Factors associated with ventilatory failure in obesity

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Abstract

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I would like to dedicate this thesis to two people who are sadly not with us anymore, Richard Madgewick and Nicky Richards. Both sadly passed away during the course of this thesis all will be truly missed

Declaration

I declare that the work in this dissertation was carried out in accordance with the requirements of the University's Regulation and Code of Practice for Research Degree Programmes and that it has not been submitted for any other academic award.

Except where indicated by specific reference in text, the work is the candidate's own work. Work done in collaboration with, or with the assistance of others, is indicated as such.

Signed: ARI MANUEL

Date: 1st July 2015

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Abbreviations

Aa	Alveolar arterial gradient
AHI	Apnoea Hypopnoea Index
BMI	Body Mass index
BP	Blood Pressure
CI	Confidence Interval
CO_2	Carbon dioxide
CPAP	Continuous Positive airway pressure
DXA	Dual energy x-ray absorptiometry
ESS	Epworth Sleepiness Scale
MRI	Magnetic Resonance imaging
NRES	National Research Ethics Service
O_2	Oxygen
Pa	Partial Pressure
PEEP	Positive end-expiratory pressure
PI	Principal Investigator
PIL	Participant/ Patient Information Leaflet
R&D	NHS Trust R&D Department
REC	Research Ethics Committee
SOP	Standard Operating Procedure

INTRODUCTION - CHAPTER 1

1.1 History of Obesity Hypoventilation Syndrome (OHS)

This chapter discusses the definition and historical perspective of OHS. It goes on to discuss the current definition of the syndrome and the possible mechanisms of the development of ventilatory failure in obesity. I will outline each potential cause in turn, before describing my hypotheses, and then place these in the clinical context of patients with OHS.

1.1.1 Definition and historical perspective

Obesity as a disease, with well-defined complications, is approximately one century old. For much of human history, corpulence was considered a sign of good health, status and increased fat regarded as an advantage. The impact of gross obesity on quality of life began to be noticed late in the 18th century and on ill health in the middle of 19th century; but it was only in the first decades of the 20th century that the morbid complications and increased mortality of obesity began to be documented by the insurance industry (Williamson, 1993).

Apart from obesity, there are a number of other well-known causes of hypoventilation in humans, such as pulmonary disease (severe obstructive or restrictive), chest wall deformities, severe hypothyroidism and neuromuscular disease.

Obesity Hypoventilation Syndrome (OHS) is characterised as the combination of obesity, daytime hypoventilation, and thus chronic daytime hypercapnia, unexplained by any other cause of hypoventilation.

The association between obesity and daytime hypoventilation has long been recognised. A famous example of this is William Howard Taft (1857–1930). A review of historical records described William Howard Taft, 27th President of the United States (1909–1913), as having progressive obesity, daytime hypoventilation, hypersomnolence and evidence of cor pulmonale at the end of his presidency. Of historical interest, OHS was described well before the more commonly known Obstructive Sleep Apnoea (OSA) was recognised as a specific clinical entity in 1969 (Kuhlo et al., 1969).

Burwell is credited with describing the first case of OHS. He described a patient who finally sought treatment after his symptoms caused him to fall asleep during a hand of poker, despite having been dealt a full house of aces over kings (Bickelmann et al., 1956). Burwell went on to popularise the term ‘Pickwickian syndrome’ in his case report by noting the similarities between his patient and the boy Joe (Fig. 1), Mr Wardle’s servant in Charles Dickens’ *The Posthumous Papers of the Pickwick Club.* Thus, since then, the terms Pickwickian and OHS have become synonymous.

Burwell’s original classification of Pickwickian Syndrome, from his case report, included the following clinical features:

1. Marked obesity

2. Hypoventilation

3. Somnolence

4. Twitching

5. Cyanosis

6. Periodic respiration

7. Secondary Polycythemia

8. Right ventricular hypertrophy

9. Right ventricular failure

Figure 1: Thomas Nast's drawing of the fat boy in '*The Pickwick Papers*'

The current definition of OHS consists of obesity (defined as a body mass index [BMI] ≥30 kg/m^2) and chronic daytime alveolar hypoventilation (defined as a $PaCO_2$ > 6kPa (45 mm Hg) and PaO_2 <70 mm Hg) (Berger et al., 2001).

Obesity is of course a key component of the definition of OHS. In epidemiological studies, obesity is associated with excessive somnolence and loud snoring (Rossner, 1987). Obesity is also closely involved in the pathogenesis of OSA, hence OSA and OHS are often regarded as closely related and often confused. Some even regard OHS as a form of sleep-disordered breathing (Borel et al., 2012) However it is vital to make the distinction that OHS and OSA are distinct entities and this thesis will explore the pathogenesis and predictors of OHS only.

Sleep-disordered breathing is not always regarded as part of the basic definition of OHS, and varies between researchers in this area (Borel et al., 2012). However, individuals with OHS often demonstrate a number of polysomnographically-defined phenotypes during sleep. Sleep hypoventilation alone does not define OHS unless daytime hypercapnia is also present. In some studies, approximately 90% of patients with OHS have predominantly OSA as their sleep-disordered breathing. The other 10% of patients are considered to have predominantly ‘sleep hypoventilation’ which is defined as a 10mm Hg increase in sleeping $PaCO_2$ versus wakefulness, or persistent oxygen desaturation during sleep, unexplained by transient obstructive apnoeas or hypopneas.

There are many other causes of ventilatory failure. OHS should be considered a diagnosis of exclusion when other causes of hypoventilation have been evaluated and excluded, such as pulmonary disease (severe obstructive or restrictive), chest

wall deformities, severe hypothyroidism or neuromuscular disease (Ayappa et al., 2002).

1.1.2 Mechanism of ventilatory failure in obesity

The development of hypoventilation in obesity is highly variable with some patients with BMIs of little over 30 going into ventilatory failure, and some with BMIs of over 60 who do not. There clearly must be other important precipitating factors that contribute to developing hypercapnia.

Understanding the development of hypoventilation and subsequent daytime hypercapnia in OHS has undergone considerable evolution since the first description of the ‘Pickwickian syndrome’ in 1956 (Bickelmann et al. (1956). The pathogenesis of hypoventilation in obesity is complex and likely multifactorial. There are several possible mechanisms which have been postulated for daytime normocapnia to develop into hypercapnia in obesity, which include:

1) Differences in measures of obesity and its distribution

2) Differences in lung function

3) Differences in muscle strength

4) Differences in sleep-related measures

5) Differences in ventilatory drive

6) Differences in hormonal, nutritional and inflammatory measures

One question however arises: why do only some obese individuals develop ventilatory failure? It appears that the majority of obese and morbidly obese (BMI>

40 kg/m^2) individuals are able to compensate for the abnormalities imposed by their excessive weight, maintaining daytime normocapnia. Consequently, it follows that OHS emerges only when compensatory mechanisms fail or become overwhelmed (Norman et al., 2006).

It is also important to realise that the above respiratory abnormalities do not occur in isolation, and that a complex interaction is likely to occur between pulmonary function, drive and sleep-disordered breathing (Mokhlesi, 2010).

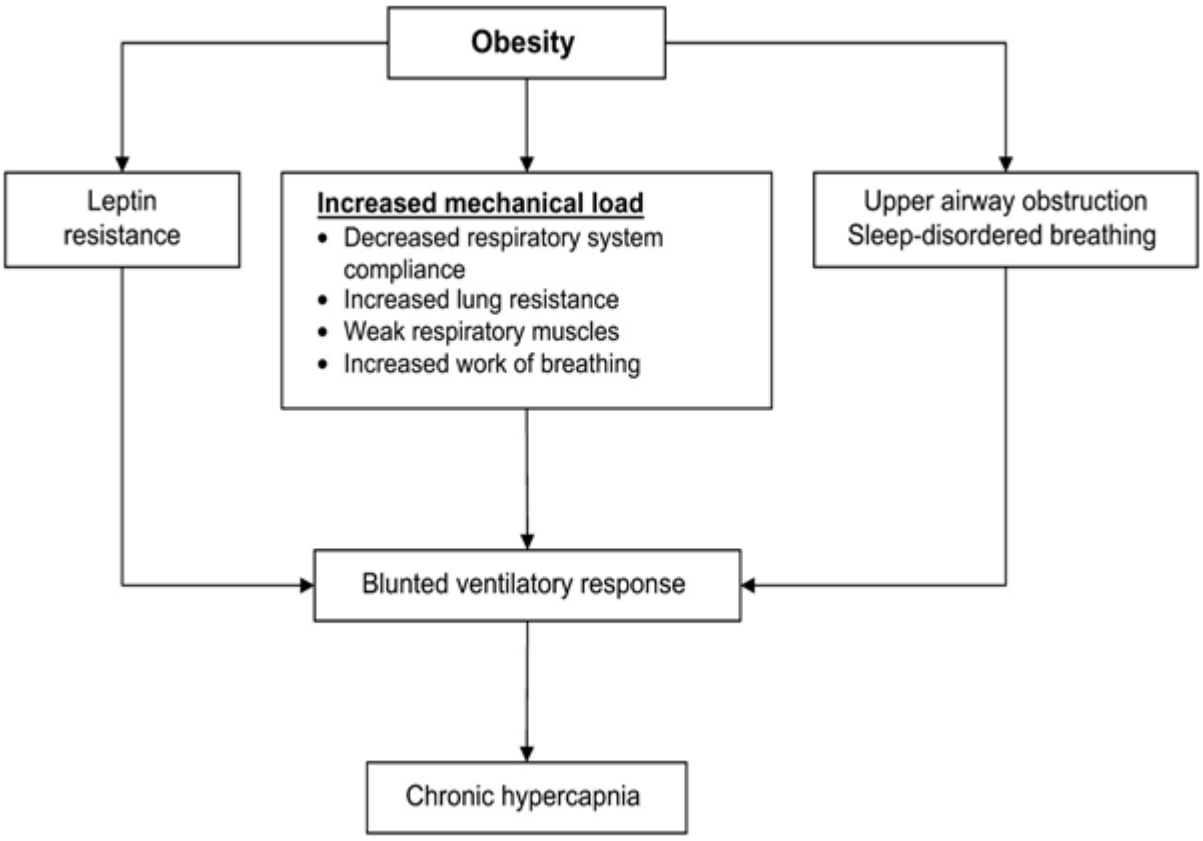


Figure 2: Possible mechanisms for the development of daytime hypercapnia in obesity (Piper and Grunstein, 2011).

Researchers have previously looked at each area in isolation, attempting to identify causation of ventilatory failure in obesity. A few of the key hypotheses are outlined below

An example of a difference in hormonal levels might be seen with leptin. Leptin infusions in a leptin-deficiency animal model cause significant improvements in minute ventilation and hypercapnic ventilatory response within three days of infusion (Tankersley et al., 1998).

Other attempts at looking at determinants of hypoventilation include looking at ventilatory response and ventilatory drive differences. This revealed that individuals with OHS suffer from a blunted central responsiveness to hypercapnia and hypoxia, both well-established tests for the assessment of ventilatory responsiveness (Zwillich et al., 1975).

Perhaps the degree of obstructive sleep apnoea does play a role. Recent observational data indicates that chronic daytime hypercapnia as a consequence of OSA may be increasing in incidence (Kaw et al., 2009).

It has been shown that obesity imposes a significant mechanical load and mass effect on an individual, leading to a reduction in total respiratory system compliance. This causes a subsequent increase in lung resistance (Naimark and Cherniack, 1960) which can lead to an increase in work of breathing and ultimately the development of hypoventilation and daytime hypercapnia.

Obesity is a well-known cause of restrictive lung function, but a recent study showed that abdominal obesity may be associated with a greater reduction in FEV_1/VC ratio,

suggesting an effect on airways resistance as well as possible increased diaphragm loading (Chen et al., 2007).

1.1.3 The role of bicarbonate and the value of the base excess measurement

Is a one-off measurement of CO_2 the best marker for the long-term hypoventilation in obesity? I have explored bicarbonate and base excess as another predictor of hypoventilation in obesity.

Respiratory acidosis from carbon dioxide retention in patients with chronic respiratory disease is corrected by the generation of bicarbonate and a sustained elevation of the threshold for renal bicarbonate reabsorption. Frequently, when these patients are admitted with acute hypercapnic respiratory failure, an elevated plasma bicarbonate concentration ($[HCO_3^-]$) is present, indicating established chronic respiratory failure (Epstein and Singh, 2001).

The control of plasma $[HCO_3^-]$ by the kidneys and the removal of carbon dioxide by the alveoli have markedly different time constants. In clinical practice, a specific situation occurs in which the plasma bicarbonate concentration, which is generated to offset and correct the respiratory acidosis that occurs with an elevated arterial partial pressure of carbon dioxide during the night, becomes superfluous when the hypercapnia resolves on awakening (following improvements in respiratory drive muscle load and/or drive).

This diurnal variation in the metabolic requirement to offset nocturnal hypercapnia results in a metabolic alkalosis during the day. Norman et al. used a computer model to simulate carbon dioxide and renal bicarbonate kinetics resulting from periods of nocturnal hypoventilation (Norman et al., 2006). Transient hypercapnia during SDB,

causing transient renal compensation, was assumed. A transition from acute hypoventilation to chronic hypoventilation occurred over several days, if either renal excretion of [HCO_3^-], or ventilatory response to hypercapnia were reduced.

Correctly recognising the whole spectrum of the obese population with impending or chronic respiratory failure is important for designing the most appropriate long-term respiratory management. There is a clinical priority to recognise the different stages and phenotypes of OHS to determine both what dictates a poorer prognosis, and which treatments to use (such and non-invasive ventilation or ventilatory stimulants (Raurich et al., 2010)).

1.1.4 Clinical issue of OHS

The morbidity of OHS patients is most commonly related to ventilatory failure. Berg and colleagues performed an observational study looking at the healthcare costs of individuals with OHS compared to normocapnic obese controls. They showed that patients with OHS use much more healthcare than obese patients without hypoventilation in the 5 years before OHS is actually confirmed (Berg et al., 2001). This confirms the increased healthcare utilisation and subsequent costs of care for this group of patients.

The prevalence of OHS in the general population is unknown, as one of the major measures to identify this disorder (namely a raised $PaCO_2$ on arterial blood gas measurements) is not routinely performed in epidemiological studies.

Another issue in the management of this group of patients is that the precise prevalence of OHS in the general population remains uncertain because no general population-based studies have been performed to examine this issue.

A current estimate suggests around 0.37% of the US population may have OHS equating to several hundred thousand individuals (Berg et al., 2001). In light of the rapidly increasing numbers of individuals becoming morbidly obese, and the significantly greater need they have for medical care, OHS needs to be considered as a significant clinical and societal problem.

1.2 Mechanism of ventilatory failure in obesity

1.2.1 Differences in measures of obesity and its distribution

Evidence for an effect of obesity on chest wall compliance varies between studies, possibly as a result of methodological differences. Measurement of chest wall compliance is difficult, since the respiratory muscles must be relaxed and inactive to allow an accurate measurement. Studies of conscious, spontaneously breathing subjects have suggested that there is a reduction in chest wall compliance in obesity. However, normal chest wall compliance has been reported in studies of anesthetized, paralyzed subjects with mild obesity (Pelosi et al., 1996), as well as in studies of conscious subjects using different measurement techniques (Sharp et al., 1964; Suratt et al., 1984).

A reduction in respiratory compliance may contribute to the development of hypoventilation in obesity. From studies showing differences when comparing obese and lean subjects, obese subjects have a reduced compliance of the respiratory system in comparison with lean seated subjects. Furthermore, the compliance declines further in the supine position in the obese subjects only. These findings suggest that both obesity and posture could have a role in reducing respiratory compliance. Most of the reduction seen was due to falls in chest wall compliance

(Sutherland et al., 2008). In another study, which looked at patients with OHS and compared them to normocapnic obese individuals, it was shown that total respiratory compliance was reduced in OHS patients to one third of the controls (Sharp et al., 1964).

The effect of reduced compliance of the respiratory system is to increase the overall work of breathing. The same study by Sharp confirmed this, showing that obese subjects have a work of breathing rate of over double that of lean subjects. This was partially attributable to elastic work on the lung, as well as chest wall compliance as mentioned above, in the obese patients (Sharp et al., 1964).

Morbidly obese patients compared with normal-weight subjects are commonly characterized by the following: (1) lower respiratory system compliance, caused by a decrease both in lung and chest wall compliance, although the former was prevalent; (2) higher lung resistance with a marked increase in airway and "additional" lung resistance; and (3) significant lower FRC as a consequence of these alterations (Pelosi et al., 1996).

A reduction in the downward movement of the diaphragm, due to increased abdominal mass, is likely to decrease TLC by limiting the room for lung expansion on inflation (Pelosi et al., 1996).

Obesity can lead to abdominal compression due to a mass loading action, which can lead to a number of effects. One of those effects could be the upward compression on the diaphragm. This compression subsequently reduces lung volumes, and this reduction leads to reduced traction on the pharynx. This increases the collapsibility of the pharynx (Owens et al., 2010). Hence the distribution of obesity, and in particular in this case of central obesity, may have a particularly provocative role in

the degree of nocturnal hypoventilation and hence development of daytime hypercapnia.

To investigate the effect of obesity on upper airway collapse, researchers have looked at the effect of weight loss on the upper airway. It was shown that weight loss reduces upper airway collapse by modifying anatomy and restoring function, with a 13% weight loss responsible for a decreased nasopharyngeal airway collapsibility in obese patients studied with OSA. This weight loss was achieved with diet alone. All the subjects had a decrease in apnoea-hypopnea index (AHI) (Suratt et al., 1987). This study was replicated with patients undergoing bariatric surgery. There was an improvement in pharyngeal and glottic function and significant decrease in AHI after a 26kg weight loss in obese patients with OSA (Sugerman et al., 1986).

Visceral adipose tissue (VAT) is thought to have important metabolic properties. It accumulates primarily deep in the peritoneal cavity within the upper part of the abdomen and infiltrates the liver, stomach, pancreas and the mesentery. It is associated with impaired non-esterified fatty acid (NEFA) metabolism, leading to a hyperlipolytic state in which adipose cells are resistant to the antilipolytic effects of insulin. The resulting NEFA flux to the liver may induce increased hepatic production of glucose. Excess VAT is associated with a risk of type 2 diabetes, dyslipidemia, hypertension, cardiovascular diseases and cancer (Ohlson et al., 1985, Seidell et al., 1997).

Sutherland et al. (2008) used a wide range of body fat variables using dual energy x-ray absorptiometry (DXA) to determine the effect of fat distribution on lung volumes in healthy adults. Lung volumes were only loosely associated with BMI but both DXA and non-DXA-derived variables reflecting upper body fat had highly significant

negative correlations with FRC and ERV in both men and women. There were no differences in the strength of these associations between the markers of abdominal obesity (waist circumference, waist-to-hip ratio, DXA waist fat, DXA abdominal fat) or thoracic fat (DXA trunk fat, DXA thoracic fat). These findings confirm the important effect of upper body fat on the lung, but the inability to differentiate the effects of abdominal and thoracic fat suggests that they may be interdependent.

1.2.2 Hypothesis I: Differences in measures of obesity and its distribution

I hypothesise that there is a difference in measures of obesity and its distribution in individuals with OHS and normocapnic obesity which contributes to its pathogenesis.

1.2.3 Lung function

1.2.3.1 Change in lung volumes with obesity and posture

In this section I will discuss the potential mechanism based on differences in fat distribution and differences due to changes in posture and lung volume.

Obesity causes a reduction in lung volumes in individuals. Patients with even mild obesity (BMI 30-35 kg/m^2) are reported to have lower forced vital capacity (FVC), Lower Total Lung Capacity (TLC), Lower Functional Residual Capacity (FRC), Residual Volume (RV) and expiratory reserve volume (ERV) (Leone et al., 2009).

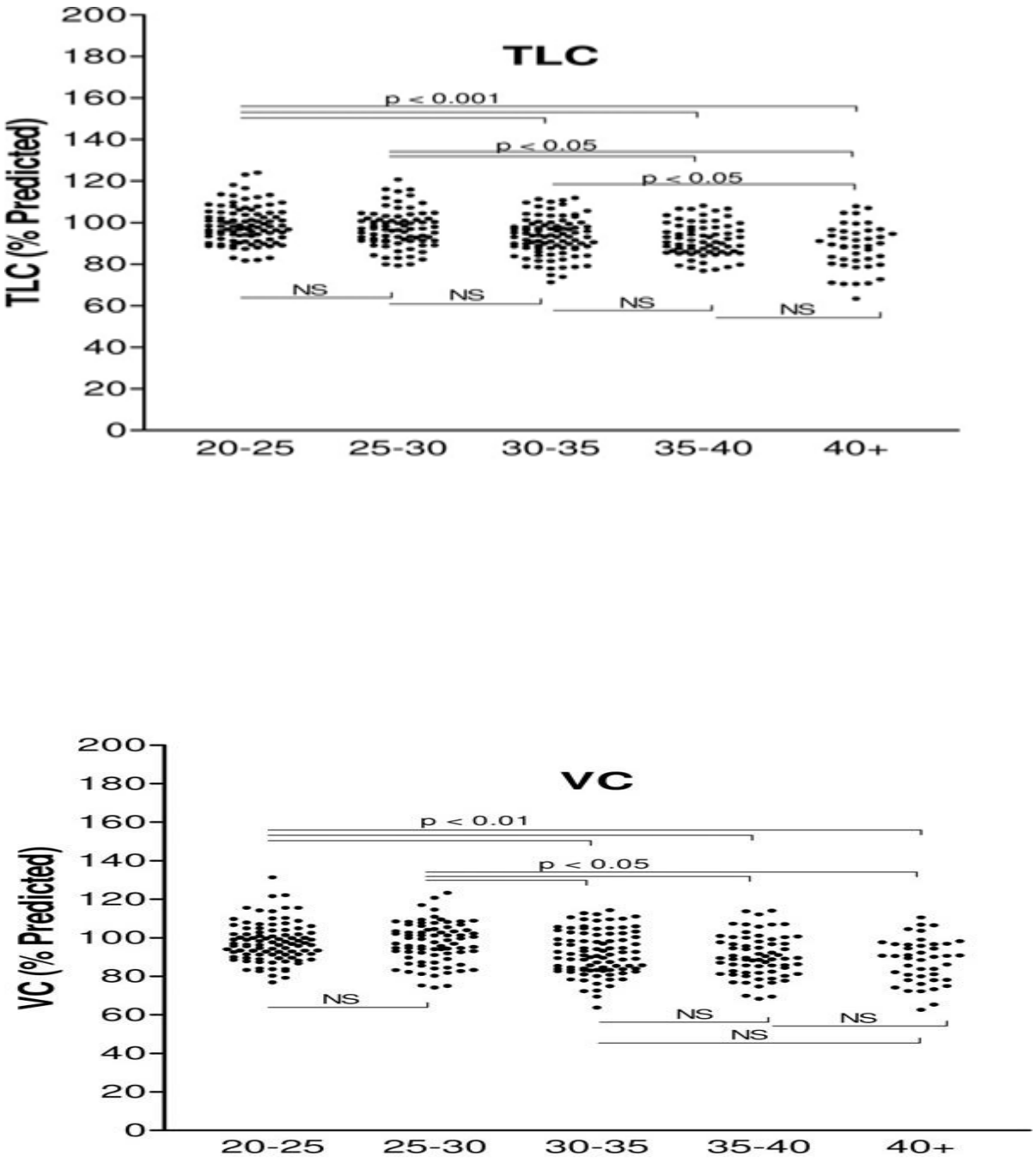


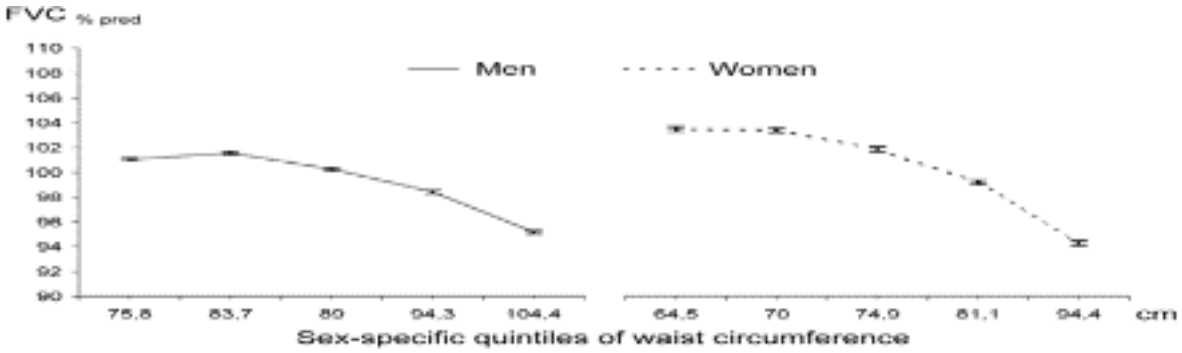
Figure 3: Effects of BMI on TLC (top), and VC (bottom). The horizontal solid lines are the between-group comparisons from ANOVA and post hoc test. NS = not significant ($p > 0.05$). (Leone et al., 2009)

The most consistently reported effect of obesity on lung function is a reduction in the functional residual capacity (FRC). This effect reflects a shift in the balance of inflationary and deflationary pressures on the lung due to the mass load of adipose tissue around the rib cage and abdomen and in the visceral cavity. The effects of obesity on the extremes of lung volumes, at total lung capacity (TLC) and RV, are often modest.

Obesity is a well-known, yet variable, cause of restrictive lung function but a study showed that abdominal obesity may be associated with a greater reduction in FEV_1/VC ratio. This suggests two possible mechanisms: one with an effect on airways resistance and another a possible increase in diaphragm loading. These conclusions were drawn from a very large population study (Chen et al., 2007).

Another cross-sectional population-based study, consisting of 121,965 subjects, showed lung function impairment was associated with metabolic syndrome (prevalence = 15.0%) and abdominal obesity was the strongest predictor of lung function impairment (Odds ratio, 1.94 [1.80–2.09] and Odds ratio, 2.11 [1.95–2.29], for FEV_1 and FVC, respectively (Leone et al., 2009))

Does gender have a role to play in the decreases of static lung function in increasing obesity? Some studies have suggested the effect of obesity on absolute lung function may be greater among men than among women, possibly attributable to greater central fat distribution in men (Chen et al., 2007).



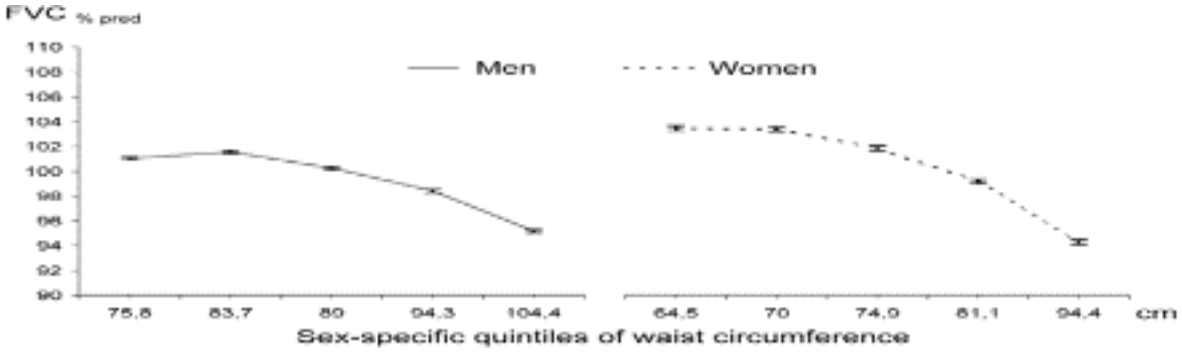


Figure 4: FVC% predicted according to sex-specific quintiles of waist circumference

What is the relationship between increasing obesity and decline in lung volumes? As outlined above, the relationship is inverse but shows a wide range of percentage predicted volumes forever increasing levels of BMI. However, in terms of the pathogenesis of OHS and the development of hypoventilation and daytime hypercapnia, the differential decrease in lung volumes may be vital. This is confirmed in a study which showed with increasing BMI, the decline in FVC, TLC, and RV is linear, but the decline in FRC and expiratory reserve volume is exponential (Jones and Nzekwu, 2006). The consequently marked decline in ERV with respect to the other lung volumes results in an effect on tidal respiration. In obesity, the reduction in FRC may become so marked that the FRC approaches

residual volume (RV). Tidal respirations are subsequently nearer to residual volume and at this point airway calibre is narrowest. As the airway calibre is narrow, there is increased airflow obstruction which needs the development of intrinsic positive end expiratory pressure to overcome, hence increasing the work of breathing.

