory measurements took place at each visit so as not to be influenced by anything involved such as methacholine.

I compared fraction of exhaled Nitric Oxide at 50ml flow rate ($FeNO_{50}$), Alveolar NO and NO flux in Obese subjects between visits for the whole group. I also explored differences between groups (dietician vs control) and

also between those that had significant weight loss vs those that did not and explored possible correlations.

7.2.1 Statistical analysis

Categorical variables are expressed as percentages of total subjects and compared using Chi squared test. Continuous variables are expressed using mean ±SD and compared between groups with the Students unpaired *t* test if normally distributed. Non-normal distributions determined by Shapiro-Wilk testing are expressed using median and interquartile range. Variables compared between visits were compared using paired-samples *t* testing. Correlations were performed between normally distributed variables using Pearson correlations two-tailed test and non-normally distributed variables using Spearman's. Any correlations were checked visually for homoscedasticity to confirm any relationship. Significance for multiple comparisons were adjusted by Bonferroni correction. Significance was determined if $p<0.05$ and alpha level adjusted by Bonferroni for number of observations studied.

7.3 Results

For consort diagram see Fig 7, (p140).

Missing data occurred in each study visit for the following reasons:

Dietician group
Baseline: Exhaled Nitric Oxide available in 24 missing in 2 subjects
Flow independent parameters available in 19 missing in 7 subjects
3 months: Exhaled Nitric Oxide available in 21 missing in 0 subjects
Flow independent parameters available in 19 missing in 2 subjects
6 months: Exhaled Nitric Oxide available in 21 missing in 1 subjects
Flow independent parameters available in 17 missing in 5 subjects

Control group
Baseline: Exhaled Nitric Oxide available in 24 missing in 1 subject
Flow independent parameters available in 20 missing in 5 subjects
3 months: Exhaled Nitric Oxide available in 12 missing in 2 subjects
Flow independent parameters available in 11 missing in 3 subjects
6 months: Exhaled Nitric Oxide available in 15 missing in 1 subject
Flow independent parameters available in 13 missing in 3 subjects

7.3.1 Exhaled nitric oxide measures for all subjects, dietician and control groups

Measurements of fraction of exhaled nitric oxide at 50ml flow rate and flow independent parameters are show here for the baseline visit, 3 and 6 months. Data is presented as median (interquartile range) due its the skewed nature.

Fraction of exhaled nitric oxide measured at 50mlflow rate for each visit

	Baseline FeNO (50ml/s)	3 months FeNO (50ml/s)	6 months FeNO (50ml/s)
All subjects	18.3 (26.2)	24 (40.1)	24.7 (26.3)
Dietician group	16.4 (26.9)	25.3 (40.6)	31 (24.6)
Control group	18.9 (24.6)	17.2 (39.1)	15.8 (36.6)

All measures expressed as median (IQR)

Table 32. Mean (SD) exhaled nitric oxide measured at 50ml/s flow rate from the mouth for all subjects and each group for each visit

Alveolar nitric oxide measured at each visit.

	Baseline Alveolar NO	3 months Alveolar NO	6 months Alveolar NO
All subjects	2.3 (1.9)	1.9 (3.13)	2.3 (3.1)
Dietician group	2.3 (1.9)	2.5(2.8)	2.9 (2.9)
Control group	2.1 (2)	1 (2.1)	1.4 (2.7)

All measures expressed as median (IQR)

Table 33. Mean (SD) calculated alveolar nitric oxide from exhaled air the mouth for all subjects and each group for each visit

Bronchial flux nitric oxide measured at each visit.

	Baseline Bronchial NO flux	3 months Bronchial NO flux	6 months Bronchial NO flux
All subjects	1000 (1300)	880 (2225)	1080 (1445)
Dietician group	940 (1000)	1040 (2460)	1460 (1440)
Control group	1030 (1365)	840 (1620)	960 (1610)

All measures expressed as median (IQR)

Table 34. Mean (SD) calculated bronchial flux nitric oxide from exhaled air the mouth for all subjects and each group for each visit

There were no significant differences for all subjects or either group for any measure of nitric oxide between visits, baseline to 3 months, 3 to 6 months or baseline to 6 months. Also there were no significant differences for all measures of nitric oxide between the dietician and control groups at any visit.

7.3.2 Correlations between measures of nitric oxide and BMI

There were no significant correlations between any measure of nitric oxide and BMI at any visit.

7.3.3 Correlations between change in measures of nitric oxide and change in weight from baseline

Change in $FeNO_{50}$, Alveolar NO and bronchial NO flux between baseline, 3 and 6 months were analysed with change in weight for all subjects, dietician and control groups. There were no significant correlations found between baseline and 3 months, 3 to 6 months or baseline to 6 months for any group.

7.3.4 ≥5% weight loss group vs <5% weight loss group – between group comparisons

As in previous chapters it is noted that some subjects lost weight in the control group just as some subjects in the dietician group gained weight. Further analysis was performed using groups that lost clinically significant weight determined as ≥5% of baseline weight vs those that did not.

Fraction of exhaled nitric oxide measured at 50mlflow rate for each visit

	Baseline FeNO (50ml/s)	3 months FeNO (50ml/s)	6 months FeNO (50ml/s)
≥5% weight loss group	16.4 (14.8)	29.8 (58.6)	31 (25.2)
<5% weight loss group	18.5 (41.2)	24.7 (39.7)	19.2 (29.3)

All measures expressed as median (IQR)

Table 35. Mean (SD) exhaled nitric oxide measured at 50ml/s flow rate from the mouth for significant weight loss group vs. non significant weight loss group for each visit

Alveolar nitric oxide measured at each visit

	Baseline Alveolar NO	3 months Alveolar NO	6 months Alveolar NO
≥5% weight loss group	1.8 (2.1)	2.5 (2.9)	2.9 (3.5)
<5% weight loss group	2.2 (1.1)	1.4 (3.8)	2.9 (3.5)

All measures expressed as median (IQR)

Table 36. Mean (SD) calculated alveolar nitric oxide from exhaled air the mouth for significant weight loss group vs. non significant weight loss group for each visit

Bronchial flux nitric oxide measured at each visit

	Baseline Bronchial NO flux	3 months Bronchial NO flux	6 months Bronchial NO flux
≥5% weight loss group	1040 (940)	740 (2460)	1000 (1435)
<5% weight loss group	960 (1150)	970 (2090)	1220 (1560)

All measures expressed as median (IQR)

Table 37. Mean (SD) calculated bronchial flux nitric oxide from exhaled air the mouth for significant weight loss group vs. non significant weight loss group for each visit

There was no significant difference for any measure of nitric oxide between those that lost ≥5% of total body weight compared to those that did not. There was also no significant difference for either group for any measure of nitric oxide between baseline and 3 or 6 months and from 3 to 6 months.

7.3.5 Medication and exhaled nitric oxide

There were no significant correlations with any markers of exhaled nitric oxide and inhaled steroid dose.

7.3.6 Other measures

Relationships between exhaled nitric oxide and quality of life and bronchial responsiveness have been explored elsewhere in the relevant chapters and no relationship between exhaled nitric oxide was found with these other variables.

Additional material for this chapter can be found in appendix C

7.4 Conclusions and discussion

There was no correlation between BMI and levels of exhaled Nitric Oxide (a measure of eosinophilic airway inflammation[317]) either at a standard flow rate or using the computed flow independent parameters of alveolar and bronchial wall flux of nitric oxide. There was no significant correlation between weight change and change in levels of all nitric oxide parameters. There was also no significant difference in all nitric oxide variables when comparing the dietician group vs the control group and those that lost over 5% of their body weight in 6 months vs those that did not.

Nitric oxide in the lung can be detected in the exhaled air of subjects and is produced by the enzyme nitric oxide synthase which exists in inducible and constitutive isoforms[71-75]. Only the expression of inducible nitric oxide correlates with levels of exhaled nitric oxide and this enzyme is expressed in increased amounts in asthma. Increased levels of exhaled nitric oxide are related to eosinophilic airway inflammation which theoretically may be increased by IL-6 like proinflammatory substances such as leptin associated with adipose tissue[239]. Adipose tissue associated with obesity also produces

increases in IL-4 and IL-5 which theoretically could increase airway inflammation along with reduced levels of the anti-inflammatory adipokine adiponectin[221, 222]. My findings do not support this and suggest that any change in the health status of obese asthmatics is not as a result of a change in eosinophilic airway inflammation but due to other possible reasons such as the mechanical effects of obesity on lung physiology[176] or non-eosinophilic airway inflammation such as a neutrophil predominant phenotype[24]. Kim et al investigated non asthmatic subjects and also found no relationship between adiposity, serum levels of leptin, adiponectin, tumour necrosis factor alpha or interleukin-6 and exhaled nitric oxide. They also suggest that inflammation due to adiposity may not have an influence on nitric oxide production in the lungs detectable by exhaled nitric oxide[318].

Maniscalco et al showed that non-asthmatic obese subjects had lower levels of exhaled nitric oxide than healthy controls which increased following weight loss induced by bariatric surgery and this was also related to FRC[4]. This suggests that I may be seeing an effect of airway calibre and breathing near to closing volume resulting in an altered characteristic of nitric oxide diffusion through the airways masking a signal from an inflammatory component in these asthmatic subjects[314, 315]. Any increased NO due to airway inflammation could be offset by the effects of airway calibre resulting in a net effect to cancel out the effect of each mechanism[240]. This could be why I did not find a significant correlation in this group of subjects and therefore did not reproduce the findings of Maniscalco who excluded asthmatic and atopic subjects. Both of these studies described have in fact excluded asthmatics which may explain why my findings differ.

Obesity causes reductions in FRC and ERV bringing the lungs close to closing volume[176] which could be involved in changes in measured levels of exhaled nitric oxide, especially alveolar levels of nitric oxide which would be 'trapped' in areas of lung which are affected by low lung volumes. Reduced airway calibre may result in decreased residence time for NO gas in the airways due to increased airflow velocity and also there may be effects of back diffusion of NO from the bronchial compartment to the alveoli. Although the protocol did not allow measures of static lung volumes to be performed and I did not measure closing volume the reproducibility of all measures of exhaled nitric oxide suggests that this is not an issue as one would expect to see changes in alveolar NO with weight loss as FRC would be expected to increase. This is reinforced by the evidence that there was no correlation between nitric oxide levels and airway specific conductance which is a marker of airway calibre and therefore a surrogate for lung volume. Another reason for a reduction in exhaled NO could be the increased blood flow to the lungs that occurs in obesity which may be acting as a sump for NO which is a highly reactive molecule[203, 205, 319].

There was no relationship between PC45 and exhaled nitric oxide and no relationship between change in PC45 and change in exhaled nitric oxide. This may again reflect the effect of obesity on lung volumes and other influences on bronchial responsiveness rather than the influence of inflammation; it may also suggest that inflammation other than eosinophilic inflammation may play a part in the obese subjects. Specific airway inflammation due to 'phenotype' was explored in the previous chapter through

the cell counting from induced sputum however and there did not appear to be a neutrophilic predominance either.

There are several potential limitations noted that may explain the negative outcomes found. The subjects were asthmatic and were taking inhaled steroids. The study design was not such that steroid medication could be withdrawn or standardised. Steroids are known to improve airway inflammation and reduce exhaled nitric oxide[107]. This may therefore reduce levels of exhaled nitric oxide in my subjects and mask the effect of BMI. However there was no significant difference in mean dose of inhaled steroids between the dietician and control groups therefore any difference would have been due to the additional effect of the intervention or weight change. Also there was no relationship between dose of inhaled steroids used and the levels of exhaled nitric oxide measured. This was the same for the significant weight loss vs non significant weight loss groups. Additionally the treatment did not change appreciably between visits and therefore change longitudinally would likely have been due to change in weight rather than a change in medication. As this was a real life study investigating many factors related to medical weight loss in obese asthmatics I wished to explore the effect of weight loss in a population that would likely be encountered in clinical practice.

Exhaled nitric oxide can also be affected by factors such as smoking, diet and other respiratory conditions which may have affected the study[94, 320]. I attempted to exclude these factors by excluding those with other significant comorbidities, subjects were asked to fast and avoid caffeinated drinks prior to their visit and all subjects were non smokers or ex-smokers.

Although this is one of the largest studies of its kind the numbers recruited are still low and therefore the power of the study may not have been sufficient to show a difference between groups.

Lastly I was unable to measure lung volumes in my patients due to the need to avoid deep inspiratory manoeuvres at the time of bronchial challenge testing and therefore I am unable to adjust for FRC in my subjects which may affect the level of exhaled nitric oxide and is also related to obesity.

7.5 Summary

Despite these limitations my results agree with other studies that have failed to show a relationship between BMI, adiposity, adipokines, airway inflammation and exhaled nitric oxide. The association between asthma and obesity is unlikely to be related to an association with eosinophilic airway inflammation linked to inflammatory substances from increased adipose tissue. This would not exclude non-eosinophilic airway inflammation however and this is explored in chapter 6 with differential cell counts obtained from induced sputum.

Chapter 8: Bronchial responsiveness and reactivity in obese asthmatics

8.1 Introduction

Obesity and asthma are related, with an increase in BMI associated with an increase in the prevalence of asthma[3]. Asthma is characterised by reversible airway obstruction as a result of hyper-responsiveness of bronchial wall smooth muscle which can be measured using bronchial provocation challenge testing, non-selectively, either directly or indirectly by exposing the airways to various stimuli[14]. The relationship of bronchial responsiveness to BMI is controversial with some suggesting that although wheeze and breathlessness is associated with obesity, bronchial responsiveness is not[213]. Others suggest otherwise and have shown an increase in bronchial responsiveness in obese subjects[211, 212].

Indirect challenges involve the use of chemical stimuli to initiate one or more of the intermediate steps leading to bronchoconstriction and direct challenges involve the use of substances such as muscarinic agonists (e.g. methacholine) to directly stimulate receptors on airway smooth muscle[14, 17, 143]. Challenge testing can be used to assist with making a diagnosis and to assess asthma control or severity, however airway hyper-responsiveness to methacholine is not synonymous with asthma and its severity is not synonymous with asthma severity[17, 149, 321]. Despite this the measurement of bronchial hyper-responsiveness to methacholine is accepted as a way of assessing asthma severity in clinical trials and a way of tracking change with intervention[144].

Historically, for diagnostic purposes, asthma challenge tests target a significant change in FEV_1 with a 20% fall in FEV_1 being considered a positive

test and an arbitrary cut off to exclude significant bronchial responsiveness for most research studies set at 8mg/ml using increasing doses of methacholine.

Standardised methods have been developed to perform methacholine challenge tests[17]. And a doubling concentration of methacholine is administered with assessment of the FEV_1. The dose of methacholine calculated to induce a 20% drop in FEV_1 is used to define bronchial responsiveness and is termed PC_{20}. Alternatively airway constriction can be measured using body plethysmography which can avoid deep inhalations to measure increase in airway resistance or its reciprocal, specific airway conductance (sGaw) and the cut off of a 45% drop in sGaw is used to produce PC_{45} which equates to PC_{20}. Two standardised methods are also described to administer methacholine, one requires deep inhalations and the other a tidal breathing method.

There are two theoretical reasons for an increase in bronchial responsiveness in obesity, an increase in the underlying airway inflammation due to an increase in proinflammatory substances produced by adipose tissue leading to an increase in bronchial smooth muscle responsiveness[305] and the mechanical effect of obesity leading to a reduction in lung volumes, decrease in airway diameter and reduced smooth muscle stretch[154, 176].

To investigate bronchial responsiveness in relation to obesity it is important to avoid methods that would require deep inhalations which may provide a bronchoprotective effect[154, 200, 319, 322]. Direct challenge with methacholine was chosen using a tidal breathing method to administer methacholine and body plethysmography to measure changes in sGaw.

We hypothesised that bronchial responsiveness would be related to BMI with increased responsiveness associated with increasing BMI which would decrease with weight reduction. To explore whether any change in bronchial responsiveness could be related to airway inflammation or a reduction in airway calibre we explored the relationship between bronchial responsiveness, exhaled nitric oxide and specific airway conductance.

As a secondary outcome measure we explored bronchial hyper-reactivity and its relationship to BMI and weight loss as the speed or intensity of response to a bronchoconstricting agent, which has been shown to have a better relationship with HRQoL related to severity of asthma[151]. The slope of the dose-response curve used in this way has been shown to be more useful in identifying patients with asthma[152] and in maintaining a better relationship with the degree of oxidative stress of patients[153]. For analysis I used the dose-response slope and also the bronchial reactivity index.

8.2 Methods

The methods have been described in detail in Chapter 2 and will be outlined in brief here.

To avoid the bronchoprotective effect of deep inspiratory manoeuvres, bronchial challenge testing was carried out using the tidal breathing method and airway responsiveness was measured by specific airway conductance (sGaw) using body plethysmography with a change in sGaw of ≥45 % used to terminate the test and calculate PC_{45}.

All procedures were carried out by the author following instruction by laboratory staff at University Hospital Aintree pulmonary function unit and the

procedure was performed on all subjects at baseline, 3 months and 6 months. The order of tests has been explained earlier in chapter 2.

Prior to testing subjects were asked to refrain from using their inhaled medication for 12 hours and any oral medication for 24 hours, They were also asked to refrain for taking caffeinated drinks for 12 hours.

The Two-minute tidal breathing dosing protocol was used adapted from the ATS guidelines[17] previously mentioned using the following 10 doubling concentrations of methacholine: Diluent, 0.03, 0.06, 0.125, 0.25, 0.5, 1, 2, 4, 8, 16, 32 mg/ml. The PC_{45} was calculated as the concentration of methacholine required to produce a 45% fall in sGaw (baseline sGaw x 0.55). sGaw was measured using a Medgraphics™ Elite Plethysmograph was used which is capable of accommodating patients up to 180 Kg and as I wished to avoid deep inspiratory manoeuvres so as not to affect bronchial reactivity I measured airway resistance only but not full lung volumes of the subjects by asking the subjects to breathe at tidal volume throughout the procedure. Between tests the patient moved outside the body box for the next nebulisation of methacholine.

Following the procedure 5mg nebulised salbutamol was administered and spirometry checked.

Determination of PC_{45}.

The concentration of methacholine required to cause a drop in sGaw of 45% or PC_{45} was calculated using a logarithmic method as follows:

$$PC_{45} = \text{antilog}\ [\ \log C1 + \frac{(\log C2 - \log C1)(45-R1)]}{R2-R1}$$

Where

C1 = second-to-last methacholine concentration (concentration preceding C2)

C2 = final concentration of methacholine (concentration resulting in a 45% or greater fall in sGaw)

R1 = percent fall in sGaw after C1

R2 = percent fall in sGaw after C2

Determination of bronchial hyperreactivity: Dose response slope & Bronchial Reactivity Index.

To calculate dose response slope and bronchial reactivity index the method described by Burrows et al[264] was used and adapted to PC_{45}. The dose response data were summarised by the expression: percent decline in sGaw / dose, where percent decline sGaw was defined as the decline in sGaw (from the post saline value) after the final methacholine dose administered, and the dose was defined as the final cumulative dose administered. This can be graphically represented as the slope of a line connecting the origin of a dose response curve with the final point of the curve referred to as the dose-response slope.

The slope was calculated by dividing the percent decline in baseline sGaw after the last methacholine challenge by the log of the last methacholine concentration given to account for skewed data. To avoid negative or zero

logarithms in the denominator, all concentrations were expressed as milligrams per decilitre.

