University of Leicester

Investigating the Relationship Between Sleep Disordered Breathing, Glycaemic Control and Inflammation.

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Metabolic Syndrome (MetS), Type 2 Diabetes (T2DM) and obesity related sleep disorders like Sleep Disordered Breathing (SDB) share common features including visceral adiposity, impaired glycaemic control and increased cardiovascular disease (CVD) risk. As sub-clinical inflammation is considered a key player in these conditions they are thought to be interrelated. We aimed to further investigate this putative interrelationship.

In a multi-ethnic population with a spectrum of glucose tolerance (sub-study of the ADDITION-study), we report that abdominal obesity underpins the association between SDB and systemic inflammation. South Asians with SDB had significantly higher levels of leptin, poorer glycaemic control but lower levels of oxidative stress than their Caucasian counterparts. These data suggest that the pathogenesis of SDB is different between these ethnic groups and may aid in understanding why South Asians are at increased risk of T2DM and CVD. Furthermore, SDB is independently associated with increased likelihood of MetS. However, no differences in cardiovascular markers, inflammatory biomarkers or anthropometric measures were observed between those with excessive daytime sleepiness or sleep disturbances as determined by the Epworth Sleepiness Scale and the Sleep Assessment Questionnaire, respectively. This suggests that these questionnaires are broad and insensive in identifying these sleep parameters.

Obstructive Sleep Apnoea (OSA) is a severe form of SDB which can be successfully treated with Continuous Positive Airway Pressure (CPAP). Reported results on the effects of CPAP therapy on glycaemic control are inconsistent thus no difinative conclusion could be made from the systematic review carried out to answer this research question. Thus 'The Leicester Sleep and Sugar Study' was conducted to further establish whether CPAP-therapy impacts glycaemic control or systemic inflammation in subjects with established T2DM and newly diagnosed OSA. We report a clinically significant improvement in glycaemic control (HbA1c -0.8%) and a significant reduction in waist circumference with improved psychological well being 6 months post CPAP-therapy.

It is evident that OSA is associated with T2DM and MetS although the direction of cause and effect has not been elucidated to date. The results reported here suggest that OSA negatively impacts on glycaemic control. Additionally we report a possible ethnic difference in the pathophysiology of SDB with inflammation playing a key role. Further research is required in this area to further establish these findings.

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List of Abbreviations

APAP	Automated Positive Airway Pressure
BMI	Body Mass Index
BSQ	Berlin Sleep Questionnaire
CGMS	Continuous Blood Glucose Monitoring
Clamp Study	Hyperinsulinaemic–Euglycaemic clamp Study
CPAP	Continuous Positive Airway Pressure
CRP	C-Reactive Protein
CVD	Cardiovascular Disease
ESS	Epworth Sleepiness Scale
EDS	Excessive Daytime Sleepiness
HADS	Hospital Anxiety and Depression Score
HbA1c	Gylcosylated Haemoglobin
IL-6	Interleukin-6
IPAQ	International Physical Activity Questionnaire
MetS	Metabolic Syndrome
OAD	Oral Antidiabetic Agent
OSA	Obstructive Sleep Apnoea
p.QOH	Perception of Quality of Health
p.QOL	Perception of Quality of Life
PGF	8-Iso-Prostaglandin-F2-alpha
PSG	Polysomnography
RAS	Renin-Angiotensin-Aldosterone System
SAQ	Sleep Assessment Questionnaire
SDB	Sleep Disordered Breathing
T2DM	Type 2 Diabetes
TNF-α	Tumour Necrosis Factor-alpha
WHO QOL-BREF	World Health Organisation Quality Of Life Brief Questionnaire

Chapter One

Introduction to Sleep, Metabolic Syndrome, Type 2 Diabetes, Inflammation and Obstructive Sleep Apnoea

In this chapter I will discuss a number of parameters of sleep, the Metabolic Syndrome (MetS), Type 2 Diabetes (T2DM), inflammation and the sleep disorder – Obstructive Sleep Apnoea (OSA). I am going to provide background to each of these topics, discuss diagnosis and treatment where applicable and evidence of any inter-relationships between them.