In obesity, mass loading shifts the balance of forces between chest wall/abdomen and lung and produces a decrease in functional residual capacity (FRC). The resulting decreased airway diameter is manifest as a reduction in expiratory reserve volume (ERV). Assessment of distal airways has been elusive to traditional testing techniques due to their large aggregate cross-sectional area and minimal contribution to total airway resistance (Torchio, 2009). Forced oscillation testing has demonstrated elevated resistance associated with reduction of resting lung volumes as demonstrated by measurement of airway conductance.

1.2.4 Hypothesis II: Differences in lung function

I hypothesise that there is a difference in lung volumes between individuals with OHS and normocapnic obesity which contributes to its pathogenesis.

1.2.5 Differences in muscle strength

It is unclear how obesity might reduce muscle strength directly but there is evidence of fatty infiltration of muscles which would directly disadvantage muscle. This is worse in patients with increasing obesity and increased insulin resistance and reduces with exercise which leads to an increase in muscle strength (Franssen et al., 2008).

Respiratory muscle strength in obesity has been variously reported as normal or reduced in those with OHS (Ladosky et al., 2001)

Although inspiratory muscle strength may be normal when measured in the upright position, this may not be the case when the individual is supine, where the efficacy of the diaphragm has been shown to be reduced in the morbidly obese (Steier et al., 2009)

Maximum voluntary ventilation, a reflection of respiratory muscle endurance and lung mechanics, has been shown to decrease as BMI increases (Ladosky et al., 2001)

The reduction in endurance reduces muscle strength. The respiratory muscles are also inefficient in obese individuals. The reduction in endurance may also reflect changes in the structure of respiratory muscles themselves. In an animal model of obesity, the diaphragms of obese Zucker rats were found to undergo remodeling such that the cross-sectional area of type I and type IIa fibers significantly increased, and the diaphragm thickened (Farkas and Rochester, 1988). Powers et al. (Powers et al., 1996) showed that obesity produced a fast-to-slow shift in myosin heavy chain phenotype with an increase in the oxidative capacity of the respiratory muscles in obese Zucker rats, a change that would promote fatigue resistance in the face of a high work of breathing.

1.2.6 Hypothesis III: Differences in muscle strength

I hypothesise that there is a difference in muscle strength between in individuals with OHS and normocapnic obesity which contributes to its pathogenesis.

1.2.7 Difference in sleep-related measures

Table 1: Prevalence of Obesity Hypoventilation Syndrome in patients with Obstructive Sleep Apnoea

Authors	N	Design	Country	BMI Kg/m^2	AHI events /hr	OHS (%)
(Verin et al., 2001)	218	Retrospective	France	34	51	10
(Laaban and Chailleux, 2005)	1,141	Retrospective	France	34	55	11
(Kessler et al., 2001)	254	Prospective	France	33	76	13
(Resta et al., 2000)	219	Prospective	Italy	40	42	17
(Golpe et al., 2002)	175	Retrospective	Spain	32	42	14

As mentioned above, OHS and OSA are intricately linked. Previous data would suggest OSA uncommonly produces ventilatory failure unless there are additional factors present (Radwan et al., 1995). These are generally thought to be lower airways obstruction (e.g. COPD or asthma) but more recent observational data indicates that hypoventilation leading to daytime hypercapnia as a consequence of OSA may be increasing in incidence (Kaw et al., 2009). Table 1 illustrates how common the incidence of OHS is within a population of patients attending sleep apnoea outpatient clinics. In terms of treatment for OHS, alleviation of the OSA alone

can reverse ventilatory failure, even with no change in airways resistance or reduction in the obesity of the individual (Salord et al., 2013). One explanation for this is that upper airway narrowing during sleep (OSA or heavy snoring) may contribute to ventilatory failure in the already obese.

Berger et al. (Berger et al., 2009) state that the conventional explanation of nocturnal hypoventilation does not fully explain why individuals with OHS remain persistently hypercapnic during the day. The authors hypothesise that chronic hypercapnia results from the inability to offload carbon dioxide during the day in response to carbon dioxide loading at night from sleep-disordered breathing. Offloading is more likely to occur in those with short episodes of hypercapnia, due to apnoeas and hypopnoeas, compared with patients with long periods of hypoventilation.

Modelling work suggests that when apnoea events become more than three times longer than the breathing interval between them, carbon dioxide will accumulate, despite maximum augmentation of tidal volume, as there is insufficient time for this hyperventilation to return carbon dioxide to normal before the next episode of carbon dioxide loading. A secondary mechanism which then impairs chemosensitivity is the increase in serum and cerebrospinal fluid bicarbonate, which is exacerbated by reduced renal bicarbonate excretion, as described previously.

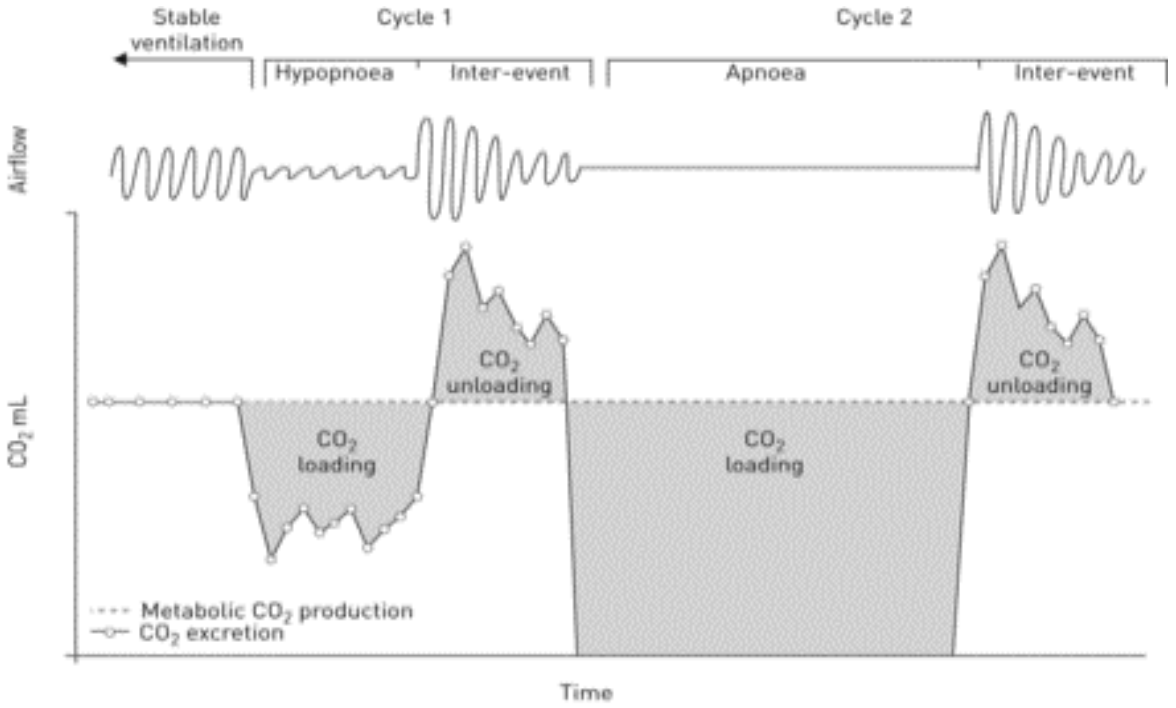


Figure 5 Illustration demonstrating carbon dioxide loading and unloading during short and long respiratory events (Berger et al., 2008)

Due to the potential role of upper airway obstruction during sleep to cause nocturnal hypoventilation and potentially diurnal ventilatory failure, it is necessary to measure the degree of OSA during sleep with a standard overnight sleep study. In addition a more sophisticated measure of the load on the upper airway from neck obesity can be gained from the pressure needed to open the airway during sleep. This is derived from the degree of auto continuous positive airway pressure device (autoCPAP) required to overcome upper airway load during sleep and reverse the apnoeas, hypoventilation and inspiratory flow limitation (Smith and Lasserson, 2009). These devices have employed breath-to-breath algorithms that raised or lowered pressure to maintain a patent airway. AutoCPAP devices have been marketed as having identical OSA-controlling efficacy to fixed-pressure CPAP while delivering lower

mean pressure, with the assumption that lower pressure application would be more comfortable, improve adherence and, thus, long-term patient outcomes (Teschler et al., 1996) Bench studies have indicated that autoCPAP devices variably respond to certain types of simulated hypopnoeas and demonstrate delays when responding to apnoeas (Rigau et al., 2006)

1.2.8 Hypothesis IV: Increased sleep-disordered breathing and nocturnal hypoventilation

I hypothesise that there is a difference in sleep-disordered breathing and nocturnal in individuals with OHS and normocapnic obesity which contributes to its pathogenesis.

1.2.9 Difference in ventilatory drive

In this section I will discuss the potential mechanism based on differences in ventilatory response to hypoxia and hypercapnia.

An experiment looked at the oxygen consumption of obese and lean individuals. Oxygen consumption (VO_2) was measured on sedated, and then anaesthetised, obese individuals and the results found were compared with those of lean subjects. The comparison showed the VO_2 required for spontaneous ventilation was substantially higher in the obese group (Zavala and Printen, 1984) and hence left less reserve in oxygen. Oxygen consumption (VO_2) and CO_2 excretion rates are increased in obesity.

Respiratory drive may have an important role in the pathogenesis of OHS in individuals. In studies which compared patients with OHS and normocapnic patients with morbid obesity, both groups were shown to have similar respiratory drives. One

of the standard measures of respiratory drive was used, using mouth occlusion over the first 100 milliseconds of inspiration (Weiner et al., 1998).

However, other studies have shown patients with OHS to have reduced ventilatory response to hypoxaemia and measurements of central ventilatory drive have also been shown to be lower in OHS patients compared to BMI matched subjects not in ventilatory failure (Han et al., 2001).

The unstimulated central drive to breathe during rest has been found to be higher in normocapnic subjects with morbid obesity than in normal-weight control individuals, presumably due to compensation for the increased mechanical load (Verbraecken and McNicholas, 2013). An impaired ventilatory response to hypercapnia is also a common feature of obesity-related hypoventilation (Verbraecken and McNicholas, 2013) as illustrated above, although hypercapnic ventilatory responses are highly variable between individuals, and are sometimes even within the normal range in patients with only mild hypercapnia.

In summary, it appears that ventilatory response to hypoxia is blunted in patients with obesity-related hypoventilation (Verbraecken and McNicholas, 2013).

The measurement of ventilatory control is fraught with difficulties. The very presence of chronic hypercapnia in a patient implies reduced ventilatory control unless the loading on the ventilatory system is very high. Any measurements of ventilatory control done in the presence of ventilatory failure are extremely difficult to interpret.

1.2.10 Hypothesis V: Difference in ventilatory response to hypercapnia and hypoxia

I hypothesise that there is a difference between ventilatory drive to hypercapnia and hypoxia in individuals with OHS and normocapnic obesity which potentially contributes to the pathogenesis of OHS.

1.2.11 Difference in Hormonal, nutritional and inflammatory measures

1.2.11.1 Role of hormonal, nutritional, inflammatory and cytokine measures

1.2.11.1.1 Leptin and adipocytes

The adipocytes within fat tissue are the source of several efferent peptide hormones. Two such hormones are leptin and adiponectin which both have important metabolic properties.

Leptin is a protein of the obese (ob) gene (Zhang et al., 1994). It is constituted of 146 amino acids and its concentration is proportional to body fat tissue. It is an adipocyte-derived cytokine predominantly produced in the white adipose tissue. However, low levels of expression are also detected in the pituitary gland (Morash et al., 1999), hypothalamus and skeletal muscle (Schwartz et al., 2000). Leptin is an appetite suppressant and is associated with satiety, weight control and obesity.

A mouse model with a genetic leptin deficiency was created to help better understand the hormone's function. In this mouse model, mice became morbidly obese and were shown to suffer with hypoventilation (Kalra, 2008) (Figure 3). The role of leptin was then further assessed following an infusion of replacement leptin in the mouse model. This showed responses with significant improvements in minute

ventilation and hypercapnic ventilatory response within three days of infusion (Tankersley et al., 1998) (Figure 6).

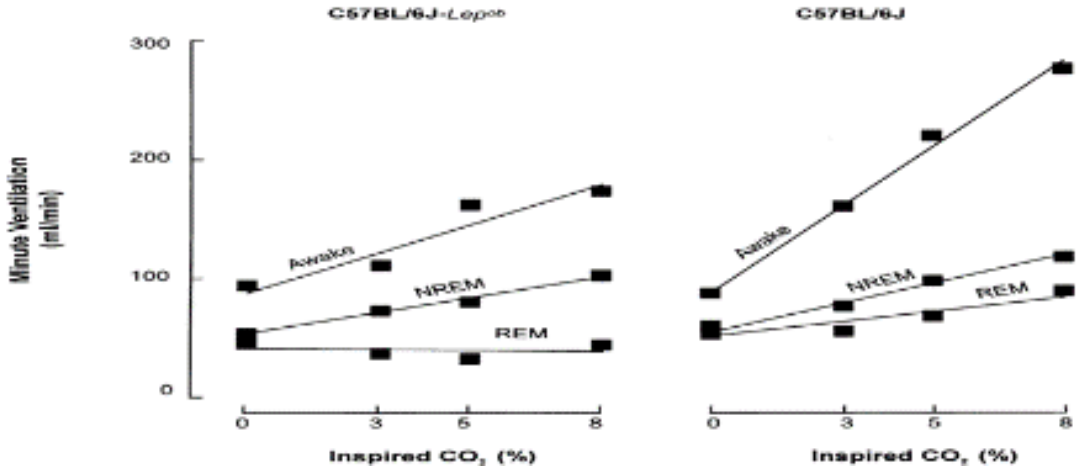


Figure 6: The relationship between minute volume and inspired CO_2 is blunted across all three sleep/wake states in one adult obese C57BL/6J- *Lep*ob (LACK LEPTIN) mouse compared with one adult lean wild-type mouse.

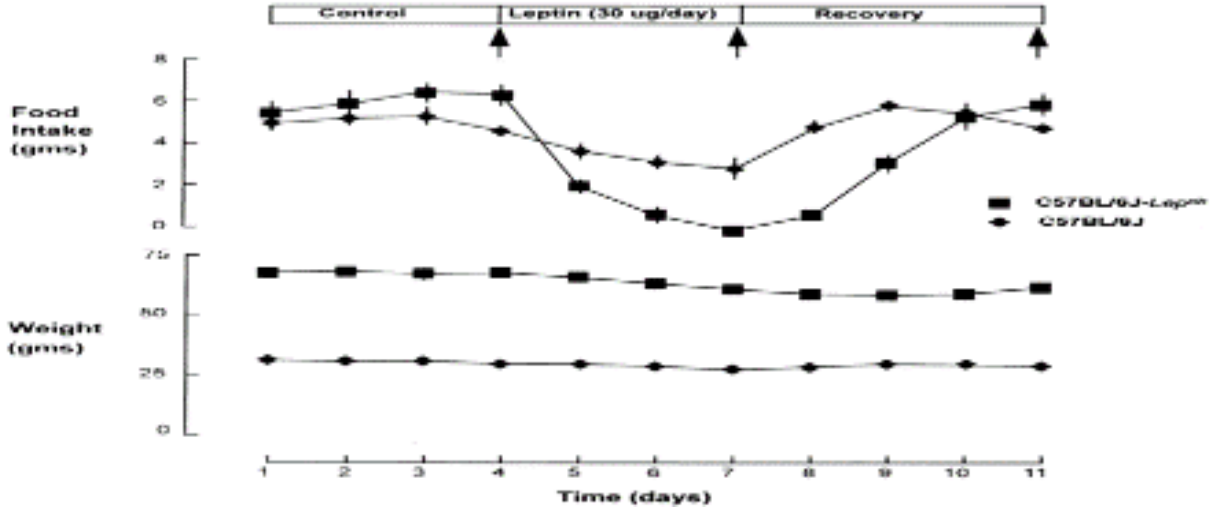


Figure 7: The effect of 3 d of leptin infusion (30 µg/d) on food intake and weight in eight C57BL/6J-*Lep*ob mice and in eight wild-type mice. The *upward arrows* mark the days on which ventilatory control data were collected. *Note*: mutant mice were weight-matched on days during which ventilatory data were collected for the leptin condition and the recovery condition. (Tankersley et al., 1998)

The role of leptin in humans is unclear and conflicting. Leptin has been shown to be associated with increased body fat and found to be higher in hypercapnic, versus eucapnic, patients with OSA, but with the same percentage of body fat (Tankersley et al., 1998). A larger study found leptin levels increased with increasing BMI and were higher in hypercapnic subjects (Kapsimalis et al., 2008). However, conflicting data showed the mean leptin level was lower in patients with OHS (but no sleep apnoea) than matched obese eucapnic subjects, and leptin levels actually increased after correction of hypercapnia with non-invasive ventilation (NIV).

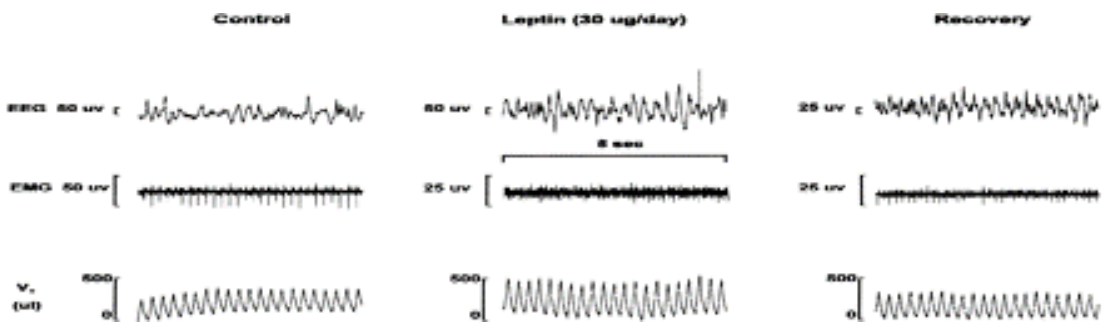


Figure 8**:** Stimulation of resting VT after leptin infusion (30 µg/d) in one C57BL/6J-*Lep*ob mouse. Sample traces were obtained while breathing 0% CO_2 in 40% O_2 during NREM sleep in control, leptin and recovery conditions. The tidal volume (VT), or volume change from trough to peak (inspiration), is larger during leptin infusion (373 µl) than during control (235 µl) or recovery (263 µl) conditions. (Tankersley et al., 1998)

1.2.11.1.2 Inflammatory and cytokine measures

Obesity is associated with systematic inflammation. Adipose tissue is no longer considered an inert tissue mainly devoted to energy storage, rather an active participant in regulating physiologic and pathologic processes including immunity and inflammation. Adipose tissue produces and releases a variety of proinflammatory and anti-inflammatory factors including adipokines, cytokines, and chemokines. Altered adipokine levels have been observed in a variety of inflammatory conditions. It appears to be associated with elevated levels of pro-inflammatory adipokines (Bastard et al., 2006) such as leptin and HS-CRP and decreased levels of anti-inflammatory adipokines such as adiponectin (Chin et al., 1999). HS-C-reactive protein (CRP), a member of the pentraxin family of proteins, is

an acute-phase reactant, increasing 1000-fold in response to ischemia and inflammatory conditions (Westhuyzen and Healy, 2000)

Adipose tissue, now considered an endocrine organ, produces inflammation and metabolism mediating cytokines. One such adipokine, adiponectin, may be an endogenous insulin sensitize, necessary for regulation of insulin sensitivity and glucose homeostasis (Tschritter et al., 2003)

Adiponectin is also thought to affect the respiratory system. This may be via affecting the small airways (Williams et al., 2012).

Obesity is a disease state characterized by chronic systemic low grade inflammation and associated inflammatory changes in the adipose tissue (Apovian et al., 2008).

Patients with OHS many have several stimuli that might increase the burden of chronic inflammation. One stimulus is hypoxia. In animal models and humans, hypoxia of the adipose tissue has been shown to be associated with local fat inflammation (Hosogai et al., 2007). It seemed reasonable to hypothesise that in OHS patients, compared to normocapnic obesity subjects, there could be an associated increase of systemic inflammation with an increased production of proinflammatory adipocytokines.

1.2.11.1.3 Nutritional measures

Vitamin D is a fat soluble vitamin that is either consumed in the diet or produced in the skin following sunlight exposure of the skin. Vitamin D is inactive and is hydroxylated twice, once in the liver and once in the kidney to make the active form of the vitamin, 1, 25 dihydroxyvitamin D (1, 25(OH)2D). Vitamin D is a key hormone in humans. Its deficiency is now considered a pandemic and has been implicated in

several diseases including cardiovascular morbidity and mortality (Siadat, Kiani et al., 2012; Abu El Maaty, Hassanein et al., 2013; Kienreich, Tomaschitz et al., 2013), metabolic syndrome and type 2 diabetes (Siadat et al., 2012).

Several clinical and epidemiological studies report that obese subjects have lower serum concentrations of vitamin D, and also show a negative correlation of vitamin D concentrations with BMI and waist circumference (Taheri, Saedisomeolia et al., 2012; De Pergola, Nitti et al., 2013). Hence, vitamin D may have a key role in the pathogenesis of obesity-related disease.

There is emerging evidence that vitamin D exerts a range of effects on skeletal muscle. Reports of profound muscle weakness and changes in the muscle morphology of adults with vitamin D deficiency have long been described. These reports have been supplemented by numerous trials assessing the impact of vitamin D on muscle strength and mass which falls in predominantly elderly and deficient populations. Vitamin D alters muscle metabolism, specifically its sensitivity to insulin, which is a pertinent feature in the pathophysiology of insulin resistance and type 2 diabetes (Girgis et al., 2013).

The presence of nutritional deficiencies in obesity may seem paradoxical (considering the excess caloric intake) but many studies have documented that several micronutrient deficiencies may be higher in prevalence in obese people, particularly in those suffering from extreme obesity (BMI > 40 kg/m^2) (von Drygalski and Andris, 2009) Generally, obesity is a risk factor for deficits of several micronutrients and, with respect to iron, the prevalence of deficiency has been reported to be 35%. Iron depletion prior to bariatric surgery (BS) among obese patients has been related to high levels of inflammatory markers and BS seems to

correct this iron depletion by means of a reduction in chronic inflammation (von Drygalski and Andris, 2009). In fact, there are several studies suggesting a potential link between obesity and altered iron metabolism (Cheng, Bryant et al., 2012).

1.2.12 Hypothesis VI: Differences in hormonal, nutritional, inflammatory and cytokine measures

I hypothesise that there is a difference in hormonal, nutritional, inflammatory and cytokine measures in individuals with OHS and normocapnic obesity subjects with the abnormal levels potentially contributing to the pathogenesis of OHS.

METHODOLOGY

2.1 Patient recruitment and study design

2.1.1 Subjects

This was an open cross-sectional study of obese subjects with and without conventionally defined OHS. The work was carried out in the Oxford Sleep Unit, Oxford Centre for Respiratory Medicine, Churchill Hospital, Oxford, UK, which is a National Health Service secondary and tertiary referral centre. Subjects were recruited and studied between June 2011 and September 2013. The study was registered prospectively with a global trials registry site (ClinicalTrials.gov NCT01380418). The study was approved by the Oxford Research Ethics Committee (Oxfordshire REC B 11/H0605/9).

After an initial approach, all subjects interested in participating in the study were invited to a screening appointment and written informed consent was obtained from all participants.

Of the eligible patients during the study period, subjects consented to enter the trial. The subjects who declined to participate did so mainly due to the time involved or distance to travel.

2.1.2 Study design

Obese subjects (BMI>30 kg/m^2) with or without conventionally defined OHS were included. Patients were referred from the following sources: (1) following a recent admission to hospital after a diagnosis of OHS had been made; (2) after referral to

the sleep and ventilation clinic for assessment of possible SDB and/or OHS; and (3) during assessment for bariatric surgery.

Participants were excluded for the following reasons:

(1) Currently being treated with respiratory stimulants or depressants, diuretics or theophylline

(2) Presence of obstructive lung disease (FEV_1/FVC ratio <70% predicted) or significant smoking history

(3) Other severe co-morbidities such as congestive cardiac failure, primary CNS or neuromuscular diseases or untreated hypothyroidism

(4) Currently on treatment for OHS including continuous positive airways pressure (CPAP) or non-invasive ventilation (NIV).

<table>
<tr><td colspan="2">Consent

Interview

Clinical examination

Based: Oxford Centre for Respiratory Medicine</td></tr>
<tr><td>Sleep study – VISI study

Fasting Overnight

Based: Oxford Sleep Unit</td><td>Hypothesis IV: Increased sleep-disordered breathing and nocturnal hypoventilation</td></tr>
<tr><td>Arterial blood gas

Fasting blood tests

Measurements of obesity

Ventilatory control

Lung function test (lying and seated)
Forced oscillation technique (lying and standing)

Muscle strength tests – quadriceps muscle testing, handheld dynamometer
Respiratory muscle: sniff, MIP/MEP

Muscle and fat biopsy

Based: Oxford Centre for Respiratory Medicine, Oxford centre for diabetes, endocrinology and metabolism</td><td>

Hypothesis VI: Difference in hormonal, nutritional, inflammatory and cytokine measures

Hypothesis I: Differences in measures of Obesity and its distribution

Hypothesis V: Difference in ventilatory response to hypercapnia and hypoxia

Hypothesis II: Differences in Lung function.

Hypothesis III: Differences in Muscle strength.</td></tr>
</table>

Figure 9: Flow-diagram showing subject pathway through research protocol

2.1.2.1 Demographic data

The following data were collected on all participants: age, sex, height, weight, BMI and resting SpO_2 measured with a standard pulse oximeter (Massimo UK, Hants, UK). Daytime somnolence was assessed using the Epworth sleepiness score (ESS) (Johns, 1991).

2.1.2.2 Arterial blood gases

Radial arterial blood gases were taken with the patient seated comfortably at between 8am to 10am and analysed immediately on a blood gas at 37°C.

One point auto-calibrations of the analyser were performed every ½ hour using a neural buffer and gases and two point calibrations every two hours using an additional acidotic buffer solution and gases. In addition, the blood gas machine was calibrated manually once a day using the neutral and acidotic aqueous solutions, all as recommended by the manufacturer.

2.2 Assessment of fat distribution

2.2.1 Skinfold measurements

2.2.1.1 Background

For more than three decades, prediction equations published by Durnin and Womersley (DW) in 1974 (Durnin and Rahaman, 1967) have been widely used to estimate percent body fat from caliper-measured skinfold thicknesses. The authors' thorough approach to equation development and provision of easy-to-use tables for estimation of percent body fat with any combination of four commonly used skinfolds

(biceps, triceps, subscapular and suprailiac) made this a seminal contribution to the field of body composition assessment. The equations were developed in a Caucasian population and were based on the linear relationship between the log of skinfolds and the hydrodensitometry-measured body density (Durnin and Rahaman, 1967).

2.2.1.2 Methods

All measurements were taken on the left side of the body with the subject seated on a stool. (Durnin and Rahaman, 1967)

The technique for skinfold measurements was the same for all positions. Harpenden Callipers were used with a constant spring pressure of 10g/mm. Subjects were asked to relax their muscles and measurements were taken on dry skin on the left side of the body. The skinfolds were grasped between the thumb and index finger, using the pads at the tip of the thumb and finger, and the skinfold was gently pulled away from the body. The callipers were placed perpendicular to the fold on the site marked, with the dial facing upwards, at approximately 1cm below the finger and thumb.

While maintaining a grasp of the skinfold, the callipers were allowed to be released so that the full tension was placed on the skinfold. The dial was read to the nearest 0.5mm, 1 to 2 seconds after the grip had been fully released.

The callipers were not placed too close to the body nor too far away at the tip of the skinfold. A minimum of 3 measurements were taken at each site and repeated if tests varied by more than 1mm. The final value recorded was the average of the readings.