The expression used therefore to obtain the dose response slope is as follows:

$$DRS = \frac{\text{percent decline in sGaw}}{\text{Log}_{10}C2}$$

C2 = Final concentration of methacholine (mg/dl)

$$\text{Where percent decline in sGaw} = \frac{\text{Baseline sGaw - Final sGaw}}{\text{Baseline sGaw}} * 100$$

Bronchial response index[151] was used to provide a continuous and relatively normally distributed variable for use in statistical analysis:

$$BRI = \text{Log}_{10}\ DRS$$

8.2.1 Statistical analysis

Categorical variables are expressed as percentages of total subjects and compared using Chi squared test. Continuous variables are expressed using mean ±SD and compared between groups with the Students unpaired *t* test if normally distributed. Non-normal distributions determined by Shapiro-Wilk testing are expressed using median and interquartile range. Variables compared between visits were compared using paired-samples *t* testing. Correlations were performed between normally distributed variables using Pearson correlations two-tailed test and non-normally distributed variables using Spearman's. Any correlations were checked visually for homoscedasticity to confirm any relationship. Significance for multiple comparisons were adjusted by Bonferroni correction. Significance was

determined if $p<0.05$ and alpha level adjusted by Bonferroni for number of observations studied.

8.3 Results

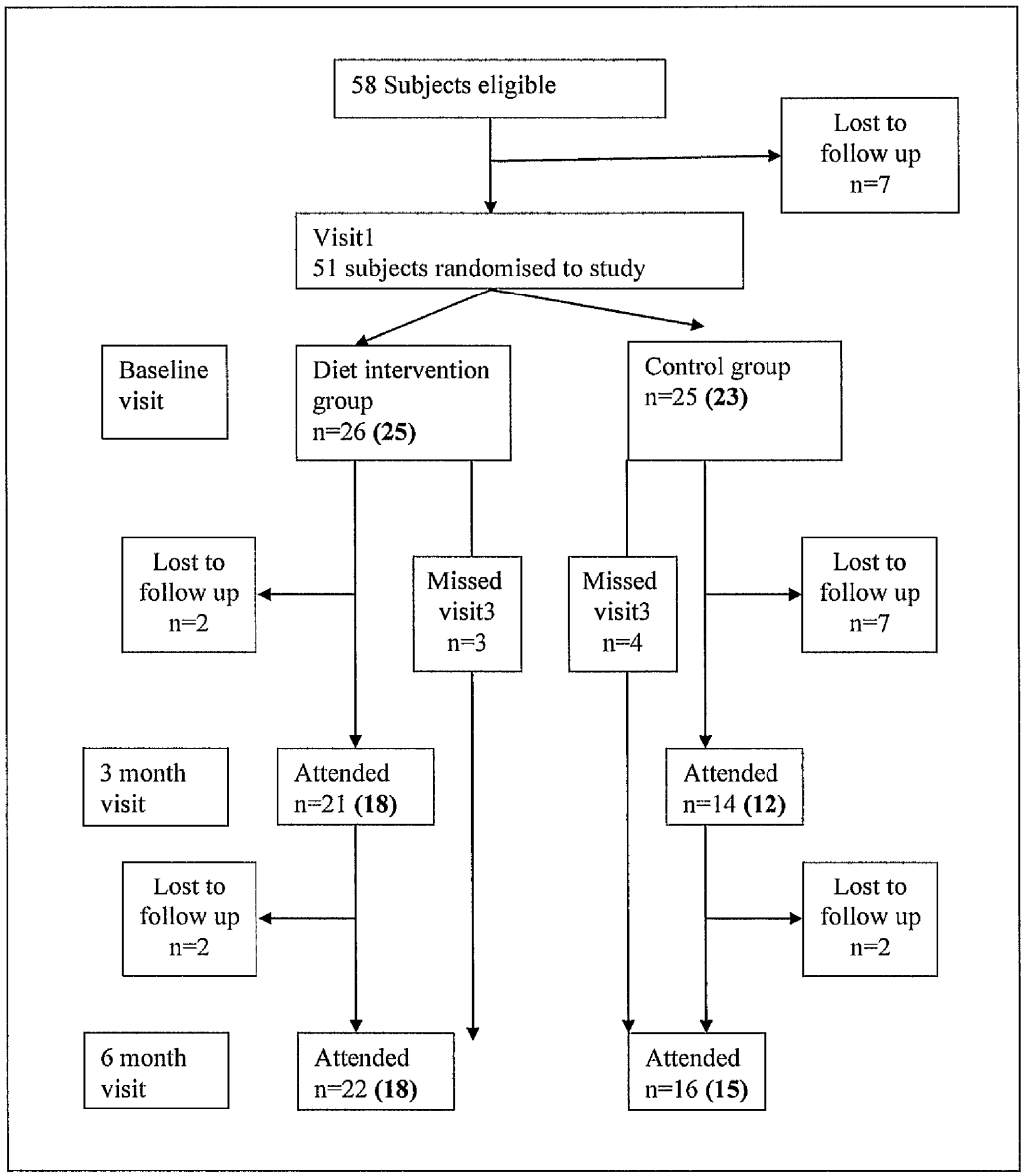


Fig 16. Consort diagram for study. (numbers in brackets = subjects with available completed questionnaires)

Missing data was present for the following reasons:

Pt12 6 months FEV1 too low (41%) therefore not done
Pt17 3 months pt refused
Pt17 6 months pt refused
Pt27 6 months Starting sGaw too low
Pt29 6 months Starting sGaw too low
Pt38 3 months Technical error
Pt60 baseline Technical error – equipment failure
Pt67 baseline Starting sGaw too low
Pt78 baseline Used screening visit as first visit
*in brackets = number of subjects with BR data available

8.3.1 Measures of specific airways conductance, bronchial responsiveness and reactivity at each visit

All subjects. Mean (sd)

Bronchial responsiveness, starting specific airway conductance and measures of bronchial reactivity for all subjects are shown below.

	Baseline	3 Months	6 Months
sGaw	0.155 (0.05)	0.156 (0.05)	0.155 (0.05)
PC_{45}	0.219 (0.3)	0.271 (0.394)	0.331 (0.619)
$LogPC_{45}$	-0.900 (0.439)	-0.827 (0.447)	-0.776 (0.458)
DRS	52.5 (24.5)	48.3 (23.8)	48.8 (22.9)
BRI	1.77 (0.15)	1.74 (0.149)	1.74 (0.149)

sGaw = Specific airway conductance; PC_{45} = Provocative concentration of methacholine to cause a 45% drop in sGaw from baseline; $LogPC_{45}$ = Log base 10 of PC_{45}; DRS = Dose response slope; BRI = Bronchial response index.

Table 38. Mean (SD) for all subjects for specific airway conductance, bronchial responsiveness and reactivity for each visit

There were no significant differences for all measures of bronchial responsiveness and reactivity or airway conductance between baseline to 3 months, baseline to 6 months and 3 months to 6 months. There was a trend towards an improvement in PC_{45} at 3 months and 6 months.

Comparing between groups. Mean (sd)

Bronchial responsiveness, starting specific airway conductance and measures of bronchial reactivity for all subjects are shown below.

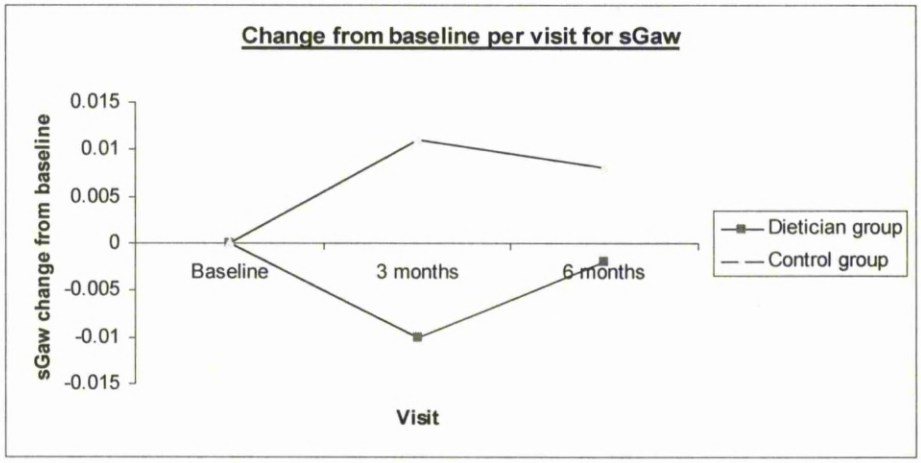
	Dietician group baseline	Control group baseline	Dietician group 3 months	Control group visit 3 months	Dietician group 6 months	Control group 6 months
sGaw	0.149 (0.054)	0.16 (0.048)	0.151 (0.056)	0.165 (0.061)	0.151 (0.054)	0.161 (0.048)
PC_{45}	0.203 (0.229)	0.236 (0.365)	0.278 (0.401)	0.261 (0.399)	0.375 (0.787)	0.276 (0.319)
$LogPC_{45}$	-0.915 (0.451)	-0.884 (0.435)	-0.799 (0.436)	-0.869 (0.48)	-0.785 (0.501)	-0.765 (0.414)
DRS	53.33 (25.77)	51.58 (23.56)	47.23 (24.23)	50.13 (23.87)	52.32 (26.6)	44.29 (14.76)
BRI	1.77 (0.16)	1.76 (0.15)	1.73 (0.15)	1.75 (0.16)	1.76 (0.17)	1.72 (0.12)

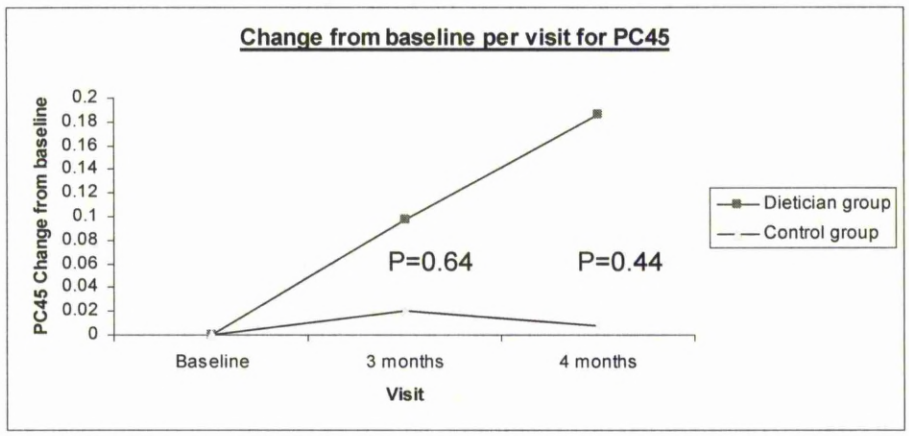
sGaw = Specific airway conductance; PC_{45} = Provocative concentration of methacholine to cause a 45% drop in sGaw from baseline; $LogPC_{45}$ = Log base 10 of PC_{45}; DRS = Dose response slope; BRI = Bronchial response index.

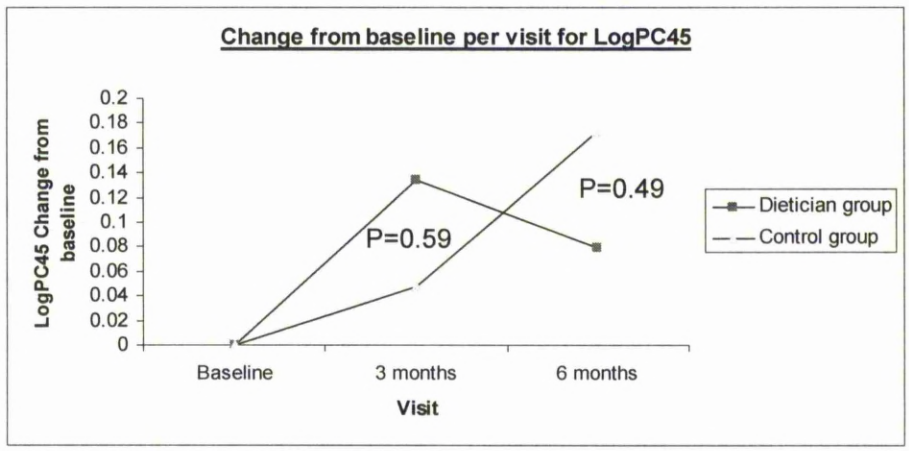
Table 39. Mean (SD) for dietician and control groups for specific airway conductance, bronchial responsiveness and reactivity for each visit

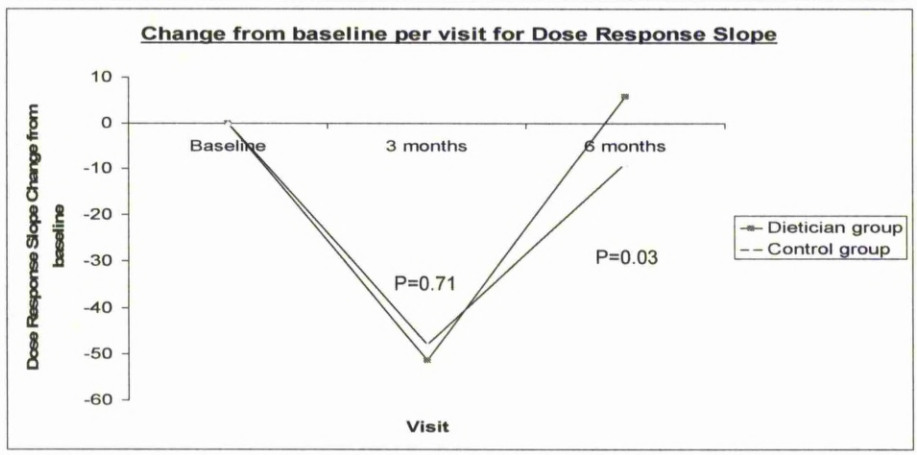
There were no significant differences for either the dietician or the control group for all measures of bronchial responsiveness and reactivity or airway conductance between baseline to 3 months, baseline to 6 months and 3 months to 6 months. Again there was a trend towards an improvement in PC_{45} at 3 and 6 months.

There were no significant differences between the control group and dietician group for any visit for any measure of bronchial responsiveness and reactivity or airway conductance.




Change from baseline per visit for PC45
PC45 Change from baseline
0.2
0.18
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
P=0.64
P=0.44
Baseline
3 months
4 months
Visit
Dietician group
Control group


Change from baseline per visit for LogPC45
LogPC45 Change from baseline
0.2
0.18
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
P=0.49
P=0.59
Baseline
3 months
6 months
Visit
Dietician group
Control group


Change from baseline per visit for Dose Response Slope
Dose Response Slope Change from baseline
10
0
-10
-20
-30
-40
-50
-60
Baseline
3 months
6 months
P=0.71
P=0.03
Visit
Dietician group
Control group

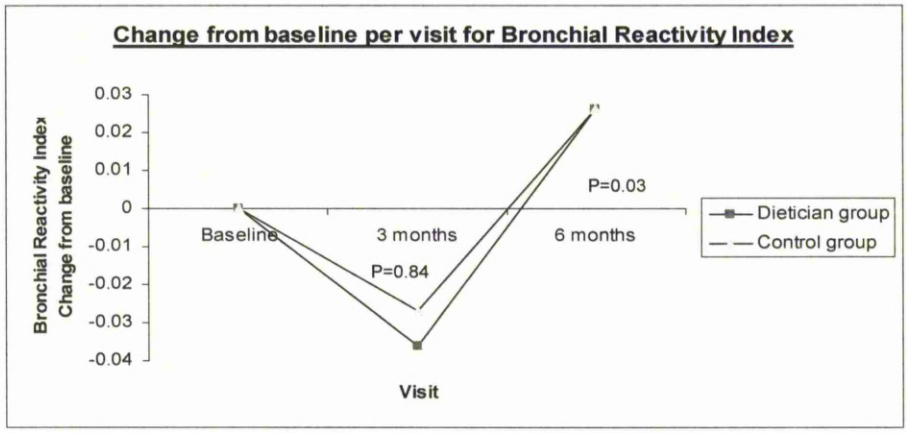


Fig 17. Graphs to show change in from baseline at each visit for Dietician and Control groups for sGaw, PC45, LogPC45, Dose Response Slope and Bronchial Reactivity Index. P values represent differences between groups at each visit from paired samples analysis

8.3.2 Correlations between BMI and specific airway conductance plus measures of bronchial responsiveness and reactivity for all subjects and each group

There were no significant correlations for all subjects between BMI at each visit and specific airway conductance or any measure of bronchial responsiveness or reactivity except for $LogPC_{45}$ at 3 months (r=0.391, p=0.025).

There were no significant correlations for either the dietician or control group between BMI at each visit and specific airway conductance or any measure of bronchial responsiveness or reactivity except for $LogPC_{45}$ at 3 months (r=0.659, p=0.014) for the control group.

8.3.3 Relationship between percentage change in weight and change in specific airway conductance and change in measures of airway responsiveness and reactivity. All subjects, dietician and control groups

There were no significant correlations between percentage weight change from starting weight and change from baseline in sGaw or change in bronchial responsiveness or reactivity except for sGaw between baseline and 3 months (r=0.528, p=0.020) although when examining the scatterplot for heteroscedasticity this did not appear true . This was also true if compared to percentage change from baseline for sGaw, bronchial responsiveness and reactivity variables.

8.3.4 ≥5% weight loss group vs <5% weight loss group – between group comparisons

	≥5% weight loss group Baseline	<5% weight loss group Baseline	≥5% weight loss group 3 months	<5% weight loss group 3 months	≥5% weight loss group 6 months	<5% weight loss group 6 months
sGaw	0.146 (0.051)	0.154 (0.055)	0.150 (0.067)	0.161(0.052)	0.147 (0.056)	0.161 (0.047)
PC_{45}	0.226 (0.276)	0.234 (0.362)	0.206 (0.219)	0.276 (0.425)	0.477 (0.941)	0.241 (0.281)
$LogPC_{45}$	-0.927 (0.516)	-0.862 (0.405)	-0.864 (0.407)	-0.813 (0.441)	-0.709 (0.545)	-0.818 (0.405)
DRS	52.31 (24.24)	50.47 (24.51)	50.70 (22.03)	48.03 (27.02)	52.05 (30.15)	46.75 (17.56)
BRI	1.764 (0.168)	1.756 (0.145)	1.762 (0.137)	1.732 (0.161)	1.755 (0.179)	1.735 (0.132)

sGaw = Specific airway conductance; PC_{45} = Provocative concentration of methacholine to cause a 45% drop in sGaw from baseline; $LogPC_{45}$ = Log base 10 of PC_{45}; DRS = Dose response slope; BRI = Bronchial response index.

Table 40. Mean (SD) for significant weight loss and non-significant weight loss groups for specific airway conductance, bronchial responsiveness and reactivity for each visit

There were no significant differences between those that lost significant weight (≥5% of baseline) and those that did not for specific airway conductance, bronchial responsiveness or reactivity at any visit.

8.3.5 Relationship between specific airway conductance, bronchial responsiveness and bronchial reactivity with exhaled nitric oxide

I have explored the relationship between measures of airway conductance and bronchial responsiveness and reactivity with exhaled nitric oxide here. Relationships between health related quality of life questionnaire scores and induced sputum differential cell counts have been discussed elsewhere in the relevant chapters. Correlations are shown below for each visit.