1.1 Sleep: an overview

Sleep was originally thought to be a passive process, the quiescent part of our daily lives. However, developments in research and technology have provided us with the means with which to explore and unveil this phenomenon. It has been understood since the 1950s that sleep is a dynamic process; it is a reversible state of decreased consciousness and perceptional disengagement from and reduced responsiveness to the environment [1] . It is clear that most parts of the brain are in an active state; this activity however is different to the activity demonstrated in the 'wake state' discussed below. Sleep is a process that is as essential to our physiological needs as food and water [2]; it affects our daily functioning on a physical and mental level and is required for our survival. For example rats have a usual life span of 2 to 3 years, but if deprived of all stages of sleep only live for 3 weeks [1]. Today's fast moving society inflicts pressure on many people to sacrifice sleep in favour of other activities, and since the invention of the light bulb in 1879 the average American sleeps for 6.9 hours on weeknights in comparison to the previous average of 10 hours [3]. Sleep deprivation is thought to have behavioural, endocrine and metabolic consequences, although, some detrimental effects of sleep deprivation can be reversed when the sleep debt is repaid. Daytime sleepiness is the major symptom of sleep deprivation and the effects of it can be catastrophic. This is an increasing issue with public

health, a recent study by the Sleep Research Laboratory at Loughborough University, UK states that 20% of serious road accidents on motorways and monotonous roads in Great Britain are due to falling asleep at the wheel [4]. The optimal sleep duration for most adults is 8 - 9 hours and this varies between individuals. Sleep disorders are a major cause of sleep deprivation, and thus provide a useful tool for the study of sleep and the physiological consequences of abnormal sleep.

Sleep disorders occur when the physiological mechanisms responsible for initiating, maintaining and terminating sleep in its various forms become disorganised, attenuated or exaggerated [5]. A critical issue in the study of sleep is its measurement. Clinicians in this field require sleep measurement methods that provide them with a foundation with which to make a diagnosis, select specific treatments and monitor treatments.

In this section I will discuss a number of parameters of sleep including sleep architecture, methods of measuring sleep, neuroendocrine mechanisms involved in sleep and the effect of age on sleep architecture.

1.1.1 Sleep architecture

It is very difficult to determine the length of nocturnal sleep as it is influenced by a number of factors of which volitional control is among the most significant in human beings [1]. In addition a number of factors can contribute to individual heterogeneity in sleep architecture including age, circadian rhythms, temperature of environment, prior sleep

history, pathology and drug ingestion [1]. Hence determining a normal sleep pattern within a population is a complex task. Using polysomnography (PSG) and a controlled environment, research has shown that the process of sleep is made up of a number of stages and that the distribution of these stages display a highly structured and well-organised cyclical pattern in the normal healthy adult.

Polysomnography (PSG)

PSG is the ‘gold standard ’electrophysiological technique for measuring sleep. It is used for determining sleep architecture and in the identification of specific sleep disorders. The practice of over-night sleep recordings by PSG became common practice in the 1960s [1] and PSG is now the reference to which the validity of other sleep measurement techniques can be measured. A full PSG combines the measurement of brain activity by electro-encephalography (EEG), eye movements by electro-occulography (EOG), muscle tone by electro-myography (EMG), thoraco-abdominal movements, nasal airflow, snoring, oxygen saturation and posture. Additional parameters can be assessed for the investigation of sleep disorders such as periodic leg movement in sleep which will not be discussed here. For the identification of other sleep disorders for example obstructive sleep apnoea, limited information set may be used, for example thoraco-abdominal movements, nasal airflow, snoring, oxygen saturation and posture.