The position of the patient and sites selected were as follows:

2.2.1.3 Triceps skinfold

The subject was asked to stand with the left arm (right arm if left handed) hanging freely. The lower border of the acromial process of the scapula was felt. This was located as the most outer and the lowest border of the flat bony prominence over the shoulder. The upper end of the measuring tape was placed over the point and ran down along the arm to touch the tip of the elbow joint. The distance was read between these two points and with a ball-point pen a mark was made mid point between them. The position of this mark was referred to as the standard midpoint.

2.2.1.4 Biceps skinfold (seated)

The subject sat on a stool and placed their arm on their thigh in the supinated position. The standard midpoint to the anterior aspect of the arm was extended. The skinfold was picked up, as above, 1cm above the midpoint directly above the centre of the cubital fossa.

2.2.1.5 Subscapular skinfold (seated)

The subject sat on a stool with their chest bare. The subject was felt for the inferior angle (lowest point) of the left scapula and a fold of skin was picked up just below it, pointing slightly downwards and outwards.

2.2.1.6 Suprailiac skinfold (seated)

The iliac crest was felt for on the left side with the subject seated on a stool. A skinfold was picked just above the iliac crest in the mid-axillary line.

2.2.1.7 Chin (seated)

The subject looked straight ahead, eyes level. Skinfold mid-chin.

2.2.1.8 Midaxillary skinfold (seated)

Subject was asked to flex their elbow and move arm and shoulder backwards. The horizontal skinfold mid-axillary line level with the Xiphi-sternal junction was pinched.

2.2.1.9 Males juxta-nipple (seated)

The diagonal skinfold was pinched halfway between the nipple and the anterior axillary (underarm) crease.

2.2.1.10 Front forearm (seated)

The vertical fold at top of forearm was pinched.

2.2.1.11 Back forearm (seated)

The vertical fold below elbow was pinched.

2.2.1.12 Abdominal (standing)

The vertical fold 2cm midpoint of navel was pinched.

2.2.1.13 Iliac crest (standing)

The top of iliac crest was located in a mid-axillary line. The diagonal skinfold was pinched 1cm above.

2.2.1.14 Buttock (standing)

The vertical skinfold in the upper outer quadrant of the buttock was pinched.

2.2.1.15 Calf (seated)

Seated with a knee bent 90°, a pinch of the vertical skinfold on the inner calf (widest part) was taken.

2.2.1.16 Circumferential measurement

A tape measure was used to measure the neck, upper arm, lower arm, hip, waist, thigh and calf circumferences.

2.2.2 DXA imaging

2.2.2.1 Background

Among several methods for studying in vivo human body composition, dual-energy X-ray absorptiometry (DXA) has emerged as one of the most commonly used clinical standards (Ellis, 2000).

To understand how DXA works it is important to appreciate that the main component of the device is an energy source. Typically, the energy source produces photons at two different energy levels, 40 and 70 kiloelectron volt (keV), which pass through

various different tissue types in the body and attenuate at rates related to their elemental composition. Bone is rich in highly attenuating minerals, calcium and phosphorous, and is readily distinguishable from soft tissues. The unique elemental profiles of bone, fat and non-bone lean tissue allow for visualization and separate analysis of each tissue type.

Two unfortunate limitations of DXA scanners have been the weight limitation of the scanning bed (typically BMI <40) and the width of the scanning area (usually <60 cm). When an individual's body dimensions exceed these limits, the accuracy of the measurement is typically compromised. This limitation has led to the development of a technique to estimate total-body composition from only being able to accurately perform a half-body scan. Half-body scans can accurately predict whole-body percent body fat, fat mass and non-bone lean mass in the thick total-body scanning mode.(Tataranni and Ravussin, 1995) Excellent precision of the DXA has also been reported as within-subject test–retest reproducibility (Tataranni and Ravussin, 1995).

Methods

Regions:

(1) Visceral

(2) Android

(3) Gynoid

(4) Total fat body composition, including total body fat (BF) were measured by DXA (GE Lunar Prodigy; Madison, WI, USA; 14.1 enCore software version with CoreScanTM) using automatic total-body scan mode.

The regions of interest (ROI) for regional body composition were defined using the software provided by the manufacturer.

The android region (Android), located over the abdomen, is roughly 10cm in height, extending from the iliac crest toward the head for 20% of the distance from the iliac crest to the base of the skull.

The hip ROI (Gynoid) was defined superiorly below the pelvis cut line as 1.5 times the height of the android ROI, inferiorly below the superior line by two times the height of the Android ROI and laterally at the outer leg cut lines.

Ethanol and water bottles with a volume of 8l, simulating fat and fat-free soft tissues respectively, were scanned monthly as soft-tissue quality control markers.

The conventional DXA trunk ROI included chest, abdomen and pelvis.

1. Visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT)

Recently, a new DXA-based VAT measurement approach, CoreScan, has been developed. The CoreScan algorithm computes VAT mass and volume within the android region of a total body DXA scan by subtracting SAT from total android fat.

The software algorithm works through detection of the width of the subcutaneous adipose tissue (SAT) layer on the lateral part of the abdomen and the anterior–

posterior thickness of the abdomen, which can be determined using X-ray attenuation (Figure 10).

Visceral fat pads all internal vital organs such as the stomach, kidney, heart and pancreas.

Increasing evidence suggests that VAT is an important measurement to make in assessing different phenotypes of obesity because its ectopic accumulation, particularly in the liver, is strongly associated with risk of metabolic diseases (Ross et al., 1995).

DXA regions of interest (ROIs) are highly correlated with total VAT as measured with MRI.

This new DXA software application provides a high degree of precision and accuracy in a single measurement (Kaul et al., 2012).

VAT computed by DXA has been validated using CT across a broad population of adults between 18 and 90 years of age and a wide range of BMI (Molarius and Seidell, 1998).

The reported relationship between waist circumference and VAT varies by gender and ethnicity, with the correlation being 0.5 to 0.7 with large inter-operator variability (Lear et al., 2007).

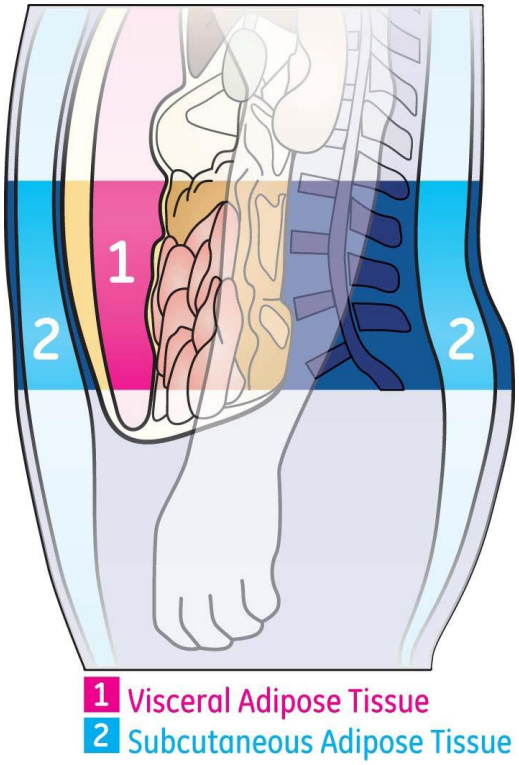


Figure 10: Diagram illustration distribution of visceral adipose tissue and subcutaneous adipose tissue

2.2.2.2 Technical concerns

There are some important factors to consider when analysing specific DXA ROIs, such as posture, overlap of upper limbs and trunk, vertebral conditions, image resolution and technician skill. If a subject is positioned incorrectly during the scan (i.e. not parallel to the DXA table edge), it is more difficult to define the rectangular points. When the upper limbs are positioned in close contact with the trunk, measuring an ROI may be affected by the overlapping regions. This potential error is especially problematic in obese subjects with a BMI of greater than 40.

2.2.3 Magnetic Resonance Imaging (MRI)

2.2.3.1 Background

It has become increasingly clear that the distribution of body fat is an important determinant of the health risks associated with obesity (Kissebah et al., 1989). Most of the research in this area is focused on anthropometric definitions of fat distribution, particularly the waist-to-hip circumference ratio and various trunk and limb skinfold thicknesses. It was suggested that MRI could be another way for determining fat distribution (Foster et al., 1984).

As a patient is placed in the MRI unit, samples of the body's protons (hydrogen atoms) align themselves to equilibrium with the external magnetic field. A radiofrequency (RF) pulse that flips the precession of the hydrogen proton away from its equilibrium position is then transmitted to the patient's body. After the RF pulse is turned off, the protons "relax" to equilibrium. Longitudinal relaxation time (T1) and transverse relaxation time (T2) vary for different body tissues, and this variation provides the basis for imaging these tissues. This information can help characterise lesions and, because MRI offers excellent contrast between various tissues, it is particularly sensitive at differentiating adipose tissue. On MRI Tl-weighted sequences, fat is readily identified by a strong signal. Use of phased array coils and stronger magnetic gradients shortens scan times somewhat and will partially overcome breathing artifacts.

MRI has the advantage over CT scanning of not exposing the subjects to ionizing radiation. Images are produced by placing the subject in a strong magnetic field and then exposing the subject to radio frequency.

MRI has become an established method for the in vivo quantification of fat tissue (Ross et al., 1992).

MRI can differentiate SAT and VAT with multi-dimensional images. However, only MRI, with its many sensitive and flexible contrast mechanisms, can quantify ectopic fat. MRI involves no ionizing radiation which allows for indefinite repeatability in longitudinal studies and in children. It is emerging as a comprehensive tool for fat quantification.

2.2.3.2 Method

In practice, multiple-slice MRI is the preferred options for volume calculation.

Accuracy is often high with MRI, but defining different adipose tissue deposits depends on the settings of the MRI scanner.

The intensity of the magnetic resonance (MR) image depends on four parameters: nuclear density, two relaxation times, called T1 and T2, and motion of the nuclei within the imaged area. As the nuclear density increases, increasing numbers of nuclei align with the magnetic field, producing a more intense MR signal. Abnormal soft tissue can be better differentiated through measurement of these four parameters than through any other imaging technique. In addition, scans can be performed in any plane because the spatial orientation of the image is determined by manipulation of magnetic fields by flowing blood and, in this study, to look at the distribution of adipose tissue.

T1 weighted image (also referred to as T1WI) is one of the basic pulse sequences in MRI and demonstrates the differences in the T1 relaxation time of tissues.

Fat has a large longitudinal and transverse magnetization and appears bright on a T1 weighted image. Thus, water has a low signal and appears dark.

Subjects were placed in an eight channel phased array head coil and MR images were taken in a 3.0 T 750 MRI Scanner (General Electric Healthcare, Milwaukee, WI, USA).

Each acquisition took approximately 3 minutes. The areas scanned were the neck, chest wall, abdomen and thigh.

The data was interpreted with the observer blinded to the subject.

The abdominal compartment was defined as extending from the superior aspect of the xiphoid process to the most inferior slice depicting the L5-S1 interspace. MR images were obtained in 1cm contiguous intervals (slice thickness of 1cm) throughout the abdominal compartment. Analysis of the abdominal MR images was performed in the Sleep Imaging Center at the University of Pennsylvania. Using image and volumetric analysis software (Amira 4.1.2, Visage Imaging Inc., and San Diego, CA), each slice of the abdominal MRI scan was manually examined.

Automated thresholding and manual segmentation by the region growing tool were used to segment SAT and VAT volumes from other abdominal anatomy by pixel valuation. Anatomical landmarks were identified and adipose tissue beds were labelled and segmented. Inter-muscular adipose tissue was included. An example of the analysis protocol is seen in Figure 9

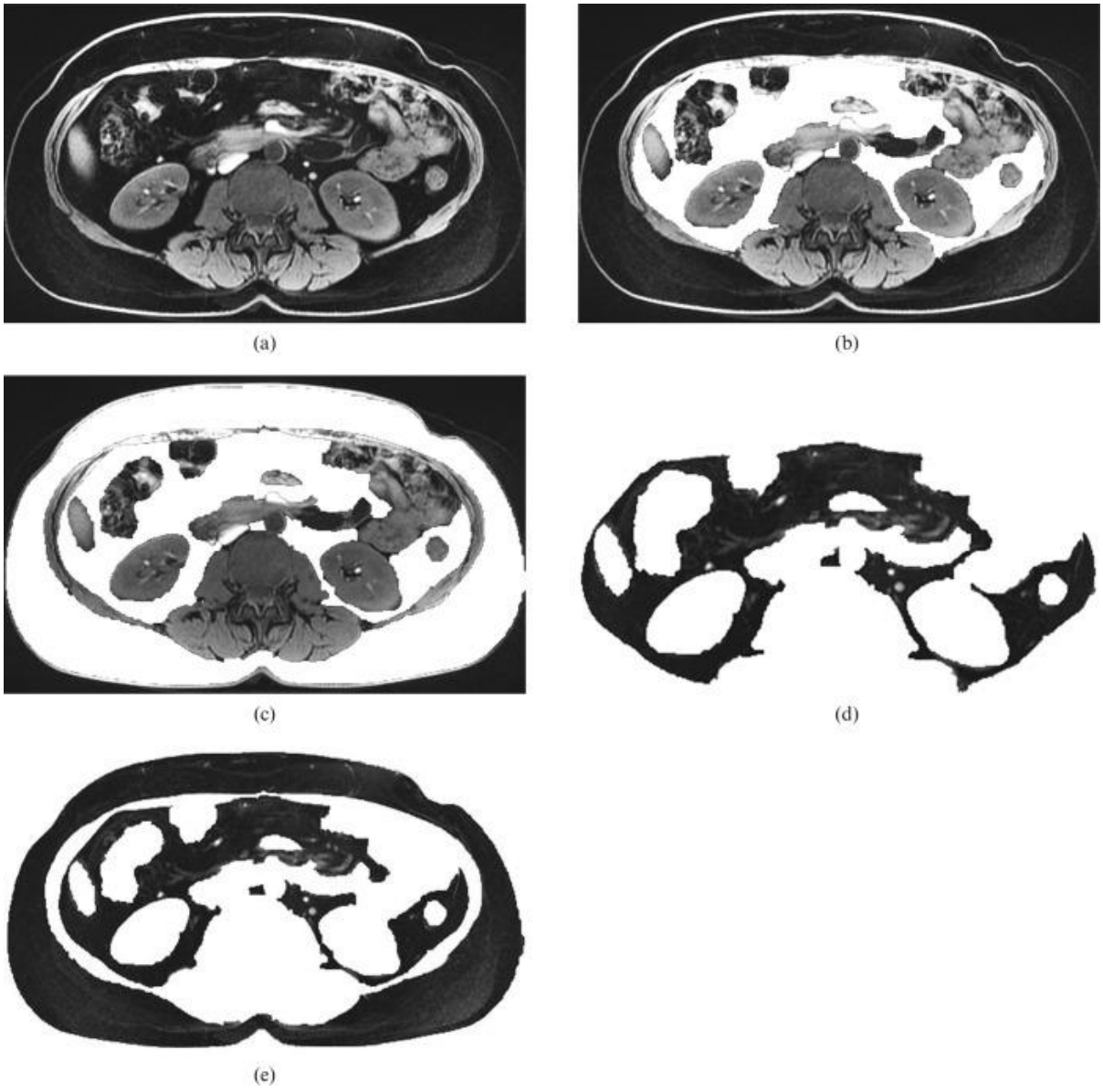


Figure 11 (Nagai et al., 2008) (a) Axial gradient-echo fat-saturated three-dimensional MRI (repetition time (ms)/echo time (ms)) of the abdomen at the level of the L3 vertebral body. (b) Visceral and (c) total fat components are highlighted. (d) Visceral and (e) total fat components are then extracted. The percentage of visceral adipose tissue is then calculated (Shuster et al., 2012).

2.2.4 Bioimpedance

2.2.4.1 Background

Compared to DXA, bioimpedance analysis (BIA) has been shown to provide a good degree of accuracy for estimating body composition in various populations of healthy subjects with stable hydration levels and within the normal range of body fat (Norgan, 2005). BIA is an accessible, safe and cost-efficient method that avoids exposure to radiation and has been widely used to measure body composition in clinical populations (Kyle et al., 2004). However, BIA lacks specificity and accuracy because it is based on differences in resistance when an electrical current is conducted through fat and lean components of the body. Thus, prediction equations are used to determine fat-free mass. While BIA can estimate whole-body fat content, recent attempts to assess the amount of abdominal subcutaneous and visceral fat by BIA indicated significant correlations when compared with precise imaging techniques such as CT (Nagai et al., 2008). BIA calculates total fat mass by subtracting fat-free mass from body weight. Lean tissue measurements are influenced by hydration status, which is often a problem in clinical populations. If lean tissue measurements by BIA are imprecise, these errors will also confound fat measures. These limitations are the probable basis for the discrepancies that have been identified with dual energy X-ray absorptiometry (DXA) total body fat comparisons with BIA (Mourtzakis et al., 2008).

2.2.4.2 Method

The BodyStat-1500 machine (Bodystat Ltd, Isle of Man, British Isles) was used for impedance. The height and weight of the subject was measured accurately and recorded.

The patient was asked to remove their right shoe and sock and lie down on a bed with their arms and legs spread slightly apart so that no part of the body was touching another.

Self-adhesive electrodes were applied as follows:

1. Behind the knuckle of the MIDDLE finger of the RIGHT hand.
2. On the dorsal surface of the RIGHT wrist mid-way between the radial and ulnar heads.
3. Behind the 2nd toe of the RIGHT foot at the level of the metatarsal-phalangeal joint.
4. In the midline on the anterior aspect of the knuckle of the RIGHT foot at the level of the medial and lateral malleoli.

The Bodystat Leads were applied as follows:

1. The red leads were attached to the electrodes by the toe and the finger i.e. RED WAS DISTAL
2. The black leads were attached to the electrodes at the ankle and wrist i.e. BLACK WAS PROXIMAL

2.2.5 Assessment of fat biology

2.2.5.1 Fat biopsy technique and sampling

2.2.5.1.1 Background

In obesity, oxygen supply cannot meet the demand of expanding adipose, resulting in relative hypoxia within adipose tissue, increased lactate production and hypoperfusion in both obese human and animal models (Regazzetti et al., 2009). Hypoxia was found to cause insulin resistance in adipocytes and human subcutaneous abdominal adipocytes (Regazzetti et al., 2009). In the obese mouse model, hypoxia occurs specifically in white adipose tissue and the response to adipose hypoxia may lead to insulin resistance (Yin et al., 2009). White adipose tissue (WAT) distribution greatly affects metabolic risk. Increased intra-abdominal/visceral fat is associated with a high risk of metabolic disease, whereas increased subcutaneous fat in the thighs and hips exerts little or no risk of metabolic disease. Recent evidence indicates that these functional differences may be developmental in origin and perhaps to the degree of infiltration of these different fat depots with other cells, which release cytokines that contribute to the insulin resistance seen in obesity.

HIF1, the main mediator of the hypoxia response in adipose tissue, is almost undetectable in lean mice but significantly increased in obese mice resulting in induction of the HIF1 target genes Glut1 and Pdk1 (Ye et al., 2007). Moreover, overexpression of a constitutively-active HIF1α in adipose tissue leads to glucose intolerance and insulin resistance (Makowski et al., 2001). As a major hypoxia-responsive gene, the protein abundance of HIF1α and its transcriptional activity in

adipose tissue are elevated in obese adipose tissue. It has been postulated that local hypoxia instigates macrophage infiltration and inflammation in obese adipose tissue through activation of HIF1α.

2.2.5.1.2 Method

Subcutaneous adipose tissue from the abdominal wall was taken at the level of the umbilicus. The subject was fasted.

The subject was asked to lie on their back. The skin was exposed and cleaned with betadine solution, then patted dry with sterile dressing towel. A sterile fenestrated was draped over the biopsy site. Lignocaine (10ml of 1% lignocaine) was injected in a fan-like pattern into the biopsy site with a grey needle (size 27G). Five minutes were allowed for the area to become anaesthetised. 10ml of normal saline was put into a 60ml syringe. A sterican biopsy needle (14G) was fitted to a 60ml syringe. The needle was passed through the skin and into the fat. Suction was applied by pulling the plunger of the syringe out and inserting a 10cm cryovial between the end of the barrel and the end of the plunger.

The needle was pushed back and forth within the anaesthetised subcutaneous tissue.

Blood-stained adipose tissue appeared in the syringe. When 5mls of fluid were collected inside the syringe, the needle was withdrawn. This normally took 1-2 minutes.

Firm pressure was applied with a sterile dressing to the biopsy area for at least 15 minutes.

The syringe was transferred with the biopsy to the bench. The plunger was pulled out of the back of the syringe and the contents poured onto a sterile gauze stretched over a beaker. The syringe was washed with sterile 0.9% sodium chloride and poured onto the gauze with the biopsy; the sample was washed with the syringe of 0.9% sodium chloride previously drawn up. Any small blood clots were removed with forceps.

A piece of aluminium foil approximately 5cm square was balanced and set to zero. A sterile spatula or forceps were used to place 150mg to 200mg of the cleaned adipose tissue sample onto the foil. This was dropped into liquid nitrogen. The samples were then transferred to a freezer maintained at -80°C. The samples were then removed and transferred to the Oxford Centre of Diabetes, Endocrinology and Metabolism.

2.3 Assessment of lung function

2.3.1 Pulmonary function testing

2.3.1.1 Background

Obesity causes a range of spirometric abnormalities. Even in mild obesity, results of spirometry might be normal or might suggest a restrictive process, with a symmetric reduction in FEV_1 and forced vital capacity (FVC) (Collins, Hoberty et al. 1995).

2.3.1.2 Method

All lung function testing was performed in the Lung Function Department of the Oxford Respiratory Unit, using standard software (Jaeger USA; Yorba Linda, CA).

Testing was performed in either the lying or seated position and the position was recorded. The patient's age, height and weight were recorded for use in calculation of the reference values. Long and short-acting bronchodilators were avoided prior to testing. Ambient temperature, barometric pressure and time of day were recorded.

Each study was performed at the same time of the day for every study patient.

Spirometry: Forced inspiratory and expiratory flow loops and lung volumes were performed (Miller et al., 2005) as per the ERS/ATS guidelines

Predicted values were taken from the published data of a European Respiratory Society working party (Miller et al., 2005).

2.3.2 Forced oscillation technique

2.3.2.1 Background

Impulse oscillometry system (IOS) is a largely non-invasive method for determination of respiratory mechanics. Impulse oscillometry measures both small and large airways resistance and resonance capacitance of the lung. Its main advantage is its ability to perform measurements in a non-invasive, relatively effort-independent and minimally-intrusive manner during spontaneous normal tidal breathing (Goldman et al., 2005).

IOS can be best explained from imagining forcing air in and out of the mouth and then measuring the pressure at the mouth that would result. There will also be some dynamic components. For example, if the airway resistance were high, then there would be some inertia to moving the air, and the pressure would transiently rise until the airflow got going. So, if all you could do was measure the pressure oscillations

while you oscillate the air in and out of the mouth, then what you get will be a complicated combination of all these three components – the actual airway resistance, the effect of inertia and the effect of the lungs' stiffness. The forced oscillation system has as its primary data the instantaneous plot of pressure and flow during sinusoidal oscillations of air at the mouth.

The higher the pressure required to produce flow, the higher the impedance to flow that must be present. Because the relationships are not linear, the maths only works when small oscillations are applied. If there were no out of phase components to the pressure/flow relationship, then it would simply be looking at airway resistance (R, also known as the real component). Because of these components that influence the relationship between pressure and flow, the overall relationship of pressure divided by flow is called impedance (or Z) rather than resistance (R). It is possible to derive from these overall impedance (Z) measurements (made during forced oscillations at the mouth) and not only ordinary flow resistance (R), but also the reactance (X, also known as the imagery component). Reactance is what causes the out of phase components and is called this because it is due to the system examined *reacting* to the pressure oscillations being applied.

$$Z \text{ (impedance)} = R \text{ (resistance)} + X \text{ (reactance)}$$

Reactance itself is made up of two components: elastance (the inverse of capacitance or compliance) describes the pressure required to change volume, thus representing lung and chest wall dispensability or compliance; and innertance, described as the "extra" pressure to accelerate flow, which produces a positive out of phase component. The more innertance, the more positive the reactance value.

Let us first consider the in phase variation in resistance with frequency (which has not been referred to yet). In a normal person, the majority of airflow is in the upper airway, and indeed extra-thoracic due to the larynx. This affects resistance at all frequencies and thus varies little across the frequencies used in IOS. The relationship between R and frequency is therefore relatively flat in normal subjects. Increased resistance in the lower airways with disease tends to affect the pressure/flow relationship mainly at low oscillation frequencies.

Thus at low oscillation (5HZ), the resistive component reflects overall resistance, both upper and lower. But at higher frequencies (20Hz), it reflects mainly central and upper airway resistance.

Measuring R5 is a sensible choice to describe the overall changes in total resistance. The work the inspiratory muscles has to do to overcome increases in both upper and lower airway resistance, is presumably worse when lying down. One could then use R20 to look at the upper airway and R5-R20 to look at the lower airway contributions.

An extra measure is to look at the change in R5 within a breath (Vt). Erect, one would expect little within-breath change, as the FRC is not so close to residual volume (and closing volume) when the resistance rises sharply; supine, one would expect a big rise in R5 when exhaling, to the new lower FRC.

Assessment of distal airways has been elusive in traditional testing techniques due to their large aggregate cross-sectional area and minimal contribution to total airway resistance (Cosio et al., 1978); (Macklem, 1998) (Mead, 1970). Large and small airways mechanics can be inferred from responses at high (20 Hz) and low (5–15 Hz) frequencies, respectively.

Low-frequency oscillations at the mouth are transmitted to the lung periphery while those at 20Hz and higher frequencies are limited to larger airways (Frantz and Close, 1985).

2.3.2.2 Method

Impulse oscillometry (IOS) was performed utilizing the Jaeger impulse Oscillation System (Jaeger USA; Yorba Linda, CA). First, measurements were obtained at baseline FRC during tidal breathing in the seated position. Subjects used their hands to support their cheeks, and breathed normally through a mouthpiece that stabilized the tongue position to minimize oral resistance.

The mouthpiece was connected to a pneumotachometer and small pressure oscillations were “forced” by a loudspeaker attached to the other end of the tube. Respiratory resistance (R) and reactance (X) were calculated from oscillatory components of flow and pressure (Goldman et al., 2005) at 5–35 cycles/s (Hz).

A minimum of 3 trials of 30 seconds’ duration were performed. Furthermore, each individual’s IOS measurements were compared with predicted values.

Parameters reported included:

R5 and R20 are measures of resistance at oscillation frequencies of 5 Hz and 20 Hz.

For the purpose of evaluating properties of the respiratory system, assumptions were made based on the models of Dubois, Otis and Mead (Dubois et al., 1956, Otis et al., 1956, Mead, 1970).

AX was measured to reflect non-uniform distribution of airflow in distal airways, and X5 was measured to reflect dynamic elastance. X5 correlates with dynamic lung compliance (Goldman et al., 2005) (Macklem and Mead, 1967, Dubois et al., 1956);(Otis et al., 1956) (Lutchen et al., 1994).

Three trials of FO (consisting of 30 seconds of tidal breathing at FRC) were performed. Force oscillation tracings were inspected to check that correlations (coherence) among pressure, flow phase and amplitude appeared acceptable (coherence >0.8). Mean values from the three trials for each oscillometry index were calculated and used for analysis.

2.4 Assessment of lung function, ventilatory capacity and muscle strength

2.4.1 Non-invasive muscle testing

2.4.1.1 Background

In contrast to the limbs, respiratory muscle strength cannot be measured directly but is inferred from pressure measurements. The simplest measurements of global respiratory muscle strength may be measurement of pressures in the upper airway (in the nose or mouth with the glottis open) during maximal manoeuvres. Upper airway measurements have the advantage of being non-invasive but only global volitional measures of muscle strength may be made, and airway narrowing may lead to poor pressure transmission.

The principal advantage of volitional tests is that they give an estimate of inspiratory or expiratory muscle strength, are simple to perform and are well tolerated by

patients. Passage of balloon catheter systems into the oesophagus and/or stomach is not usually required.