	FeNO50ml Baseline	FeNO50ml 3 months	FeNO50ml 6 months
sGaw	R= 0.043 P= 0.774	R= 0.199 P= 0.293	R= 0.155 P= 0.375
PC_{45}	R= -0.178 P= 0.241	R= -0.405* P= 0.027	R= -0.252 P= 0.156
$LogPC_{45}$	R= -0.203 P= 0.181	R= -0.533* P= 0.002	R= -0.351* P= 0.045
DRS	R= 0.301* P= 0.045	R= 0.500* P= 0.004	R= 0.408* P= 0.018
BRI	R= 0.312* P= 0.037	R= 0.557* P= 0.001	R= 0.446* P= 0.009

* significant correlation $p<0.05$
sGaw = Specific airway conductance; PC_{45} = Provocative concentration of methacholine to cause a 45% drop in sGaw from baseline; $LogPC_{45}$ = Log base 10 of PC_{45}; DRS = Dose response slope; BRI = Bronchial response index.

Table 41. Correlations between exhaled nitric oxide, specific airway conductance, bronchial responsiveness and measures of bronchial reactivity at each visit for all subjects

There were significant correlations between measures of bronchial reactivity and the fraction of exhaled nitric oxide at baseline, 3 months and 6 months. There were also correlations between PC45 and $FeNO_{50}$ at 3 months and $logPC_{45}$ and $FeNO_{50}$ at 3 and 6 months. However there were no significant correlations between change in $FeNO_{50}$ from baseline and the corresponding change in specific airway conductance and measures of

bronchial responsiveness or reactivity between baseline to 3 months and baseline to 6 months.

8.3.6 Inhaled steroids and bronchial responsiveness

There were no significant correlations with any measures of bronchial responsiveness and inhaled steroid dose.

Additional material for this chapter can be found in appendix D

8.4 Conclusions and discussion

I investigated the presence of bronchial responsiveness and reactivity in a group of obese asthmatic subjects. Although there was a trend towards a decrease in bronchial responsiveness at 3 and 6 months for the group as a whole this did not reach significance. This was also true when exploring individual groups and was seen in both the dietician and control groups. There was a larger decrease in responsiveness from baseline in the dietician group compared to the control group although the difference between groups at each visit did not reach significance. There was no significant change in airway specific conductance between visits for all subjects or between groups. There was also no significant change for all subjects and in either group at 3 or 6 months for measures of bronchial reactivity, i.e. the dose response slope or bronchial reactivity index. There was a trend towards a decrease in weight at 3 and 6 months in the group as a whole and an increased weight loss in the dietician group which may explain this change in bronchial responsiveness, however when exploring the relationship between BMI and bronchial reactivity

there was no significant correlation between BMI and bronchial responsiveness at any visit for all subjects or for either group. There was also no correlation between weight loss as a percentage of original weight at 3 and 6 months and changes in measures of bronchial responsiveness or reactivity over this period which would suggest that BMI is less likely to be linked to any changes in reactivity or responsiveness in this population of obese asthmatics. This was further reinforced when comparing those subjects that lost ≥5% of their original weight compared to those that did not achieve this significant weight loss as no significant differences were found between these two groups.

It has been suggested that bronchial responsiveness and reactivity can be affected by lung volumes by becoming increased by lower lung volumes[154, 200]. I was unable to measure lung volumes directly as the method required the avoidance of deep inspiratory manoeuvres, however I was able to measure airway calibre by specific airway conductance (sGaw) and did not find a relationship between sGaw and BMI in either group or the group as a whole. I did not see changes in sGaw at 3 or 6 months as I did with PC45 and there were no correlations between change in weight and change in sGaw or any significant differences in sGaw between those that lost significant weight i.e. ≥5% of their original weight compared to those that did not achieve significant weight loss.

It is unlikely therefore that any trend towards improvement in bronchial responsiveness is related to changes in specific airway conductance or changes in baseline airway calibre which may be a surrogate for lung volumes.

There was a possible relationship between airway reactivity and airway inflammation as measured by the fraction of exhaled nitric oxide as there were significant correlations between the dose response slope and bronchial reactivity index at each visit, although there were no correlations with PC45. Despite this however there were no correlations between change in $FeNO_{50}$ between visits and change in measures of bronchial responsiveness or reactivity. This suggests that any change in bronchial responsiveness or reactivity in our obese asthmatic population is unlikely to be due to the influence of weight through an immunological mechanism. As discussed in chapter 7 the possible effect of airway calibre on measured FeNO must be bourne in mind however and this may have masked an association with this variable[240, 314, 315].

Airway hyper-responsiveness (AHR) is a characteristic of asthma, and histamine and methacholine bronchoprovocation challenges have been widely used to document and quantitate AHR[17, 143]. We assessed bronchial responsiveness in our subjects using a direct stimulus acting directly on airway smooth muscle; methacholine. Bronchial hyper-responsiveness is responsible for recurrent episodes of wheezing, breathlessness, chest tightedness, and coughing in asthma[14]. The methacholine challenge is the most commonly performed. I used a commonly used method standardised to American Thoracic Society Guidelines to ensure comparability of the data and also to ensure comparability between visits for the subjects[17]. The 2-min tidal breathing method was chosen to avoid deep inspiratory manoeuvres and it should be noted that this provides maximum diagnostic sensitivity when methacholine is inhaled by non-deep inhalation methods[322]. It was previously

thought that deep inhalation and breathhold probably resulted in greater retention of aerosol, better deposition of aerosol or both but further studies have shown this not to be the case. When the methods are performed as per the ATS guidelines, the tidal breathing method repeatedly produces a greater response due to the greater dose administered in the tidal breathing method, and also due to the lack of brochodilatation and bronchoprotective effect of deep inhalation[323, 324]. There is a correlation between asthma severity and the severity of AHR[144, 145] that improves with anti-inflammatory therapeutic strategies such as inhaled steroids[146]. There is a modest correlation between the severity of direct AHR and airway inflammation with mainly eosinophils or metachromatic cells[147]. There is also an increased response to direct stimuli with nonasthmatic airflow obstruction closely related to the severity of chronic bronchial obstruction felt to represent a geometric issue with regard to airway diameter[148]. Therefore the method chosen for this study is appropriate for investigating obese subjects. Future studies of obese subjects are needed to compare methods of performing challenge testing to report on how a raised BMI can influence the sensitivity of these different methods including those that involve deep inspirations and those that don't.

There is felt to be two components to AHR, a variable and fixed component with the variable component being able to change with improvement in airway inflammation and the fixed component being related to structural and functional changes in the airway termed airway remodelling[143]. My subjects had fairly well preserved FEV_1 which would suggest that airway remodelling and fixed airflow obstruction was unlikely to be an important

factor and I was measuring the variable component which should track airway inflammation.

Although AHR is felt to be related to eosinophilic airway inflammation some studies have dissociated the relationship between eosinophils and AHR. Studies using Mepolizumab an anti-interleukin-5 agent found that patients that had a reduction in eosinophils continued to have AHR and symptoms[134]. This helps to explain the lack of a response to weight loss in terms of any change in immunological response through measurements of exhaled nitric oxide. Dixon et al have also demonstrated a lack of a relationship between airway inflammation measured by airway differential cell counts and bronchial responsiveness in obese subjects undergoing surgical weight loss suggesting that another mechanism is involved[2].

One of the difficulties inherent in assessing bronchial responsiveness and reactivity is the fact that specificity is low and bronchial responsiveness can be present in non-asthmatic conditions such as allergic and nonallergic rhinitis[325]. As there is no gold standard confirmation of the presence of asthma it is also difficult to determine whether my population had bronchial responsiveness as a consequence of the asthma syndrome, however all the subjects had symptoms, had a doctor diagnosis of asthma and were on significant amounts of treatment. Changes in bronchial reactivity could be due to a number of reasons, including the mechanical effects of obesity. I strived to exclude patients with other reasons for AHR although I cannot completely exclude gastroesophageal reflux disease which is common in obese persons and may be related to AHR[326].

It is important to note limitations to the study in terms of measuring bronchial responsiveness. Due to the nature of the subjects' obesity it is difficult to perform measurements in these patients accurately especially using body plethysmography as the subjects' size presents problems with volumes in the body box. I used a plethysmograph which is rated to 180Kg and paid careful attention to calibration, subject technique and allowed time for the equipment to settle before performing measurements. Despite this there may have been some errors introduced in measurements due to subjects having to move between locations to nebulise methacholine and perform measurements.

Another source of potential error may be introduced from the requirement to avoid deep inhalations[322]. During the measurement of specific airway conductance the subject is required to pant at a predetermined frequency and during the process of doing this subjects may wish to deepen their inspiration. I tried to avoid this by carefully explaining to each subject what was required from them and providing feedback from the graphical representation of the equipment software.

Bronchial responsiveness can be altered by a number of factors and there is a natural variability in repeatability of the test. I tried to reduce these extra factors to a minimum by avoiding exacerbations, using standardised recommendations for avoiding medication, all visits were in the morning and at the same time of day. Co-morbidities can also affect bronchial responsiveness such as gastroesophageal reflux disease[326], however as this was a longitudinal study this effect should have less impact.

Lastly there is some controversy over the best measurement of bronchial responsiveness or reactivity. I have used PC_{45} to methacholine with sGaw as this is recommended in international guidelines, is therefore standardised and can be compared with other studies[17]. I have also used the slope of the response in the bronchial responsiveness index and dose response slope which some suggest is a more sensitive measure[151, 152].

8.6 Summary

Weight loss in obese asthmatics appears to have no significant effects on objective measures of airway obstruction or airway reactivity as a marker of severity. There was no correlation between airway responsiveness and BMI or change in weight. Measuring airway responsiveness in the obese population is difficult and further studies are required to establish the optimal method to allow comparison of different studies.

Chapter 9: Conclusions and Discussion

9.1 Summary of results

In this thesis I have shown that when recruiting obese asthmatic subjects, of 91 subjects tested for bronchial responsiveness, approximately one third did not demonstrate increased bronchial responsiveness that would be consistent with a diagnosis of asthma by most accepted definitions[12, 15, 327]. There was no significant difference between those with and those without bronchial responsiveness who were well matched for asthma medication, age and gender, for health related quality of life as measured by generic, respiratory specific and weight specific quality of life questionnaires, There was no significant difference between exhaled nitric oxide either. There was a significant correlation between BMI and HRQoL but not with other measures of asthma severity.

51 subjects were enrolled into the study, 26 were randomised into the dietician group and 25 into the control group. Both groups lost weight overall with a greater mean weight loss in the dietician group (-5% at 3 months and -4.9% at 6 months) than the control group (-3% at 3months and -2.7% at 6 months) although the two groups did not differ significantly. In addition there was a significant weight loss between baseline and 3 months ($p<0.05$) and 6 months ($p<0.05$) in the dietician group which was not seen in the control group. This was seen with intention to treat analysis which did not differ with last observation carried forward. Therefore further analysis in the study used intention to treat. It is accepted by expert opinion that a clinically important weight loss is ≥5%[286, 287], there were subjects that achieved this level of weight loss in the control group as well as the dietician group, therefore

further analysis was carried out for each variable by using two further groups, significant weight loss vs non significant weight loss.

Health related quality of life measured using the generic SF36[160], disease specific SGRQ[163] and IWQOL-Lite[244] showed improvement in quality of life scores from baseline to 3 months and 6 months in the dietician group but not the control group. There was no significant difference at any visit between groups although there was a trend towards a greater improvement in the dietician group compared to the control group that did not reach significance. In contrast to the screening visit cross sectional analysis, there was no relationship between generic and respiratory specific quality of life and BMI or change in weight, although as would be expected there were some weak correlations between the IWQOL-Lite weight specific questionnaire domains, BMI and weight change. When comparing those that lost ≥5% of baseline weight there was a trend towards a greater improvement in HRQoL vs those that did not lose weight although this did not reach significance. Although the HRQoL scores were better in the ≥5% weight loss group compared to the <5% weight loss group the two did not differ statistically. There was no significant effect of gender on the HRQoL scores and no significant correlation between other measures of asthma severity (FeNO, PC_{45}) and HRQoL.

There were no significant correlations between BMI and differential cell counts from induced sputum and no significant differences between dietician and control groups or those that lost ≥5% weight and those that did not. There was also no significant correlation with weight change and any cell line and no

relationship between eosinophil predominant or neutrophil predominant inflammation and BMI.

Similarly when using exhaled nitric oxide as a marker of eosinophilic airway inflammation, no significant relationships were found with BMI, weight change and no significant differences were found between dietician and control groups or those that lost ≥5% baseline weight vs. those that did not.

Lastly there was no significant correlation with bronchial responsiveness, reactivity or specific airway conductance vs. BMI or weight change and no differences between the dietician vs. control group or ≥5% weight loss vs. <5% weight loss group.

Using fat% measured by bioimpedence technique did not show any additional significant results and there were no significant correlations with body fat% and any of the markers of asthma severity in addition to those already described with BMI. Therefore body fat% has not been explored further in the analysis.

Inhaled steroid dose had no effect on any measures of asthma severity.

Although I intended to do so I was unable to analyse serum inflammatory markers or adipokines due to technical difficulties. Due to poor reply rates and poor completion I was unable to include peak flow and symptoms diaries in the analysis.

9.2 Interpretation

I have shown that of the subjects that attended and had been given a physician diagnosis of asthma on significant amounts of treatment, around

one third did not demonstrate bronchial hyper-responsiveness, suggesting a mis-classification of diagnosis possibly due to the effects of obesity on respiratory mechanics producing similar symptoms to asthma. These subjects have a significant health impairment with HRQoL having a greater impact than other traditional markers of asthma severity i.e. airway responsiveness (PC_{20}), lung function (FEV1% and FVC% predicted) or airway inflammation ($FeNO_{50}$). As the negative correlation between BMI and HRQoL was found in all questionnaires used it is likely to be a generic effect of the impact of obesity on HRQoL rather than an effect on the respiratory system in asthma per se as there was no significant correlation between BMI and the symptoms domain of the SGRQ which includes questions on the frequency of cough, sputum, breathlessness, wheeze and exacerbations. Although patients can have asthma without the presence of bronchial hyper-responsiveness many studies require the presence of bronchial responsiveness defined as a PC_{20} of <8% mg/ml or reversibility of FEV1 to inhaled bronchodilators of 15%[17, 143]. I therefore used this criteria towards making the diagnosis of asthma which was supported by the evidence of less airway inflammation, less airway obstruction and less atopy in those that did not show bronchial hyper-responsiveness. It is interesting that one third of subjects did not fulfil these criteria despite their diagnosis and this may reflect the possibility that symptoms of breathlessness associated with the effects of increased BMI on airway physiology may be misinterpreted to be consistent with asthma in an obese population. This has important implications for interpreting studies that have not included these objective tests to confirm a diagnosis of asthma in a population.

Of those subjects that fulfilled the inclusion criteria for the study I demonstrated that it was possible to achieve a significant weight loss with dietary intervention and meal replacement strategy over a 6 month period albeit mild when compared to surgical weight loss studies. Although there was a trend towards greater weight loss in the dietician group vs. the control group the two groups did not differ significantly at 3 or 6 months. It is noted that not all subjects lost weight in the dietician group and also some of the subjects in the control group lost significant weight therefore two further groups were created i.e. a significant weight loss vs. no significant weight loss. Although the study was powered to show a significant difference with the numbers of subjects recruited, and this is one of the largest trials of dietary intervention in obese asthmatics, it is likely that the study was underpowered to demonstrate a significant difference between groups as we had aimed to recruit 40 subjects into each group with the hope of retaining 25 per group. There may also have been a study effect on the control group, the so called Hawthorne effect[292], resulting in weight loss occurring due to the fact that the subject was involved in a clinical study. Although there were trends to suggest that the dietician group differed from the control group this was not shown statistically and more numbers are likely to be needed in any future studies of this nature.

There were significant improvements seen in all subjects and the dietician group for HRQoL from baseline to 3 and 6 months although the dietician and control group did not differ significantly. This may be due to a lack of power once again for the study and too few subjects enrolled in each group. Again there was a trend for an improvement in HRQoL in the control group as well as the dietician group which could be due to the effect of

enrolment into a clinical trial rather than the effect of diet or weight loss in itself. There was not a similar correlation between BMI and measures of HRQoL as was seen in the screening visit in the generic and respiratory specific questionnaires although there were significant correlations seen for the weight specific questionnaire which would be expected to be more sensitive to changes in weight[244]. There was a trend towards an improvement in HRQoL with lower BMI. The same was true for change in weight and change in HRQoL with significant correlations seen only in the weight specific questionnaire. When comparing those that lost significant weight (≥5% of baseline weight) with those that did not, again there was a trend towards a greater improvement in HRQoL in the weight loss group although not significant. There were no relationships found between HRQoL and bronchial hyper-responsiveness or measures of inflammation. Although non significant when taking into account results from the screening patients HRQoL is affected in obese asthmatic subjects mainly by BMI and excess weight rather than other traditional measures of asthma severity. Weight loss has the potential to improve the quality of life of these patients which may account for improvements in asthma severity and asthma control[161].

When investigating the effect of BMI on inflammation there were no relationships found between BMI and any cell type in the differential cell count from induced sputum, no significant differences between either dietician or control groups or significant vs non significant weight loss groups. This is similar to findings of other groups who have failed to show a relationship between BMI and airway inflammation as measured by induced sputum[2, 236, 241]. This does not support the theory that obesity alters the inflammatory

profile of the lung or that increase in BMI is associated with an increase in airway inflammation. There did not appear to be a specific sputum phenotype associated with obesity in this group of subjects either. This was also supported by the lack of a relationship between exhaled nitric oxide and BMI, changes in weight or when comparing between dietician vs. control groups or significant weight loss vs. no weight loss groups. It is important to note that many of the subjects were taking significant amounts of inhaled steroid medication which may have an effect on reducing exhaled nitric oxide levels[108] and reducing eosinophil counts[116], however, longitudinally there was no apparent change in markers of inflammation with change in weight. As medication was not altered this helps to support the suggestion that BMI does not affect the inflammatory milieu of the airways as has been suggested in animal studies and in vitro. Kim et al studied the effect of BMI and airway inflammation measured by exhaled nitric oxide in healthy adults and failed to find a significant relationship but do note that there are significant differences in the possible confounding factors that become important in investigating these measures in asthmatic individuals such as atopy, ethnicity, medication, combined respiratory diseases and other clinical variables[318]. Although FeNO measures eosinophilic airway inflammation and does not reflect noneosinophilic airway inflammation, it may be that obesity could affect airway inflammation without recruitment of eosinophils. However I failed to show any association with neutrophils in sputum differential cell counts and BMI in this study. Sutherland et al showed that in asthmatics, no significant interaction was observed between systemic and airway inflammation supporting my findings[241]. The theory that systemic inflammation spilling over into the blood

from adipose tissue can cause local inflammation in distant organs including the airways appears unlikely.

Finally there were no correlations between BMI or change in weight and measures of bronchial responsiveness, reactivity or airway calibre measured as specific airway conductance. There were also no differences between groups. This therefore suggests that BMI does not influence bronchial responsiveness in obese asthmatics and weight loss does not improve this marker of asthma severity in this group of subjects. Obesity may affect bronchial responsiveness due to its effect on lung volumes and therefore airway calibre[154, 176]. I was careful to avoid deep inspiratory manoeuvres prior to challenge testing to avoid the bronchoprotective effect of deep inspiration[322] but as a result was unable to describe the FRC of the subjects which may have a significant relationship with bronchial responsiveness or reactivity. I was able to measure specific airway conductance which may act as a surrogate for airway calibre and this had no effect.