PSG is time consuming and expensive to administer because it requires an over night supervised stay in the sleep laboratory. It additionally requires technical expertise for the

analysis of the study results and a certain amount of preparation before the subject is tested in order to achieve the most accurate representation of the subject‘s habitual sleep period. A sleep diary is usually used so that the subject’s habitual sleep time can be simulated. Due to the expense of a full PSG for subjects admitted for diagnostic purposes, a standard battery of subjective tests will have been completed including the Epworth Sleepiness Scale (Section 1.1.2) thus the results of PSG may confirm the sleep expert’s intuitive diagnosis. One of the draw backs of PSG is that false conclusions on a subjects sleep pattern can be drawn due to the ‘first night’ effect, where a subject’s sleep may be disrupted due to sleeping in an unfamiliar and restricted environment. Therefore in the case of research and the investigation of certain sleep disorders, PSG should be undertaken on two consecutive nights with the results of the first night disregarded.

A hypnogram (Figure 1) is a graphical representation of the sleep phase. The graph is derived from EEG readings during a PSG study. Due to the sporadic nature of the EEG readings they are divided up into epochs of 20-30 seconds thus providing enough time for a sleep stage to be characterised. Each epoch over the sleep period is scored according to the sleep stage present either using a trained sleep specialist or automatically with specific software. The resulting scores are then plotted to produce the hypnogram and the individual’s sleep architecture can be determined to provide information on total sleep time, sleep latency, number and length of non- Rapid Eye Movement (NREM) and Rapid Eye Movement (REM) periods.

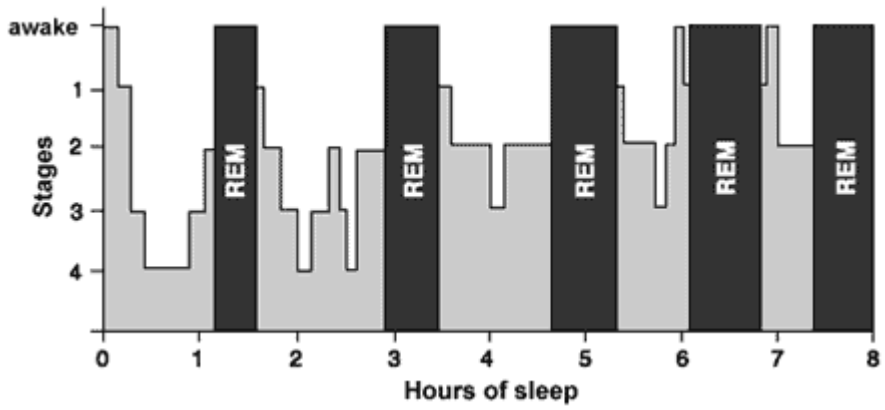


Figure 1: A typical hypnogram from a young, healthy adult. Light-gray areas represent NREM sleep [6]

Sleep stages

There are two main phases of sleep the NREM phase and the REM phase. These two phases and wakefulness are continuously cycled throughout the twenty-four hours of the day with all three having characteristic EEG activity.

Sleep onset occurs through NREM sleep in the healthy adult. There are four stages to this sleep phase. Stages 3 and 4 of NREM are referred to as slow wave sleep (SWS) because their EEG pattern consists of slow-waves with large-amplitude and slow-activity, sleep spindles and k-complexes In this part of the sleep phase there is low muscle tone and large movements occur i.e. rolling over in bed. By comparison the REM phase movements are less pronounced and generally small i.e. rapid movements of the eyes, and twitching fingers and toes, additionally movements of the limbs can occur in short bursts. Therefore the EEG activity pattern displays low-voltage, fast-activity or de-synchronisation.