However, it can be difficult to ensure that the subject is making a truly maximal effort. Although normal subjects can potentially activate peripheral and respiratory muscles fully during voluntary efforts (Gandevia and McKenzie 1985) even experienced physiologists cannot always do this reliably for respiratory efforts (Bigland-Ritchie, Furbush et al. 1992) and naive subjects have even greater difficulty (Allen, Gandevia et al. 1995).

Thus, it is hard to be certain whether low mouth pressure measurements truly represent reduced strength, or merely reduced neural activation. Indeed, there may be some activation of agonist muscles simultaneously (De Troyer, Legrand et al. 1998). However, in practice, a normal result can be of value in precluding clinical weakness.

The relationship between the tension (force) generated by a respiratory muscle (strength) and the pressure produced in the thorax or mouth is complex. The diaphragm is a curved structure and acts as a piston so that the pressure or force per unit area output is only indirectly related to muscle tension. In addition, the mechanical linkage of each individual respiratory muscle within the chest wall and with other inspiratory or expiratory muscles influences the net pressure produced. Thus, even though activation may be maximal, the pressure produced is derived from a complex set of interactions within and between muscles and the chest wall and its contents. Nevertheless, it is the pressure developed by the inspiratory muscles that drives ventilation and, in spite of the many assumptions, these

measures can usefully reflect global respiratory muscle strength for clinical evaluation as well as physiological studies.

2.4.1.2 Sniff Tests

2.4.1.2.1 Background

A sniff is a short, sharp voluntary inspiratory manoeuvre performed through one or both unoccluded nostrils. It involves contraction of the diaphragm and other inspiratory muscles. To be useful as a test of respiratory muscle strength, sniffs need to be maximal, which is relatively easy for most willing subjects, but may require some practice.

The sniff was described in 1927 as a radiological test of diaphragm paralysis because, in normal subjects, it was associated with crisp diaphragm descent during inspiration.

Esau and co-workers (Esau, Bye et al., 1983) suggested that a short, sharp sniff would approximate the diaphragm contraction elicited by a brief stimulation of the phrenic nerves (Esau, Bellemare et al., 1983; Esau, Bye et al., 1983). Miller and co-workers (Miller, Moxham et al., 1985) showed that normal subjects generated greater diaphragmic pressure (Pdi) during maximal sniffs than during maximal static inspiratory efforts, perhaps because the manoeuvre achieves rapid, fully coordinated recruitment of the inspiratory muscles (Laroche, Mier et al., 1988).

The detailed respiratory mechanics of this dynamic manoeuvre have been little studied, but numerous studies using the sniff in normal subjects and patients have found it to be a robust measure. The nose appears to act as a Starling resistor, so

that nasal flow is low and largely independent of driving, oesophageal pressure (Pes) (Pertuze, Watson et al., 1991). Pdi measured during a sniff (Pdi, sniff max) reflects diaphragm strength

It has been suggested that pressures measured in the mouth, nasopharynx or one nostril give a clinically useful approximation to oesophageal pressure during sniffs (Koulouris, Vianna et al., 1989; Heritier, Rahm et al. 1994) These measurements do not require the passage of oesophageal or gastric balloons and they are easier for operator and subject. However, pressure transmission may be impaired, particularly when there is significant disease of the lungs (Uldry and Fitting, 1995).

The force–length relationship and the varying contribution of airway resistance (Prs), maximal inspiratory pressure (PImax) and maximal expiratory pressure (PEmax) vary markedly with lung volume (Black and Hyatt 1971) so subjects find it easier to maximize their inspiratory efforts at low lung volumes and expiratory efforts at high volumes. By convention, therefore, and to standardize measurement, PImax is measured at or close to residual volume (RV) and PEmax at or close to total lung capacity (TLC). It is a useful voluntary test for evaluating diaphragm strength in the clinical setting, giving equal or greater pressures than maximal static efforts (Miller, Moxham et al., 1985).

2.4.1.3 Method

Flanged mouthpieces were used and are readily available in pulmonary function laboratories. These mouthpieces are easy for patients to use, especially those with muscular weakness. The test was performed with the patient strongly urged subjects to make maximum inspiratory (Mueller manoeuvre) and expiratory (Valsalva

manoeuvre) efforts at or near RV and TLC, respectively. Subjects were normally seated and nose clips were not required. Because these are unfamiliar manoeuvres, careful instruction and encouraged motivation was essential. Subjects often needed coaching to prevent air leaks around the mouthpiece and to support the cheeks during the expiratory efforts; this was helped by having them pinch their lips around the mouthpiece. Once the operator was satisfied, the maximum value of three manoeuvres that varied by less than 10% was recorded.

For measurement of maximal sniff pressures, patients were encouraged to make maximum efforts.

Subjects were instructed to sit comfortably and to make sniffs using maximal effort. Detailed instruction on how to perform the manoeuvre was not necessary, and found to be counterproductive.

However, subjects were encouraged to make maximal efforts with a rest between sniffs. Most subjects achieved a plateau of pressure values within 5–10 attempts and patients required little practice.

This method is relatively reproducible and has a smaller range of normal values than mouth pressures (Miller, Moxham et al., 1985)

2.4.2 Peripheral muscle testing

2.4.2.1 Quadriceps Strength

2.4.2.1.1 Background

The quadriceps muscle is of significant functional importance and may be affected by disuse, local disorders or systematic problems. The force generated by a contracting muscle depends upon a number of factors, including the number of fibres stimulated and the muscle length. It is important that the same procedure is always followed to obtain a Quadriceps Maximal Voluntary Contraction (QMVC) to ensure consistent, reproducible and standardised measurements. Although lower limb muscles are largely responsible for limiting activities such as walking and going up inclines or flights of stairs, it is recognized that the activities of daily living (ADL) that involve the upper limbs, especially those that involve unsupported upper limbs (upper limbs at shoulder level without support), are also poorly tolerated by patients with chronic lung conditions such as COPD (Dolmage et al., 1992).

In the chronic lung condition COPD, the degree of functional impairment appears to differ between the upper and lower limbs and can be explained by the compartment theory. In brief, respiratory muscle function and lower limb muscle function are impaired by structural and functional changes. Upper limb muscle structure and function are relatively preserved which is due to the maintenance of ADLs that involve the arms or due to the use of some of those muscles during the work of breathing (Gea et al., 2001).

Structural changes in the lower limb muscles result in reduced overall exercise capacity, progressing to deconditioning caused by a reduction in the performance of physical ADLs in order to avoid dyspnoea (Gosker and Schols, 2008). The performance of physical ADLs appears to be a determining factor in muscle changes (Doucet et al., 2010). However, such changes are also thought to be modulated by

other local and systemic factors, such as inflammation, oxidative stress, pharmacological treatment and nutritional disorders.

2.4.2.2 Method

Precautions were taken prior to the procedure. Patients did not have the maximal voluntary contraction recorded on the right leg if they reported any known confounding musculoskeletal or neuromuscular pathology affecting the right leg. This included:

1) Severe osteoarthritis of either the right hip or knee joint causing pain when positioned or performing the manoeuvre.
2) Compressive spinal pathology affecting the lumbar nerve roots.
3) Polyneuropathy or mononeuropathy affecting the femoral nerve.
4) Previous stroke (thrombo-emboli or haemorrhagic).
5) Critical peripheral vascular disease affecting proximal leg blood flow.

Quadriceps strength of the right leg was measured using an apparatus similar in principle to that described by Edwards and co-workers (Edwards, Hill et al., 1975).

The procedure was explained to the subject. The subject rested their hands on top of their thighs to avoid the use of their upper limbs and to increase force. Supervision prevented the patient using other muscle groups to increase contraction force, such as holding on with hands, arching the back, lifting the buttocks off the chair or pushing the pelvis against the hip strap. The subject was asked to perform a warm-up consisting of four contractions at approximately 50% effort, followed by four

contractions at approximately 75% effort, before commencing QMVC measures. Strong encouragement was provided for maximal effort. The maximal measurement was maintained for several seconds. There was at least a twenty second interval between maximal contractions to allow for recovery. Five QMVC contractions were performed, with the best three efforts recorded. If the force was increasing, additional manoeuvres were permitted.

The subjects were studied seated in the quads chair, with hip and knee flexion of 90 degrees. An inextensible strap was placed around the ankle, immediately proximal to the malleoli, adjusted to ensure the knee remained at 90 degrees flexion. The ankle was connected to a strain gauge with integrated transducer (MicroFET 2; Hoggan, West Jordan, UT) by an inextensible ankle strap. A seatbelt was secured across the subject's hips to stabilise the pelvis.

The signal was then passed via a carrier amplifier (constructed by the Medical Engineering Department at the University of Oxford) to a handheld display.

The patients were then asked to make three maximum voluntary contraction (MVC) manoeuvres while lying supine; the largest of these was selected for analysis.

2.4.2.3 Handheld dynamometer (HHD)

2.4.2.3.1 Background

Muscle-strength tests using a handheld dynamometer (O'Shea, Taylor et al., 2007) have been demonstrated to be valid and reliable measures of arm function in patients with COPD (O'Shea, Taylor et al., 2007; Stark, Walker et al., 2011).

Some commonly used upper limb (UL) tests include the unsupported upper limb exercise test (UULEX) and the 6-minute pegboard and ring test (6PBRT).

Results demonstrate moderate to strong correlations among the muscle-strength test, upper limb exercise test (UULEX) and 6-minute pegboard and ring test (6PBRT), as well as the responsiveness of all three tests to a pulmonary rehabilitation programme that included arm training and values obtained with a handheld dynamometer.

Hand held dynamometer (HHD) was first described by Lovett and Martin in 1916 (Bohannon, 2006) and is a convenient device that can be placed between the hand of the practitioner and the patient's tested body part, similar to how a practitioner would perform a manual muscle test.

In their study, Kolber (Kolber, Beekhuizen et al., 2007) and his team concluded there was concurrent validity when comparing HHD with isokinetic dynamometry, the gold standard of muscle strength assessment.

However, a common theme noted with this method was the weaknesses found in the lack of standardised methods when performing the manual muscle test while using an HHD.

Eight of the 19 studies qualified that the practitioner testing the patient was trained in the technique. In addition, eight of the 19 studies qualified the manner in which the force was applied to the HHD (e.g., patient-initiated vs. practitioner-initiated, direction of force, etc.).

One reason for the poor interrater outcome may be attributable to the fact that the examiners in these studies were allowed to use any technique with which they were most comfortable.

However, when standardised techniques are used, manual muscle testing reliability is identified to be prominently greater in the healthy control population (Frese, Brown et al., 1987).

Considering the cost of isokinetic testing devices and the impracticality of daily or routine clinical testing, however, the 19 studies that compared HHD to isokinetic testing were demonstrated in general to have a moderate-to-good reliability and validity when compared with isokinetic testing.

2.4.2.4 Method

Isometric muscle force of elbow and shoulder flexion was measured on the dominant side using a handheld dynamometer (MicroFET 2; Hoggan, West Jordan, UT, USA).

Participants performed up to five trials with a 1-minute rest between trials; the average of the highest three measures within 5% of one another was used for analysis.

2.4.3 Activity measures

2.4.3.1 Activity monitor

2.4.3.1.1 Background

An imbalance of energy intake and energy expenditure leads to obesity which is associated with increased morbidity and mortality.

Total energy expenditure (TEE) consists of resting energy expenditure (REE), the thermic effect of food (TEF) and activity thermogenesis (AT).

So far, mainly physical activity recall questionnaires have been used to quantify physical activity in daily life in both research and clinical settings (Sallis and Saelens, 2000). Energy equivalents of the logged activities are determined from the physical activity recall questionnaires, multiplied by the duration of activity and then summed to derive an estimate of AT. The accuracy of the activity logs and the determination of energy equivalents are the major limitations of this approach.

Mahabir et al. (King et al., 2004) demonstrated a deviation of up to 60% when evaluating physical activity by using four different questionnaires in comparison to the doubly-labelled water method, the gold-standard of looking at evaluating physical activity. In particular, obese participants tended to overestimate their activity to a higher degree than normal-weight subjects. This is confirmed by a recent review by Prince et al. (Welk et al., 2004) that addressed the poor correlations between self-report and direct measures of physical activity.

2.4.3.2 Method

The SenseWear Pro 2 Armband (SWA; Body Media, Inc., Pittsburgh, PA, USA) is a device that, in addition to movement data, also collects physiologic data from multiple sensors and estimates total energy expenditure (TEE) and physical activity by using specific algorithms. Therefore, SWA appears to provide a suitable approach for measuring the different components of TEE.

The SWA utilises a multi-sensor array including a 2-axis accelerometer, skin temperature sensor and a near-body ambient temperature sensor.

The subjects wore the SWA on the non-dominant upper arm over the triceps muscle. The SWA was attached securely with an adjustable elastic strap for a period of 5-10 minutes before data collection to allow for acclimatisation of the SWA to skin temperature.

TEE was continuously measured for at least three days (preferably two weekdays and one weekend day) with the SWA. Patients were asked to wear the SWA as described above at least 23 hours per day.

For the analysis of the SWA data, the appropriate software was used (InnerView Research Software, version 4.0, build 0-610; BodyMedia, Inc., Pittsburgh, PA, USA). The subject's gender, age, height and weight were programmed into the SWA before each analysis.

The number of steps, metabolic equivalents (MET), the duration of energy expenditure to distinct levels of physical activity graded in METs, and the time spent in supine position as well as the duration of sleep were calculated. METs define the energy expenditure related to bodyweight. One MET is equivalent to 1 kcal/kg of bodyweight/hour. Moderate physical activity is usually classified as between 3 and 5 METs.

2.4.4 Assessment of muscle biology

2.4.4.1 Background

In obese individuals, lipids excessively accumulate in adipose tissues and ectopically accumulate in non-adipose tissues including skeletal muscle (Unger et al., 2010). Lipids in skeletal muscle have been extensively studied in the context of insulin sensitivity. However, lipid overload in muscle appears to affect not only insulin signalling, but also muscle maintenance and regeneration. The underlying mechanisms are not fully understood but recent experimental data suggest that multiple factors, such as accumulation of toxic lipid metabolites and low-grade inflammation, result in impaired muscle regeneration under conditions of obesity.

The impact of obesity on skeletal muscle maintenance and physiology has been addressed in rodent models of obesity to date.

Obesity is characterised by elevated adipose storage in subcutaneous and visceral adipose deposits and non-adipose organs, a phenomenon called ectopic lipid accumulation (van Herpen and Schrauwen-Hinderling, 2008). In addition, obese individuals have increased circulating fatty acids (Boden and Shulman, 2002) and high ectopic lipid deposition in skeletal muscle partially resulting from increased fatty acid uptake from the circulation (Goodpaster and Wolf, 2004).

Lipids within skeletal muscle are comprised of two pools: extramyocellular lipids, (EMCL) localised in adipose cells between myofibres, and intramyocellular lipids, (IMCL) located within muscle cells (Zehnder et al., 2006). A portion of EMCL comprises adipose tissue closely associated with the muscle, referred to as intermuscular adipose tissue (IMAT). IMAT accumulation in obese patients is positively correlated with insulin resistance and reduced muscle performance (Timmers et al., 2008).

In skeletal muscle, lipotoxic species interfere with insulin signalling and are thought to be partly responsible for insulin resistance in obesity (Bosma et al., 2012). However, it remains largely unknown as to what other physiologic processes are impaired by these lipid metabolites in skeletal muscle.

Insulin resistance and mitochondrial and metabolic dysfunction are perhaps the most prominent muscle abnormalities that negatively impact whole-body metabolism and physical performance in states of obesity and type 2 diabetes. Skeletal muscle maintenance depends on ongoing repair and regeneration.

As has been mentioned above, the association between intramyocellular triglyceride (IMTG) content and insulin resistance is now well established in animals and obese humans and most studies investigating the mechanisms of insulin resistance in muscle use high-fat diet rodent models.

Much research has concentrated on the quadriceps because it is readily accessible and is a primary locomotor muscle. However, it may not represent skeletal muscle function as a whole because it is a chronically underused muscle in obesity. Controversy therefore remains over whether the main mechanisms involved in skeletal muscle dysfunction are local or systematic. Loss of muscle function should be equally distributed if the underlying pathology is a generalised process, such as inflammation or derangement of blood gases.

What is the best method of muscle sampling? Percutaneous needle biopsy was introduced by Duchenne as early as 1868 (Duchenne, 1867) for the investigation of patients with muscular dystrophy. The method changed very little during the following 90 years until 1957, when Reiffel and Stone suggested that the

percutaneous needle biopsy be used for the study of muscle electrolytes in man by neutron activation analysis (Reiffel and Stone, 1957).

2.4.2.2 Methods

In 1960 a percutaneous muscle biopsy technique was developed, using a biopsy needle (Bergstrom et al., 1963).

It consists of a sharp tipped hollow outer needle with a small opening near the tip. A cylinder with a sharp edge fits tightly into the needle.

The technique is as follows. The quadriceps lateralis is identified using anatomical landmarks. Percutaneous biopsies of the right vastus lateralis muscle were performed at mid-thigh level (15cm above patella). The needle is inserted through a small incision made in locally-anaesthetised skin into the quadriceps muscle and one or more pieces of tissue bulging into the ‘window’ are punched out with the sharp cylinder. The method of sampling is rapid and only slightly traumatic and can therefore be repeated several times on the same individual.

Only slight discomfort was experienced by most subjects, with more severe pain during the procedure or severe change during the following days occurring only rarely. With a biopsy needle of 4.5mm in diameter, about 20-100mg of muscle tissue was obtained. The sample was immediately weighed repeatedly on a sensitive electrobalance and the weight curve was extrapolated to zero time to compensate for water evaporation. One of the samples was frozen as quickly as possible (by using liquid nitrogen). After freeze-drying, connective tissue was able to be removed by dissection at room temperature and the remaining sample accurately weighed. The samples were then transferred to Kings College, London for analysis.

2.5 Assessment of sleep-disordered breathing, hypoventilation and upper airway

2.5.1 Sleep studies

2.5.1.1 Background

The majority of OHS patients (approximately 80–90%) have OSA but a minority have pure nocturnal hypoventilation with a rise in PCO_2 by 10mmHg at night. Chronic hypercapnia results from the inability to offload carbon dioxide during the day in response to carbon dioxide loading at night from sleep-disordered breathing. Offloading is more likely to occur in those with short episodes of hypercapnia, due to apnoeas and hypopnoeas, compared with patients with long periods of hypoventilation.

Modelling work suggests that when apnoea events become more than three-times longer than the breathing interval between them, carbon dioxide will accumulate despite maximum augmentation of tidal volume, as there is insufficient time for this hyperventilation to return carbon dioxide to normal before the next episode of carbon dioxide loading (Berger et al., 2008).

A secondary mechanism, which then impairs chemosensitivity, is the increase in serum and cerebrospinal fluid bicarbonate, which is exacerbated by reduced renal bicarbonate excretion. It is this combination of mechanical and sleep-related effects that potentially causes chronic alveolar hypoventilation.

2.5.1.2 Methods

Sleep-disordered breathing (SDB) was assessed from a one-night, in-hospital respiratory polygraphic sleep study. Subjects' body movements (derived automatically from the video signal), heart rate and pulse transit time changes were routinely recorded as markers of arousal from sleep. The sleep study equipment measured nasal pressure via nasal cannula, SpO_2, pulse rate and respiratory effort via thoracic and abdominal bands (Win-Visi monitoring system; Stowood Scientific Instruments, Oxford, UK). From these measurements, AHI per hour in bed and oxygen desaturation events per hour in bed were automatically calculated, with manual review and editing (including removal of clear awake periods) to ensure accuracy of the data as previously described (Stradling et al., 1997). The variables derived for this study were: (1) number of oxygen desaturations (≥4%) per hour of study (ODI); (2) mean SaO_2 overnight apnoea-hypopnoea index (AHI); and (3) time, or % of time, during the overnight study spent with a SpO_2 below 90%.

2.5.2 Epworth sleepiness score

2.5.2.1 Background

The Epworth sleepiness score (ESS) was used as a measure of sleepiness. Being a subjective test, it is susceptible to reporting bias due to factors such as patients' expectations following an intervention, mood, eagerness to please by reporting favourable changes and, in some cases, a reluctance to admit to dozing whilst driving.

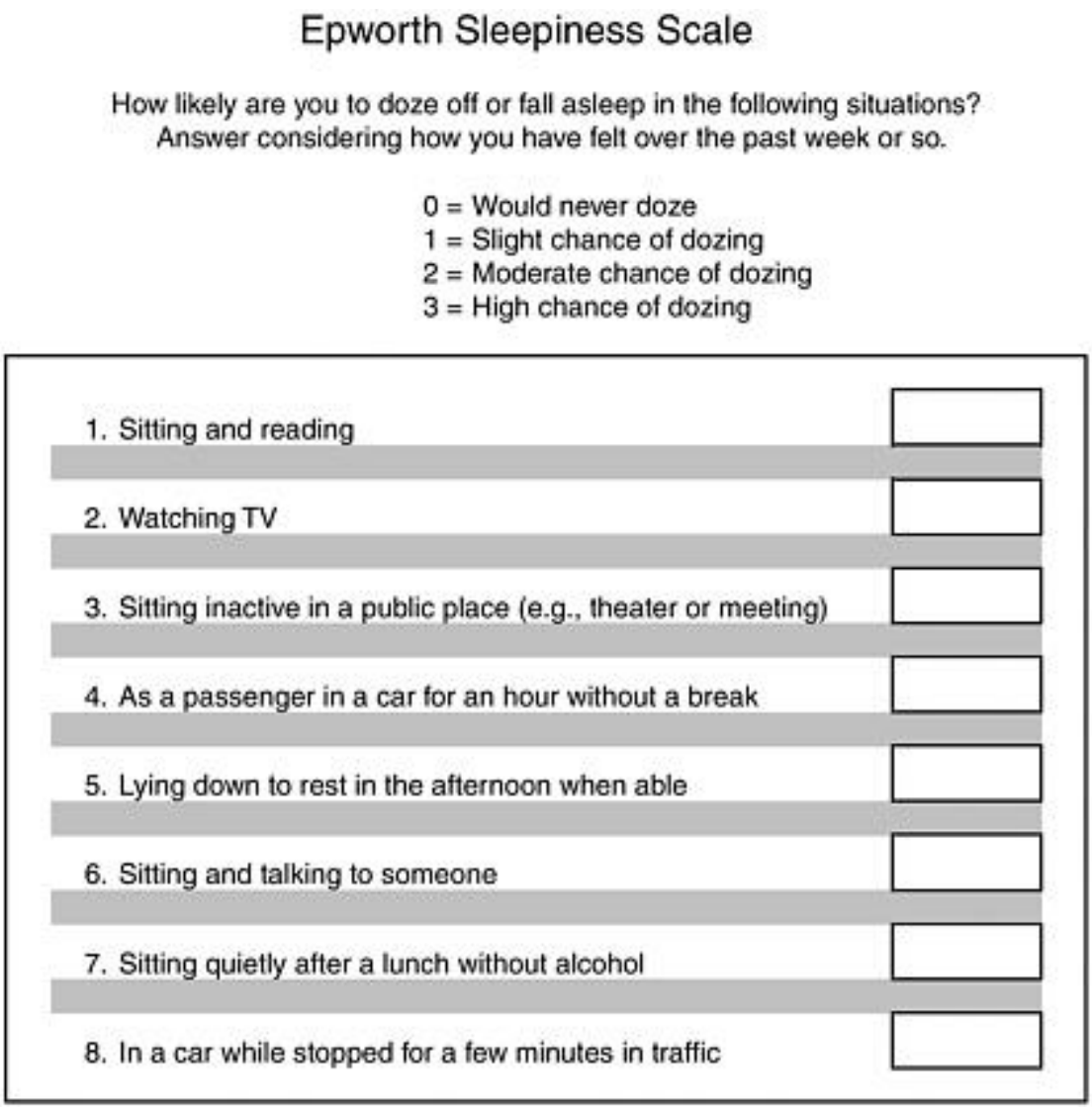
A further disadvantage of the ESS is that inter-individual variability is large, with patients' motivation to stay awake and level of activity playing a key role in frequency of daytime dozing. In addition, the ESS was developed in patients with OSA and narcolepsy and has not been specifically validated in patients with respiratory failure. I might have obtained a more accurate measure of sleepiness had I performed objective studies, such as the maintenance of wakefulness test, but these tests are time consuming and it was not considered practical to lengthen the study protocol for my subjects. The ESS has the advantage of being quick and easy to perform and, due to its subjective nature, it perhaps gives a better reflection of inconvenience to the patient caused by daytime dozing.

The ESS was originally validated against sleep latency measured during the multiple sleep latency tests and during overnight polysomnography in normal subjects, obstructive sleep apnoea syndrome, narcolepsy and idiopathic hypersomnia. It has subsequently been used in other conditions associated with sleep-disordered breathing, for example the ESS was found to be high in patients with motor neuron disease (Lyall et al., 2001).

2.5.2.2 Method

Patients were asked to complete the Epworth Sleepiness Scale (ESS) (Johns, 1991) as shown in Figure 12. This is a simple, self-administered questionnaire providing a subjective measurement of general levels of daytime sleepiness. Patients were asked to describe how likely they would be to doze in eight everyday situations. A maximum score of 24 is possible; a score of greater than 10 is thought to be abnormally sleepy. Results depend greatly on a patient's motivation to stay awake;

some abnormally sleepy people purposefully keep themselves busy to avoid sleeping during the day.



Epworth Sleepiness Scale

How likely are you to doze off or fall asleep in the following situations?
Answer considering how you have felt over the past week or so.

0 = Would never doze
1 = Slight chance of dozing
2 = Moderate chance of dozing
3 = High chance of dozing

1. Sitting and reading	
2. Watching TV	
3. Sitting inactive in a public place (e.g., theater or meeting)	
4. As a passenger in a car for an hour without a break	
5. Lying down to rest in the afternoon when able	
6. Sitting and talking to someone	
7. Sitting quietly after a lunch without alcohol	
8. In a car while stopped for a few minutes in traffic	

Figure 12: Epworth Sleepiness Score

2.5.2.3 Background

Auto-adjustable positive airway pressure (APAP) devices are a treatment alternative to fixed pressure continuous positive airway pressure (CPAP) therapy for the upper airway obstruction in OSA. The APAP devices aim to deliver optimised pressure throughout sleep and make therapy more comfortable and tolerable.

Upper airway resistance represents a dynamic property, dependent on numerous factors including body position, body weight, sleep stage, sleep deprivation, alcohol consumption and the use of other sedatives, nasal resistance and airway humidification (Marrone and Bonsignore, 2002) (Cartwright, 1984, Cartwright et al., 1991) (Dolly and Wynne, 1982). Variation in these factors can occur within a single night or between nights causing changes in airway resistance.

APAP devices can automatically detect changes in surrogates of upper airway collapse and adjust the pressure delivered accordingly, allowing delivery of an optimal pressure throughout the night.

Numerous markers of upper airway collapse such as snoring, apnoeas/hypopnoeas, flow limitation and the forced oscillation technique have been utilised in devices to auto-adjust the pressure requirements.

Other techniques used to detect apnoeas/hypopnoeas include analysis of the pressure generated in the anterior nares through standard nasal cannulae, detection of differences between maximal inspiratory and expiratory flow and the use of pneumotachographs (Teschler et al., 1996).

2.5.2.4 Method

The Autoset S9 used in the research study reviews the shape of the inspiratory flow curve on a breath-by-breath basis. A normal unobstructed breath gives a smooth rounded curve shape but, as the upper airway narrows, flattening of the curve occurs, altering the shape. The degree of flattening determines the response of the device.

The pressure required by the Autoset S9 is recorded and this value was used as a measure of pressure required to overcome upper airway resistance in these subjects.

Patients assigned to CPAP were instructed in the use of an auto-adjusting CPAP machine (Autoset S9, ResMed, Abingdon, UK). Induction was by trained staff who were not involved in outcome assessments or data analysis. Humidification and interface choices were made on an individual basis. All patients had one or more follow-up visits to download compliance data, check for residual apnoea/hypopnoeas and mask leakage and to make any necessary adjustments.

2.6 Assessment of Ventilatory drive: the hypercapnic ventilatory response and hypoxic challenge

2.6.1 Measurement of response to hypoxia and hypercapnia challenge

2.6.1.1 Background

Healthy people respond to hypoxaemia by increasing their minute ventilation reducing the impact of the fall in inspired oxygen. The ventilatory response to

hypoxia is mediated by the peripheral chemoreflex, a reflex arc from the carotid body sensor to the respiratory muscle effectors (Torrance, 1996).