I have shown that asthma severity associated with an increase in BMI is unlikely to be due to an effect of obesity on bronchial responsiveness or airway inflammation in these patients. There is a possible relationship with worsening of health related quality of life which had an inverse correlation with BMI with a trend to improve with weight loss but this may be due to a generic effect of obesity on HRQoL rather than from improvements in asthma severity. The effect of obesity on airway physiology can result in symptoms that are similar to those of asthma such as wheeze and shortness of breath[249] and

reduced quality of life can be associated with an increase in asthma symptoms that may be due to the generic effects of obesity on quality of life that could alter the patients' perception of their symptoms. This could therefore explain the results of some studies of the effects of surgical weight loss in obese asthmatics that have reported improvement in symptoms but have not included any measures of asthma severity such as bronchial responsiveness, spirometry or measures of airway inflammation.

One of the problems in making a diagnosis of asthma is that there is no universally accepted definition[15]. It is generally accepted that asthma consists of appropriate symptoms, airway inflammation and reversible airway obstruction and I have selected a population of subjects with airway hyper-reactivity and symptoms although not all were found to have markers of increased airway inflammation. This population differs from some studies that have included subjects simply on the basis of symptoms and a physician diagnosis of asthma. I have shown that these types of populations may possibly include subjects that do not fulfil all the criteria for a diagnosis of asthma and no evidence of airway inflammation or bronchial hyper-responsiveness. Improvements in these subjects with weight loss may have been due to the improvement in airway mechanics rather than improvements in asthma severity. I have been unable to investigate the relationship of airway mechanics to HRQoL, airway inflammation or bronchial hyper-responsiveness in this study as the protocol was not designed to do so and this should be explored further in future studies.

Another study by Aaron et al[249] has found similar results and reported in an uncontrolled study of weight loss in 58 obese women with 24 subjects having a physician made diagnosis of asthma showed that weight loss improved respiratory function independently of the severity of airway responsiveness. Others have also found similar findings in measuring induced sputum differential cell counts. Todd et al have reported no difference in differential cell counts between obese and non-obese subjects and also did not find a correlation between cell counts and BMI[236].

It is likely that the relationship between asthma and obesity remains complex. There are theoretical reasons that the inflammation associated with increased adipose tissue in obesity should influence the inflammatory state of the lungs in asthma with a resultant increase in bronchial responsiveness leading to increased airway obstruction, symptoms and a decline in control[1, 232, 234, 309, 318]. I have shown that although health related quality of life is worse in obesity this does not appear to be related to any change in airway inflammation or bronchial responsiveness. It is likely that obesity has an influence on the mechanics of breathing and this combined with its generic effect on quality of life leads to an increase in the perception of asthma in obese individuals rather than a worsening of the severity of asthma per se.

Recent studies in asthmatic and non asthmatic subjects undergoing surgical weight loss who achieved larger reductions in weight than this study have found improvements in bronchial responsiveness with weight loss and improvements in serum and adipose markers of inflammation but no change in inflammatory cell type[2, 328]. When comparing asthmatics to control subjects

there was less airway inflammation in the obese group at baseline which increased with weight loss. This supports my findings of not showing an increase in airway inflammation in this group of subjects who were all asthmatic.

Significance

These findings add to the body of evidence of the complexities of the relationship between obesity and asthma. I have shown the importance of confirming a diagnosis in the obese population with asthma like symptoms by obtaining objective evidence of airway inflammation or bronchial responsiveness. I have also highlighted that previous studies reporting the effects of BMI on asthma and the effect of weight loss based on self reported diagnosis of asthma must be interpreted in the light that a third of these subjects may not have true asthma with bronchial hyper-responsiveness by definition. I have demonstrated that there is no particular inflammatory asthma "phenotype" associated with obesity as has been suggested previously.

I was unable to report on the importance of alterations in lung volumes and further work is required to explore how the relationship, particularly with FRC and closing volume affect symptoms, bronchial responsiveness and the measurement of exhaled nitric oxide. I was also unable to report on levels of serum adipokines, leptin and adiponectin and how they may relate to airway inflammation and bronchial reactivity due to problems with measuring these markers. Others have failed to show relationships between these markers and airway inflammation. Due to the nature of the subject visits it would be impractical to include more investigations however these variables could be investigated separately. Although there was a lengthy recruitment process

with an extensive advertisement for subjects I was unable to reach the recruitment target and the study has likely lacked the power to produce a significant result. As trends towards improvements in some areas were seen it would be important to repeat the study with more subjects.

I have shown that significant weight loss can be achieved in obese asthmatics and although it is unlikely to improve asthma severity in terms of airway inflammation or improvements in bronchial responsiveness, improvements in quality of life and respiratory mechanics can be gained which have a significant clinical impact in these patients.

More recently it has been shown that with larger improvements in weight loss changes in bronchial responsiveness, serum and adipose inflammatory markers plus airway inflammatory markers can occur although there is no change in airway inflammatory cells[2, 328]. There were some trends towards this which did not reach significance likely due to the smaller weight loss in this study. There are also important differences in the changes seen in obese asthmatics vs non asthmatics.

9.3 Limitations and future recommendations

Limitations have been discussed in detail in each chapter relating to individual variables measured and I will discuss limitations which are pertinent to the study as a whole here.

There are several limitations to the study which could have potentially affected the outcomes. Although I recruited relatively large numbers of subjects compared to previously published prospective studies, numbers were still relatively low and therefore likely to be underpowered to find significant

relationships between variables measured or differences between groups. There was also a significant drop out rate which was greater in the control group which also decreased the power of the study. A longer period of recruitment may have helped to increase numbers.

As this was a clinical study investigating the effect of weight loss in the real world, asthma medication was not withdrawn prior to screening the subjects and this may have excluded some subjects that may have had asthma that was well controlled however as one of the outcomes was to detect a possible improvement in bronchial responsiveness it was important to include only those with detectable bronchial responsiveness. Interestingly steroid dose showed no correlations with markers of airway inflammation or airway responsiveness.

Full lung volumes were not measured which may have been altered in obesity which could have had a significant effect on symptoms and other measured variables. As it is possible to induce a bronchoprotective effect by deep inspiratory manoeuvres it was decided that the protocol would avoid techniques requiring a deep inspiration to total lung capacity prior to measuring bronchial responsiveness. After performing methacholine challenge testing, lung volumes were likely to be altered and therefore measuring these after would not likely represent the subject's normal situation. Adding further visits to obtain lung volumes was not practical as the visits for each subject were lengthy and intensive. Subjects were unlikely to accept further investigations as evidenced by the drop out rate in the study.

Although this was a randomised trial, it was an open design and the investigators were aware which group the subject was in at the time of the 3 and 6 month visits.

The exclusion and inclusion criteria were designed to exclude subjects with major comorbidities associated with obesity, that could have affected the study. However objective testing to investigate the presence of gastroesophageal reflux or cardiac disease was not performed and subjects may have had comorbidities that I was not aware of. As this was a longitudinal study these effects should have been minimised. One significant comorbidity in particular that is commoner in the obese population is obstructive sleep apnoea. I was unable to screen for this condition and therefore I may have included subjects with this although none of the subjects were using continuous positive airway pressure overnight. This potentially could be a confounding factor as it is known that obstructive sleep apnoea may contribute to worse asthma control and also may contribute to leptin resistance[329]. Weight loss may lead to improvement in sleep apnoea meaning that the longitudinal effects may not be minimised although I would not expect any obstructive sleep apnoea to have improved with the amount of weight loss achieved. The possible contribution of obstructive sleep apnoea to quality of life, asthma control and airway inflammation should be considered in further studies on weight loss in asthma.

Ex smokers were also included in the study with a definition of having stopping smoking >2 years prior to inclusion. I did not specify a pack year history as a cut off for inclusion and all subjects had a physician diagnosis of asthma. I accept however that this may have allowed the inclusion of some

subjects that may have had chronic obstructive pulmonary disease which may have affected the inflammatory profile of the airways. Subjects however gave a good history of reversible airway obstruction and only those with significant bronchial hyperresponsiveness were included in the study.

Subjects were taking medication and this was not withdrawn, subjects were advised on asthma treatment at the screening visit and medication was not altered by the investigators for the duration of the study, however the subjects may have altered their use of medication through the study. Also asthma exacerbations were not formally recorded but subjects may have had exacerbations and treatment for them. If a subject reported an exacerbation within 3 weeks of a study visit that visit was postponed to avoid the effect of the acute inflammation.

The two groups studied were designed so that one group lost significant weight through dietician input and a control group that did not lose weight. Unfortunately there were some subjects that lost weight in the control group that were given a healthy eating leaflet. This would be difficult to avoid as it would be unethical to have a control group that was given no advice or even encouraged to increase weight. The weight loss achieved was also moderate when compared to surgical intervention trials and this may not have been sufficient to show significant changes as have been reported in recent studies[2, 328].

Lastly, the order of investigations was designed so as to have the least impact on each test from the one previous to it however inevitably there may have been some interaction. For example sputum induction had to follow bronchial responsiveness testing as sputum induction would likely cause

bronchoconstriction, however inhalation of methacholine and subsequent inhalation of salbutamol may have affected the cellular content of the sputum. An alternative would be to increase the number of visits and do these on different days, however as noted previously this was not reasonable for the subjects who had volunteered their time. Also as asthma is a variable condition it would be more difficult to attribute any relationships or lack of thereof by measuring different variables at different time points.

Further work is required to investigate the complex relationship between obesity, inflammation, quality of life, airway physiology and asthma. There is difficulty in determining the diagnosis of asthma in the obese population and work is required to investigate further the best method to measure bronchial responsiveness in obesity. In this study I avoided deep inhalations in my subjects so as to avoid its bronchoprotective effect but this effect may not be present in obesity. The influence of obesity on lung volumes and how this affects measures of asthma severity also need to be explored further. I was unable to explore the effect of change in ERV in obesity on symptoms, bronchial reactivity and exhaled nitric oxide. This may be an important factor in determining how to investigate these subjects in future trials. Further exploration into how obesity affects the symptoms of patients with and without asthma is required to explore why many patients without bronchial responsiveness are diagnosed and treated for asthma in the first place. As I failed to find significant change in weight loss between the dietician and control groups larger numbers are required in future trials to achieve greater power.

The inclusion of a non-asthmatic group would also add further detail on why the inflammatory connections behave differently in asthmatics vs non-asthmatics. Since the design of this study it has been shown that there is likely to be a clearer association between systemic and local airway inflammation in non-asthmatic obese subjects which is not seen in asthmatic subjects[2]. This may be due to the overriding local effects of exogenous airborne factors such as aeroallergens which overrides the effect of the systemic influence of adipose tissue or the effect of inhaled medication. Future studies should include a non-asthmatic group of subjects to explore these differences further to compare local factors with systemic effects of obesity. In view of the number of investigations and numbers of subjects required it was not possible to include a non-asthmatic group or non-obese subjects in this study.

9.4 Final statement

This study presents evidence that obese patients can have symptoms that mimic asthma and it is important to confirm the diagnosis with objective clinical investigations. This has important implications for the interpretation of some previous epidemiological studies and also clinically to ensure these patients do not receive unnecessary treatment. The patients in whom asthma is confirmed may benefit from a different approach aimed at improving their quality of life using a multidisciplinary approach to asthma management which includes attention to the impact of weight and encouraging weight loss rather than increasing anti-inflammatory medication.

Appendix A

Questionnaires.

SGRQ

Subject Number

St. George's Hospital Respiratory Questionnaire

This questionnaire is designed to help us learn more about how your breathing affects your life. Do not spend too long deciding about your answers. Read each question carefully and answer by ticking (✓) the response that best applies to you. Please answer **ALL** questions, as honestly as you can. This questionnaire will remain confidential.

PART 1

	most days a week	several days a week	a few days a month	only with chest infections	not at all
1) Over the last month, I have coughed:	☐	☐	☐	☐	☐
2)Over the last month, I have brought up phlegm: (sputum)	☐	☐	☐	☐	☐
3) Over the last month, I have had shortness of breath:	☐	☐	☐	☐	☐
4) Over the last month, I have had attacks of wheezing:	☐	☐	☐	☐	☐

5) During the last month, how many severe or very unpleasant attacks of chest trouble have you had?

Please tick (✓) one

- more than 3 attacks ☐
- 3 attacks ☐
- 2 attacks ☐
- 1 attack ☐
- no attacks ☐

6) How long did the last attack of chest trouble last for?
(go to question 7 if you had no severe attacks)

Please tick (✓) one

- a week or more ☐
- 3 or more days ☐
- 1 or 2 days ☐
- less than a day ☐

7) Over the last month, in an average week, how many good days (with little chest trouble) have you had?

Please tick (✓) one

- no good days ☐
- 1 or 2 good days ☐
- 3 or 4 good days ☐
- nearly every day is good ☐
- every day is good ☐

8) If you have a wheeze, is it worse in the morning?

Please tick (✓) one

- No ☐
- Yes ☐

PART 2

Section 1

How would you best describe your chest condition?

	Please tick (✓) one
The most important problem I have	☐
Causes me quite a lot of problems	☐
Causes me a few problems	☐
Causes no problem	☐

If you have ever had paid employment:

	Please tick (✓) one
My chest trouble made me stop work altogether	☐
My chest trouble interferes with my work or made me change my work	☐
My chest trouble does not affect my work	☐

Section 2

Questions about what activities make you feel breathless these days?

	Please tick (✓) in each box that applies to you these days: True	False
Sitting or lying still	☐	☐
Getting washed or dressed	☐	☐
Walking around the home	☐	☐
Walking outside on the level	☐	☐
Walking up a flight of stairs	☐	☐
Walking up hills	☐	☐
Playing sports or games	☐	☐

Section 3

Some more questions about your cough and breathlessness these days?

	Please tick (✓) in each box that applies to you these days: True	False
My cough hurts	☐	☐
My cough makes me tired	☐	☐
I am breathless when I talk	☐	☐
I am breathless when I bend over	☐	☐
My cough or breathing disturbs my sleep	☐	☐
I get exhausted easily	☐	☐

Section 4

Questions about other effects that your chest trouble may have on you these days?

	Please tick (✓) in each box that applies to you these days:

	True	False
My cough or breathing is embarrassing in public	☐	☐
My chest trouble is a nuisance to my family or friends	☐	☐
I get afraid or panic when I cannot get my breath	☐	☐
I feel that I am not in control of my chest problem	☐	☐
I do not expect to get my chest any better	☐	☐
I have become frail or invalid because of my chest	☐	☐
Exercise is not safe for me	☐	☐
Everything seems too much of an effort	☐	☐

Section 5

Questions about medication, if you are receiving no medication go straight to section 6.

Please tick (✓) in each box that applies to you these days:	True	False
My medication does not help me very much	☐	☐
I get embarrassed using my medication in public	☐	☐
I have unpleasant side effects from my medication	☐	☐
My medication interferes with my life a lot	☐	☐

Section 6

These are questions about how your activities might be affected by your breathing.

Please tick (✓) in each box that applies to you these days:	True	False
I take a long time to get washed or dressed	☐	☐
I cannot take a bath or shower, or I take a long time	☐	☐
I walk slower than other people, or I stop for rests	☐	☐
Jobs such as housework take a long time, or I have to stop for rests	☐	☐
If I walk up one flight of stairs, I have to go slowly or stop	☐	☐
If I hurry or walk fast, I have to stop or slow down	☐	☐
My breathing makes it difficult to do things such as walk up hills, carry things up stairs, light gardening such as weeding, dance, play bowls or play golf	☐	☐
My breathing makes it difficult to do things such as carry heavy loads, dig the garden or shovel snow, jog or walk at 5 miles per hour, play tennis or swim	☐	☐
My breathing makes it difficult to do things such as Very heavy manual work, run, cycle, swim fast or play competitive sport	☐	☐

Section 7

We would like to know how your chest usually affects your life.

	Please tick (✓) in each box that applies to you because of your chest trouble: True	False
I cannot play sports or games	☐	☐
I cannot go out for entertainment or recreation	☐	☐
I cannot go out of the house to do the shopping	☐	☐
I cannot do housework	☐	☐
I cannot move far from my bed or chair	☐	☐

Here is a list of other activities that your chest trouble may prevent you doing. (You don't have to tick these, they are just to remind you of ways in which your breathlessness may affect you):

Going for walks or walking the dog

Doing things at home or in the garden

Sexual intercourse

Going out to church, pub, club or place of entertainment

Going out in bad weather or smoky rooms

Visiting family or friends or playing with children

Please write any other important activities that your chest trouble may stop you doing:

.....

.....

.....

.....

Now would you tick in the box (one only) which you think best describes how your chest affects you:

	Please tick (✓) one
It does not stop me doing anything I would like to do	☐
It stops me doing one or two things I would like to do	☐
It stops me doing most of the things I would like to do	☐
It stops me doing everything I would like to do	☐

Thank you for filling in this questionnaire. Before you finish would you please check that you have answered all the questions.

SF-36

SF 36 Health Survey

Instructions:

This survey asks for your views about your health. This information will keep track of how you feel and how well you are able to do your usual activities.

Answer every question by marking the answer as indicated. If you are unsure about how to answer a question please give the best answer you can.

1. **In general, would you say your health is:** (*tick one*)
   Excellent.....☐ 1
   Very Good.....☐ 2
   Good.....☐ 3
   Fair.....☐ 4
   Poor.....☐ 5

2. **Compared to one year ago, how would you rate your health in general now?**
   (*tick one*)
   Much better than one year ago.....☐ 1
   Somewhat better than one year ago.....☐ 2
   About the same as one year ago.....☐ 3
   Somewhat worse than one year ago.....☐ 4
   Much worse than one year ago.....☐ 5

3. **The following questions are about activities you might do during a typical day. Does your health now limit you in these activities? If so, how much?**
   (*tick one number on each line*)

Activities	Yes, limited a lot	Yes, limited a little	No, not limited at all
a. **Vigorous activities**, such as running, lifting heavy objects, participating in strenuous sports	☐ 1	☐ 2	☐ 3
b. **Moderate activities**, such as moving a table pushing a vacuum cleaner, bowling or playing golf	☐ 1	☐ 2	☐ 3
c. Lifting or carrying groceries	☐ 1	☐ 2	☐ 3
d. Climbing **several** flights of stairs	☐ 1	☐ 2	☐ 3
e. Climbing **one** flight of stairs	☐ 1	☐ 2	☐ 3
f. Bending, kneeling, or stooping	☐ 1	☐ 2	☐ 3
g. Walking **more than one mile**	☐ 1	☐ 2	☐ 3
h. Walking **half a mile**	☐ 1	☐ 2	☐ 3
i. Walking **one hundred yards**	☐ 1	☐ 2	☐ 3
j. Bathing or dressing yourself	☐ 1	☐ 2	☐ 3

4. **During the <u>past 4 weeks</u>, have you had any of the following problems with your work or other regular daily activities <u>as a result of your physical health?</u>**

(tick one number on each line)

	YES	NO
a. Cut down on the **amount of time** you spend on work or other activities	☐ 1	☐ 2
b. **Accomplished less** than you would like	☐ 1	☐ 2
c. Were limited in the **kind** of work or other activities	☐ 1	☐ 2
d. Had **difficulty** performing the work or other activities (for example it took extra effort)	☐ 1	☐ 2

5. **During the <u>past 4 weeks</u>, have you had any of the following problems with your work or other regular daily activities <u>as a result of any emotional problems</u> (such as feeling depressed or anxious)?**

(tick one number on each line)

	YES	NO
a. Cut down the **amount of time** you spend on work or other activities	☐ 1	☐ 2
b. **Accomplished less** than you would like	☐ 1	☐ 2
c. Didn't do work or other activities as **carefully** as usual.	☐ 1	☐ 2

6. **During the <u>past 4 weeks</u>, to what extent has your physical health or emotional problems interfered with your normal social activities with family, friends, neighbours, or groups?**

(tick one)

- Not at all ☐ 1
- Slightly ☐ 2
- Moderately ☐ 3
- Quite a bit ☐ 4
- Extremely ☐ 5

7. **How much <u>bodily</u> pain have you had during the <u>past 4 weeks</u>?** *(tick one)*

- None ☐ 1
- Very mild ☐ 2
- Mild ☐ 3
- Moderate ☐ 4
- Severe ☐ 5
- Very severe ☐ 6

8. **During the <u>past 4 weeks</u>, how much did <u>pain</u> interfere with your normal work (including both work outside the home and housework)?** *(tick one)*

- Not at all ☐ 1
- A little bit ☐ 2
- Moderately ☐ 3
- Quite a bit ☐ 4
- Extremely ☐ 5

9. **These questions are about how you feel and how things have been with you <u>during the past 4 weeks</u>. For each question, please give one answer that comes closest to the way you have been feeling. How much of the time during the <u>past 4 weeks</u>:**

(tick one number on each line)

	All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	None of the time
a. Did you feel full of life?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
b. Have you been a very nervous person?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
c. Have you felt so down in the dumps that nothing could cheer you up?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
d. Have you felt calm and peaceful?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
e. Did you have a lot of energy?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
f. Have you felt downhearted and low?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
g. Did you feel worn out?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
h. Have you been a happy person?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
i. Did you feel tired?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6

10. **During the <u>past 4 weeks</u>, how much of the time has your <u>physical health or emotional problems</u> interfered with your social activities (like visiting with friends, relatives, etc.)?** *(tick one)*

All of the time.....☐ 1
Most of the time.....☐ 2
Some of the time.....☐ 3
A little of the time.....☐ 4
None of the time.....☐ 5

11. **How TRUE or FALSE is each of the following statements for you?**

(tick one number on each line)

	Definitely true	Mostly true	Don't know	Mostly false	Definitely false
a. I seem to get ill more easily than other people	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
b. I am as healthy as anybody I know	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
c. I expect my health to get worse	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
d. My health is excellent	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

IWQOL-Lite

Impact of weight on quality of life questionnaire-lite version (IWQOL-LITE)

Please answer the following statements by circling the number that best applies to you in the past week. Be as honest as possible. There are no right or wrong answers.