Non-REM sleep

Seventy-five percent of sleep is spent in the NREM phase [7] (figure 1). The distribution of time spent in each of these four stages is not equal: ~ 5% stage 1, ~ 50% stage 2, ~ 20% stages 3 and 4 of total sleep time. The cerebral cortex is the site of analysis and interpretation of the sensory information, the generation of all voluntary motor action and of higher cognitive functions such as learning, memory and conscious perception. In the NREM stage there is an absence of most of the higher functions of the cerebral cortex. Therefore we see loss of motor activity and depression of somatic and visceral reflexes in this phase.

Stage 1 and 2

Stage 1 is entered through a state of drowsiness and exhibits the lowest amount of slow wave activity; the arousal threshold is at its lowest at this point in sleep. This state appears to be a transitional stage between wakefulness and defined sleep. Stage 2 follows from stage 1 and lasts for approximately 10-15 minutes in the first cycle. This is the point that is considered to be the official onset of consolidated sleep.

Stage 3 and 4 (SWS)

Stages 3 and 4 last between 20-40 minutes in the first sleep cycle. It is these stages that represent the deepest stages of sleep.

REM sleep

The REM phase exhibits de-synchronized EEG activity patterns of a wide range. In comparison to NREM, the activity of the cerebral cortex is intensely active, with simultaneous intense motor inhibition. It is this phase that is associated with dreaming, based on vivid dream recall being reported in ~ 80% of arousals from this sleep phase [8]. NREM and REM cycle continuously throughout the night. Initially the length of NREM increases as the length of REM does. As the sleep episode continues, REM sleep becomes longer with stages 3 and 4 of NREM sleep occupying less time in the second and third cycles and they can disappear from the later cycle's altogether (Figure 1). Stage 2 of NREM is extended to occupy this portion of the cycle. Throughout the night an average sleep cycle will last between 90-100 minutes with an average of 5 cycles per night in the healthy adult.

1.1.2 Methods of measuring sleep

Sleep is a complex physiological process and in order to gain an insight into this field for diagnostic and/or research purposes a number of tools have been developed. The measurement of sleep can be divided into two main categories; subjective and objective measures. Generally subjective measures are used for the investigation of sleep disorders with the objective measures used for their clinical diagnosis in both the research and clinical setting. Tables 1a and 1b summarise some of the more common of the two methods for investigating sleep (copies of the questionnaires can be found in Appendix 1). Following this I will give a brief overview of the neuroendocrine mechanisms involved in sleep and what effect sleep deprivation has on plasma glucose levels.

Objective Measure	Description
Polysomnography (PSG)	• Gold standard electrophysiological technique • Measures -Brain activity by EEG and EOG, Muscle tone by EMG, Thoracoabdominal movements, posture, Nasal airflow, snoring, oxygen saturation. • Additional sensors can be attached for recording leg movements etc. • Not restricted on what parameters of sleep can be measured can measure all or a subset. • Requires an overnight stay in sleep laboratory, a sleep technician and trained sleep clinician to analyze results. • Preferable if the patient has two overnight stays to rule out 'first night effect'.
Home Sleep Respiratory Kit	• Ambulatory polygraphic device for the diagnosis of sleep disorders like OSA, periodic leg movement. • Measures - Respiratory effort, snoring and air flow, oxygen saturation levels and body position and movement • Requires 20 minute set up time with a health care professional • Patient goes home for the night • Device returned and results uploaded and sent to sleep clinician for analyses • Rules out 'first night effect'
Multiple Sleep Latency Test (MSLT)	• Polygraphic EEG 'gold standard' for measuring physiological sleepiness • Measures the mean sleep onset latency (and REM latency) in a sleep-promoting environment • Subjects lay down, eyes closed, in a PSG setting, in a dark and quiet room. The patient is instructed NOT to resist sleep during the four or five 20 minute daytime nap opportunities. These napping opportunities are scheduled 2 hours apart. • Cannot determine the cause of sleepiness - only assess the degree of sleepiness • Requires the presence of a sleep technician but carried out during the daytime • Expensive and time consuming to administer i.e. approximately 22 hours of testing
Multiple Wakefulness Test (MWT)	• Polygraphic EEG measure of physiological sleepiness • Measures the ability of the subject to stay awake in a sleep-promoting environment • Cannot determine the cause of sleepiness - only assess the degree of sleepiness • Subject remains in a quiet recumbent position in a dimly lit to dark room in the absence of anything physically or mentally stimulating for 20-40 minutes every 2 hours • Requires the presence of a sleep technician but carried out during the daytime • Expensive and time consuming to administer i.e. approximately 22 hours of testing