The response to hypoxaemia in patients with OHS is likely to represent a more complex relationship between changes in ventilation and perfusion matching and the ability to increase ventilation (BaHammam, 2010).

Patients with OHS are at risk of cor pulmonale and right heart failure under hypoxic conditions inducing more pulmonary vasoconstriction.

Obese subjects have increased rates of oxygen consumption (VO_2) and carbon dioxide production (VCO_2), even when they are at rest. Thus, obese persons must increase their minute ventilation to meet the increased oxygen requirements and maintain adequate alveolar ventilation (Steier et al., 2009).

OHS patients fail to augment drive to compensate for the added load created by excess weight, permitting a gradual rise in CO_2 to take place (Sampson and Grassino, 1983).

Central responsiveness to hypercapnia and hypoxia is blunted in OHS patients compared to normal-weight subjects and eucapnic obese patients with or without OSA (Zwillich et al., 1975).

The slope of the hypercapnic ventilatory response has been to shown <1l/min/mmHg in OHS, between 1.5 and 2.5l/min/mmHg in eucapnic obese individuals and 2-3l/min/mmHg in healthy subjects (Verbraecken et al., 1995).

2.6.1.2 Ventilatory response testing

2.6.1.3 Method

Instrument calibrations preceded the study of each patient and the studies were performed after the overnight sleep study between 8am and 10am. Both the hypoxic and the hypercapnic challenge tests were performed on the same day with the subject having a 30min interval between each test. Subjects were seated comfortably in a chair at rest, wearing a full face mask (Respironics Gel Comfort, Philips, UK) initially delivering air only, through a two-way, non-rebreathing valve (T-shape, Hans Rudolph Inc, Kansas City, MO, USA). Baseline measurements of resting SpO_2, end-tidal carbon dioxide ($EtCO_2$) levels and resting ventilation (NICO 2, Philips-Respironics, UK) were made over at least a 15-minute period until stable. The expiratory limb of the circuit was attached to the NICO 2 capnograph and oximeter, whose digital output was connected to a PC (Dell, USA), allowing breath-by-breath measurements to be taken continuously over this period.

2.6.1.4 Hypoxic ventilatory response test

After this baseline period, subjects first inhaled a hypoxic gas mix of 15% oxygen and balance nitrogen (BOC Ltd, Guildford, UK) through the two way, non-rebreathing valve from a gas reservoir (100 litre non-diffusing Douglas collection bag). The effect of this hypoxic gas mixture on SpO_2, $EtCO_2$, and ventilation was followed over a period of at least 15 minutes until the subject's ventilation was stable. During the hypoxic challenge, the subject's SpO_2 was monitored continuously to prevent severe hypoxemia, with the test being terminated if SpO_2 values fell below 70% (1 subject),

or if the patient became distressed (no subjects). Thus, a two-point steady-state, poikilocapnic estimate of the hypoxic response was made.

2.6.1.5 Hypercapnic ventilatory response test

The ventilatory response to 5% CO_2, balance oxygen (BOC Ltd, Guildford, UK), was then measured using the same technique as described above. The subjects inhaled the hypercapnic gas mix through the two-way, non-rebreathing valve from the gas reservoir. The effect of this hypercapnic gas mixture on SpO_2, $EtCO_2$ and ventilation was followed over a period of at least 15 minutes until the subject's ventilation was stable, unless the patient became distressed (no subjects). Thus a two-point steady-state, hyperoxic estimate of the hypercapnic response was made.

In addition to the two-point ventilatory responses to hypoxia and hypercapnia (l/min increase per fall in SpO_2, or rise in PCO_2), other simpler derivatives were calculated. The degree to which the SpO_2 was defended during the hypoxic challenge test was taken as a simple measure of poikilocapnic hypoxic sensitivity (this measure can be obtained during a simple 'fitness to fly' assessment (Seccombe et al., 2004) without the need to measure the minute ventilation). Similarly, the end-tidal PCO_2 reached during the challenge was recorded. The average of the final stable five-minute period of each study was used for all these analyses.

2.7 Assessment of hormonal, nutritional and inflammatory cytokine measures

2.7.1 Blood samples

2.7.1.1 Background

The adverse intracellular consequences of nutrient toxicity in the adipocyte, including mitochondrial (oxidative) stress, also have systemic consequences. Systemic mediators of adipocyte dysfunction include adipokines, free fatty acids, and inflammatory mediators adipokines, including adiponectin, leptin, resistin, and ghrelin, are circulating molecules produced by adipocytes that affect energy use and production and appear central to the pathophysiology of obesity and its systemic health effects, including nonalcoholic fatty liver disease, insulin resistance, atherosclerosis, and type 2 diabetes. Obesity is associated with increased release of nonesterified fatty acids and triglycerides into the circulation. Most nonesterified fatty acids, commonly termed “free” fatty acids, circulate in the bloodstream bound to albumin. Higher concentrations of circulating free fatty acids and triglycerides are associated with lipid accumulation in multiple tissues, including liver, skeletal muscle, heart, and pancreatic β-cells. In older adults, peripheral (skeletal muscle) insulin resistance correlates with intracellular accumulation of lipids and decreased mitochondrial function as assessed by nuclear magnetic resonance spectroscopy(Petersen and Shulman, 2002) CRP is a circulating marker of inflammation produced predominantly by the liver in response to IL-6. Circulating CRP concentrations are also higher in adults with metabolic syndrome, and increased CRP is an independent risk factor for type 2 diabetes and CVD (Cook et al., 2000)

2.7.1.2 Methods

Blood samples were taken in the early morning (usually from the right antecubital fossa). The blood was taken into plain, sodium citrate, lithium heparin and EDTA tubes. The samples were either submitted for routine analysis in the clinical laboratory or immediately frozen either as whole blood (clotted and EDTA) or immediately centrifuged at 2000 x gravity for 5 minutes. Plasma was then aliquoted and stored at -70C until assayed.

Subjects were fasted overnight during the sleep study to reduce the burden to them. All subjects had fasting blood samples taken. Blood samples were immediately sent to the hospital laboratory for estimation of routine biochemistry and haematology – glucose, folate and lipids, iron studies (including ferritin), fasting insulin, thyroid function test, cholesterol, fatty acids, vitamin D and BNP and glucose – while a specimen of clotted blood was spun and frozen at -80°C and subsequently sent in batches for serum leptin, Adipokines, HS-CRP in duplicate, using a highly sensitive radioimmunoassay or ELISA. Leptin assays (R&D Systems Europe, Abingdon, UK), adiponectin assay (R&D Systems Europe, Abingdon UK) and HS-CRP (CardioPhase HS-CRP, Siemens Healthcare, Erlangen, Germany) were all performed at the NIHR Core Biochemistry Assay Laboratory, Cambridge Biomedical Research Centre.

2.7.1.3 Statistical methods

Data analyses were performed using SPSS™ (version 22, IBM Corporation Ltd, USA). Data are presented as the mean and standard deviation for normally distributed data. Data analysis for the role of bicarbonate looked at any differences between the three groups (normal, raised base excess only, and raised PaCO2)

were explored using analysis of variance (ANOVA) with post hoc assessment of any significant inter-group differences using Duncan's post hoc comparison test. For the ANOVA, any non-normally distributed data were first converted using a logarithmic transformation.

For the predictors analysis, data was tested for normality and parametric analyses or log transformation were conducted when appropriate. Statistical analysis was a two-stage process. First, within each area of potential areas of mechanism, linear regression analysis was used to ascertain the most predictive measure of both CO_2 levels and base excess. Once the best representative of each mechanistic area or domain had been identified, these were then entered into a final linear regression model to identify the ranking of predictors of CO_2 and BE.

2.7.1.4 Variables measured

The variables measured were as followed:

Measures of Obesity - Weight (kg) BMI (kg/m^2)

Circumferences - Neck, Waist Hip Ratio, Hip Waist Ratio, Upper arm, Forearm, Thigh, Calf

Skinfold measurements - Chin, Triceps, Biceps, Forearm front, Forearm back, Midaxillary, Iliac Crest, Supra-Iliac chest, Abdomen, Thigh, Calf, Buttock,

DXA measurements - Visceral adipose tissue volume (l), Visceral adipose tissue mass (kg) Legs % of total (Kg), Leg fat (%), Leg lean muscle (%), Trunk of total (%), Trunk total (KG), Trunk fat (%), Trunk lean muscle (%), Fat (%), Gynoid (%), Gynoid (kg), Gynoid (fat), Ratio Trunk/total, Android lean, Android fat, Android tissue

Pulmonary Function test - FEV1 %PN, seated, FVC %PN, seated, VC%PN, seated, ERV seated (ml), FEV1 %PN, lying, FVC%PN, lying, VC %PN, lying, ERV (ml), lying

IOS - R5%PN, seated, R20%PN, seated, Change Z5 at FRC, seated (KPa/L/sec), R5%PN, lying, R20%PN, lying, Z5%PN, lying, Change Z5 at FRC, lying (KPa/L/sec)

Muscle strength - MIP Max (cmH_20), MEP Max (cmH_20), Sniff Max (cmH_20), Max Handgrip (kg), Max Quadriceps (kg)

Sleep study data - ESS, ODI (events/hr), Mean overnight oxygen saturation, AHI, Time spent below 90% SaO_2 (hrs: mins: sec), Time spent below 90% SaO_2 (% of sleep study time), Arousals per hour, Average size of arousals, No PTT arousals, Av size of Respiratory swing, Obstructive, Central

AutoCPAP measurements

Variable Ventilatory responses to 5% CO_2 - Ventilatory drive (l/min/kPa), Change in minute ventilation (l), Final end-tidal $PaCO_2$ (kPa), Final SaO_2 (%), Ventilatory responses to 15% oxygen, Ventilatory drive (l/min/%), Change in minute ventilation (l), Fall in SaO_2 (%), Final SaO_2 (%)

Ventilatory responses to 15% oxygen - Ventilatory drive (l/min/%), Change in minute ventilation (l), Fall in SaO_2 (%), Final SaO_2 (%)

Blood measures - BNP (ng per Litre), Glucose (mmol), Insulin (IU), Vitamin D (IU), Ferritin (mmol), Total Iron (mmol), Transferrin (mmol), B12 (IU), Folate (mmol), TSH, HbA1C, Leptin ng/ml, Adiponectin µg/ml, HS CRP mg/l

Actigraphy - Physical activity (MET 3.0), Daily steps, Average MET*Impedance (ohms)* - Fat %, Fat kg, Lean %, Lean kg, Water %, Water kg

MRI, Fat and muscle biopsy findings were not included in results

RESULTS

The results section is divided into the experiment looking at the role of a raised bicarbonate in the physiology of obesity related ventilatory failure and the section looking at experiments looking at predictors of ventilatory failure, with discussion in Chapter 4.

3.1 Is a raised bicarbonate, without hypercapnia, part of the physiological spectrum of obesity-related hypoventilation?

Obesity hypoventilation syndrome (OHS) conventionally includes awake hypercapnia, but an isolated raised bicarbonate, even in the absence of awake hypercapnia, may represent evidence of "early" OHS. I investigated whether such individuals exhibit certain features characteristic of established OHS. To test this hypothesis I divided the subjects from the study on the basis of their BE and CO_2 and whether it was normal or elevated.

3.1.1 Subject Grouping

Following arterial blood gas analysis, subjects were divided a priori into three groups according to the following criteria

1)	Normal daytime PaCO_2 (≤6kPa) and base excess (<2mmol/l)
2)	Normal daytime PaCO_2 (≤6kPa) and elevated base excess (≥2mmol/l)
3)	Elevated daytime PaCO_2 (>6kPa)

Seventy-one obese subjects (37, 52%, males) were studied during the 27-month recruitment period. Demographic and anthropometric data are reported for each group in Table 2. There were no differences in age, weight or BMI between the groups, although the p-value for BMI approached significance (p=0.056) between groups.

The ventilatory drive measurements at baseline and after hypoxic and hypercapnic challenge testing are shown in Table 3, 4 and 5, respectively. Table 6 shows the sleep data for each group.

For most of the ventilatory response and sleep measurements, the group with only a raised BE are a middling group, between the normal and OHS groups. The ANOVA and Duncan's post hoc comparison tests suggest for some measures that the raised BE group is more similar to the OHS group, rather than to the normal group; for example, the fall in SaO_2 and the rise in $ETCO_2$ during the hypoxic and hypercapnic challenges respectively and the time spent below 90% SaO_2 overnight (despite similar values for the awake baseline SaO_2, Table 6). This will be discussed further in section 4.

Table 2: Demographic, anthropometric and gas exchange data for each group

	Group 1, 2 and 3 Combined	**Group 1 Normal $PaCO_2$ and Normal BE (n = 33)**	**Group 2 Normal $PaCO_2$ and Elevated BE (n = 22)**	**Group 3 Elevated $PaCO_2$ (n = 16)**	**ANOVA p-value**
Age (years)	52.1 (8.8)	53.6(9.4)	48.7(7.9)	53.7(7.6)	0.09

	Group 1, 2 and 3 Combined	Group 1 Normal $PaCO_2$ and Normal BE (n = 33)	Group 2 Normal $PaCO_2$ and Elevated BE (n = 22)	Group 3 Elevated $PaCO_2$ (n = 16)	ANOVA p-value
Weight (Kg)	136 (29.5)	130(28.9)	135(22.6)	148(35.1)	0.09
BMI (kg/m^2)	47.2 (9.80)	45.2(9.1)	46.5(7.9)	51.6(11.7)	0.056
pH (log $[H^+]$	7.42 (0.03)	7.41(0.02)[a]	7.44(0.01)[b]	7.41(0.03)[a]	<0.001
PaO_2 (kPa)	9.87 (1.39)	10.26(1.38)[a]	9.77(1.35)[a,b]	9.17(1.26)[b]	0.031
$PaCO_2$ (KPa)	5.57 (0.79)	5.15(0.47)[a]	5.42(0.32)[a]	6.62(0.91)[b]	<0.001
Base excess (mmol/l)	+2.08 (2.41)	+0.12(1.38)[a]	+3.01(0.98)[b]	+4.78(2.10)[c]	<0.001
Standard bicarbonate (mmol/l)	26.2 (2.11)	24.4(1.18)[a]	27.0(0.87)[b]	28.5(2.11)[c]	<0.001
Creatinine (mmol/l)	70.6 (12.9)	82.1(28.7)	70.6 (12.9)	76.8 (17.9)	0.19
Haematocrit (fraction)	0.43 (0.04)	0.42 (0.04) [a]	0.45 (0.04)[b]	0.44 (0.05)[a,b]	0.02

Data are expressed as mean (SD). [a, b, c] Grouping of data as determined by Duncan's post hoc comparison.

Abbreviations: $PaCO_2$ = partial pressure of arterial carbon dioxide; PaO_2 = partial pressure of arterial oxygen; BMI = body mass index; BE = Base excess.

NOTE: Normal daytime $PaCO_2$ was a $PaCO_2$ ≤6kPa, and a normal BE was a BE <2mmol/l

Table 3: Baseline ventilation data

	Group 1 Normal $PaCO_2$ and Normal BE (n = 33)	Group 2 Normal $PaCO_2$ and Elevated BE (n = 22)	Group 3 Elevated $PaCO_2$ (n = 16)	ANOVA p-value
Minute ventilation (l/min)	8.05 (1.84)	8.33 (2.26)	7.54 (1.61)	0.47
Respiratory rate (bpm)	14.7 (4.01)	16.1 (4.93)	14.3 (5.89)	0.48
$\%SpO_2$	96.0[a] (1.83)	96.3[a] (1.62)	92.4[b] (1.69)	0.007
$EtCO_2$ (kPa)	5.3[a] (0.40)	5.4[a] (0.35)	6.2[b] (0.6)	<0.001

Data shown from average of the final five minutes of the challenge.

Data are expressed as mean (SD). [a, b, c] Grouping of data as determined by Duncan's post hoc comparison.

Abbreviations: bpm = breaths per minute; $\%SpO_2$ = % arterial oxygen saturation from oximetry; $EtCO_2$ = end-tidal partial pressure of carbon dioxide; kPa = kilopascals.

NOTE: Normal daytime $PaCO_2$ was a $PaCO_2 \leq 6$kPa, and a normal BE was a BE < 2 mmol/l

Table 4: Results from the hypoxic ventilatory response test

	Group 1 Normal $PaCO_2$ and Normal BE (n = 33)	Group 2 Normal $PaCO_2$ and Elevated BE (n = 22)	Group 3 Elevated $PaCO_2$ (n = 16)	ANOVA p-value
Minute ventilation (L/min)	10.12 (3.27)	9.26 (2.31)	9.21 (2.28)	0.45
Respiratory rate (bpm)	14.8 (3.91)	15.6 (4.90)	13.8 (5.11)	0.55
$\%SpO_2$	93.4[a] (2.36)	92.6[a] (2.50)	87.2[b] (1.50)	0.001
$EtCO_2$ (kPa)	5.18[a] (3.01)	5.21[a] (2.60)	6.03[b] (0.54)	<0.001
Decrease in $\%SpO_2$	2.50[a] (1.64)	3.68[b] (2.07)	5.01[b] (0.40)	0.002
Rise in minute ventilation (l/min)	2.12 (1.01)	0.97 (0.96)	1.39 (1.02)	0.20
Ventilatory response (l/min/$\%SpO_2$)	2.42 (4.48)	0.40 (0.41)	0.66 (1.20)	0.058

Data shown from average of the final five minutes of the challenge.

Data are expressed as mean (SD). [a, b, c] Grouping of data as determined by Duncan's post hoc comparison.

Abbreviations: bpm = beats per minute; $\%SpO_2$ = % arterial oxygen saturation from oximetry; $EtCO_2$ = end-tidal partial pressure of carbon dioxide; kPa = kilopascals.

NOTE: Normal daytime $PaCO_2$ was a $PaCO_2 \leq 6kPa$, and a normal BE was a BE < 2 mmol/l.

Table 5: Results from the hypercapnic ventilatory response test

	Group 1 Normal $PaCO_2$ and Normal BE (n = 33)	**Group 2 Normal $PaCO_2$ and Elevated BE (n = 22)**	**Group 3 Elevated $PaCO_2$ (n = 16)**	**ANOVA p-value**
Minute ventilation (L/min)	14.6[a] (5.43)	11.96[b] (2.31)	11.76[b] (3.30)	0.035
Respiratory rate (bpm)	14.3 (4.09)	16.6 (4.90)	15.0 (5.11)	0.15
$\%SpO_2$	99.0 (1.61)	98.4 (2.20)	98.3 (2.30)	0.40
$EtCO_2$ (kPa)	6.03[a] (0.44)	6.03 [a] (0.47)	7.09[b] (0.81)	<0.001
Rise in end-tidal CO_2 (kPa)	0.73 (0.33)	0.65 (0.39)	0.80 (0.46)	0.14
Rise in minute ventilation (l/min)	6.73[a] (0.72)	3.64[b] (0.37)	3.51[b] (0.43)	0.026
Ventilatory response to CO_2 (l/min/kPa)	1.57 (0.91)	0.87 (0.69)	0.70 (0.59)	0.50

Data shown from average of the final five minutes of the challenge.

Data are expressed as mean (SD). [a, b, c] Grouping of data as determined by Duncan's post hoc comparison.

Abbreviations: bpm = beats per minute; %SpO_2 = % arterial oxygen saturation from oximetry; $EtCO_2$ = end-tidal partial pressure of carbon dioxide; kPa = kilopascals.

NOTE: Normal daytime $PaCO_2$ was a $PaCO_2 \leq 6$kPa, and a normal BE was a BE < 2 mmol/l.

Table 6: Sleep Data

	Group 1 Normal $PaCO_2$ and Normal BE (n = 33)	**Group 2 Normal $PaCO_2$ and Elevated BE (n = 22)**	**Group 3 Elevated $PaCO_2$ (n = 16)**	**ANOVA p-value**
Epworth sleepiness score	12.2 (5.5)	12.1 (5.0)	11.9 (6.2)	0.99
4% Oxygen desaturation index (ODI, events/h)	40.6 (26.3)	53.9 (44.5)	55.7 (40.8)	0.28
Apnoea/hypopnoea index (events/h)	20.6 (26.0)	34.0 (33.7)	29.5 (28.5)	0.25
Mean %SpO_2	92.6[a] (2.6)	90.7[a,b] (5.24)	88.0[b] (7.17)	0.007
Time spent with %SpO_2 < 90% (h:m:s)	1:14:20[a] (1:23:35)	1:59:20[b] (2:02:20)	2:43:49[b] (2:28:46)	0.04
Percentage of time spent with %SpO_2 <90	17.3[a] (19.3)	27.7[b] (28.7)	42.3[b] (34.7)	0.012

Data are expressed as mean (SD). [a, b, c] Grouping of data as determined by Duncan's post hoc comparison.

Abbreviations: %SpO_2 = % arterial oxygen saturation from oximetry.

NOTE: Normal daytime $PaCO_2$ was a $PaCO_2 \leq 6kPa$, and a normal BE was a BE < 2 mmol/l.

3.2 Predictors of obesity-related chronic ventilatory failure

3.2.1 Introduction

This sections shows all the correlations between the dependant variables and either BE or $PaCO_2$ with the corresponding significance. Significance for the correlations in the research was set at a $p<0.05$. All data shown includes mean, standard data and 0–100% range. There are thirteen results tables and one summary table. All the tables are looking at measures who potentially correlate with BE and/or $PaCO_2$, which are the two physiological variables of ventilatory failure from Section 3.1. The results will take the form a series of tables, outlining correlations between individual predictors in each physiological domain before a final table looking at the best predictors in a multiple linear regression. Each table is separated with a brief legend outlining its contents.

Table 7 shows the Arterial blood gases and acid base balance results for the group as a whole

3.2.2 Measures of obesity and its distribution

Table 8 shows the correlation between ventilatory failure (BE and $PaCO_2$) and measures of obesity and distribution. Despite BMI being the standard measure of obesity, it correlates poorly with ventilatory failure ($r=0.2$, $p>0.75$). The highest correlation is with the DXA measurements of visceral fat, although these were only available from the 43 subjects with a BMI ≤40.

3.2.3 Lung function and oscillometry data

Table 9 and Table 10 show the correlation between the dependant variables and measures of lung function and oscillometry. The lung volume with the highest correlation was the lying FEV_1, although all the conventional lung function measures were significantly correlated. The best oscillometry measures were those done lying, and R20 %PN (reflecting more the upper airway resistance) was the best predictor although the other derivatives were not too dissimilar.

3.2.4 Muscle strength

Table 11 shows the correlations between ventilatory failure and measures of muscle strength. The sniff max was the best correlate although the maximum inspiratory pressure (MIP) was also significantly correlated.

3.2.5 Sleep-related measures

Table 12 and 13 shows the correlations between ventilatory failure and the sleep-related measures and autoCPAP. The measures of overall nocturnal hypoxia were the best correlates, although the ODI was also significantly correlated whereas AHI was not. There appeared to be no correlation to our measures of upper airway resistance

3.2.6 Ventilatory drive

Table 14 shows the correlations between ventilatory failure and the results obtained during breathing 5% CO_2 (in oxygen), and 15% oxygen. The ventilatory responses to 15% O_2 measures tended to be more predictive than the ventilatory responses to 5% CO_2.

3.2.7 Hormonal, nutritional and inflammatory measures

Table 15 and 16 shows the correlations between ventilatory failure and various hormonal, nutritional and inflammatory measures. Vitamin D levels were generally low, with 19% having levels which are determined deficient. There were only a few significant correlates: BNP, vitamin D and transferrin.

3.2.8 Bioimpedance and activity

Table 17 and 18 shows the correlations between bioimpedance and activity and ventilatory failure. The only significant correlation is between bioimpedance and both measures of ventilatory failure.

3.2.9 Linear regression of all domains

The top part of Table 19 shows the best correlates from each of the individual domains, and then the result of the linear regression of all of these six predictors. The bottom part of table 19 shows the only two remaining significant predictors: the fall in SaO_2 during the 15% oxygen challenge, and the DXA measure of intra-abdominal (or visceral) fat. All the other individually significant predictors drop out.

Table 7: Arterial blood gases and acid base balance

Variable	Mean	SD	Range (0–100%)
pH	7.42	0.03	7.35–7.48
PO_2 kPa	9.95	1.21	6.55–12.10
PCO_2 kPa	5.56	0.79	4.16–9.63
HCO_3^-mmol/l	26.2	2.11	21.50–33.30
Base excess mmol/l	2.08	2.41	-3.5 - +10

Mean (SD) values for arterial blood gas measurement for the whole group with range (0–100%).

Seventy-one obese subjects (37, 52%, males) were studied during the 27-month recruitment period. Arterial blood gas data are shown in Table 7.There is a considerable spread of values for the dependant variables, $PaCO_2$ and base excess.