Physical function	Always true	Usually true	Sometimes true	Rarely true	Never true
1. Because of my weight I have trouble picking up objects.	1	2	3	4	5
2. Because of my weight I have trouble tying my shoelaces.	1	2	3	4	5
3. Because of my weight I have difficulty getting up from chairs.	1	2	3	4	5
4. Because of my weight I have trouble using stairs.	1	2	3	4	5
5. Because of my weight I have difficulty putting on or taking off my clothes.	1	2	3	4	5
6. Because of my weight I have trouble with mobility (getting around)	1	2	3	4	5
7. Because of my weight I have trouble crossing my legs.	1	2	3	4	5
8. I feel short of breath with only mild exertion (e.g. climbing a single flight of stairs).	1	2	3	4	5
9. I am troubled by painful or stiff joints.	1	2	3	4	5
10. My ankles and lower legs are swollen at the end of the day.	1	2	3	4	5
11. I am worried about my health.	1	2	3	4	5

Self-esteem	Always true	Usually true	Sometimes true	Rarely true	Never true
1. Because of my weight I am self-conscious.	1	2	3	4	5
2. Because of my weight my self-esteem is not what it could be.	1	2	3	4	5
3. Because of my weight I feel unsure of myself.	1	2	3	4	5
4. Because of my weight I don't like myself.	1	2	3	4	5
5 Because of my weight I am afraid of being rejected.	1	2	3	4	5
6. Because of my weight I avoid looking in mirrors or seeing myself in photographs.	1	2	3	4	5
7. Because of my weight I am embarrassed to be seen in public places.	1	2	3	4	5

Impact of weight on quality of life questionnaire-lite version (IWQOL-LITE)

Sexual Life	Always true	Usually true	Sometimes true	Rarely true	Never true
1. Because of my weight I do not enjoy sexual activity.	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
2. Because of my weight I have little or no sexual desire.	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
3. Because of my weight I have difficulty with sexual performance.	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
4. Because of my weight I avoid sexual encounters whenever possible.	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

Public Distress	Always true	Usually true	Sometimes true	Rarely true	Never true
1. Because of my weight I experience ridicule, teasing, or unwanted attention.	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
2. Because of my weight I worry about fitting into seats in public places (e.g. theatres, cinemas, restaurants, cars, or aeroplanes).	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
3. Because of my weight I worry about fitting through aisles or turnstiles.	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
4. Because of weight I worry about finding chairs that are strong enough to hold my weight.	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
5. Because of my weight I experience discrimination by others.	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

Work *(Note: for those not in paid employment, answer with respect to your daily activities.)*	Always true	Usually true	Sometimes true	Rarely true	Never true
1. Because of my weight I have trouble getting things done or carrying out my responsibilities.	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
2. Because of my weight I am less productive than I could be.	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
3. Because of my weight I don't receive appropriate pay rises, promotions or recognition at work.	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
4. Because of my weight I am afraid to go for job interviews.	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

Impact of weight on quality of life questionnaire-lite version (IWQOL-LITE)

Food Craving A craving is defined as an intense desire for a particular food that is difficult to resist. Over the past week,	Always	Often	Sometimes	Rarely	Never
1. How often did you experience a craving for any of the following foods: high fat foods (such as fried foods, sausages), sweet things (such as chocolate, ice cream, biscuits) or carbohydrates (such as pasta, potatoes, bread)?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
2. How often did you feel intense hunger no matter what or how much you ate?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
3. How often were you able to eat your favorite foods and still feel in control of your eating?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
4. How often did you experience an inability to stop eating once you started, even if you felt full?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

Sleep Over the past week,	Every day	5-6 Days	3-4 Days	1-2 Days	No days
1. How often did you wake up feeling fresh and rested?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
2. How often did you experience difficulty falling asleep?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
3. How often did you experience difficulty staying asleep (other than for going to the toilet)?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
4. How often were you satisfied with the quality of your sleep?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

Appendix B

Additional material for HRQoL chapter

Differences between dietician and control groups for SF36, SGRQ and IWQOL-Lite questionnaires.

	Baseline	3 months	6 months
Phys Functioning	3.037 (0.088)	0.101 (0.753)	0.233 (0.632)
Role Physical	6.665 (0.013)*	0.313 (0.580)	0.222 (0.640)
Bodily Pain	1.812 (0.184)	0.800 (0.378)	0.236 (0.630)
General Health	2.939 (0.093)	5.284 (0.028)*	2.077 (0.158)
Vitality	6.123 (0.017)*	0.191 (0.665)	0.391 (0.536)
Social functioning	7.393 (0.009)*	0.071 (0.791)	1.009 (0.322)
Role Emotional	0.238 (0.628)	0.077 (0.783)	0.492 (0.488)
Mental Health	2.538 (0.118)	5.842 (0.022)*	1.769 (0.192)
Physical Health summary	7.202 (0.010)*	0.465 (0.500)	0.225 (0.638)
Mental Health Summary	4.712 (0.035)*	1.213 (0.279)	1.509 (0.227)
Total	6.383 (0.015)*	0.496 (0.486)	1.148 (0.291)

Values are F statistic & (p values) when comparing between groups for each questionnaire domain. ANOVA
*p≤0.05

Table 42. Comparison of means (ANOVA) at each visit between dietician and control groups for each domain for the SF-36 HRQoL questionnaire

With Bonferroni correction (adjustment of alpha level: p<0.00625) there were no significant differences seen.

	Baseline	3 months	6 months
Symptoms	2.416 (0.127)	1.843 (0.184)	0.414 (0.524)
Activity	3.340 (0.074)	0.274 (0.604)	2.323 (0.136)
Impacts	2.178 (0.146)	2.411 (0.130)	0.711 (0.405)
Total	3.597 (0.064)	1.724 (0.198)	1.352 (0.253)

Values are F statistic & (p values) when comparing between groups for each questionnaire domain. ANOVA
*p≤0.05

Table 43. Comparison of means (ANOVA) at each visit between dietician and control groups for each domain for the SGRQ HRQoL questionnaire

There were no significant differences between groups for any scores of any domains for the SGRQ questionnaire.

	Baseline	3 months	4 months
Physical Function	5.208 (0.027)*	0.410 (0.526)	0.074 (0.788)
Self Esteem	2.278 (0.138)	0.144 (0.707)	0.122 (0.729)
Sexual Life	0.001 (0.974)	0.209 (0.651)	1.024 (0.319)
Public Distress	0.782 (0.381)	0.001 (0.974)	0.445 (0.509)
Work	4.036 (0.050)	0.119 (0.733)	0.147 (0.704)
Total	3.228 (0.079)	0.093 (0.762)	0.003 (0.954)

Values are F statistic & (p values) when comparing between groups for each questionnaire domain. ANOVA
*p≤0.05

Table 44. Comparison of means (ANOVA) at each visit between dietician and control groups for each domain for the IWQOL-Lite HRQoL questionnaire

HRQoL Scores Change from baseline

SF 36

	3 months Dietician group	3 months Control group	6 months Dietician group	6 months Control group
Phys Functioning	10.2 (12.9)	1.1 (14.3)	13.2 (14.1)	5.9 (12.8)
Role Physical	37.5 (39.3)*	-1.8 (38.6)*	28.4 (47.7)	1.6 (35.9)
Bodily Pain	2 (26.1)	-3.1 (18.7)	4 (25)	2.8 (17.7)
General Health	1.4 (15.3)	3.5 (14.1)	6.6 (16.7)	3.2 (12.1)
Vitality	10.8 (13.4)*	-1.1 (17.8)*	11.8 (16.7)	1.9 (12.5)
Social functioning	18 (23.7)*	-3.6 (23.6)*	10.3 (22.9)	1.5 (17)
Role Emotional	13.4 (36.6)	14.4 (56.5)	6 (40.7)	16.7 (51.6)
Mental Health	-3.2 (13.6)	4.9 (14.6)	0.4 (15.1)	-0.3 (14.9)
Physical Health summary	12.5 (13.3)*	-0.2 (14.1)*	12.9 (16.8)	3 (11.7)
Mental Health Summary	8 (13)	3.5 (17.5)	6.9 (14.3)	4.6 (16.4)
Total	11.3 (13.2)	1.6 (16.7)	8.2 (19.2)	4.1 (13.9)

* Significant correlation p<0.05. With Bonferroni adjustment these were no longer significant

Table 45. Change in domain score from baseline for dietician and control group at 3 and 6 months for the SF-36 HRQoL questionnaire

	3 months Dietician group	3 months Control group	6 months Dietician group	6 months Control group
Symptoms	-8.2 (11.2)*	0.7 (10.4)*	-11.3 (10.5)	-2.7 (21.2)
Activity	-8.9 (11.7)	-2.3 (9.1)	-9 (11.4)	-7.7 (12.3)
Impacts	-6.5 (11)	-4.8 (9.7)	-2.9 (11.8)	-4.1 (14.5)
Total	-7.5 (8.7)	-3 (8.2)	-6.1 (9.5)	-4.9 (12)

* Significant correlation p<0.05. With Bonferroni adjustment this was no longer significant.

Table 46. Change in domain score from baseline for dietician and control group at 3 and 6 months for the SGRQ HRQoL questionnaire

	3 months Dietician group	3 months Control group	6 months Dietician group	6 months Control group
Physical Function	7.8 (8.5)	1.3 (15.8)	8.2 (14.9)	0.3 (14)
Self Esteem	6.8 (19.9)	0.5 (16.6)	8.6 (17.5)	3.6 (21.7)
Sexual Life	5.6 (14.3)	9.8 (18.3)	8.2 (17.2)	2.5 (15.3)
Public Distress	3.6 (16.5)	1.8 (17.5)	7.3 (14.5)	5 (12)
Work	7.2 (11.7)	4 (14.4)	7.7 (16.4)	3.3 (18.9)
Total	7.3 (8.2)	3.4 (14.6)	8.7 (11.2)	2.3 (11.9)

* Significant correlation p<0.05. With Bonferroni adjustment this was no longer significant.

Table 47. Change in domain score from baseline for dietician and control group at 3 and 6 months for the IWQOL-Lite HRQoL questionnaire

Correlations between BMI and HRQoL

	Baseline All subjects	Baseline Dietician group	Baseline Control group	3 months All subjects	3 months Dietician group	3 months Control group	6 months All subjects	6 months Dietician group	6 months Control group
Phys Functioning	R= -0.215 P= 0.129	R= -0.236 P= 0.246	R= -0.159 P= 0.447	R= -0.244 P= 0.165	R= -0.102 P= 0.669	R= -0.410 P= 0.146	R= -0.140 P= 0.400	R= 0.124 P= 0.581	R= -0.535* P= 0.033
Role Physical	R= -0.015 P= 0.917	R= 0.021 P=0.917	R= 0.015 P= 0.941	R= -0.317 P= 0.068	R= -0.090 P= 0.706	R= -0.553* P= 0.040	R= -0.080 P= 0.635	R=0.233 P= 0.296	R= -0.693* P= 0.003
Bodily Pain	R= -0.232 P= 0.101	R= -0.248 P= 0.222	R= -0.187 P= 0.371	R= -0.192 P= 0.276	R= 0.049 P= 0.838	R= -0.545* P= 0.044	R= -0.251 P= 0.128	R= -0.016 P= 0.944	R= -0.609* P= 0.012
General Health	R= -0.285* P= 0.043	R= -0.168 P= 0.411	R= -0.387 P= 0.056	R= -0.117 P= 0.510	R= 0.044 P= 0.854	R= -0.599* P= 0.024	R= -0.093 P= 0.578	R= -0.002 P= 0.992	R= -0.299 P= 0.260
Vitality	R= -0.079 P= 0.581	R= -0.090 P=0.662	R= -0.003 P= 0.988	R= -0.271 P= 0.121	R= -0.226 P= 0.339	R= -0.348 P= 0.223	R= -0.233 P= 0.160	R= -0.138 P= 0.540	R= -0.382 P= 0.144
Social functioning	R= -0.116 P=0.417	R= -0.194 P= 0.343	R= 0.054 P= 0.798	R= -0.335 P= 0.053	R= -0.083 P= 0.728	R= -0.695* P= 0.006	R= -0.125 P= 0.454	R= 0.077 P= 0.735	R= -0.554* P= 0.026
Role Emotional	R= 0.004 P= 0.979	R= 0.090 P= 0.661	R= -0.075 P= 0.721	R= -0.338 P= 0.051	R= -0.090 P=0.706	R= -0.677* P= 0.008	R= 0.009 P= 0.958	R= 0.291 P= 0.189	R= -0.527* P= 0.036
Mental Health	R= -0.175 P= 0.221	R= -0.249 P= 0.219	R= -0.047 P= 0.823	R= -0.145 P= 0.415	R= -0.142 P= 0.550	R= -0.215 P= 0.460	R= -0.249 P= 0.131	R= -0.127 P= 0.575	R= -0.426 P= 0.100
Physical Health summary	R= -0.184 P= 0.196	R= -0.166 P= 0.417	R= -0.157 P= 0.454	R= -0.319 P= 0.066	R= -0.092 P=0.701	R= -0.601* P= 0.023	R= -0.195 P= 0.240	R= 0.087 P= 0.702	R= -0.629* P= 0.009
Mental Health Summary	R= -0.145 P= 0.308	R= -0.117 P= 0.570	R= -0.133 P= 0.526	R= -0.319* P= 0.048	R= -0.128 P= 0.590	R= -0.682* P= 0.007	R= -0.144 P= 0.389	R= 0.089 P= 0.693	R= -0.519* P= 0.039
Total	R= -0.158 P= 0.267	R= -0.150 P= 0.466	R= -0.119 P= 0.570	R= -0.358* P= 0.038	R= -0.113 P= 0.637	R= -0.666* P= 0.009	R= -0.205 P= 0.217	R= 0.063 P= 0.782	R= -0.613* P= 0.012

* Significant correlation p<0.05

Table 48. Correlations r(p) between BMI and all domains for all subjects, dietician and control groups at each visit for the SF-36 HRQoL questionnaire

	Baseline All subjects	Baseline Dietician group	Baseline Control group	3 months All subjects	3 months Dietician group	3 months Control group	6 months All subjects	6 months Dietician group	6 months Control group
Symptoms	R= -0.066 P=0.645	R= -0.200 P= 0.326	R= 0.014 P= 0.947	R= 0.105 P= 0.548	R= -0.269 P= 0.238	R= 0.545* P= 0.044	R= 0.210 P= 0.206	R=-0.073 P= 0.0748	R= -0.640* P= 0.008
Activity	R= 0.111 P= 0.436	R= 0.204 P= 0.317	R= -0.009 P= 0.966	R= 0.263 P= 0.127	R= 0.176 P= 0.444	R= 0.351 P= 0.219	R= 0.290 P= 0.077	R= 0.160 P= 0.476	R= 0.476 P= 0.062
Impacts	R= -0.111 P= 0.437	R= -0.182 P= 0.373	R= -0.069 P= 0.743	R= 0.096 P= 0.583	R= 0.029 P= 0.900	R= 0.232 P= 0.426	R= 0.178 P= 0.284	R= 0.085 P= 0.707	R= 0.331 P= 0.211
Total	R= -0.031 P= 0.827	R= -0.079 P= 0.703	R= -0.035 P= 0.869	R= 0.185 P= 0.287	R= 0.029 P= 0.902	R= 0.386 P= 0.173	R= 0.248 P= 0.133	R= 0.095 P= 0.676	R= 0.496 P= 0.051

* Significant correlation p<0.05

Table 49. Correlations r(p) between BMI and all domains for all subjects, dietician and control groups at each visit for the SGRQ HRQoL questionnaire

	Visit 2 All subjects	Visit 2 Dietician group	Visit 2 Control group	Visit 3 All subjects	Visit 3 Dietician group	Visit 3 Control group	Visit 4 All subjects	Visit 4 Dietician group	Visit 4 Control group
Physical Function	R= -0.437* P= 0.001	R= -0.419* P= 0.033	R= -0.448* P= 0.025	R= -0.538* P= 0.001	R= -0.426 P= 0.061	R= -0.680* P= 0.011	R= -0.482* P= 0.002	R= -0.413 P= 0.056	R= -0.646* P= 0.009
Self Esteem	R= -0.282* P= 0.045	R= -0.154 P= 0.453	R= -0.397* P= 0.049	R= -0.381* P= 0.029	R= -0.336 P= 0.147	R= -0.474 P= 0.101	R= -0.359* P= 0.029	R= -0.304 P= 0.170	R= -0.475 P= 0.074
Sexual Life	R= -0.175 P= 0.220	R= -0.092 P= 0.653	R= -0.255 P= 0.218	R= -0.235 P= 0.188	R= -0.209 P= 0.378	R= -0.265 P= 0.382	R= -0.246 P= 0.142	R= -0.165 P= 0.464	R= -0.457 P= 0.086
Public Distress	R= -0.715* P= 0.000	R= -0.603 P= 0.01	R= -0.826* P= 0.000	R= -0.704* P= 0.000	R= -0.657* P= 0.002	R= -0.752* P= 0.003	R= -0.581* P= 0.000	R= -0.606* P= 0.003	R= -0.531* P= 0.042
Work	R= -0.220 P= 0.121	R= 0.071 P= 0.732	R= -0.502* P= 0.011	R= -0.402* P= 0.020	R= -0.151 P= 0.524	R= -0.631* P= 0.021	R= -0.217 P= 0.196	R= 0.026 P= 0.910	R= -0.592* P= 0.020
Total	R= -0.460* P= 0.001	R= -0.328 P= 0.102	R= -0.586* P= 0.002	R= -0.581* P= 0.000	R= -0.479* P= 0.033	R= -0.705* P= 0.007	R= -0.471* P= 0.003	R= -0.409 P= 0.059	R= -0.633* P= 0.011

* Significant correlation p<0.05

Table 50. Correlations r(p) between BMI and all domains for all subjects, dietician and control groups at each visit for the SGRQ HRQoL questionnaire

Correlations between change in weight and change in domain scores between visits.