Table 1a: Overview of objective methods of measuring sleep

Objective Measure	Description
Pittsburgh Sleep Quality Index (PSQI)	• Measures subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleep medication, day time dysfunction • 19 individual items that generate seven 'component scores', the sum of which provides a global score. • Test-retest format (1 month) • Each component is scored between 0 - 3 resulting in a global score that lies between 0 - 21. The higher the global score the poorer the quality of sleep • Economical, easy and comprehensible for use by subjects and professionals • Cannot be used as a single diagnostic tool • It is valuable in identifying subjects with poor sleep and therefore further clinical investigations are still required for an accurate diagnosis • Validated
Epworth (ESS)	• Measures the propensity of the subject to fall asleep under certain environmental conditions in daily life over the last 2 weeks • 8 items rated on a scale of 0 - 3, 3 = highly soporific • Score 0-9 No-daytime sleepiness, 10-12 Borderline, >12 Excessive daytime sleepiness • ESS usefulness in quantifying changes in the EDS after treatment of OSA via CPAP therapy • Economic and very quick to complete and score • Commonly used in the clinical setting in conjunction with the BSQ to determine if a subject requires an overnight sleep study for the diagnosis of OSA. • Validated
Berlin Sleep Questionnaire (BSQ)	• Identified people at high risk of OSA from those at low risk • Focuses on the risk factors of OSA including obesity, hypertension, snoring and the subsequent daytime sleepiness • The questionnaire is split into three categories based on these common risk factors • 2 or more positive categories = high risk of OSA • Typically used in conjunction with the ESS • Not diagnostic but is a quick, economic, non-invasive tool for assessing a person's risk of OSA • Validated
Sleep Assessment Questionnaire (SAQ)	• The Sleep Assessment Questionnaire • 17 item self-administered questionnaire • Six sleep factors measures – Insomnia, Non-restorative Sleep, Restlessness, Daytime Sleepiness, Sleep Apnoea, Sleep Schedule Disorder • Provides global score – Identifies people with sleep disturbances ($\geq$10) • Easy to complete • Expensive to score – web based pay per questionnaire • Validated

Table 1b: Overview of subjective methods of measuring sleep

1.1.3 Neuroendocrine mechanisms of sleep

The regulation of a large number of endocrine components is controlled by the sleep-wake cycle. This control is not simply restricted to the Hypothalamic-Pituitary-Adrenal axis (HPA-axis) but further to the hormones that control carbohydrate metabolism, appetite, water and electrolyte balance. This balance is detrimentally affected by diminished sleep quality and sleep loss both partial and chronic. Table 2 summarises some of the normal 24 hour profiles of a number of these hormones and the effect that acute partial sleep deprivation have on their regulation. It is clear from the overview of these neuroendocrine components that partial sleep deprivation has a large impact on their normal 24 hour profiles. Disruptions in the regulation of such compounds could have profound effects on the metabolism and thus render these individuals at increased risk of developing conditions associated with metabolic dysfunction. This is further supported by alterations in blood glucose levels that have been reported in otherwise healthy adults who are subjected to short term partial sleep deprivation as I have discussed below.