Table 8: Obesity indices

Variable	Mean	SD	Range (0–100%)	Correlation to BE (p value)	Correlation to $PaCO_2$ (p value)
Weight (kg)	136.3	29.5	77.8–229	0.12 (0.11)	0.18 (0.13)
BMI (kg/m^2)	47.2	9.8	32.3–73.9	0.20 (0.86)	0.21 (0.78)
Neck (cm)	46.5	4.8	38–58	0.15 (0.22)	0.28 (0.21)
Waist (cm)	136.3	17.4	102–180	0.16 (0.20)	0.20 (0.97)
Hip (cm)	133.9	20.2	101–170	0.18 (0.13)	0.18 (0.15)

Variable	Mean	SD	Range (0–100%)	Correlation to BE (p value)	Correlation to $PaCO_2$ (p value)
Impedance (ohms)	382.3	86.9	194–710	-0.27 (0.03)	-0.18 (0.15)
Visceral adipose tissue volume (l)	**3.6**	**1.2**	**3.6–6**	**0.50 (<0.001)**	**0.35 (0.02)**
Visceral adipose tissue mass (kg)	**3.4**	**1.2**	**1.3–5.6**	**0.50 (<0.001)**	**0.36 (0.02)**
Legs DXA %	41.17	8.91	22.4–56.8	-0.13 (0.32)	-0.15 (0.25)
Legs Kg DXA	38.81	7.78	21.20–59.60	0.19 (0.15)	0.17 (0.19)
Leg fat Kg DXA	15.71	5.23	6.76–29.20	0.01 (0.92)	-0.02 (0.86)
Leg lean kg DXA	**22.36**	**5.38**	**12.47–41.80**	**0.24 (0.07)**	**0.26 (0.04)**
Trunk DXA %	54.32	5.14	41.5–63.5	-0.08 (0.53)	-0.13 (0.34)
Trunk Kg DXA	66.35	11.53	42.50–94.30	0.12 (0.37)	0.16 (0.24)
Trunk fat Kg DXA	35.69	8.00	21.97–55.69	0.04 (0.74)	0.05 (0.71)
Trunk lean Kg DXA	29.65	5.12	20.06–45.30	0.18 (0.16)	0.26 (0.05)
Gynoid DXA %	48.20	9.48	25.30–68.50	-0.16 (0.21)	-0.23 (0.08)
Gynoid kg DXA	20.82	9.36	12.00–58.90	-0.15 (0.25)	-0.22 (0.09)
Gynoid fat kg DXA	8.80	2.89	3.22–16.40	-0.04 (0.75)	-0.13 (0.31)
Ratio Trunk/total DXA	0.61	0.04	0.51–0.72	-0.03 (0.78)	0.07 (0.61)
Android lean Kg DXA	**4.60**	**0.91**	**3.09–7.60**	**0.23 (0.08)**	**0.25 (0.05)**
Android fat Kg DXA	6.71	1.58	4.05–11.02	0.02 (0.85)	-0.02 (0.87)

Variable	**Mean**	**SD**	**Range (0–100%)**	**Correlation to BE (p value)**	**Correlation to $PaCO_2$ (p value)**
Android tissue Kg DXA	13.62	11.39	3.94–66.00	-0.19 (0.89)	0.05 (0.64)
Legs total DXA %	41.16	8.91	22.4–56.8	-0.13 (0.32)	-0.15 (0.25)
Legs total DXA (Kg)	38.81	7.78	21.20–59.60	0.19 (0.15)	0.17 (0.19)
Leg fat DXA Kg	15.71	5.23	6.76–29.20	0.01 (0.92)	-0.02 (0.86)
Leg lean DXA Kg	**22.36**	**5.38**	**12.47–41.80**	**0.24 (0.07)**	**0.26 (0.04)**
Trunk total DXA %	54.32	5.14	41.5–63.5	-0.08 (0.53)	-0.13 (0.34)
Trunk DXA Kg	66.35	11.53	42.50–94.30	0.12 (0.37)	0.16 (0.24)
Trunk fat DXA Kg	35.69	8.00	21.97–55.69	0.04 (0.74)	0.05 (0.71)
Trunk lean DXA Kg	**29.65**	**5.12**	**20.06–45.30**	**0.18 (0.16)**	**0.26 (0.05)**
Gynoid total DXA %	48.20	9.48	25.30–68.50	-0.16 (0.21)	-0.23 (0.08)
Gynoid DXA kg	20.82	9.36	12.00–58.90	-0.15 (0.25)	-0.22 (0.09)
Gynoid fat DXA kg	8.80	2.89	3.22–16.40	-0.04 (0.75)	-0.13 (0.31)
Android lean DXA kg	**4.60**	**0.91**	**3.09–7.60**	**0.23 (0.08)**	**0.25 (0.05)**
Android fat DXA kg	6.71	1.58	4.05–11.02	0.02 (0.85)	-0.02 (0.87)
Android tissue DXA kg	13.62	11.39	3.94–66.00	-0.19 (0.89)	-0.05 (0.64)
Neck cm	46.47	4.84	38–58	0.15 (0.22)	0.22 (0.07)
Waist cm	102	17.36	102–180	0.15 (0.19)	0.20 (0.09)

Variable	Mean	SD	Range (0–100%)	Correlation to BE (p value)	Correlation to $PaCO_2$ (p value)
Hip cm	101	20.17	101–177	0.18 (0.13)	0.17 (0.14)
Waist Hip Ratio cm	0.33	0.13	0.33–1.38	0.05 (0.64)	0.07 (0.53)
Hip Waist Ratio cm	0.72	0.26	0.72–3	-0.10 (0.37)	-0.09 (0.45)
Chin cm	21.24	7.25	0–51	-0.13 (0.27)	-0.08 (0.50)
Triceps cm	49.63	19.15	7–97	0.03 (0.78)	0.01 (0.90)
Biceps skinfold cm	33.81	15.62	8–80	-0.08 (0.47)	0.00 (0.88)
Forearm front skinfold cm	13.91	9.21	3–49	-0.05 (0.67)	-0.21 (0.08)
Forearm back skinfold cm	18.60	12.87	4–62	-0.07 (0.56)	0.06 (0.60)
Midaxillary skinfold cm	42.39	14.04	0–89	0.07 (0.53)	0.15 (0.20)
Iliac Crest skinfold cm	52.57	20.22	0–96	-0.11 (0.37)	-0.06 (0.62)
Supra–Iliac crest skinfold cm	54.93	21.74	0–99	-0.10 (0.41)	-0.07 (0.56)
Abdomen skinfold cm	57.89	29.95	0–100	-0.21 (0.12)	-0.17 (0.20)
Calf skinfold cm	24.60	27.42	0–85	-0.14 (0.34)	-0.25 (0.07)
Buttock skinfold cm	52.43	34.06	0–100	-0.12 (0.36)	-0.12 (0.37)
Upper arm circumference cm	40.64	6.08	23–57	-0.11 (0.36)	-0.14 (0.24)
Forearm circumference cm	31.03	4.60	13–54	0.03 (-0.76)	0.00 (0.97)
Thigh circumference cm	64.22	11.83	0–94	-0.22 (0.06)	-0.39 (0.09)

Variable	Mean	SD	Range (0–100%)	Correlation to BE (p value)	Correlation to $PaCO_2$ (p value)
Calf circumference cm	46.01	8.29	0–58	-0.14 (0.34)	-0.25 (0.07)

Table 9: Lung function indices

Variable	Mean	SD	Range (0–100%)	Correlation to BE (p value)	Correlation to $PaCO_2$ (p value)
Lung volumes					
FEV_1 %PN, seated	**96.3**	**19.7**	**54–163**	**-0.36 (0.01)**	**-0.48 (<0.001)**
FVC %PN, seated	**98.3**	**19.1**	**69–166**	**-0.30 (0.01)**	**-0.43 (<0.001)**
VC%PN, seated	**98.8**	**18.1**	**63–161**	**-0.30 (0.01)**	**-0.41 (<0.001)**
ERV seated (ml)	494	292	0–1250	0.05 (0.69)	0.05 (0.68)
FEV_1 %PN, lying	**82.2**	**18.9**	**40.9–139**	**-0.40 (<0.001)**	**-0.46 (<0.001)**
FVC%PN, lying	**84.6**	**18.9**	**42.7–146**	**-0.31 (0.01)**	**-0.44 (<0.001)**
VC %PN, lying	**84.8**	**20.5**	**37.1–148**	**-0.32 (0.01)**	**-0.40 (<0.001)**
ERV lying (ml)	143.9	188.0	0–800	-0.13 (0.92)	-0.08 (0.53)

Table 10: Oscillometry results

Variable	Mean	SD	Range (0–100%)	Correlation to BE (p value)	Correlation to PaCO2 (p value)
R5%PN,seated	145.2	52.9	64–290.3	0.08 (0.50)	0.09 (0.46)
R20%PN,seated	114.2	41.9	50.8–297.4	0.01 (0.95)	-0.01 (0.94)
Z5%PN, seated	155.4	58.6	64.2–300.7	0.13 (0.29)	0.14 (0.25)
Change Z5 at FRC, seated (KPa/L/sec)	0.72	0.80	0–4.2	0.12 (0.32)	0.06 (0.66)
R5%PN, lying	**198.9**	**65.0**	**26.5–401.1**	**0.25 (0.04)**	**0.27 (0.02)**
R20%PN, lying	**145.5**	**40.0**	**30–247.5**	**0.29 (0.02)**	**0.23 (0.05)**
Change Z5 at FRC, lying (KPa/L/sec)	**1.59**	**0.95**	**0.1–5.4**	**0.26 (0.04)**	**0.22 (0.78)**
R5%_Seat	145.15	52.93	64.0–290.3	0.09 (0.45)	0.08 (0.49)
R20%_Seat	114.08	41.93	50.8–297.6	0.00 (0.95)	-0.09 (0.95)
R5%_Lying	**198.92**	**65.00**	**26.5–401.1**	**0.25 (0.03)**	**0.27 (0.02)**
R20%_Lying	**145.53**	**40.03**	**30.0–247.5**	**0.29 (0.01)**	**0.23 (0.05)**

Mean values (SD) for variables in the lung function domain with range (0–100%). Correlations are shown with base excess (BE) and $PaCO_2$ (p–values in brackets). ERV = expiratory reserve volume.

Table 11: Muscle strength indices

Variable	Mean	SD	Range (0–100%)	Correlation to BE (p value)	Correlation to CO_2 (p value)
MIP Max (cmH_20)	**81.9**	**31.3**	**26–156**	**-0.22 (0.05)**	**-0.20 (0.09)**
MEP Max (cmH_20)	86.5	32.1	38–178	-0.06 (0.64)	0.02 (0.84)
Sniff Max (cmH_20)	**79.9**	**34.2**	**22–176**	**-0.28 (0.02)**	**-0.22 (0.05)**
Max Handgrip (kg)	36.0	12.1	16–67	-0.04 (0.75)	0.04 (0.77)
Max Quadriceps (kg)	39.1	23.1	14–72.6	-0.13 (0.41)	-0.22 (0.17)

Mean values (SD) for variables in the muscle strength domain with range (0–100%).

Correlations are shown with base excess (BE) and $PaCO_2$ (p–values in brackets).

MIP=maximal inspiratory mouth pressure, MEP=maximal expiratory mouth pressure, Sniff= maximal sniff pressure.

Table 12: Sleep–related measures

Variable	Mean	SD	Range (0–100%)	Correlation to BE (p value)	Correlation to CO_2 (p value)
ESS	12.2	5.2	2–22	0.15 (0.20)	-0.15(0.90)
ODI (events/hr)	**48.4**	**36.7**	**2.44–155.85**	**0.34 (0.004)**	**0.39 (<0.001)**
Mean overnight oxygen saturation	**90.95**	**5.07**	**65.04–97.64**	**-0.50 (<0.001)**	**-0.60 (<0.001)**
AHI (events/hr)	27.0	29.5	0–100	0.15 (0.21)	0.09 (0.46)

Variable	Mean	SD	Range (0–100%)	Correlation to BE (p value)	Correlation to CO_2 (p value)
Time spent below 90% SaO_2 (hrs:mins:sec)	**1:53:16**	**2:04:50**	**00:00:05–07:51:00**	**0.33 (0.01)**	**0.29 (0.01)**
Time spent below 90% SaO_2 (% of sleep study time)	**27.2**	**29.2**	**0.0–100**	**0.46(<0.001)**	**0.50(<0.001)**

Mean values (SD) for variables in the sleep domain with range (0–100%). Correlations are shown with base excess (BE) and $PaCO_2$ (p–values in brackets). ESS= Epworth Sleepiness Score. ODI= oxygen desaturation index. AHI= apnoea/hypopnoea index.

Table 13: AutoCPAP measures

Variable	Mean	SD	Range (0–100%)	Correlation to BE (p value)	Correlation to $PaCO_2$ (p value)
AutoCPAP	12.0	2.8	5–19.8	0.136 (0.31)	0.083 (0.54)
Arousals per hour	40.3	21.5	0–83.1	-0.034 (0.78)	-0.083 (0.50)
Average size of arousals	32.8	6.2	23.9–65.9	-0.050 (0.68)	0.099 (0.42)
No PTT arousals	294.1	166.6	10.0–609	-0.03 (0.81)	-0.026 (0.83)
Av size of Respiratory swing	16.0	10.8	5.6–72.9	0.02 (0.98)	0.073 (0.55)
Obstructive	79.5	130.2	0–531	0.093 (0.44)	0.058 (0.63)

Variable	Mean	SD	Range (0–100%)	Correlation to BE (p value)	Correlation to $PaCO_2$ (p value)
Central	29.8	56.4	0–235	0.080 (0.50)	-0.06 (0.95)

Mean values (SD) for variables in the sleep domain with range (0–100%). Correlations are shown with base excess (BE) and $PaCO_2$ (p–values in brackets).

Table 14: Ventilatory drive measures

Variable	Mean	SD	Range (0–100%)	Correlation to BE (p value)	Correlation to CO_2 (p value)
Ventilatory responses to 5% CO_2					
Ventilatory drive (l/min/kPa)	1.37	1.70	0.01–10.46	-0.05 (0.70)	-0.04 (0.73)
Change in minute ventilation (l)	**5.16**	**4.14**	**0.12–12.2**	**-0.29 (0.02)**	**-0.22 (0.07)**
Final end–tidal $PaCO_2$ (mmHg)	47.1	5.26	37.3–68.4	0.01 (0.96)	0.16 (0.20)
Final SaO_2 (%)	**98.6**	**1.97**	**89.53–100.0**	**-0.33 (0.01)**	**-0.23 (0.06)**
Ventilatory responses to 15% oxygen					
Ventilatory drive (l/min/%)	**1.44**	**3.29**	**0.004–16.80**	**-0.28 (0.02)**	**-0.14 (0.26)**
Change in minute ventilation (l)	**9.64**	**2.78**	**5.00–21.45**	**-0.33 (0.001)**	**-0.30 (0.01)**
Fall in SaO_2 (%)	**3.44**	**2.33**	**0.15–11.28**	**0.46 (<0.001)**	**0.30 (0.01)**

Variable	Mean	SD	Range (0–100%)	Correlation to BE (p value)	Correlation to CO_2 (p value)
Final SaO_2 (%)	**91.8**	**5.49**	**57.81–97.53**	**-0.56 (<0.001)**	**-0.65 (<0.001)**

Mean values (SD) for variables in the ventilatory drive domain with range (0–100%). Correlations are shown with base excess (BE) and $PaCO_2$ (p–values in brackets). The derivatives shown are those that were significant only.

Table 15: Hormonal, nutritional and inflammatory measures

Variable	Mean	SD	Range (0–100%)	Correlation to BE (p value)	Correlation to CO_2 (p value)
BNP (ng per Litre)	**7.10**	**14.4**	**0.6–102**	**0.43 (<0.001)**	**0.62 (<0.001)**
Glucose (mmol)	6.33	2.76	4.1–21.8	-0.18 (0.15)	-0.15 (0.21)
Insulin (IU)	**261**	**204**	**64–1185**	**-0.23 (0.06)**	**-0.13 (0.31)**
Vitamin D (IU)	**42.6**	**19.3**	**12.5–102.3**	**-0.30 (0.01)**	**-0.16 (0.19)**
Ferritin (mmol)	**137**	**129**	**7.7–532**	**-0.19 (0.12)**	**-0.24(0.05)**
Total Iron (mmol)	14.6	5.8	2.4–33.3	-0.12 (0.31)	-0.21 (0.08)
Transferrin (mmol)	2.94	0.44	2.03–4.23	0.36 (0.002)	0.33 (0.01)
B12 (IU)	389	244	145–2000	-0.12 (0.35)	-0.16 (0.21)
Folate (mmol)	6.96	4.04	1.6–24	-0.19 (0.12)	-0.107(0.39)
TSH	2.65	1.54	0.2–7.8	-0.14 (0.22)	0.05 (0.64)
HbA_1C	6.42	1.22	5–9.9	-0.21 (0.08)	-0.11(0.37)
Leptin ng/ml	74.8	65.2	9–510	-0.19 (0.11)	-0.01 (0.95)

Variable	Mean	SD	Range (0–100%)	Correlation to BE (p value)	Correlation to CO_2 (p value)
Adiponectin µg/ml	5.15	2.37	1.81–11.9	-0.03 (0.84)	-0.14 (0.26)
HS CRP mg/l	8.52	6.48	0.44–29.8	0.18 (0.14)	0.14(0.26)

Mean values (SD) for hormonal, nutritional and inflammatory measures, with range (0–100%). Correlations are shown with base excess (BE) and $PaCO_2$ (p–values in brackets).

BNP–Brain natriuretic peptide, B12–vitamin B12, TSH–Thyroid–stimulating hormone, HbA_1C–Glycated haemoglobin.

Table 16: Blood test variables (hormones, vitamin and cytokines) and activity

Variable	Mean	SD	Range (0–100%)	Correlation to BE (p value)	Correlation to $PaCO_2$ (p value)
SBP (mmHg)	134.3	20.1	71–176	0.07 (0.56)	-0.06 (0.60)
DBP (mmHg)	82.5	13.3	51–106	0.04 (0.74)	-0.09 (0.49)
Hb (g/L)	14.1	1.7	9.3–17.2	0.20 (0.08)	0.11 (0.35)
Hct (%)	**0.43**	**0.05**	**0.32–0.56**	**0.41 (<0.001)**	**0.31 (<0.001)**
Albumin (g/dl)	44.5	2.76	36–51	0.13 (0.24)	0.06 (0.56)
Creatinine (µmol/L)	77.4	22.96	43–177	-0.19 (0.11)	-0.11 (0.34)
ALP (IU/l)	185.5	58.8	80–434	-0.21 (0.07)	-0.27 (0.20)
ALT (IU/l)	34.8	17.5	13–101	-0.09 (0.43)	-0.14 (0.22)
BNP (ng/L)	**7.1**	**14.4**	**0.6–102**	**0.43 (<0.001)**	**0.61 (<0.001)**
Fasting glucose (mmol/l)	6.3	2.7	4.1–21.8	-0.17 (0.14)	-0.15 (0.20)
Fasting insulin (mIU/L)	261.2	204.5	64–1185	-0.23 (0.05)	-0.12 (0.30)
Vitamin D (IU)	**42.6**	**19.3**	**12.5–102.3**	**-0.30 (0.01)**	**-0.15 (0.19)**
Calcium (µmol/L)	2.31	0.08	2.15–2.51	0.05 (0.67)	0.05 (0.70)
Ferritin µmol/L	137.0	129.1	7.7–531.8	-0.18 (0.12)	**-0.24 (0.04)**

Variable	Mean	SD	Range (0–100%)	Correlation to BE (p value)	Correlation to $PaCO_2$ (p value)
Total Iron	14.6	5.8	2.4–33.3	-0.12 (0.31)	-0.20 (0.08)
Transferrin	**2.94**	**0.4**	**2.03–4.23**	**0.35 (0.01)**	**0.32 (0.01)**
B12	389.2	243.9	145–2000	-0.11 (0.34)	-0.15 (0.21)
Folate	6.7	4.0	1.6–24	-0.19 (0.12)	-0.10 (0.38)
Cholesterol	4.9	1.4	2.2–11	-0.19 (0.09)	-0.28 (0.01)
Triglycerides	**2.02**	**1.1**	**0.67–5.96**	**-0.29 (0.01)**	**-0.22 (0.03)**
Fatty acids	**0.61**	**0.24**	**0.16–1.23**	**-0.41 (0.01)**	**-0.43 (0.01)**
TSH	2.7	1.5	0.19–7.8	-0.14 (0.22)	0.05 (0.65)
HbA1c	6.4	1.2	5–9.9	-0.20 (0.08)	0.01 (0.95)

Mean values (SD) for hormonal, nutritional and inflammatory measures, with range (0–100%). Correlations are shown with base excess (BE) and $PaCO_2$ (p–values in brackets).

Table 17: Bioimpedance variables

Variable	Mean	SD	Range (0–100%)	Correlation to BE (p value)	Correlation to $PaCO_2$ (p value)
Fat %_Bio	44.87	10.07	21.1–63.9	-0.01 (0.91)	-0.06 (0.61)
Fat kg_Bio	61.55	22.04	22.3–118.4	0.09 (0.46)	0.05 (0.66)
Lean % _Bio	54.92	13.92	5.0–101.6	0.13 (0.27)	0.16 (0.18)
Lean kg_Bio	75.96	25.50	20.6–220.0	0.18 (0.13)	0.24 (0.04)
Water %_Bio	44.98	16.36	12.5–129.5	-0.02 (0.81)	0.03 (0.79)
Water_Bio	56.86	14.89	32.8–111.2	0.11 (0.36)	0.15 (0.21)
Impedance	**382.3**	**86.86**	**194–704**	**-0.18 (0.14)**	**-0.27 (0.02)**

Mean values (SD) for hormonal, nutritional and inflammatory measures, with range (0–100%). Correlations are shown with base excess (BE) and $PaCO_2$ (p–values in brackets).

Table 18: Activity variables

Variable	Mean	SD	Range (0–100%)	Correlation to BE (p value)	Correlation to $PaCO_2$ (p value)
Physical activity (MET 3.0)	9:15:39.46	12:38:15.87	0:14:00.00 – 77:02:24.00	-0.08 (0.53)	-0.05 (0.69)
Daily steps	4169.5	3293.1	644–19345	-0.03 (0.77)	-0.03 (0.78)
Average MET	1.0	.23	0.70–1.9	-0.02 (0.86)	0.04 (0.74)

Table 19: Linear regression analysis using best predictors from each domain

	Pearson correlation coefficient	P-value
BEST PREDICTORS FROM EACH DOMAIN		
OBESITY		
Visceral adipose tissue volume by DXA scan	0.50	0.002
LUNG FUNCTION		
FEV_1 %PN, lying	-0.40	0.001
SLEEP VARIABLES		
Mean overnight oxygen saturation	-0.50	<0.001
VENTILATORY CONTROL		
Fall in SaO_2 (%) during 15% O_2 challenge	0.46	<0.001

	Pearson correlation coefficient	**P-value**
RESPIRATORY MUSCLE STRENGTH		
Sniff Max (cmH_20)	-0.28	0.02
Hormonal, nutritional and inflammatory measures		
Vitamin D	-0.30	0.01
SIGNIFICANT INDEPENDENT PREDICTORS		
	Cumulative correlation coefficient	
Fall in SaO_2 (%) during 15% O_2 challenge	0.49	<0.001
Visceral adipose tissue volume by DXA scan	0.71	0.01

DISCUSSION

The results and discussion section exclude any data gained from the MRI scans, fat and muscle biopsies.

4.1 The role of Base excess in obesity-related hypoventilation

4.1.1 Is a raised bicarbonate, without hypercapnia, part of the physiological spectrum of obesity-related hypoventilation?

In this prospective observational cohort study, I have demonstrated that obese subjects with a raised base excess, but with a normal daytime arterial partial pressure of carbon dioxide, have a ventilatory response between those of normal obese subjects (without evidence of awake hypoventilation) and those with hypercapnia and thus conventionally-defined obesity hypoventilation syndrome. This evidence suggests that these subjects are in the middle of a spectrum, and indeed they could be considered ‘early OHS’ patients, though I do not have longitudinal data showing that this will progress and if so over what timescale. These data challenge the conventional threshold-based definition of obesity hypoventilation syndrome, in so much as patients with daytime hypercapnia may represent only the severe end of the obesity-related hypoventilation spectrum.

The most striking example of the similarity between the group with overt OHS and the group with only a raised BE occurred in the hypoxic ventilatory response test. The subjects with a raised base excess level, but daytime normocapnia, showed a greater fall in oxygen saturation than was observed in the normocapnic obese subjects without an elevated base excess. Furthermore, there was no significant difference between those subjects with only an elevated base excess and the

patients with hypercapnia. It is possible that the failure to increase ventilation and defend the oxygen level, in the face of nocturnal hypoxia, could be a contributory cause to the gradual development of daytime hypercapnia. However, it is also conceivable that the reverse might be true, and my interpretation remains a hypothesis that needs to be tested.

Although the results of the hypercapnic ventilatory response tests also showed that the obese subjects with only a raised base excess have a similarity to those fulfilling the conventional criteria for OHS, causality is particularly difficult to establish; once there is a raised plasma bicarbonate level, the ventilatory response to inhaled carbon dioxide will be attenuated (Fahey and Hyde, 1983). Therefore, I can only comment that there was greater similarity between the subjects with metabolic compensation, with and without chronic hypercapnia, than between the subjects with normocapnia and a normal acid-base balance.

The unstimulated central drive to breathe during rest has been found to be higher in normocapnic subjects with morbid obesity than in normal-weight control individuals, presumably due to compensation for the increased mechanical load (Verbraecken and McNicholas, 2013). An impaired ventilatory response to hypercapnia is also a common feature of obesity-related hypoventilation (Verbraecken and McNicholas, 2013), although hypercapnic ventilatory responses are highly variable between individuals, and are sometimes even within the normal range in patients with only mild hypercapnia. The ventilatory response to hypoxia is also known to be blunted in subjects with obesity-related hypoventilation (Verbraecken and McNicholas, 2013). Thus, my observations of generally reduced hypoxic and hypercapnic ventilatory responses across the spectrum of obesity-related hypoventilation – from

normocapnia with some metabolic compensation through to metabolically compensated chronic hypercapnia – are supported by previous data.

There was no difference across the groups in terms of daytime somnolence, desaturation frequency (ODI), apnoeas and hypopnoeas (AHI) and overall oxygenation (mean SpO_2 overnight). Despite this, there were, however, differences observed in both the absolute time and the percentage of time spent below a SpO_2 level of 90% between the three groups (the raised BE only, and chronic hypercapnia groups, being the most similar). Although this greater nocturnal hypoventilation would be expected in the chronic hypercapnia group as a consequence of their lower baseline oxygen saturation level, this would not be the explanation for the subjects with normocapnia and metabolic compensation, as their awake SpO_2 values were similar. Again, it is difficult to infer the direction of causality in a cross-sectional study, but the group members with an elevated base excess, but normal daytime carbon dioxide levels, are likely to represent those patients at an early stage in the obesity-related hypoventilation spectrum; consequently, they would be those patients at risk of developing chronic hypercapnic respiratory failure if there were further deteriorations in the balance between neural respiratory drive, respiratory muscle load and respiratory muscle capacity (as would be expected, for example, with a further increase in weight). These findings suggest that sustained hypoventilation may be more relevant than intermittent hypoxia, such as that which occurs with purely obstructive or central sleep apnoea. This is physiologically plausible as brief transient periods of hypoventilation seen in upper airways obstruction only leads to a rise in carbon dioxide to mixed venous levels, with the rise thereafter very much slower (Mahutte et al., 1978). More prolonged hypoventilation, with attendant rises

in the partial pressure of arterial carbon dioxide, is required to promote bicarbonate generation and renal reabsorption of bicarbonate.

4.2 Predictors of ventilatory failure in obesity

In the next section I will discuss the predictors, which correlate either to PCO_2 and BE being statistically significantly in each physiological domain, and compare my findings with the current understanding is in the field. Currently, there is very little work looking at predictors of ventilatory failure in obesity, and more work has looked at either patients with pure OSA or those with OSA who have accompanying hypercapnia: this literature, although not strictly related, is used to help refute or support my findings, which is presented in the discussion below.

4.2.2 Measures of obesity and its distribution

Findings show that visceral adipose tissue, leg, android, trunk lean (all from DXA scan) significantly correlated to either BE or CO_2.

Obesity is a key component in diagnosis of OHS. However, there are issues surrounding the most accurate way to calculate obesity, and perhaps, upon reflection, DXA gives the best measurement of fat distribution. Traditional simple methods, such as body mass index (BMI), are known to be an inaccurate indicator of body fat, and as such are not a good predictor of comorbidities (Okorodudu et al., 2010). Most adult patients with a sleep disorder (classically OSA) have central obesity and increased visceral fat (Grunstein et al., 1993).

A very contentious topic at the moment is the importance of which areas fat is deposited, in relation to obesity. From this study, the visceral adipose tissue (VAT)

area seems to be pivotal. No work has been done in this area with patients with OHS. In OSA patients, VAT, which is measured at the level of the umbilicus, and its ratio to total adipose tissue area is shown to be both significantly greater in OSA patients than in non-OSA patients. All subjects whose VAT area measured more than 220cm^2 manifested OSA. A multiple linear regression analysis revealed that the visceral AT area significantly correlated to the apnoea index, when age was taken into consideration (Shinohara et al., 1997). These three findings suggest that VAT is important in the development and occurrence of sleep-disordered breathing.

Other studies in adults have confirmed these findings, and have found that visceral adiposity correlated more strongly than BMI with the presence of OSA (Vgontzas et al., 2000).

In another study, the same authors demonstrated that several measures of abdominal adiposity (waist circumference, waist to-hip ratio and percentage fat mass measured by bioelectric impedance) correlated with oxygen desaturation, associated with brief respiratory pauses but not OSA (Verhulst et al., 2007). These findings may be in keeping with the conclusion that the distribution of fat is key to the development of sleep-disordered breathing.

Abdominal fat is thought to result in, and to lead to, cranial displacement of the diaphragm, decreasing longitudinal tracheal traction on the upper airway and leading to increased propensity for upper airway collapse (Rowley et al., 1996).

Recently, gender roles have been implicated in the issue of fat distribution? Women tend to distribute fat peripherally around the hips, buttocks and thighs, whereas men tend to distribute excess fat more centrally on the abdomen and neck (Whittle et al., 1999)

Traditional anthropometric measures used in studies of OSA include body mass index (BMI), waist and neck circumferences, neck-to waist ratio (NWR), waist-to-hip ratio (WHR) and skin-fold anthropometry, which are of limited accuracy in determining fat distribution (Davies et al., 1992). This is confirmed by the studies' findings, which show no correlation between these predictors or BE and CO_2.

In both men and women, it is centrally-located fat rather than that which is peripherally-located that contributes to the pathogenesis and severity of OSA. However, there are substantial sex-based differences in the association between fat distribution and severity of OSA found within the study.

4.2.3 Bioimpedanc

Within this study, bioimpedance has been found to correlate with PCO_2 (-0.27) significantly, but none of the other derivative of bioimpedance, as listed within the results section, correlated with BE or PCO_2. Bioimpedance analysis (BIA) is a reliable, non-invasive, safe and effective technique to measure body composition (Segal et al., 1988). BIA analysis is based on the principle that electric current flows at different rates through the body depending upon its' overall composition of fat, water and muscle. BIA determines the overall electrical resistance of the body. Adipose tissue is significantly less conductive than muscle or bone: additional parameters assessed using BIA include total body water (TBW), body cell mass (BCM), body fat mass (FM), lean fat free mass (FFM), body cell mass index (BCMI) and muscle mass (MM). It is interesting that only bioimpedance correlates with BE and CO_2, and probably reflects the inaccuracies of this method, especially in the super obese.