	Baseline to 3 months All subjects	Baseline to 3 months Dietician group	Baseline to 3 months Control group	3 to 6 months All subjects	3 to 6 months Dietician group	3 to 6 months Control group	Baseline to 6 months All subjects	Baseline to 6 months Dietician group	Baseline to 6 months Control group
Phys Functioning	R= -0.310 P= 0.074	R= -0.116 P= 0.625	R= -0.384 P= 0.175	R= -0.148 P= 0.435	R= -0.167 P= 0.508	R= -0.242 P= 0.449	R= -0.241 P= 0.145	R= -0.121 P= 0.593	R= -0.294 P= 0.268
Role Physical	R= -0.217 P= 0.217	R= -0.096 P= 0.687	R= -0.193 P= 0.508	R= -0.080 P= 0.673	R= 0.236 P= 0.345	R= -0.352 P= 0.262	R= 0.047 P= 0.780	R= 0.144 P= 0.524	R= 0.092 P= 0.735
Bodily Pain	R= -0.155 P= 0.381	R= 0.002 P= 0.993	R= -0.325 P= 0.257	R= -0.231 P= 0.219	R= -0.320 P= 0.196	R= -0.249 P= 0.434	R= -0.102 P= 0.542	R= -0.118 P= 0.600	R= -0.090 P= 0.741
General Health	R= -0.224 P= 0.203	R= 0.137 P= 0.564	R= -0.607* P= 0.021	R= 0.081 P= 0.671	R= 0.380 P= 0.119	R= -0.144 P= 0.656	R= -0.020 P= 0.904	R= 0.243 P= 0.276	R= -0.304 P= 0.253
Vitality	R= -0.034 P= 0.850	R= 0.353 P= 0.127	R= -0.145 P= 0.622	R= -0.211 P= 0.264	R= -0.108 P= 0.670	R= -0.347 P= 0.270	R= -0.010 P=0.953	R= 0.281 P= 0.205	R= -0.213 P= 0.428
Social functioning	R= -0.164 P= 0.354	R= -0.262 P= 0.265	R= 0.047 P= 0.872	R= -0.027 P= 0.886	R= 0.069 P= 0.786	R= -0.162 P= 0.615	R= -0.032 P= 0.850	R= 0.068 P= 0.764	R= -0.060 P= 0.826
Role Emotional	R= -0.070 P= 0.694	R= -0.112 P= 0.639	R= -0.054 P= 0.854	R= 0.218 P= 0.248	R= 0.0270 P= 0.278	R= 0.292 P= 0.357	R= 0.206 P= 0.215	R= 0.330 P= 0.134	R= 0.085 P= 0.753
Mental Health	R= 0.016 P= 0.929	R= 0.108 P= 0.650	R= -0.162 P= 0.579	R= -0.133 P= 0.484	R= -0.040 P= 0.876	R= -0.262 P= 0.411	R= -0.065 P= 0.700	R= 0.022 P= 0.924	R= -0.143 P= 0.598
Physical Health summary	R= -0.282 P= 0.107	R= 0.025 P= 0.918	R= -0.420 P= 0.135	R= -0.178 P= 0.346	R= 0.081 P= 0.748	R= -0.524 P= 0.080	R= -0.050 P= 0.764	R= 0.131 P= 0.560	R= -0.141 P= 0.602
Mental Health Summary	R= -0.151 P= 0.393	R= -0.044 P= 0.852	R= -0.176 P= 0.548	R= 0.076 P= 0.689	R= 0.218 P= 0.385	R= -0.118 P= 0.714	R= 0.095 P= 0.569	R= 0.331 P= 0.133	R= -0.061 P= 0.824
Total	R= -0.233 P= 0.184	R= -0.066 P= 0.781	R= -0.261 P= 0.368	R= -0.044 P= 0.816	R= 0.073 P= 0.774	R= -0.356 P= 0.256	R= 0.062 P= 0.713	R= 0.220 P= 0.326	R= -0.067 P= 0.806

* Significant correlation p<0.05

Table 51. Correlation r(p) of change in weight (Kg) and change in domain scores for all subjects, dietician and control groups between baseline to

3 months, 3 to 6 months and baseline to 6 months for the SF-36 HRQoL questionnaire

	Visit 3-2 All subjects	Visit 3-2 Dietician group	Visit 3-2 Control group	Visit 4-3 All subjects	Visit 4-3 Dietician group	Visit 4-3 Control group	Visit 4-2 All subjects	Visit 4-2 Dietician group	Visit 4-2 Control group
Symptoms	R= -0.090 P= 0.606	R= -0.300 P= 0.187	R= -0.104 P= 0.723	R= 0.022 P= 0.905	R= -0.096 P= 0.696	R= 0.079 P= 0.806	R= 0.008 P= 0.962	R= -0.094 P= 0.679	R= -0.026 P= 0.925
Activity	R= 0.058 P= 0.740	R= 0.001 P= 0.995	R= -0.005 P= 0.985	R= -0.052 P= 0.780	R= -0.036 P= 0.885	R= -0.010 P= 0.976	R= 0.114 P= 0.494	R= 0.049 P= 0.829	R= 0.172 P= 0.523
Impacts	R= 0.151 P= 0.386	R= -0.151 P= 0.513	R= 0.431 P= 0.124	R= -0.106 P= 0.569	R= 0.152 P= 0.534	R= -0.250 P= 0.433	R= 0.123 P= 0.460	R= -0.057 P= 0.800	R= 0.281 P= 0.291
Total	R= 0.108 P= 0.537	R= -0.162 P= 0.482	R= 0.264 P= 0.362	R= -0.096 P= 0.609	R= 0.085 P= 0.730	R= -0.164 P= 0.611	R= 0.125 P= 0.456	R= -0.035 P= 0.877	R= 0.227 P= 0.398

* Significant correlation p<0.05

Table 52. Correlation r(p) of change in weight (Kg) and change in domain scores for all subjects, dietician and control groups between baseline to 3 months, 3 to 6 months and baseline to 6 months for the SGRQ HRQoL questionnaire

	Visit 3-2 All subjects	Visit 3-2 Dietician group	Visit 3-2 Control group	Visit 4-3 All subjects	Visit 4-3 Dietician group	Visit 4-3 Control group	Visit 4-2 All subjects	Visit 4-2 Dietician group	Visit 4-2 Control group
Physical Function	R= -0.399* P= 0.022	R= -0.213 P= 0.367	R= -0.470 P= 0.105	R= 0.007 P= 0.972	R= -0.108 P= 0.671	R= 0.111 P= 0.746	R= -0.313 P= 0.060	R= -0.344 P= 0.117	R= -0.240 P= 0.390
Self Esteem	R= -0.511* P= 0.002	R= -0.383 P= 0.096	R= -0.704* P= 0.007	R= -0.157 P= 0.416	R= 0.241 P= 0.335	R= -0.451 P= 0.164	R= -0.296 P= 0.075	R= -0.101 P= 0.655	R= -0.435 P= 0.105
Sexual Life	R= -0.169 P=0.347	R= -0.443 P= 0.051	R= -0.014 P= 0.965	R= 0.244 P= 0.193	R= 0.111 P= 0.662	R= 0.342 P= 0.276	R= -0.286 P= 0.086	R= -0.297 P= 0.179	R= -0.251 P= 0.366
Public Distress	R= -0.237 P= 0.183	R= -0.090 P= 0.707	R= -0.346 P= 0.247	R= 0.183 P= 0.343	R= 0.281 P= 0.258	R= 0.094 P= 0.784	R= -0.004 P= 0.983	R= 0.104 P= 0.646	R= -0.108 P= 0.701
Work	R= -0.332 P= 0.059	R= -0.030 P= 0.899	R= -0.504 P= 0.079	R= 0.125 P= 0.517	R= 0.218 P=0.385	R= 0.021 P= 0.950	R= 0.015 P= 0.928	R= 0.146 P= 0.516	R= -0.065 P= 0.819
Total	R= -0.551* P= 0.001	R= -0.421 P= 0.065	R= -0.621* P= 0.024	R= 0.006 P= 0.975	R= 0.152 P= 0.548	R= -0.155 P= 0.650	R= -0.290 P= 0.082	R= -0.170 P= 0.451	R= -0.359 P= 0.189

* Significant correlation p<0.05

Table 53. Correlation r(p) of change in weight (Kg) and change in domain scores for all subjects, dietician and control groups between baseline to 3 months, 3 to 6 months and baseline to 6 months for the IWQOL-Lite HRQoL questionnaire

Comparing groups that achieved ≥5% weight loss at 6 months with those that did not

	Baseline ≥5%weight loss	Baseline <5% weight loss	3 months ≥5% weight loss	3 months <5% weight loss	6 months ≥5% weight loss	6 months <5% weight loss
Phys Functioning	61.8 (21.9)	61.6 (21.6)	70 (21.4)	65 (19.5)	73.4 (20.1)	70.7 (21.2)
Role Physical	62.5 (39.8)	52.3 (44.3)	91.7 (20.4)*	61.7 (45.2)*	75 (39.8)	72.7 (36.1)
Bodily Pain	74.7 (26.3)	60.6 (23.2)	72.5 (23.7)	64.7 (20.2)	80 (20.7)*	62.7 (25.3)*
General Health	49.1 (19.8)	51.7 (19.5)	55.2 (21.4)	51.1 (209)	54 (22.4)	57.1 (21)
Vitality	51.3 (21.9)	40.9 (21)	58 (23.4)	47 (19.3)	60.3 (18.8)	47.5 (19.8)
Social functioning	74.4 (23.5)	67.8 (23.6)	85.9 (23.1)	70.1 (24.9)	81.4 (27.6)	74 (25.2)
Role Emotional	66.7 (43.9)	60.6 (39.4)	91.1 (26.6)*	62.3 (39.6)*	74.9 (39.5)	72.7 (43.2)
Mental Health	67.8 (19.10	66.9 (15.3)	70.1 (20.5)	63.7 (21)	70.5 (21.2)	65.1 (20.1)
Physical Health summary	59.8 (20)	53.5 (20.7)	69.3 (16.7)	58 (17.9)	68.4 (18.8)	62.2 (19.4)
Mental Health Summary	61.8 (18.8)	57.7 (20.2)	72.1 (17.3)	58.7 (18.6)	68.2 (21.6)	63.3 (20.6)
Total	63.5 (19.5)	57.8 (20.9)	74.3 (17.2)*	60.7 (17.8)*	68.4 (22.8)	65.4 (19.5)

* significant difference p<0.05

Table 54. Mean (SD) scores for all domains for those that lost clinically significant weight and those that did not for each visit for the SF-36 HRQoL questionnaire

	Baseline ≥5%weight loss	Baseline <5% weight loss	3 months ≥5% weight loss	3 months <5% weight loss	6 months ≥5% weight loss	6 months <5% weight loss
Symptoms	56.2 (21.7)	66 (18)	51.8 (19.4)	59.2 (18.6)	50.6 (18.7)	56.9 (19.6)
Activity	48.5 (24.2)	51.6 (19)	39.8 (23.5)	48.6 (17.1)	39.2 (23.4)	43.7 (20.2)
Impacts	26 (15.3)	33 (16.5)	20 (12.6)	28.5 (16)	21.9 (14.6)	30 (20.9)
Total	37.8 (15.8)	44.1 (15.4)	31.3 (14.1)	39.8 (15)	31.9 (15.2)	38.7 (18.7)

* significant difference p<0.05

Table 55. Mean (SD) scores for all domains for those that lost clinically significant weight and those that did not for each visit for the SGRQ HRQoL questionnaire

	Visit 2 ≥5%weight loss	Visit 2 <5% weight loss	Visit 3 ≥5% weight loss	Visit 3 <5% weight loss	Visit 4 ≥5% weight loss	Visit 4 <5% weight loss
Physical Function	65.5 (24)	65 (19.1)	71.6 (21.6)	65.3 (17)	74.3 (19.2)	65.6 (20.6)
Self Esteem	50.4 (28.2)	54 (33)	62 (25)	48.2 (25.1)	61.4 (26.9)	55.1 (28.5)
Sexual Life	72.3 (26.8)	61.6 (32)	81.3 (25.9)	62.9 (26.6)	82.8 (25.4)	64 (29.8)
Public Distress	73.8 (27.5)	76.1 (24.1)	81.7 (21.9)	70 (26.1)	81.3 (24.4)	80.5 (24)
Work	85.2 (17.4)	74.4 (23.8)	90.8 (12.7)*	73.8 (17.4)*	90.6 (15.8)	79.5 (21.8)
Total	66.8 (20.3)	64 (22.5)	74.8 (17.2)	63 (17)	75.7 (17.3)	66.6 (22.4)

* significant difference p<0.05

Table 56. Mean (SD) scores for all domains for those that lost clinically significant weight and those that did not for each visit for the IWQOL-Lite HRQoL questionnaire

	Baseline to 3 months ≥5%weight loss	Baseline to 3 months <5% weight loss	Baseline to 6 months ≥5%weight loss	Baseline to 6 months <5% weight loss
Phys Functioning	8.3 (10.3)	5.7 (17.9)	11.6 (13.5)	9.1 (14.4)
Role Physical	26.7 (32)	16.7 (45.9)	12.5 (50.8)	20.5 (40.6_
Bodily Pain	-3.1 (18.5)	2.2 (21.2)	5.3 (25.6)	2.1 (19.5)
General Health	5.7 (12)	0.8 (16.9)	4.9 (12.8)	5.4 (16.5)
Vitality	5.3 (10.6)	7.3 (14.6)	9.1 (14.7)	6.6 (16.6)
Social functioning	11.6 (18.5)	6.5 (22.4)	7.1 (20.3)	6.2 (21.6)
Role Emotional	26.7 (38.3)	11.2 (46.6)	8.3 (46.4)	12.1 (45.5)
Mental Health	0.3 (9.7)	-0.3 (16.4)	2.8 (15.6)	-1.8 (14.4)
Physical Health summary	8.6 (7.2)	6.7 (16.5)	8.6 (16.6)	8.8 (15)
Mental Health Summary	10 (9)	4.9 (15.3)	6.4 (14.5)	5.6 (15.8)
Total	10.2 (6.7)	6.3 (16.5)	4.9 (19.2)	7.5 (15.7)

* significant difference p<0.05

Table 57. Change in domain score mean (sd) from baseline to 3 and 6 months for the SF-36 HRQoL questionnaire comparison of dietician and control groups.

	Baseline to 3 months ≥5%weight loss	Baseline to 3 months <5% weight loss	Baseline to 6 months ≥5%weight loss	Baseline to 6 months <5% weight loss
Symptoms	-4.5 (10)	-6.5 (12.4)	-5.6 (12.1)	-9.2 (18.9)
Activity	-9.1 (7.6)	-5.9 (13.1)	-9.2 (12.7)	-7.9 (13.1)
Impacts	-6.4 (9.3)	-6.1 (11.2)	-4 (11.4)	-2.9 (14)
Total	-6.9 (6.2)	-6 (9.9)	-5.9 (10.3)	-5.4 (10.8)

* significant correlation p<0.05

Table 58. Change in domain score mean (sd) from baseline to 3 and 6 months for the SGRQ HRQoL questionnaire comparison of dietician and control groups

	Baseline to 3 months ≥5%weight loss	Baseline to 3 months <5% weight loss	Baseline to 6 months ≥5%weight loss	Baseline to 6 months <5% weight loss
Physical Function	5.6 (6.8)	5.1 (15.9)	8.8 (15.4)	2.1 (14.1)
Self Esteem	8.6 (12.9)	-0.9 (21.9)	10.9 (17.6)	3.2 (20.1)
Sexual Life	6.3 (10.6)	9.8 (19.2)	10.5 (15.6)	2.4 (16.6)
Public Distress	4.3 (16.4)	0.6 (16.5)	7.5 (15.6)	5.5 (11.7)
Work	4.2 (10.2)	7 (15.1)	5.5 (14.9)	6.3 (19.3)
Total	5.9 (6.5)	5.3 (14)	8.9 (11.4)	3.9 (11.9)

* significant correlation $p<0.05$

Table 59. Change in domain score mean (sd) from baseline to 3 and 6 months for the IWQOL-Lite HRQoL questionnaire comparison of dietician and control groups

Exhaled Nitric oxide vs HRQoL scores.