Perhaps it isn't just the fat distribution but also fluid that plays a part in OHS. Authors have suggested that overnight rostral fluid displacement from the legs could play a role in the development of OSAS, independent of body weight (Redolfi et al., 2009). Fluid shifts could play a role in OHS, also.

Although BIA is a reliable method of body composition measurement, it could be noted that BIA has a few limitations. Some authors suggest that in obese patients (especially with BMI >34), the levels of extracellular water and fat-free mass may be overestimated, and fat mass underestimated. Moreover, in the OHS group, there were patients with comorbidities, which could have had an influence upon the results achieved (Redolfi et al., 2005).

The BIA measurement has many practical advantages: the instrumentation is relatively inexpensive and requires minimal maintenance and operator training. The measurements can be repeated as frequently as necessary, and the results are available immediately. I should also be noted that the level of active participation requited of the subjects being examined was relatively low, which highlights a high positive cost benefit ratio enabling this form of testing to be highly accessible, with a relative ease of use, on a small budget.

4.2.4 Differences in lung function and muscle strength

This subsection describes activity, muscle strength and lung function and the correlations to BE or CO_2.

No correlations were between physical activity (MET 3.0), daily steps and BE with CO_2.

Reduced physical activity is often a hallmark of patients with OHS, and often one of the limiting features is breathlessness. Breathlessness on exertion is a very common symptom in obese individuals (Gibson, 2000). In studies of obese patients with type II diabetes mellitus, one third reported abnormal breathing that became more prevalent with increasing BMI (Bulpitt et al., 1998). In healthy obese subjects, the sensation of dyspnoea may originate from an increase in the work of breathing to overcome decreases in pulmonary compliance and increases in resistance. Airway obstruction at low lung volumes may also stimulate flow receptors to increase the sensation of breathlessness (Pankow et al., 1998). However, within the study it was

found that the lack of physical activity within obese subjects does not seem to contribute to the development of OHS.

In a series of 20 obese women, looking at why activity is affected in obesity, a negative deviation of actual, from predicted resting metabolic rates (RMR) related to a lower lipid oxidation. This has been associated with a lower amount of weight loss during the 12 months following a gastric bypass (Bobbioni-Harsch et al., 2000).

Another method of testing employed was to measure physical activity (PA). Questions could be raised as to the validity of the chosen method of measuring PA due to the mechanism of calculation - an electronic device, strapped to the patients' upper arm, is relied upon to correlate movement into a predetermined sum of what kCals have been expended. It should be noted that using accelerometers to measure PA in bariatric patients involves special considerations and certain limitations: accelerometer count cut-offs for identifying different intensities of PA in the severely obese have not yet been established. Consequently, currently available thresholds that were determined in leaner samples may affect the validity of PA estimates in the bariatric population.

Varying degrees of bias have been observed when validated in healthy older obese adults (Papazoglou et al., 2006). However, limitations regarding validity have been reported during various activity types (Berntsen et al., 2010) - the majority of validation studies have been conducted in healthy adults (St-Onge et al., 2007). Two of these studies have utilised the devices used within this present study (sensewear activity) as a valid tool for estimation of cumulated daily energy expenditure, in comparison with doubly-labelled water (the gold standard) (St-Onge et al., 2007).

4.2.4.1 Strength

No correlation was found between hand grip or quadriceps strength and BE and CO_2.

For the quadriceps strength test the quadriceps femoris muscle was assessed, which consists of the articular rectus femoris and monoarticular vastii, which are responsible for knee extensor torque production and are therefore supposed to play a key role during ambulatory and functional activities. As such, this makes it an ideal muscle to assess when making a judgement on lower limb strength.

Obese adults have been found to generate higher absolute strength and power with the lower extremity muscles than their non-obese counterparts, probably because of the larger amounts of fat-free mass they carry - specifically, quadriceps muscle strength and power is relative to body mass; (Hulens et al., 2001).

As an explanation, many researchers have suggested that the extra weight chronically carried by obese individuals might serve as a favourable training stimulus to increase muscle mass and consequently muscle performance. Interestingly, a significant enhancement in muscle power has been experimentally demonstrated in athletes following three weeks of extra-load conditioning achieved by wearing special vests containing weights (an increase of 7-8% of their body mass) (Bosco et al., 1986).

When voluntarily activating the quadriceps femoris muscle, obese subjects showed reduced fatigue resistance compared to their lean counterparts, while no significant divergence was observed during the stimulated fatigue test. The observed quadriceps muscle function impairments probably contribute to the reduced

functional capacity of obese individuals during activities of daily living (Hulens et al., 2001).

In a previous human muscle biopsy study, results from histology of samples collected demonstrated that obese individuals have an increased proportion of glycolytic type IIb fibres in their vastus lateralis muscle (Kriketos et al., 1997).

4.2.4.2 Non-invasive muscle strength test

A correlation between SNIP and MIP non-invasive respiratory muscle strength and BE and CO_2 was found within this study.

Respiratory muscle strength is likely to be important in the maintenance of normocapnia in obesity. However, little is known about the “fit and obese” individuals. In addition, the biomechanical mechanism by which this group is able to generate the high ventilatory demand associated with increased physical demands is unclear.

Improving understanding comes from studying the physiologic changes in endurance-trained obese individuals, which may provide valuable new insights. One study showed that during exercise, the increment in diaphragm activity per unit change in carbon dioxide tension in the arterial blood was above normal in the obese-normal subjects but just normal in the obese-hypoventilation subjects (Lourenco, 1969). Therefore, it was postulated that the differences in diaphragm activity probably represent dissimilarities in respiratory centre output between obese subjects with and without respiratory failure. In order to produce the same amount of ventilation, obese subjects needed more diaphragmatic activity than normal subjects.

An alternative explanation of the ventilatory failure and the lesser diaphragmatic response in patients with OHS might be an increased bicarbonate concentration, secondary to chronic hypercapnia, which would decrease the responsiveness to carbon dioxide at the level of the central chemoreceptors, in a way similar to that postulated for patients with chronic obstructive lung disease (Lourenco and Miranda, 1968).

As mentioned previously, respiratory muscle function, and in particular unloading of muscle, seem to be key processes. Unloading of the respiratory muscles with treatment using non-invasive ventilation (NPPV) has been reported in other disease processes, such as chest wall restriction, due to kyphoscoliosis (Carrey et al., 1990).

It could be hypothesised that unloading of the respiratory muscles might be achieved by NPPV in massive obesity and that this effect could contribute to the beneficial clinical effects of NPPV in obese patients who have an increased impedance of the respiratory system due to morbid obesity.

In obesity, the basis for the increased impedance is that an increased abdominal load can shift the diaphragm in an upward direction, thereby decreasing expiratory reserve volume and FRC (Ray et al., 1983). As a result, obese subjects breathe at lower lung volumes along the very flat portion of the already flattened pressure-volume curve. This requires more work in breathing and places more pressure on the respiratory muscles.

It is speculated that in OHS the combination of obesity-related alterations of chest wall and lung mechanics, and increased inspiratory load due to functional upper airway obstruction during sleep, may induce respiratory muscle fatigue (Martin and Sanders, 1995). This concept is supported by the observation that in some patients

with OHS, nocturnal splinting of the upper airway with CPAP results in rapid normalisation of daytime blood gases (Shivaram et al., 1993).

4.2.4.3 Differences in lung function

Correlations exist between FEV_1 %PN, seated, FVC %PN, seated, VC%PN, FEV_1 %PN, lying FVC%PN, lying and BE and CO_2.

Obesity is well known to be associated with decrements in lung volumes, with functional residual capacity (FRC) being reduced more than residual volume (RV) (Biring et al., 1999), thus expiratory reserve volume (ERV) markedly decreases and tidal breathing takes place at a low lung volume. Under these conditions, some airways tend to narrow or even close during expiration, a fact expected to cause ventilation heterogeneities (Thomas et al., 1989).

Several investigations reported an increase in lung elastic recoil with a reduction of lung compliance both in awake (Behazin et al., 2010) and anesthetised-paralyzed obese subjects. In only one study was lung compliance found to be normal in obesity (Naimark and Cherniack, 1960) but this does not disprove that lung elastic recoil was increased. Indeed, if RV is decreased as reported in several studies (Pelosi et al., 1996) then a normal lung compliance would reflect a parallel shift of the pressure volume curve, thus suggesting higher lung recoil pressure at all lung volumes (Jones and Nzekwu, 2006).

According to principles of lung mechanics, a decrease in FRC is associated with a decrease in airway size proportional to the square root of lung volume (Hughes et al., 1972). Therefore, obesity should be associated with airway narrowing within the tidal breathing range.

In theory, there are mechanisms that could counter the effects of obesity on ventilation. An increase in tidal volume could have partly restored the equilibrium between airways of different calibre, (Anafi and Wilson, 2001) thus allowing them to distend during inspiration, although lung volumes correlate strongly with BE and CO_2.

4.2.4.4 Impulse oscillometry (IOS)

There are correlations between R5%PN lying, R20%PN lying, Z5%PN lying, change Z5 at FRC lying (KPa/L/sec), R5% lying, R20%lying and BE and CO_2.

IOS employs small-amplitude pressure oscillations superimposed on quiet breathing to measure the impedance of the respiratory system. It can distinguish impedance of central and peripheral airway to reflect the extent of airway obstruction indirectly. IOS has begun to be used to evaluate airway obstruction in OSAS patients in recent years (Navajas et al., 1998; Oostveen et al., 2003).

In healthy individuals, changing from the sitting to supine position results in an increase in airway resistance. The narrowing of pharyngeal space may be more evident in the supine position, compared to the sitting posture. So, supine posture itself may contribute to the increase in airway resistance during sleep in OSAHS patients

The present study showed that airway resistance increased when body position changed from sitting to supine: the significance increased at all frequencies.

Forced oscillation testing has previously demonstrated elevated resistance associated with reduction of resting lung volumes as demonstrated by measurement of airway conductance (Zerah et al., 1993).

Frequency dependence of resistance was also detected in obese subjects. This oscillometric parameter has been generally attributed to non-uniformity of airflow distribution in obesity, which may reflect regional functional abnormalities in the distal lung (Macklem and Mead, 1967).

In obesity, distal airway dysfunction may occur due to either mass loading induced airway compression and/or intrinsic airway disease.

The residual abnormality suggests distal airway dysfunction may relate to residual bronchial hyperreactivity (Zerah-Lancner et al., 2011).

4.2.4.5 Posture

From studies in patients with OSA, it has been observed that the avoidance of the supine position leads to a decrease in the number and severity of obstructive episodes during sleep (Cartwright, 1984). In the supine posture, the upper airway calibre and resistance are greater. Some previously published studies have classified OSA patients into two groups: positional and non-positional. There is a previous association of body position with severity of apnoeic events in patients with severe non-positional obstructive sleep apnoea (Oksenberg et al., 2000). The effect of posture on REM sleep is also seen and is different from that of non-REM sleep. It has been reported that the increase in AHI in the supine position compared to lateral position was evident only in NREM sleep (George et al., 1988).

Lung volume is an important determinant of upper airway collapsibility. Increasing lung volume increases upper airway calibre (Burger et al., 1992) and reduces its resistance and tendency to collapse (Series et al., 1990).

Potential mechanisms by which an increase in lung volume may elevate upper airway patency include 1) an increase in transmural distending pressure, induced by thoracic expansion, is associated with increased pressure gradients at the thoracic inlet and/or 2) stabilization of the airway wall through caudal traction on the upper airway from mediastinal tension generated by diaphragm descent (Kairaitis et al., 2012).

A decrease in lung volume potentially reduces the degree of axial tension (caudal traction) exerted on the upper airway (UA) and, therefore, the propensity for UA collapse.

4.2.5 Role of hormonal, nutritional, inflammatory and cytokine measures

4.2.5.1 BNP

BNP correlates significantly to both CO_2 and BE (0.43 and 0.62 respectively).

BNP is well known to correlate with both CCF and pulmonary arterial hypertension but as far as is known, has not been shown to correlate to OHS. To explain the possible correlation, other respiratory conditions could be studied, where BNP is diagnostically useful. One such condition is pulmonary hypertension (PH). Perhaps obese subjects with ventilatory failure suffer with undiagnosed PH?

There is limited data on the prevalence of PH in the obese. One retrospective single centre study reported that 5% of otherwise healthy individuals with a BMI >30kg/m^2 had moderate or severe pulmonary hypertension (with pulmonary arterial systolic pressure (PASP) greater than 50mmHg on echocardiogram; normal pressure 15–25 mmHg) (McQuillan et al., 2001).

In terms of what is known from OHS patients, pulmonary hypertension occurs in approximately 50% of them, as compared to approximately 20% of a comparable group of OSA patients (Kessler et al., 1996).

The diurnal hypoxemia, hypercapnia and acidosis associated with OHS could be all mediators of PH and could be attributed to being one of the reasons for PAH developing in OHS.

Secondary contributors to PH in patients with OHS could be restrictive lung disease, which occurs in severe obesity. Obesity causes an increase in upper airway resistance and subsequently the wide intrathoracic pressure shifts in the respiratory cycle (negative intrathoracic pressures during inspiration, up to −70 mm Hg), which may contribute to the development of PAH. These negative intrathoracic pressures augment right ventricle filling, causing a leftward shift of the intraventricular septum, which impedes left ventricle filling and thus elevates pulmonary venous pressures and also lowers left ventricle stroke volume.

Recently, increased BNP values have been found correlate with both pulmonary hypertension, and with other chronic lung diseases of different aetiologies, which serves as a risk factor for mortality (Leuchte et al., 2006).

Other evidence emerging from cell culture studies, and animal experiments, which could potentially explain the link between BNP and OHS is that hypoxia of cardiomyocytes triggers the release of BNP (Arjamaa and Nikinmaa, 2011). The physiological in vivo relevance of this cell culture finding remains unclear, as tissue oxygen levels may never reach such low and long-standing severe hypoxic values as is generally used with in vitro studies. Nevertheless, normobaric hypoxic exposure

for 60 minutes has been shown to induce a slight increase in N-terminal BNP. However, this study was performed in a highly selected group of subjects that showed either high or low renin-angiotensin system activity (Due-Andersen et al., 2008).

This study finds that levels of vitamin D correlate to BE (-0.3) significantly. In a clinical context this correlation to OHS patients has already been seen. A recent study showed increasing numbers of patients admitted to intensive care units (ICU) (Venkatram et al., 2011), with hypercapnic respiratory failure and multisystem organ dysfunction related to obesity, had low vitamin D levels. Similarly, 61 patients (8% of all ICU admissions) in another study met the same inclusion criteria as this study, for the level of vitamin D deficiency (Quraishi et al., 2014).

Vitamin D deficiency is associated with myopathy that could be associated with diaphragmatic and respiratory muscle weakness (Ceglia, 2008). Vitamin D deficiency is also associated with increased incidence of diabetes, poor glycaemic control in diabetics as well as more micro- and macrovascular complications of diabetes; all these factors could promote the development of ventilatory failure in obesity. Vitamin D deficiency has well-established links to other respiratory conditions. It has been shown to increase exacerbations in chronic obstructive lung disease (COPD), increase hospitalization rates in children with asthma and increase the risk of development of upper respiratory tract infections.

The pathogenesis underpinning its role may perhaps relate to the pleiotropic actions of vitamin D in immunity, endothelial/mucosal functions and glucose metabolism, as well as calcium homeostasis. These factors all appear to be very important in maintaining overall health. Recent evidence also links vitamin D deficiency with the

systemic inflammatory response syndrome (SIRS), septicaemia, organ failure and metabolic dysfunction in critically ill patients (Lee et al., 2009).

4.2.5.2 Transferrin

Within this study, low levels of transferrin correlate to BE and CO_2 significantly. Hypoxia appears to be key to the pathology in OHS. However, transferrin may be simply a marker of the presence of OHS rather than the cause of OHS. Metabolism of iron and oxygen is known to be interconnected by a complex, and an incompletely understood mechanism.

Sleep deprivation or other physiological consequences of sleep-disordered breathing (all seen in OHS) could conceivably influence the metabolism of iron. Absorption, iron loss, and iron stores, (from consumption of iron containing nutrients) (Pinhas-Hamiel et al., 2003), gastrointestinal function (Mieda and Yanagisawa, 2002) and key metabolic pathways are also affected by sleep quality. Another issue with ferritin is that, although its serum level is a well-accepted marker for total body iron stores, this can be variable and may not reflect ferritin levels in the cerebrospinal fluid (Earley et al., 2000).

OSA, which is often found in OHS patients, is often associated with chronic inflammation, which, in turn, could increase ferritin levels (Arnardottir et al., 2009).

The hypoxia in OHS could stimulate production of red blood cells and subsequently increase haematocrit levels to a mild extent, which could potentially explain this studies findings related to the increased level of ferritin (Hoffstein et al., 1994).

Metabolism of iron and oxygen are interconnected by complex and incompletely understood mechanisms. Oxygen metabolism is key to the pathogenesis in OHS. Mammalian cells adapt to a low oxygen environment by activating hypoxia inducible factor (HIF), a transcriptional factor which subsequently regulates expression of over 60 genes (Wang and Semenza, 1993). HIF can directly regulate gene expression by binding to hypoxia response elements (HRE), located upstream of transcriptional start sites of target genes (Semenza and Wang, 1992). HREs have been found on many genes involved in iron transport and homeostasis, including transferrin (Tf) and transferrin receptor (TfR). There is no consensus on how hypoxia affects expression of ferritin, transferrin and TfR. Most studies, often contradictory, have been conducted on cells with high iron storage capacity. Hypoxia has profound effects on systemic iron metabolism through the regulation of expression of hepcidin, the main factor controlling central iron homeostasis. There is extensive literature on the relationship between oxygen concentration and Tf iron. It is therefore unclear if this correlation is actually scientifically relevant.

4.2.5.3 Insulin

Withing this study, fasting insulin levels correlate to BE significantly. To explain the correlation between fasting insulin and BE one needs to consider the interaction of sleep and metabolism. From epidemiology studies, sleep duration has decreased over the last several decades, and also from this data a link between short sleep

duration and the prevalence of type 2 diabetes has been suggested. This has been emphasised in studies since forced decreased sleep duration in healthy individuals has been linked to impaired glucose homeostasis. Moreover, short sleep duration has been suggested to lead to obesity although, this is less conclusive since psychological and social factors also considerably impact food intake.

Little is known as to whether OHS is a risk for diabetes mellitus, so I one must look to OSA for any potential causation. OSA is a risk factor for cardiovascular disease and type 2 diabetes (Bradley and Floras, 2009). Several cross-sectional and longitudinal studies, however, were unable to conclude unequivocally that OSA is an independent risk factor for diabetes (Bradley and Floras, 2009).

Similarly to what was observed for type 2 diabetes, the prevalence of impaired fasting glucose (IFG) and of impaired glucose tolerance (IGT) increases with the severity of OSA (Seicean et al., 2008).

Again, hypoxia seen in OHS may have a key role to play. Acute hypoxemia and prolonged exposure to high altitude hypobaric hypoxia both alter glucose metabolism in healthy subjects (Oltmanns et al., 2004). Further, glucose tolerance is impaired in patients with chronic obstructive pulmonary disease and improves with oxygen supplementation (Hedenstierna and Santesson, 1976). Insulin and glucose levels have been found to be associated with AHI and/or ODI and/or nocturnal SaO_2 but this was often dependent on graduations of obesity (Bulcun et al., 2012).

Hypoxemia is an important stimulus for increasing sympathetic activity, which in turn can impair glucose homeostasis by increasing glycogen breakdown and gluconeogenesis and for releasing inflammatory factors with deleterious effects on insulin synthesis and its peripheral action.

No correlation was found between leptin levels and BE and PCO_2. Leptin is a protein hormone produced by mammalian adipocytes. It acts within the hypothalamus via a specific receptor to reduce appetite and increase energy expenditure (Considine et al., 1996).

There is evidence, for example, that obese humans have a relative deficiency of CNS leptin compared with lean controls (Caro et al., 1996). Recent data has shown, however, that leptin replacement reverses the hypoventilation that occurs in a leptin deficient mouse model of obesity (O'Donnell C et al., 1999).

Through the duration of the mouse model of obesity study, it was observed that serum leptin is a better predictor than % body fat for the presence of hypercapnia in patients with OHS.

In the ob/ob mouse, - a genetically mutated mouse model of obesity that is leptin deficient - there is an increased $PaCO_2$ and a reduced hypercapnic ventilatory response (O'Donnell C et al., 1999). Leptin administration reversed hypoventilation in this model, presumably by stimulation of central respiratory control centres (O'Donnell C et al., 1999). In humans, leptin levels can fall by up to 53% after a 10% loss of body weight, and in eucapnic obese patients the hypercapnic respiratory drive also falls after weight loss. These results suggest that leptin may act to maintain alveolar ventilation to compensate for the increased ventilatory load in obesity (O'Donnell et al., 2000).

Similarly to what was observed for type 2 diabetes, the prevalence of impaired fasting glucose (IFG) and of impaired glucose tolerance (IGT) increases with the severity of OSA (Seicean et al., 2008).

4.2.5.4 Fatty acids and triglycerides

This study found a correlation between fatty acids and triglycerides and BE and PCO_2; Little is known about the association between FA and triglycerides with OHS

OSA is associated with insulin resistance and hyperlipidemia and both conditions are associated with non-alcoholic fatty liver disease (NAFLD) (Minoguchi et al., 2006). OSA can lead to an increase in insulin resistance and to an alteration in lipid metabolism (Savransky et al., 2007). Thus, it is plausible that a high-fat diet that occurs in the presence of hypoxia with OSA promotes NAFLD. Therefore, hypoxia can increase lipogenesis and inhibit fat oxidation.

Adipose tissue hypoxia (ATH) is a new concept in understanding the pathogenesis of insulin resistance and inflammation in OSA. The concept suggests that inhibition of adipogenesis and triglyceride synthesis by hypoxia might be a mechanism for the increased free fatty acid (FFA) concentrations in obesity that occurs with insulin resistance. Gross obesity might be associated with ATH and adipocyte death. However, the exact cause of adipocyte death in obesity is not known. It has been suggested that gross obesity, per se, is associated with a reduction in blood flow to adipocytes due to diminished angiogenesis or vasoconstriction (Ye, 2009).

In rats, Repeated exposure to hypobaric hypoxia causes a significant increase in the concentration of cholesterol, fatty acids, chylomicron, LDL-cholesterol and very low-density beta-lipoproteins (VLDL), whereas the level of HDL-cholesterol decreases (Tomasova et al., 1987).

4.2.6 Difference in ventilatory response to hypercapnia and hypoxia

Ventilatory drive in the hypoxic challenge test (l/min/%) significantly correlates to BE, and changes in minute ventilation (l), with falls in SaO_2 (%) in the hypoxic challenge test. All of these factors correlate with BE and CO_2.

At baseline, obese subjects have increased rates of oxygen consumption (VO_2) and carbon dioxide production (VCO_2), even when they are at rest. Thus, obese persons must increase their minute ventilation to meet the increased oxygen requirements and maintain adequate alveolar ventilation (Steier et al., 2009).

In terms of ventilatory drive, studies of normocapnic obese individuals demonstrate an increase in respiratory drive (Lopata and Onal, 1982) which would aid in the maintenance of ventilation despite abnormal ventilatory mechanics.

Moving to patients with OHS, both hypoxic and hypercapnic ventilatory responsiveness has been shown to be attenuated in patients with OHS (Berger et al., 2001). However, in another study, central responsiveness to hypercapnia and hypoxia is blunted in OHS patients compared to normal-weight subjects and normocapnic obese patients with or without OSA (Zwillich et al., 1975).

Looking at ventilatory drive more closely, in a recent study (Chouri-Pontarollo et al., 2007), patients with OHS were divided into normal or low responders to carbon dioxide: lower ventilatory responses to carbon dioxide were associated with more time in hypoventilation in REM sleep.

The central drive to breathe is significantly higher in normocapnic subjects with morbid obesity, than in normal-weight individuals, and therefore OHS patients fail to

augment drive to compensate for the added load created by excess weight, permitting a gradual rise in CO_2 to take place (Lopata and Onal, 1982).

The decrease in ventilatory response is attributed to an inadequate increase in tidal volume as a result of a blunt neural response to hypercapnia (Macavei et al., 2013). The slope of the hypercapnic ventilatory response is <1l/min/mmHg in OHS, between 1.5 and 2.5l/min/mmHg in eucapnic obese individuals and 2-3l/min/mmHg in healthy subjects (Verbraecken et al., 1995).

The hypoxic ventilatory response is also blunted in subjects with OHS. This abnormality is, however, not familial and improves with treatment (Han et al., 2001).

Previous studies suggested that an increase in chemical drive can elevate genioglossus activity before airway opening (Berry et al., 1997), indicating that upper airway muscles can respond to increasing chemical drive prior to arousal (Carlson et al., 1995). The role of ventilatory drive in the pathophysiology of OSAS is controversial. Several studies in adults have assessed the ventilatory response to hypercapnia in patients with OSAS, but the results have been inconsistent. The ventilatory response to hypercapnia during wakefulness in adults with OSAS has been reported to be higher (Makinodan et al., 2008), lower (Garay et al., 1981) and the same (Foster et al., 2009).

Similarly, studies have shown that the occlusion pressure in 100 milliseconds (P0.1), a measurement of ventilatory drive independent of upper airway resistance, does not correlate with measures of upper airway collapsibility during wakefulness (although there is a correlation during sleep) (Marcus et al., 1999).

Obesity has been thought to result in a decreased ventilatory response to CO_2 due to changes in pulmonary mechanics, so this increased HCVR is unexplained (Ge et al., 2005).

It is possible that obese patients have different responses to hyperoxia ,compared with lean patients. In addition, it is controversial as to whether HCVR should be adjusted for body size (Hirshman et al., 1975).

The lower ventilatory response to CO_2 during sleep, compared with that during wakefulness, (Marcus et al., 1999), could be due to a decrease in the central ventilatory drive during sleep. The blunted response to CO_2 during sleep could also be due to increased upper airway collapsibility; i.e. patients with OSAS may have a normal central ventilatory drive, but ventilation may be impeded by increased upper airway resistance and/or collapse. It has previously been shown that ventilatory drive is sleep state-specific.

Patients with OSAS may have a blunted cerebrovascular response to CO_2 due to chronic nocturnal hypercapnia and hypoxemia, or to inflammation related to OSAS (Gozal et al., 2007).

It has been suggested that hypercapnic OSAS patients experience greater oxygen desaturation during sleep than do those with eucapnic OSAS.

Abnormal hypoxic and hypercapnic respiratory drive has been reported in hypercapnic OSAS patients, in contrast to normal hypoxic and hypercapnic respiratory drive in eucapnic patients (Zwillich et al., 1975): severe sleep hypercapnia will blunt the waking hypercapnic ventilatory response (Falchuk et al., 1966).

Kunitomo et al. (Kunitomo et al., 1989) demonstrated that the waking hypoxic ventilatory drive is inversely correlated with the magnitude of maximal oxygen desaturation during sleep, as well as the duration of oxygen desaturation as a percentage of total sleep time. Thus, it is possible that repetitive apnoea and exposure of the central chemoreceptors to hypercapnia causes adaptation and resetting of the ventilatory response to hypercapnia.(Kunitomo et al., 1989). Hypoxia may interfere with synthesis and turnover of a wide range of neurotransmitters.

4.2.7 Increased sleep-disordered breathing and nocturnal hypoventilation

This study found that both mean %SpO_2 and time spent with %SpO_2 < 90% both correlated with BE and CO_2.

Previous studies have shown that the majority of patients with OHS have concomitant OSA of 90%. The remaining 10% of patients have non-obstructive sleep hypoventilation characterised by an AHI <5, which is defined as either an increase in $PaCO_2$ of >10 mmHg above that of wakefulness or significant oxygen desaturation, neither of which is the result of apnoea or hypopnea (Mokhlesi and Tulaimat, 2007).

Previous studies that attempted to determine risk factors or predictors of hypercapnia in cohorts of patients with OSA reported mixed results (Mokhlesi, 2010). Mokhlesi et al. defined an AHI of 100/h as a highly specific threshold for the detection of patients with OHS. Nevertheless, the severity of AHI is not universally accepted as a good predictor of OHS (Mokhlesi et al., 2007).

A practical tool used for the screening of OHS when interpreting sleep studies, seems to be the amount of time spent with SpO_2 < 90% (Mokhlesi, 2010).

Other authors have found this sleep variable to be an independent predictor for OHS, accounting for 24% variance of pCO_2 (Resta et al., 2000).

It is thought that, in obese individuals, fat deposits in any part of the upper airway increase the total volume of soft tissue within the maxillomandibular enclosure which narrows the pharynx and increases collapsibility of the upper airways (Isono, 2012).

Interestingly, the amount of adipose tissue adjacent to the pharyngeal airway and in the intrathoracic space directly associates with AHI (Vgontzas et al., 2008, Vgontzas et al., 2000) but not with BMI. Enlarged neck circumference has often been attributed to increased adipose tissue in the neck; however, larger studies have not directly measured neck fat (Katz et al., 1990).

As previously discussed, the role of abdominal fat in upper airway instability is increasingly recognized: recumbent abdominal obesity is likely to be associated with increased cranial displacement of the diaphragm, decreasing longitudinal tracheal traction and increasing propensity for upper airway collapse (Rowley et al., 1996).

Accumulation of fat in the chest wall (abdominal and thoracic) also decreases functional residual capacity, particularly when the individual is recumbent and asleep. This increases intrathoracic pressure and thereby extramural tissue pressure at the thoracic inlet, further increasing upper airway collapsibility (Stanchina et al., 2003).

Intermittent hypoxemia predisposes animals and humans to insulin resistance (Oltmanns et al., 2004) sleep fragmentation, as a result, increases insulin resistance in healthy controls.

Total body water was not measured in the current study but there is recent data suggesting that a rostral shift of tissue fluid, as a result of lying overnight in the supine position may contribute to the occurrence of apnoeas (Redolfi et al., 2005). Several previous studies have pointed to neck size as an arbitrary measure of sleep apnoea severity (Davies et al., 1992) – this was not confirmed in the current study.

4.3 Critique of creditability of findings

4.3.1 Study design

The most important advantage of cross-sectional studies is that in general they are quick and cheap. As there is no follow-up, fewer resources are required to run the study. Cross-sectional studies are the best way to determine prevalence and are useful at identifying associations that can then be more rigorously studied using a cohort study or randomised controlled study. The most important problem with this type of study is differentiating cause and effect from simple association. Often there are a number of plausible explanations. Cross-sectional studies do not provide an explanation for their findings.

4.3.2 Patient characteristics

These data were collected from a single centre specialising in the management of patients with sleep-disordered breathing and chronic respiratory failure. However, I feel the heterogeneous nature of the whole study group reduced any selection bias and reflected the clinical picture seen in obese subjects with and without OHS. On the other hand, the exclusion of subjects with the potential for a raised plasma

bicarbonate, for reasons other than obesity-related hypoventilation, lowers the general applicability of an isolated raised base excess level to identify early OHS.

4.3.3 Assessment of hormonal, nutritional or cytokine levels

Leptin levels were not predictive; however, leptin receptor levels were not assessed and resistance rather than total leptin may be more important (Verbraecken and McNicholas, 2013).

The novel finding that vitamin D correlates with base excess is interesting but possibly not robust since the correlation with $PaCO_2$ is absent. A stronger relationship between OHS severity and transferrin has no hypothesized mechanistic basis. The interesting novel link between OHS severity and BNP suggests there may be consequences for cardiac stretch, yet this finding does not contribute insight into the causal pathway for OHS.

4.3.4 Assessment of ventilatory drive

While several methods have been developed to measure the ventilatory response to hypoxia, a standard method capable of comparing test results between subjects and within the same subject under different conditions remains elusive.

This is the criticism of the technique which was employed here as it may over-represent the effect of hypoxia on ventilation.

The normal ventilatory response to hypoxia is mediated by the peripheral chemoreflex, a reflex arc from the carotid body sensor to the respiratory muscle effectors (Torrance, 1996).

The hypoxic ventilatory response (HVR) can be measured, as in this study, with CO_2 tensions allowed to change (poikilocapnic) or, as in other studies, in which the level of CO_2 is fixed (isocapnic).

Hyperventilation, as seen here, induced by hypoxia, produces hypocapnia and alkalosis and both are potent inhibitors of ventilation. Hence studies of the ventilatory response to simple administration of low oxygen gases describe a response which is a resultant of hypoxic stimulation and hypocapnic-alkalotic inhibition and therefore may not reflect the true effect of hypoxic stimulation. This issue has been addressed by other groups. The effect of hypoxia on ventilation, when PCO_2 is uncontrolled, has been shown not to be large (Steinback and Poulin, 2007). This was thought to be because any increase in ventilation increases the elimination of CO_2, thereby lowering PCO_2 and reducing both the central and peripheral chemoreflex contributions to ventilation.

The central chemoreflex contribution is affected by changes in central PCO_2 resulting from changes in cerebral blood flow that occur in response to both hypoxia and the resulting hypocapnia (Poulin et al., 2002, Poulin et al., 1998).

Why did I use a poikilocapnic hypoxic exposure? Measuring ventilation during a poikilocapnic hypoxic exposure, it can be argued, simulates the response that would be observed in an environmental exposure to hypoxia, for example at altitude, and so is a more valid test than performing the test in isocapnic surroundings.

Another issue is that there is considerable variability in hypercapnic and hypoxic ventilatory responses among normal people. Many factors are responsible for these

individual differences in chemosensitivity**.** Women generally have lower responses than men, possibly due to their smaller size (Patrick and Howard, 1972). The influence of age, sex, body size and lung size on the control and pattern of breathing during CO_2 inhalation in Caucasians is variable (Bosma et al., 2012, Patrick and Howard, 1972).

Age also appears to be a factor. Diminished ventilatory responses to hypoxia and hypercapnia have been reported to occur with increasing age. To reinforce this finding, occlusion pressure responses were measured in the elderly. Occlusion pressure responses can be used to assess the changes with hypoxia and hypercapnia. Occlusion pressure responses to hypoxia and hypercapnia are reduced in elderly subjects, and this finding, along with the diminished responses to hypoxia and hypercapnia, probably reflect a true loss of ventilatory drive rather than any effect of aging on lung mechanics (Kronenberg et al., 1973, Peterson et al., 1981).

Comparing OSA and OHS, patients with daytime somnolence (who are found to have sleep apnoea only) have normal ventilatory responses to CO_2 and hypoxia during wakefulness (Guilleminault et al., 1973). This emphasises the importance of making the distinction between the two groups of patients.

In summary, acute changes in pulmonary ventilation are predominantly under the control of the carbon dioxide-hydrogen ion system. However, chronic adjustments of ventilation often appear to be dictated mainly by oxygen requirements of the patient. It would appear that chronic changes in ventilation are dictated primarily by hypoxic ventilatory drive while renal mechanisms maintain normal hydrogen ion concentration. There was little attempt to hold carbon dioxide tension at any particular level in this study.

In spite of its apparent importance in chronic adjustment of ventilation, hypoxic ventilatory drive has not been commonly studied directly because it is technically difficult to measure.

The fall in $Sp0_2$, when the subject is exposed to 15% 0_2 (the measure of hypoxic responsiveness), is almost certainly a product of the starting $Sp0_2$ more than anything else. The starting $Sp0_2$ could relate directly to $PaC0_2$ in patients with little to no lung disease. Thus this could be an auto-correlation and a poor measure of hypoxic responsiveness. The issue with the HCT probable means a point change in hypoxic response is sustained over 15 minutes. That is, this is not related to the acute hypoxic response at all since, by 15 minutes, the roll-off or HVD has occurred. So, the simple references to acute hypoxic measurement are misleading.

The absence of a correlation between minute ventilation and $PaCO_2$ is intriguing and warrants further explanation. Perhaps OHS is better characterised by increased deadspace or metabolism vs. obese controls. A greater fall in SaO_2 with hypoxia is expected in those with more severe hypoventilation. A higher $PaCO_2$ caused by hypoventilation will occur alongside elevated alveolar PCO_2, reduced alveolar PAO_2, a reduced baseline SaO_2 and, thus, a greater fall in SaO_2 is expected for any reduction in inspired oxygen due to the curvilinear shape of the oxyhaemoglobin dissociation curve. The fall in SpO_2 should therefore not be reported as the primary summary variable to indicate that diminished hypoxic responsiveness is a putative cause of OHS, as the greater fall in SpO_2 is arguably a trivial consequence of the disease. The slope of the ventilatory response to hypoxia is not susceptible to such confounding.

4.3.5 Assessment of sleep-disordered breathing and the upper airway

In this study I assessed sleep-disordered breathing (SDB) with overnight oximetry only. An alternative method would have been to use polysomnography (PSG).

Polysomnography, which typically records sleep state, apnoeas, hypopneas, cardiac rhythm and leg movements, is recommended for diagnosing obstructive sleep apnoeas.

The monitors used in this study record primarily oxygen saturation, can be used at home without supervision and are cheaper. The validity of these devices has been assessed by comparing them with polysomnography using recordings made either in the laboratory on the same night or at home on a different night.

The apnoea–hypopnea index (AHI) (number of events/hour of sleep) obtained from polysomnography was compared with the AHI and oxygen desaturation index (ODI) (number of events/hour of recording time) obtained from the monitors used in this study. Using AHI as the variable, portable monitors recorded with polysomnography on the same night showed sensitivities from 97 to 84% and specificities from 97 to 67% (Flemons et al., 2003).

It can be argued that accuracy in classifying patients as having or not having sleep-disordered breathing, defined by a cut-off value of AHI determined by polysomnography, is not particularly relevant for clinical practice. Both polysomnography and portable monitors have considerable night-to-night variation, which accounts for some loss of agreement between single-night observations of the two tests. As far as I know at present, AHI by itself has limited clinical significance, correlating poorly with symptoms or with outcome of treatment (Adams et al., 2001).

A number of other studies have evaluated the use of oximetry in simplifying the diagnostic strategy in SDB. Some studies have shown a sensitivity as low as 50% and specificity of up to 95% (Douglas et al., 1992, Gyulay et al., 1993, Williams et al., 1991).

Age, pulmonary function and degree of obesity are different factors influencing nocturnal desaturation which may lead to differences in terms of specificity and sensitivity.

One comment could be an assessment of hypoventilation overnight would have been preferable. This could have been done with either end tidal CO_2 or transcutaneous CO_2 assessment. Even nocturnal inspiratory time measurements could have given some insight as to the work of breathing.

Central factors in control ventilation have not been evaluated in this model. Long-term facilitation failure may help to further understand the discriminating factors in this group (Gerst et al., 2011).

4.3.6 Assessment of fat distribution

One concern in using skinfold thickness as a measure of subcutaneous fatness has been the fact that the skinfold represents the compressed double thickness of subcutaneous fat plus skin. Several methodologic considerations limit the use of skinfold thickness measurement in the obese. Another factor that is harder to evaluate, but that was clear to the investigators, is that raising an adequate skinfold for measurement is more difficult in obese people. If there was a greater increase in intraabdominal fat than in subcutaneous fat with increasing body fatness, skinfold thickness measurements would tend to underestimate total body fatness. The

possibility that bioelectrical impedance might also underestimate total body fatness in the presence of increasing intraabdominal fat is suggested by the fact that the greatest proportion of body resistance is accounted for in the arm and leg with a much smaller fraction in the trunk (Chumlea and Baumgartner, 1989).

Thus, whole-body impedance may be relatively insensitive to changes in intraabdominal fatness. On the other hand, if increasing body fatness was associated with an increase in extracellular fluid, the density of the lean body mass would be reduced below the assumed value of 1.10 (Siri, 1956) and percent body fat would tend to be overestimated when this value is used.

BIA lacks specificity and accuracy because it is based on differences in resistance when an electrical current is conducted through fat and lean components of the body. Thus, prediction equations are used to determine fat-free mass. Caution should be applied when interpreting these results as they are based on measurements of fat-free tissue and BIA calculates total fat mass by subtracting fat-free mass from body weight. Lean tissue measurements are influenced by hydration status, which is often a problem in clinical populations (Kyle and Pichard, 2000). If lean tissue measurements by BIA are imprecise, these errors will also confound fat measures.

DXA can only provide estimations of visceral adiposity as they cannot distinguish between different adipose tissue deposits. Intraabdominal fat estimated from DXA and anthropometric data in obese women was compared with VAT measurements from single-slice CT images at the fourth to fifth lumbar vertebrae and was found to be inaccurate (Ball et al., 2004). In obese women with a greater proportion of upper body fat distribution, the “narrow” location of the WC was difficult to discern on the

trunk (Ball et al., 2004). In these instances, the umbilicus circumference, which is easy to identify but located inferior to the waist, is typically recorded despite it being a larger value than the waist measurement. This inconsistency may limit the validity of the waist measurement in obese women. The inconsistency in sagittal diameter measurements is another source of inaccuracy because no standard procedures exist for measuring sagittal diameter whereby any difference in body position may also affect the measurement value (Ball et al., 2004). Nonetheless, DXA is a far more precise method than anthropometric techniques, including WC, supine sagittal diameter or BIA. MRI continues to provide a valuable method for studying fat deposition and can be used to assess abdominal fat distribution (Seidell et al., 1990). In practice, multiple-slice MRI is one of the preferred options for volume calculation, but its use is limited by accessibility and cost. Accuracy is often high with MRI, but defining different adipose tissue deposits depends on the settings of the MRI scanner. Thomas et al. (Thomas et al., 1998) found significant variation in the individual percentage of visceral and total adipose tissue based on the MRI single-slice technique with no relation to simple anthropometric measurements in a representative group of women.

4.3.7 Assessment of lung volumes, muscle biology, strength and ventilatory capacity

I performed a muscle biopsy as the technique to analysis muscle biology in this study. However, molecular genetic studies have largely replaced muscle biopsy as the diagnosing modality for dystrophies and myotonia congenita among others (Jackson, 2008).

Sampling errors can occur when taking muscle biopsies and for both open and “semi-open” biopsies false-negative results are common, due to the patchy nature of some myopathic processes.

I chose to sample the vastus lateralis, which has been established as the muscle of choice for conditions affecting the lower extremities.

My biopsy technique did not use image guidance. MRI and ultrasonography have both been used to guide needle muscle biopsy, though MRI has gained importance clinically given the patchy distribution of muscle pathology (Scott, 2004).

Spirometry is considered the gold standard in lung function. It cannot, however, provide information on, for example, lung residual volume (RV) and total lung capacity (TLC). Body plethysmography allows for the determination of these and other characteristics, such as airway resistance and intrathoracic gas volume (ITGV) (Coates et al., 1997). Moreover, these measures are recorded during breathing at rest and not by forced manoeuvres. Given the differences in measuring conditions and information provided, body plethysmography and spirometry add to each other, and a complete measurement cycle of plethysmography even includes spirometry. Body plethysmography is a technically demanding, physiologically nontrivial, highly informative, non-invasive method to obtain information on airway obstruction and lung volumes that is not available through spirometry.

The most widely used test of global inspiratory and expiratory muscle strength are the static maximum pressures measured at the mouth (PImax and PEmax). There are a few drawbacks to measurements of PImax and PEmax. One problem with static pressures is that they are volitional; thus, for the result to be a true measure of strength, the subject must make maximal contraction (Aldrich and Spiro, 1995).

Furthermore, there is wide inter-subject variability in PImax and PEmax in normal individuals, and significant variation in reported normal values for PImax (Smyth et al., 1984). This may be due to subject motivation, number of pre-measurement runs (learning effect) and peri-oral leaks, especially at higher pressures and in older subjects.

The PImax manoeuvre is both demanding and unpleasant. Low PImax may also be due to lack of motivation or co-ordination.

The sniff manoeuvre is volition-dependent, and submaximal efforts are most likely to be recorded in patients who are ill or breathless (Mulvey et al., 1991).

Other limitations of SNIP include anatomical abnormalities such as nasal polyps, nasal septal defects and septal deviation that may impair pressure transmission from the rhinopharynx. Nasal mucosal congestion in patients with rhinitis predisposes to the same kind of limitation. In the described conditions, there is underestimation of sniff oesophageal pressure by SNIP. Furthermore, SNIP measurements are unreliable in the presence of airway obstruction that prevent accurate transmission of pleural pressure to the upper airways.

Muscle function was evaluated with strength testing but cardio-pulmonary exercise testing may have given another dimension in the understanding of strength and ventilatory control in these patients (Lin and Lin, 2012).

4.4 Causes of ventilatory failure in obesity

4.4.1 Conclusion

In this prospective observational cohort study of a wide range of obese individuals, I have confirmed previous observations that BMI alone is a poor predictor of the likelihood of developing ventilatory failure. In addition, age was also not a significant predictor, suggesting that it is not simply that it also takes time for a given level of obesity to provoke ventilatory failure. I have also demonstrated that there are a number of strong predictors for the presence of ventilatory failure in obese individuals, as characterised in this paper by either a raised base excess or $PaCO_2$. Significant predictors were found in each of the biological domains I studied, suggesting that the cause of ventilatory failure in obesity is likely to be multifactorial. However, the main independent predictors for the development of daytime hypercapnia in obesity were the reduction in hypoxic sensitivity and the presence of intra-abdominal fat on DXA scan. This suggests that many of the other significant predictors in each domain may have been secondary to these two factors.

In studies such as ours, there is a significant problem deciding whether a correlate is causal, or present merely as a consequence of the ventilatory failure. A good example of this dilemma is that of the overnight SaO_2 levels. They could be predictive, by reflecting an abnormal abdominal load that limits ventilation during sleep when ventilatory responses to loaded breathing and hypoxia are normally reduced anyway; or they could reflect an abnormally reduced hypoxic drive; or they could simply be the result of starting with a lower $\%SaO_2$ due to the ventilatory failure and reduced PaO_2. Furthermore, in any study trying to assess the relative importance of associated factors, variability in the precision of the different

measurements introduces a confounding factor. For example, a truly predictive factor may fail to assert itself in a linear regression model if it is measured imprecisely. In each of the domains, where relevant, the alternative possibilities will be discussed.

I have demonstrated that a reduced hypoxic drive independently correlates with the degree of ventilatory failure. In an earlier paper, based on this cohort of patients, even those with a raised BE, without awake hypercapnia, had a diminished hypoxic response (4). Other studies have also shown that patients with OHS have a reduced ventilatory response to hypoxia (Han et al., 2001). Reduced hypoxic sensitivity, and thus the failure to maintain ventilation in the face of nocturnal hypoventilation, is an entirely plausible contributory cause to the gradual development of diurnal hypoventilation. The extra-nocturnal hypoventilation seen in these obese individuals is not necessarily directly due to the reduced hypoxic sensitivity alone, but is presumably provoked by some other factor, with the reduced hypoxic drive playing a permissive role in allowing this greater hypoventilation. Removal of carotid bodies in humans and animals reduces hypoxic drive and leads to a gradual development of chronic ventilatory failure (Teppema and Dahan, 2010). Repeated periods of nocturnal hypoventilation will lead to renal bicarbonate retention that may not be fully cleared during waking hours, leading to a gradual increase over time with the alkalosis suppressing awake ventilation. This was the explanation advanced by Berger et al. (Berger et al., 2009) who pointed out that if nocturnal hypoventilation alone occurred, then this by itself could not fully explain why individuals with OHS remain persistently hypercapnic during the day. Berger hypothesised that chronic hypercapnia results from the failure to offload carbon dioxide during the day and thus reverse any bicarbonate retention, following carbon dioxide loading at night (from ‘sleep-disordered breathing’) and the consequent bicarbonate retention. The kinetics

of this has also been modelled by Norman et al. (Norman et al., 2006). One possible therapeutic implication is that, if OHS is due to an acquired reduction in ventilatory drive rather than simply pump overload beyond the limits of compensation, then to some extent it may be reversible with ventilatory stimulation and/or bicarbonate reduction. There is some preliminary evidence for this, but no randomised controlled trial in OHS (DeBacker et al., 1995).

There is, however, an alternative possible explanation for the reduced ventilatory drive to hypoxia. It is conceivable that the development of OHS, with its attendant diurnal hypoxia, leads itself to a gradual reduction in the ventilatory response to hypoxaemia. The evidence for a reduced ventilatory drive to hypoxia, following a prolonged exposure to hypoxia, is inconsistent; following long-term exposure to altitude in lowlanders with normal lung mechanics, there have been variable findings regarding hypoxic sensitivity (Joseph and Pequignot, 2009, Weil et al., 1987, Prabhakar et al., 2009, Hupperets et al., 2004). However, even if acquired blunting is the explanation in my patients, it would still imply that restoration of ventilatory drive should be considered as a therapeutic target. Finally, it may be that the blunted hypoxic drive in my patients was simply the inability to increase ventilation in the face of hypoxia, due to the fat load on their respiratory pump. This argument has been rehearsed and explored extensively in chronic obstructive pulmonary disease (COPD) in the ‘can’t breath’ vs. ‘won’t breath’ debate. I think this is unlikely in my patients: those in whom only bicarbonate retention was present had clearly been able to return their $PaCO_2$ to normal during the day, yet still exhibited reduced hypoxic drive (4) and there were patients in my cohort with normal lung function and muscle strength who exhibited reduced hypoxic drive and normal hypercapnic drive. Furthermore, there was very little correlation between hypoxic and hypercapnic

drives (r=0.08, p=0.53), which one would expect if ventilatory drives were both dominantly affected by pump overload.

Preferential abdominal obesity is a plausible contributory cause to the gradual development of daytime hypercapnia. A cross-sectional population-based study including 121,965 subjects showed that abdominal obesity was the strongest predictor of lung function impairment (odds ratio, 1.94 [1.80–2.09] and, 2.11 [1.95–2.29], for FEV_1 and FVC, respectively (Leone et al., 2009). My findings contrast with similar work in the area. Sutherland et al. (Sutherland et al., 2008) used a wide range of body fat variables including DXA to determine the effect of fat distribution on lung volumes in healthy adults. Lung volumes were only loosely associated with BMI but variables derived from both DXA and non-DXA measurements reflecting upper body fat had highly significant negative correlations with FRC and ERV in both men and women. However, there were no differences in the strength of these associations between the markers of abdominal obesity (waist circumference, waist-to-hip ratio, DXA waist fat, DXA abdominal fat) as against those quantifying thoracic fat (DXA trunk fat, DXA thoracic fat).

An interesting observation in this dataset was that the neck circumference was a better predictor of DXA visceral fat than either waist circumference or waist/hip ratio, thus underlining the problem that OSA may indirectly code for the metabolic syndrome in cross-sectional studies.

My study is the first study of which I am aware that has looked at the role of visceral fat as measured by DXA and its effect on ventilatory failure. My findings further suggest that a greater amount of fat mass inside the abdominal cavity may contribute to worsening ventilatory failure. Particularly on lying down, the weight of

abdominal fat will alter the pressure–volume characteristics of the thorax and restrict the descent of the diaphragm, the main muscle of ventilation, and also narrow small airways with an increase in airflow resistance and intrinsic PEEP (Steier et al., 2009). The diaphragm may even be overstretched to the point of reduced efficiency of muscle fibre contraction (Sharp et al., 1986). As I have shown, this abdominal load may also lower reduce supine FEV_1 and VC, more than when sitting, and explain this correlation with ventilatory failure. This effect of a raised intra-abdominal pressure on ventilation is well recognised (Hedenstierna and Larsson, 2012) (Sugerman, 2001). In individuals with other pathologies that raise intra-abdominal pressure (particularly seen in acutely-ill patients in intensive care), additional obesity is a significant extra risk factor in these situations (Holodinsky et al., 2013). It may be that intra-abdominal fat is more significant than subcutaneous abdominal wall fat at embarrassing the diaphragm, if the abdominal compartment expansion is limited and thus effectively under pressure.

Interpreting the sleep data correlates of ventilatory failure as evidence of any primary causal role is also difficult. As discussed above, nocturnal hypoventilation may be secondary to impaired ventilatory mechanics, with poor hypoxic drive permissive of yet further hypoventilation, leading eventually to bicarbonate retention and diurnal ventilatory failure. There was no suggestion within these data that the presence of obstructive sleep apnoea (measured either as AHI or ODI) was contributory. This does not mean of course that CPAP therapy would not be expected to help as it has effects other than reducing upper airway resistance. For example, it will increase lung volumes, thereby reducing lower airways obstruction – PEEP – and the work of breathing (Steier et al., 2009). Indeed, CPAP has been shown to improve ventilatory

failure in obesity, even in those without overt OSA (Piper et al., 2008), but nonetheless can still fail in the very obese (Banerjee et al., 2007).

Because marked obesity is likely to lead to a sedentary indoor lifestyle, and perhaps be the result of consuming poor quality food, I wondered if any nutritional deficiencies might contribute to the ventilatory failure, through weaker respiratory muscles for example. Many within my obese cohort had low vitamin D levels, possibly contributing to a low grade proximal myopathy (Janssen et al., 2002). However, in the final linear regression modelling, its initial significance in correlating with ventilatory failure disappeared although, if the fall in SaO_2 during 15% O_2 breathing was removed, then vitamin D levels reappeared as predictive. Thus it is unclear whether vitamin D deficiency is playing a role in producing a clinically important proximal or diaphragmatic myopathy, for example, so a pilot trial of vitamin D replacement in those deficient would seem appropriate. The explanation for higher transferrin levels correlating with worse ventilatory failure is not clear. Higher transferrin levels would normally suggest iron deficiency, but no other iron status measure corroborated this. Isolated raised transferrin can occur with raised oestrogen levels (Horne et al., 1971) also found in the obese (Anty et al., 2008). However, other markers of poor diet such as folate did not suggest a correlation with ventilatory failure.

The main drawbacks of the study are outlined below although there is a dedicated section later on.

I chose to estimate ventilatory drives in a simple way, with two-point steady-state measurements that could be easily reproduced in most clinical pulmonary physiology laboratories. Although simple, they were able to clearly distinguish those with and

without ventilatory failure and thus I feel that they are able to characterize the hypoxic and hypercapnic drives in these obese patients. The interpretation of measurements of ventilatory control is fraught with difficulties. The very presence of chronic hypercapnia in a patient implies reduced ventilatory drive unless the loading on the ventilatory system is very high.

The estimation of visceral adipose tissue by DXA was not available from subjects with a BMI >40, as the relevant algorithms have not been verified beyond this level of obesity. Despite the reduced number of subjects with this measurement, it was still the obesity measure most predictive of ventilatory failure. Thus it is possible that this relationship might not continue to be true for BMI values above 40.

4.5 Future Research

The increasing levels of obesity mean that clinicians will see more and more patients with ventilatory failure due to this cause. At present, the usual management is either attempting to reduce weight or non-invasive ventilation. However, this work suggests that reversing the gradual accumulation of bicarbonate due to depressed ventilatory drive and overnight hypoventilation may be an alternative strategy in managing obesity hypoventilation. This has not been considered safe in most other examples of ventilatory failure, where the respiratory pump is failing or overwhelmed, such as neuromuscular disease or severe COPD, when increasing drive is likely to be ineffective and potentially dangerous. Thus it would seem reasonable to perform a randomised controlled trial of acetazolamide or equivalent in patients with obesity hypoventilation, who are not in acute decompensation, despite not being currently recommended for this purpose (Selim et al., 2012). There are small uncontrolled

trials of acetazolamide in obesity looking at hypercapnic drive that are suggestive of improvement (Raurich et al., 2010).

4.6 Summary Conclusion

Only a third of obese patients develop chronic ventilatory failure and previous data have shown a poor correlation between obesity and daytime hypercapnia. We performed an observational cohort study with comprehensive characterization of multiple factors predicted to be associated with obesity-related chronic ventilatory failure.

In this prospective, observational, hypothesis-generating, cohort study I have confirmed that BMI is a poor predictor of chronic ventilatory failure, and from the multivariate analysis the most important independent correlates were visceral fat, and hypoxic ventilatory response.

This study also shows that obese individuals with a raised standard bicarbonate, but without awake hypercapnia, represent an intermediary stage between no ventilatory failure and overt obesity-hypoventilation syndrome, as evidenced by reduced ventilatory drive and nocturnal hypoventilation. The recognition of such individuals may allow earlier intervention to prevent further deterioration, but this should be the subject of randomised controlled trials.

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