Questionnaire	Baseline			3 months			6 months		
SF36	FeNO 50ml	Alveolar Nitric oxide	Bronchial Nitric oxide	FeNO 50ml	Alveolar Nitric oxide	Bronchial Nitric oxide	FeNO 50ml	Alveolar Nitric oxide	Bronchial Nitric oxide
Phys Functioning	R=-0.252 P= 0.084	R= -134 P= 0.415	R= -0.225 P= 0.169	R= -0.269 P= 0.143	R= 0.038 P= 0.846	R= -0.417* P= 0.027	R= -0.023 P= 0.894	R= 0.127 P= 0.503	R= -0.041 P= 0.828
Role Physical	R= 0.075 P= 0.611	R= -0.037 P= 0.825	R= 0.069 P= 0.675	R= -0.161 P= 0.388	R= -0.119 P= 0.546	R= -0.182 P= 0.355	R= 0.014 P= 0.936	R= 0.055 P= 0.774	R= 0.070 P= 0.714
Bodily Pain	R= -0.089 P= 0.548	R= -0.201 P= 0.221	R= -0.088 P= 0.594	R= - 0.196 P= 0.291	R= -0.337 P= 0.080	R= -0.249 P= 0.201	R= -0.251 P= 0.141	R= 0.128 P= 0.500	R= -0.276 P= 0.139
General Health	R= -0.127 P=0.391	R= 0.115 P= 0.484	R= -0.098 P= 0.551	R= -0.065 P= 0.728	R= -0.160 P= 0.416	R= -0.186 P= 0.344	R= 0.095 P= 0.583	R= -0.049 P= 0.795	R= 0.138 P= 0.466
Vitality	R= -0.144 P= 0.328	R= -0.114 P= 0.489	R= -0.054 P= 0.743	R= -0.267 P= 0.147	R= 0.074 P= 0.709	R= -0.348 P= 0.070	R= 0.047 P= 0.786	R= 0.213 P= 0.258	R= 0.036 P= 0.850
Social functioning	R= -0.286* P= 0.049	R= -0.129 P= 0.433	R= -0.234 P= 0.151	R= -0.212 P= 0.253	R= 0.141 P= 0.475	R= -0.300 P= 0.122	R= 0.028 P= 0.872	R= 0.174 P= 0.357	R= -0.029 P= 0.877
Role Emotional	R= -0.039 P= 0.791	R= 0.079 P= 0.634	R= -0.054 P= 0.743	R= -0.043 P= 0.817	R= -0.091 P= 0.645	R= -0.050 P= 0.802	R= 0.020 P= 0.906	R= 0.229 P= 0.223	R= -0.065 P= 0.734
Mental Health	R= -0.176 P=0.232	R= -0.010 P= 0.952	R= -0.184 P= 0.262	R= -0.059 P= 0.753	R= 0.124 P= 0.528	R= -0.202 P= 0.302	R= 0.169 P= 0.324	R= 0.206 P= 0.274	R= 0.176 P= 0.351
Physical Health summary	R= -0.100 P=0.498	R= -0.093 P= 0.575	R= -0.074 P= 0.654	R= -0.247 P= 0.181	R= -0.149 P= 0.450	R= -0.355 P= 0.064	R= -0.033 P= 0.851	R= 0.118 P= 0.536	R= -0.016 P= 0.932
Mental Health Summary	R= -0.181 P= 0.218	R= -0.003 P= 0.986	R= -0.149 P= 0.365	R= -0.161 P= 0.388	R= 0.009 P= 0.964	R= -0.268 P= 0.168	R= 0.077 P= 0.655	R= 0.206 P= 0.275	R= 0.039 P= 0.839
Total	R= -0.137 P= 0.353	R= -0.061 P= 0.713	R= -0.114 P= 0.488	R= -0.211 P= 0.255	R= -0.073 P= 0.711	R= -0.314 P= 0.104	R= 0.049 P= 0.775	R= 0.199 P= 0.293	R= 0.039 P= 0.838
SGRQ									
Symptoms	R= 0.192 P= 0.190	R= 0.138 P= 0.400	R= 0.153 P= 0.351	R= 0.146 P= 0.425	R= 0.154 P= 0.426	R= 0.179 P= 0.353	R= -0.036 P= 0.837	R= -0.181 P= 0.339	R= -0.175 P= 0.356
Activity	R= 0.124 P= 0.403	R= 0.039 P= 0.813	R= 0.084 P= 0.611	R= 0.213 P= 0.241	R= -0.014 P= 0.942	R= 0.309 P= 0.103	R= -0.009 P= 0.961	R= -0.145 P= 0.443	R= -0.035 P= 0.856
Impacts	R= 0.395* P= 0.005	R= 0.217 P= 0.185	R= 0.419* P= 0.008	R= 0.417* P= 0.018	R= 0.236 P= 0.218	R= 0.505* P= 0.005	R= 0.036 P= 0.834	R= -0.180 P= 0.342	R= -0.041 P= 0.828
Total	R= 0.308* P= 0.033	R= 0.164 P= 0.317	R= 0.295 P= 0.068	R= 0.346 P= 0.053	R= 0.157 P= 0.415	R= 0.446* P= 0.015	R= 0.011 P= 0.951	R= -0.192 P= 0.308	R= -0.066 P= 0.730
IWQOL-Lite									
Physical Function	R= -0.165 P= 0.263	R= -0.304 P= 0.059	R= -.247 P= 0.129	R= -0.089 P= 0.635	R= -0.280 P= 0.149	R= -0.159 P= 0.419	R= 0.119 P= 0.495	R=0.164 P= 0.394	R= 0.180 P= 0.350
Self Esteem	R= 0.058 P= 0.696	R= 0.015 P= 0.927	R= 0.063 P= 0.704	R= -0.098 P= 0.598	R= 0.177 P= 0.368	R= -0.321 P= 0.096	R= 0.198 P= 0.254	R= 0.141 P= 0.467	R= 0.342 P= 0.070
Sexual Life	R= -0.202 P= 0.168	R= -0.170 P= 0.300	R= -0.284 P= 0.080	R= -0.297 P= 0.105	R= -0.032 P= 0.870	R= -0.429* P= 0.023	R= 0.008 P= 0.962	R= 0.144 P= 0.456	R= 0.078 P= 0.688
Public Distress	R= -0.129 P= 0.382	R= -0.108 P= 0.515	R= -0.244 P= 0.134	R= 0.174 P= 0.349	R= 0.061 P= 0.757	R= 0.094 P= 0.634	R= 0.110 P= 0.530	R= 0.041 P= 0.834	R= 0.167 P= 0.385
Work	R=-0.039 P= 0.790	R= -0.131 P= 0.427	R= -0.126 P= 0.444	R= -0.092 P= 0.621	R= -0.118 P= 0.549	R= -0.157 P= 0.424	R= -0.138 P= 0.429	R= 0.069 P= 0.724	R= =0.097 P= 0.615
Total	R= -0.157 P= 0.286	R= -0.190 P= 0.247	R= -0.240 P= 0.140	R= -0.096 P= 0.608	R= -0.062 P= 0.754	R= -0.246 P= 0.207	R= 0.122 P= 0.485	R= 0.142 P= 0.462	R= 0.215 P= 0.262

* significant correlation $p<0.05$

Table 60. Correlation r(p) between measures of exhaled nitric oxide and domain scores at each visit for the SF-36, SGRQ and IWQOL-Lite HRQoL questionnaires

	FeNO 50ml Baseline to 6 months	Alveolar NO Baseline to 6 months	Bronchial flux NO Baseline to 6 months
SF36			
Phys Functioning	R= -0.172 P= 0.331	R= -0.287 P= 0.184	R= 0.023 P= 0.919
Role Physical	R= -0.125 P= 0.481	R= 0.098 P= 0.655	R= -0.139 P= 0.528
Bodily Pain	R= -0.101 P= 0.570	R= 0.120 P= 0.586	R= -0.157 P= 0.474
General Health	R= -0.016 P= 0.927	R= 0.185 P= 0.399	R= 0.212 P= 0.332
Vitality	R= 0.122 P= 0.493	R= 0.239 P= 0.272	R= 0.128 P= 0.559
Social functioning	R= -0.082 P= 0.644	R= -0.045 P= 0.837	R= -0.175 P= 0.425
Role Emotional	R= -0.068 P= 0.701	R= -0.026 P= 0.907	R= 0.079 P= 0.720
Mental Health	R= 0.132 P= 0.456	R= 0.018 P= 0.935	R= 0.211 P= 0.333
Physical Health summary	R= -0.110 P= 0.534	R= 0.110 P= 0.617	R= -0.052 P= 0.814
Mental Health Summary	R= -0.014 P= 0.938	R= 0.047 P= 0.830	R= 0.106 P= 0.629
Total	R= -0.088 P= 0.620	R= 0.056 P= 0.801	R= -0.006 P= 0.978
SGRQ			
Symptoms	R= 0.161 P= 0.363	R= 0.181 P= 0.408	R= 0.184 P= 0.401
Activity	R= -0.176 P= 0.319	R= -0.011 P= 0.962	R= -0.297 P= 0.169
Impacts	R= -0.103 P= 0.561	R= 0.028 P= 0.899	R= -0.249 P= 0.252
Total	R= -0.085 P= 0.633	R= 0.055 P= 0.803	R= -0.227 P= 0.297
IWQOL-Lite			
Physical Function	R= 0.135 P= 0.453	R= -0.237 P= 0.289	R= 0.179 P= 0.426
Self Esteem	R= 0.410* P= 0.018	R= 0.061 P= 0.787	R= 0.503* P= 0.017
Sexual Life	R= 0.113 P= 0.532	R= -0.345 P= 0.116	R= 0.060 P= 0.790
Public Distress	R= 0.089 P= 0.624	R= -0.025 P= 0.912	R= 0.133 P= 0.556
Work	R= 0.154 P= 0.392	R= -0.173 P= 0.441	R= 0.157 P= 0.486
Total	R= 0.193 P= 0.283	R= -0.233 P= 0.296	R= 0.210 P= 0.349

* significant correlation $p<0.05$

Table 61. Correlations between change in domain score and changes in measures of exhaled nitric oxide between baseline and 6 months for the SF-36, SGRQ and IWQOL-Lite HRQoL questionnaires

Bronchial responsiveness and specific airway conductance

	Baseline		3 months		6 months	
SF36	Log_{10} PC45	sGaw	Log_{10} PC45	sGaw	Log_{10} PC45	sGaw
Phys Functioning	R= 0.006 P= 0.969	R= -0.110 P= 0.451	R= -0.058 P= 0.753	R= -0.137 P= 0.456	R= -0.252 P= 0.150	R= -0.237 P= 0.164
Role Physical	R= 0.065 P= 0.659	R= -0.073 P= 0.620	R= -0.160 P= 0.382	R= -0.197 P= 0.280	R= 0.071 P= 0.688	R= -0.073 P= 0.671
Bodily Pain	R= -0.082 P= 0.579	R= -0.075 P= 0.606	R= -0.216 P= 0.235	R= -0.281 P= 0.119	R= 0.022 P= 0.902	R= -0.154 P= 0.369
General Health	R= 0.032 P= 0.827	R= -0.002 P= 0.988	R= -0.014 P= 0.940	R= 0.235 P= 0.195	R= 0.021 P= 0.907	R= 0.149 P= 0.385
Vitality	R= 0.083 P= 0.577	R= 0.117 P= 0.424	R= -0.035 P= 0.849	R= -0.139 P= 0.448	R= -0.090 P= 0.611	R= -0.226 P= 0.185
Social functioning	R= 0.178 P= 0.225	R= -0.021 P= 0.885	R= -0.078 P= 0.673	R= -0.236 P= 0.193	R= -0.167 P= 0.345	R= -0.258 P= 0.129
Role Emotional	R= 0.092 P= 0.535	R= -0.244 P= 0.091	R= -0.307 P= 0.088	R= -0.288 P= 0.110	R= -0.235 P= 0.182	R= -0.271 P= 0.110
Mental Health	R= 0.285* P= 0.049	R= -0.083 P= 0.572	R= -0.114 P= 0.535	R= -0.029 P= 0.874	R= -0.332 P= 0.055	R= -0.371* P= 0.026
Physical Health summary	R= 0.033 P= 0.824	R= -0.048 P= 0.741	R= -0.136 P= 0.458	R= -0.154 P= 0.400	R= -0.037 P= 0.836	R= -0.137 P= 0.426
Mental Health Summary	R= 0.162 P= 0.272	R= -0.101 P= 0.491	R= -0.178 P= 0.330	R= -0.159 P= 0.386	R= -0.211 P= 0.230	R= -0.260 P= 0.126
Total	R= 0.103 P= 0.484	R= -0.104 P= 0.478	R= -0.189 P= 0.300	R= -0.214 P= 0.240	R= -0.151 P= 0.394	R= -0.128 P= 0.456
SGRQ						
Symptoms	R= -0.092 P= 0.534	R= 0.004 P= 0.979	R= 0.010 P= 0.955	R= 0.153 P= 0.396	R= -0.116 P= 0.513	R= -0.029 P= 0.864
Activity	R= 0.022 P= 0.884	R= 0.066 P= 0.652	R= 0.078 P= 0.665	R= 0.154 P= 0.392	R= 0.248 P= 0.157	R= 0.091 P= 0.598
Impacts	R= -0.140 P= 0.344	R= 0.027 P= 0.855	R= -0.213 P= 0.234	R= -0.094 P= 0.604	R= -0.007 P= 0.970	R= 0.210 P= 0.219
Total	R= -0.091 P= 0.540	R= 0.041 P= 0.777	R= -0.071 P=0.696	R= 0.054 P= 0.765	R= 0.067 P= 0.705	R= 0.147 P= 0.392
IWQOL-Lite						
Physical Function	R= -0.016 P= 0.916	R= -0.006 P= 0.965	R= -0.258 P= 0.162	R= -0.236 P= 0.201	R= -0.120 P= 0.507	R= -0.081 P= 0.645
Self Esteem	R= 0.349* P= 0.015	R= 0.200 P= 0.168	R= 0.354 P= 0.050	R= 0.193 P= 0.297	R= 0.329 P= 0.062	R= 0.049 P= 0.782
Sexual Life	R= 0.010 P= 0.945	R= -0.017	R= 0.139 P= 0.455	R= 0.074 P= 0.692	R= 0.156 P= 0.386	R= -0.274 P= 0.112

		P= 0.908				
Public Distress	R= 0.191 P= 0.193	R= 0.067 P= 0.645	R= - 0.184 P= 0.322	R= - 0.143 P= 0.444	R= 0.122 P= 0.499	R= 0.200 P= 0.250
Work	R= 0.017 P= 0.908	R= - 0.105 P= 0.472	R= - 0.110 P= 0.558	R= - 0.151 P= 0.418	R= 0.227 P= 0.203	R= -0.144 P= 0.411
Total	R= 0.169 P= 0.251	R= 0.056 P= 0.700	R= - 0.025 P= 0.896	R= - 0.073 P= 0.696	R= 0.126 P= 0.486	R= -0.062 P= 0.722

* significant correlation $p<0.05$

Table 62. Correlation r(p) between bronchial responsiveness, specific airway conductance and domain scores at each visit for the SF-36, SGRQ and IWQOL-Lite HRQoL questionnaires

	Log_{10} PC45 Baseline to 6 months	sGaw Baseline to 6 months
SF36		
Phys Functioning	R= -0.250 P= 0.176	R= -0.187 P= 0.296
Role Physical	R= 0.155 P= 0.404	R= -0.040 P= 0.826
Bodily Pain	R= 0.061 P= 0.743	R= 0.131 P= 0.468
General Health	R= 0.089 P= 0.635	R= 0.224 P= 0.209
Vitality	R= -0.185 P= 0.319	R= 0.109 P= 0.545
Social functioning	R= -0.021 P= 0.910	R= -0.044 P= 0.810
Role Emotional	R= 0.094 P= 0.616	R= 0.183 P= 0.309
Mental Health	R= -0.176 P= 0.344	R= -0.127 P= 0.482
Physical Health summary	R= 0.041 P= 0.828	R= 0.040 P= 0.826
Mental Health Summary	R= -0.008 P= 0.968	R= 0.131 P= 0.468
Total	R= 0.035 P= 0.854	R= 0.135 P= 0.453
SGRQ		
Symptoms	R= 0.159 P= 0.393	R= 0.261 P= 0.143
Activity	R= 0.273 P= 0.137	R= 0.156 P= 0.387
Impacts	R= 0.181 P= 0.329	R= 0.034 P= 0.849
Total	R= 0.265 P= 0.150	R= 0.156 P= 0.386
IWQOL-Lite		
Physical Function	R= -0.175 P= 0.347	R= -0.072 P= 0.696
Self Esteem	R= -0.065 P= 0.730	R= -0.188 P= 0.302
Sexual Life	R= -0.469* P= 0.008	R= -0.385* P= 0.029
Public Distress	R= -0.101 P= 0.590	R= -0.068 P= 0.712
Work	R= -0.261 P= 0.157	R= -0.123 P= 0.502
Total	R= -0.302 P= 0.099	R= -0.209 P= 0.250

* significant correlation $p<0.05$

Table 63. Correlations between change in domain score and changes in bronchial responsiveness and specific airway conductance between baseline and 6 months for the SF-36, SGRQ and IWQOL-Lite HRQoL questionnaires

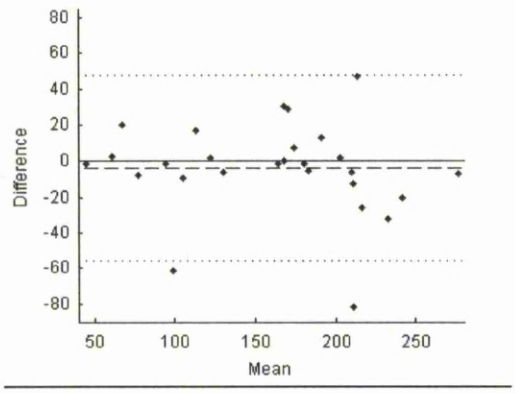
Appendix C

Additional information for differential cell counts.

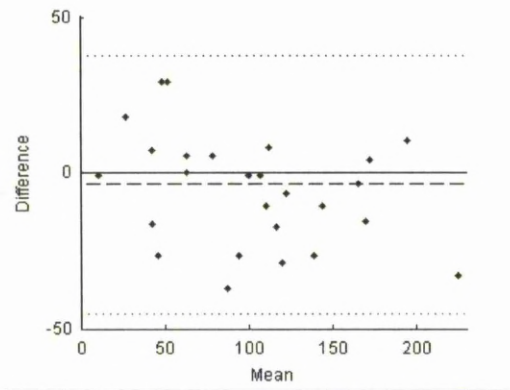
Reproducibility. Each individual slide of subjects' sputum was counted twice on separate occasions by the same individual. The following Bland Altman plots are based on these two separate counts to check for quality of reproducibility.

Baseline

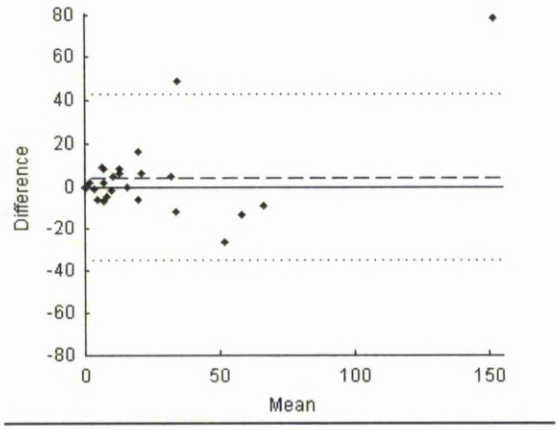
Neutrophils



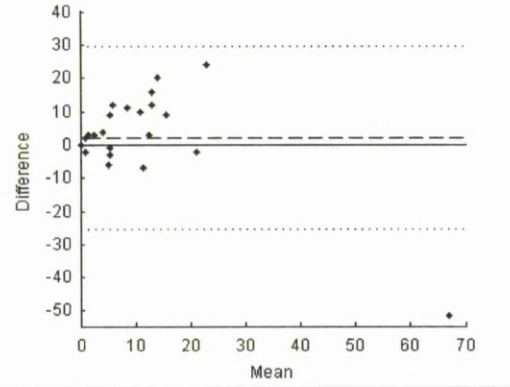
Macrophages



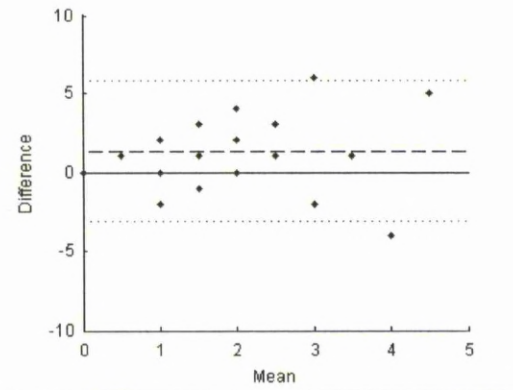
Eosinophils



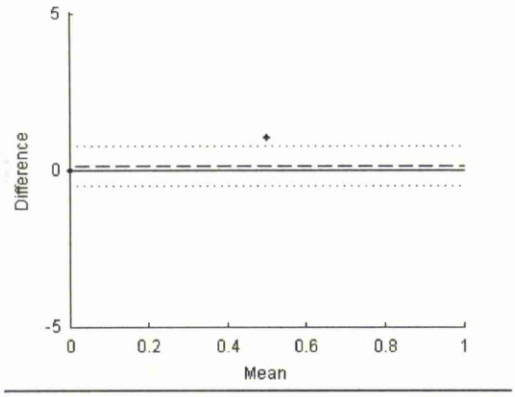
Epithelial cells



Lymphocytes

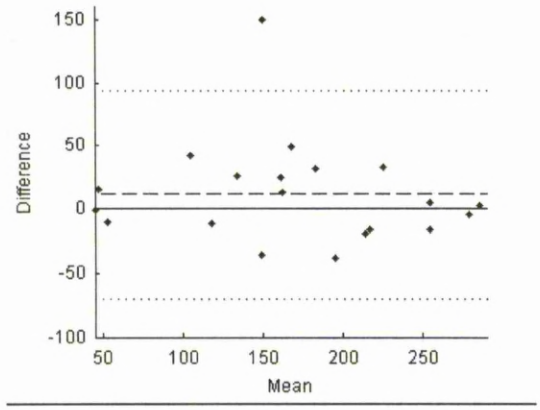


Metachromatic cells

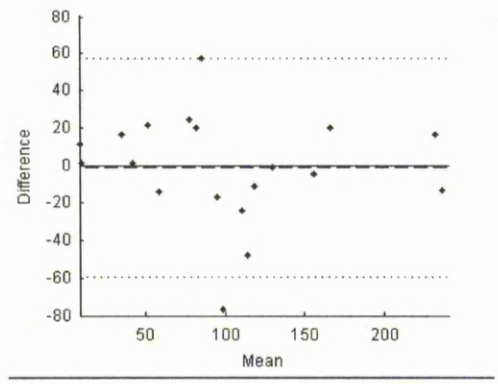


3 Months

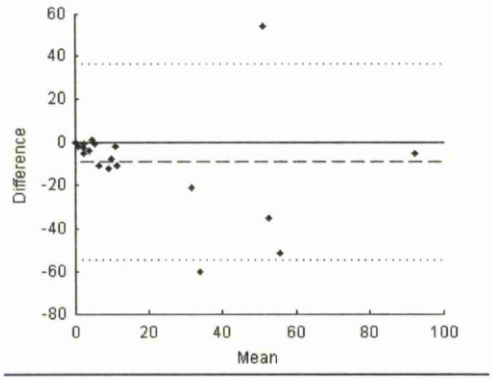
Neutrophils



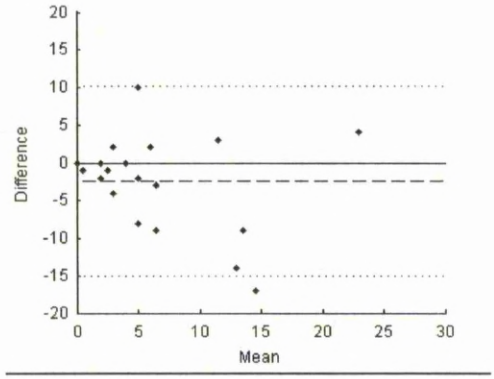
Macrophages



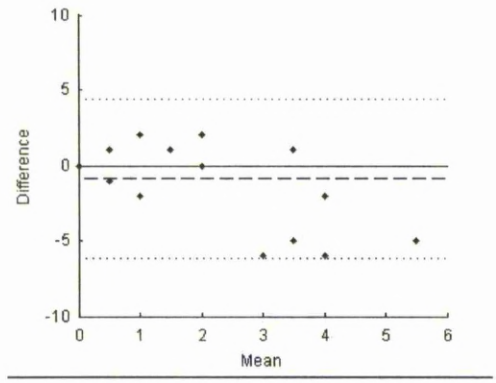
Eosinophils



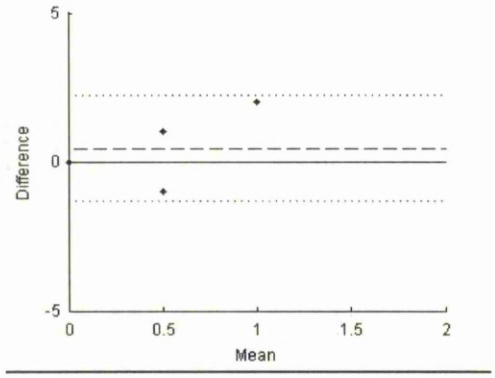
Epithelial cells



Lymphocytes

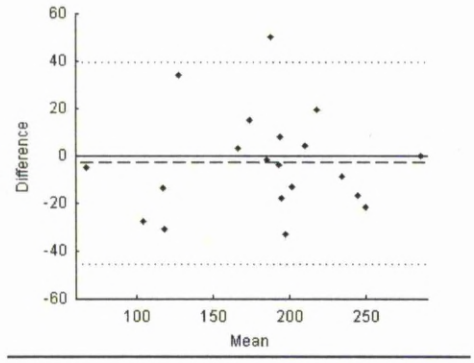


Metachromatic cells

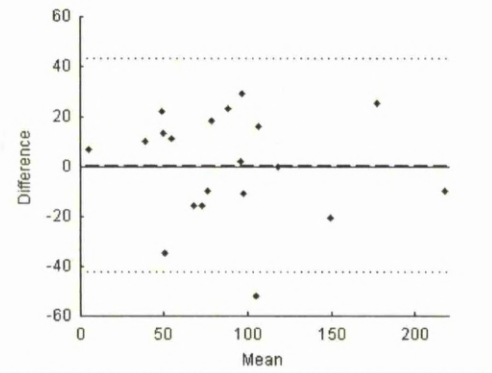


6 Months.

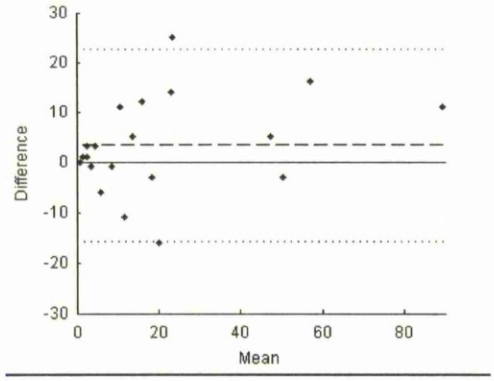
Neutrophils



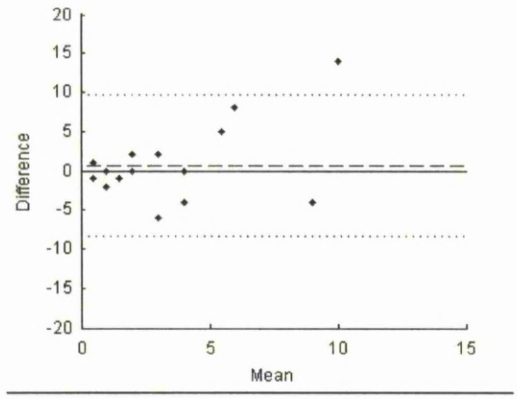
Macrophages



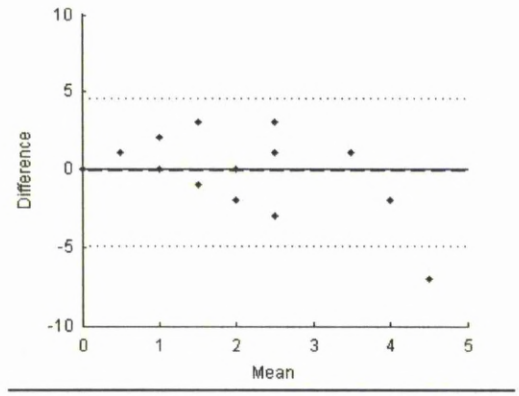
Eosinophils



Epithelial cells



Lymphocytes



Metachromatic cells

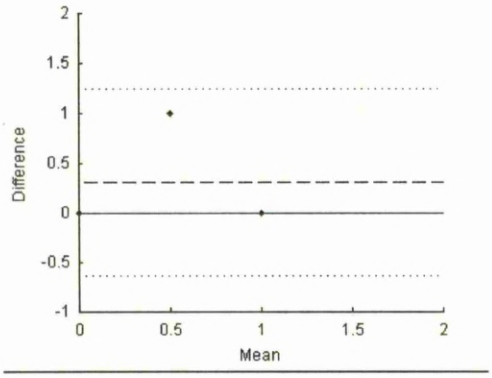


Fig 18. Bland Altman plots for differential cell counts of all subjects at each visit. Each individual cell type is shown

Correlation between change in differential cell count between visits and change in weight % between visits.

Whole group

	Baseline to 3 months	3 months to 6 months	Baseline to 6 months
Neutrophils	-0.364 (0.201)	0.163 (0.561)	0.144 (0.609)
Macrophages	0.396 (0.161)	-0.199 (0.477)	-0.025 (0.929)
Eosinophils	-0.007 (0.981)	0.170 (0.545)	-0.090 (0.750)
Epithelial	-0.177 (0.544)	-0.169 (548)	-0.026 (0.926)
Lymphocytes	0.157 (0.592)	-0.140 (0.620)	-0.151 (0.590)
Metachromatic	0.100 (0.735)	-0.359 (0.189)	0.200 (0.475)

Table 64. R (p value) shown for investigation of correlations of a change in weight as percentage of starting weight and change in cell count for each cell type for all subjects

Dietician Group

	Baseline to 3 months	3 months to 6 months	Baseline to 6 months
Neutrophils	-0.770 (0.009)	0.193 (0.569)	-0.311 (0.382)
Macrophages	0.674 (0.033)	-0.093 (0.785)	-0.094 (0.797)
Eosinophils	0.013 (0.972)	-0.003 (0.994)	0.503 (0.138)
Epithelial	-0.162 (0.655)	-0.233 (0.490)	-0.413 (0.235)
Lymphocytes	0.250 (0.486)	0.014 (0.967)	-0.285 (0.425)
Metachromatic	0.117 (0.748)	-0.173 (0.612)	-0.078 (0.831)

Table 65. R (p value) shown for investigation of correlations of a change in weight as percentage of starting weight and change in cell count for each cell type for the dietician group

Control Group

	Baseline to 3 months	3 months to 6 months	Baseline to 6 months
Neutrophils	0.655 (0.345)	0.229 (0.771)	0.289 (0.638)
Macrophages	-0.425 (0.575)	-0.300 (0.700)	-0.091 (0.884)
Eosinophils	-0.051 (0.949)	0.206 (0.794)	-0.283 (0.644)
Epithelial	-0.269 (0.731)	-0.295 (705)	0.496 (0.396)
Lymphocytes	-0.041 (0.959)	-0.482 (0.518)	0.165 (0.791)
Metachromatic	NA*	-0.407 (0.593)	0.630 (0.255)

*could not be computed as at least one of the variables is constant

Table 66. R (p value) shown for investigation of correlations of a change in weight as percentage of starting weight and change in cell count for each cell type for the dietician group.

Appendix D

Additional information for bronchial responsiveness

Comparing dietician and control groups.

	Baseline	3 months	6 months
sGaw	0.579 (0.450)	0.513 (0.479)	0.345 (0.561)
PC_{45}	0.145 (0.706)	0.013 (0.910)	0.206 (0.653)
$LogPC_{45}$	0.057 (0.812)	0.186 (0.669)	0.016 (0.901)
DRS	0.059 (0.809)	0.116 (0.736)	1.032 (0.317)
BRI	0.039 (0.844)	0.153 (0.698)	0.682 (0.415)

Table 67. F statistic (p values) when comparing dietician and control groups for each variable at each visit. ANOVA

	Baseline to 3 months Dietician group	Baseline to 3 months Control group	Baseline to 6 months Dietician group	Baseline to 6 months Control group
sGaw	-0.01 (0.046)	0.011 (0.029)	-0.002 (0.045)	0.008 (0.042)
PC_{45}	0.097 (0.339)	0.02 (0.595)	0.186 (0.651)	0.04 (0.223)
$LogPC_{45}$	0.134 (0.35)	0.047 (0.541)	0.079 (0.394)	0.173 (0.329)
DRS	-51.25 (26.17)	-47.93 (18.55)	5.83 (18.91)*	-8.87 (18.35)*
BRI	-0.036 (0.097)	-0.027 (0.153)	0.026 (-0.064)*	0.026 (0.102)*

* significant difference between groups $p<0.05$

Table 68. Mean (sd) change from baseline for sGaw, Bronchial responsiveness and Bronchial reactivity from baseline to 3 and 6 months for each group. * denotes significant difference between groups

There were no significant differences between dietician and control groups for changes from baseline for any variables of airway responsiveness or reactivity or specific airway conductance except for the dose response slope and bronchial reactivity index between visit 2 and visit 4.

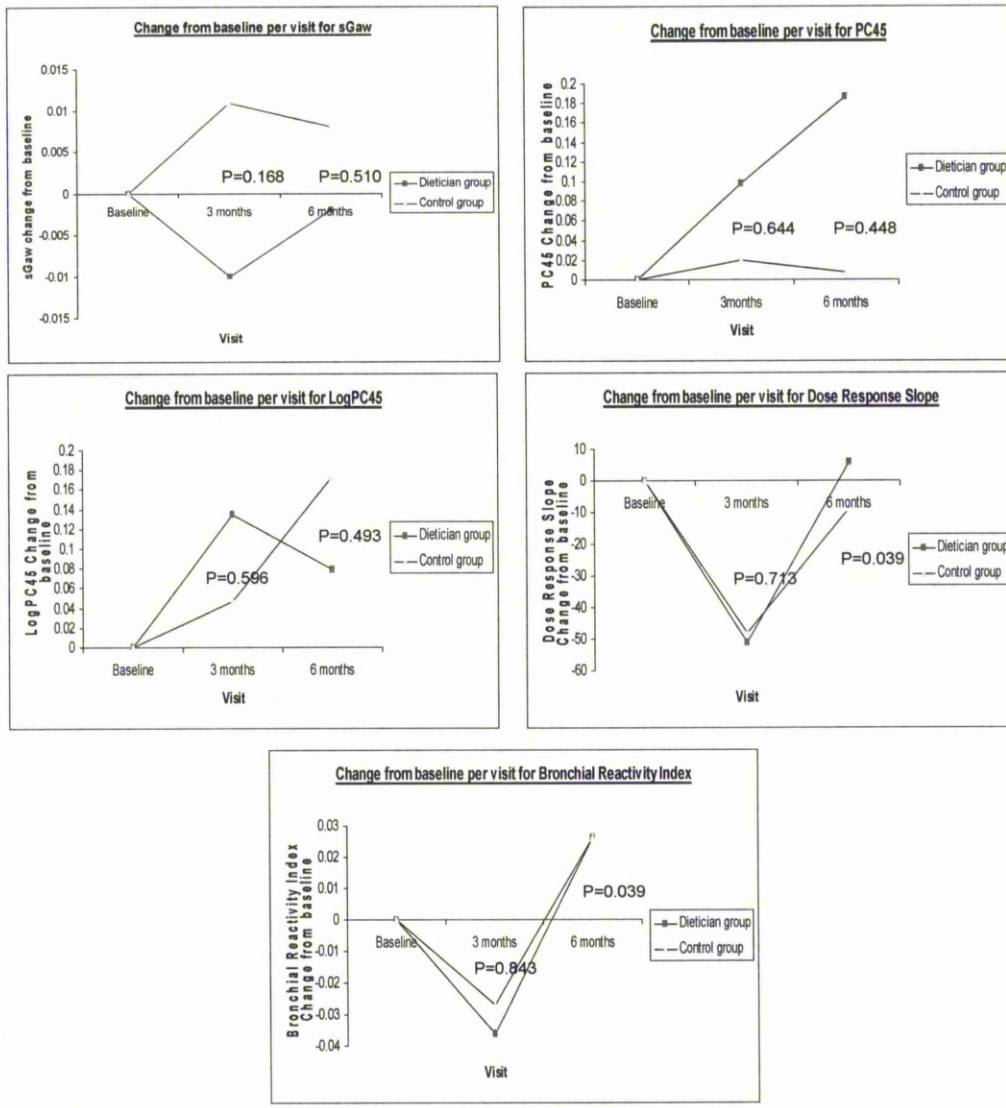


Fig 23. Graphs showing change from baseline for sGaw, PC_{45}, $LogPC_{45}$, DRS and BRI for each visit for dietician and control groups. P values show comparison of means between the groups at each visit

	Baseline	3 months`	6 months
sGaw¥	R= -0.045 P= 0.758	R= -0.002 P= 0.993	R= -0.163 P= 0.342
PC_{45} ¥	R= 0.100 P= 0.497	R= 0.274 P= 0.123	R= 0.005 P= 0.977
$LogPC_{45}$	R= 0.002 P= 0.990	R= 0.391 P= 0.025*	R= -0.012 P= 0.947
DRS	R= -0.140 P= 0.342	R= -0.229 P= 0.192	R= 0.005 P= 0.976
BRI	R= -0.158 P= 0.285	R= -0.274 P= 0.117	R= -0.018 P= 0.922

¥ = Skewed data therefore Spearman's Correlation used

* significant correlation p<0.05

Table 69. Correlations between measures of s Gaw, bronchial responsiveness, bronchial reactivity and BMI for all subjects

	Dietician group baseline	Control group baseline	Dietician group 3 months	Control group 3 months	Dietician group 6 months	Control group visit 6 months
sGaw ¥	R= -0.177 P= 0.397	R= 0.109 P= 0.611	R= -0.133 P= 0.575	R= 0.138 P= 0.654	R= -0.267 P= 0.243	R= 0.029 P= 0.918
PC_{45} ¥	R= 0.007 P= 0.974	R= 0.241 P= 0.269	R= 0.203 P= 0.391	R= 0.280 P= 0.354	R= -0.011 P= 0.963	R= -0.014 P= 0.960
$LogPC_{45}$	R= -0.189 P= 0.365	R= 0.235 P= 0.133	R= 0.160 P= 0.500	R= 0.659* P= 0.014	R= -0.020 P= 0.936	R= 0.003 P= 0.990
DRS	R= 0.000 P= 0.998	R= -0.323 P= 0.133	R= -0.166 P= 0.471	R= -0.322 P= 0.283	R= 0.008 P= 0.973	R= -0.035 P= 0.900
BRI	R= -0.007 P= 0.972	R= -0.347 P= 0.105	R= -0.129 P= 0.576	R= -0.460 P= 0.114	R= -0.001 P= 0.996	R= -0.069 P= 0.807

¥ = Skewed data therefore Spearman's Correlation used

* significant correlation p<0.05

Table 70. Correlations between measures of s Gaw, bronchial responsiveness, bronchial reactivity and BMI for dietician and control groups

	Baseline to 3 months			Baseline to 6 months		
	All subjects	Dietician group	Control group	All subjects	Dietician group	Control group
sGaw	R= 0.257 P= 0.162	R= 0.528* P= 0.020	R= -0.159 P= 0.622	R= 0.069 P= 0.699	R= 0.268 P= 0.254	R= -0.200 P= 0.493
PC_{45}	R= 0.050 P= 0.795	R= -0.030 P= 0.904	R= 0.112 P= 0.758	R= -0.221 P= 0.240	R= -0.445 P= 0.074	R= 0.253 P= 0.405
$LogPC_{45}$	R= 0.130 P= 0.493	R= -0.203 P= 0.405	R= 0.375 P= 0.256	R= -0.038 P= 0.838	R= -0.334 P= 0.175	R= 0.239 P= 0.431
DRS	R= -0.017 P= 0.927	R= -0.035 P= 0.883	R=-0.028 P= 0.934	R= -0.137 P= 0.462	R= 0.157 P= 0.534	R= -0.325 P= 0.279
BRI	R= -0.109 P= 0.558	R= 0.376 P= 0.102	R= -0.432 P= 0.184	R= -0.165 P= 0.375	R= 0.284 P= 0.254	R= -0.411 P= 0.163

* significant correlation p<0.05

Table 71. Correlation between weight loss as a percentage of starting weight and change in sGaw, PC_{45}, LogPC45, DRS and BRI at 3 and 6 months

Corresponding change	Change in FeNO50ml between baseline and 3 months	Change in FeNO50ml between baseline and 6 months
sGaw	R= 0.122 P= 0.553	R= 0.132 P= 0.478
PC_{45}	R= -0.077 P= 0.714	R= -0.075 P= 0.697
$LogPC_{45}$	R= -0.002 P= 0.991	R= -0.050 P= 0.794
DRS	R= -0.077 P= 0.707	R= 0.218 P= 0.248
BRI	R= 0.069 P= 0.738	R= 0.202 P= 0.283

Table 72. Correlations between change in exhaled nitric oxide and measures of bronchial responsiveness and reactivity

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