	Site of secretion & function	24 hour profile	Effects of sleep deprivation
Growth Hormone	Secreted by the anterior lobe of the pituitary Secretion stimulated by Growth Hormone Releasing Hormone (GHRH) & acylated-ghrelin [1] Anabolic peptide hormone – involved in metabolism of proteins, carbohydrates and lipids [9] Essential for growth & renewal of tissue Involved in maintenance of muscle and bone mass [9]	Maintained at a low and steady level over 24 hours – interrupted by short 'bursts' of secretion Major pulse of secretion occurs at sleep onset [1] Females exhibit more frequent daytime 'bursts' of secretion thus sleep-onset pulse does not account for the majority of the 24 hour levels [1]	Sleep interruptions inhibit the sleep-onset pulse [10] Partial sleep deprivation shifts the timing of the major pulse from sleep-onset to pre-sleep onset [11]
Corticotropic Axis –neuroendocrine system associated with the stress response			
Adrenocorticotropic Hormone (ACTH)	Secreted by the anterior lobe of the pituitary Stimulates secretion of cortisol & other hormones of the adrenal cortex	Corticotropic axis can be measured via plasma levels of either ACTH or cortisol Major pulse in the early hours of the morning – levels decrease steadily throughout the daytime Levels are lowest prior to sleep onset – sleep initiated when cortisol activity is dormant Thought to be predominately controlled by circadian rhythmicity [12]	Partial sleep deprivation associated with short-term inhibition of cortisol secretion [12] Amplitude of overall cortisol levels is reduced by ~15% [12] The 24 hour profile shifts with the major secretion and lowest levels occurring at earlier times [12]
Cortisol	Steroid hormone secreted by the adrenal cortex Stimulates the formation of glycogen in the liver & simultaneously decreases rate of glucose utilization in peripheral cells Involved in the release of fatty acids for metabolic use		

Hypothalamic-Pituitary-Thyroid (HPT) Axis			
Thyroid Stimulating Hormone (TSH)	Hormone released by the thyroid following stimulatory signals from the hypothalamus Increase body's basal metabolic rate Regulate protein, fat and carbohydrate metabolism	HPT-axis can be measured via plasma levels of the thyroid hormones i.e. TSH Daytime levels remain at a relatively constant low level with a pulse in the early evening. [13] Highest levels are observed around the time of sleep-onset[13]	Sleep deprivation reported to result in a ~200% increase in nocturnal TSH secretion thus sleep thought to have an inhibitory effect [13] Interruptions of nocturnal sleep suppresses the sleep related inhibition of TSH secretion [1]
Other			
Ghrelin	Predominately Synthesised and released by the stomach Orexigenic hormone (appetite-stimulating) Regulates the synthesis and secretion of a number of neuropeptides in the hypothalamus that regulate feeding Negatively associated with percentage body fat and fasting insulin levels [14] Negatively correlated with leptin levels [14]	N/A	Partial sleep deprivation results in a 71% increase in the Leptin: Ghrelin ratio [15] Increase in this ratio was proportional to the observed increase in hunger [15]
Leptin	Hormone secreted by adipocytes thus is correlated with measures of fat Leptin has both central and peripheral effects Regulates satiety and food intake via the HPA axis and peripherally effects a range of processes involved in the homeostasis of glucose[10] Circulating levels respond to acute caloric shortages to promote appetite [16] Levels are raised in response to caloric surplus [16]	Predominately dictated by food-intake However evidence of a nocturnal rise in levels which is only partially dependent on food intake [17]	Partial sleep deprivation associated with a 20-30% reduction in mean diurnal leptin levels

Table 2: Overview of main components involved in the neuroendocrine mechanisms of sleep

1.1.4 Plasma glucose levels and sleep

Plasma glucose levels within the healthy adult are maintained tightly (4 - 7 mmol/L) throughout the day via a number of metabolic and endocrine functions. This is also true for the sleep period which is concomitant with a fasting state. Glucose and insulin levels have been monitored in a number of laboratory sleep studies in humans, and glucose levels were found to remain stable across the night and only fall minimally in those cases where levels differed across this fasting period [18, 19]. Conversely when glucose and insulin levels were measured over a 12 hour period, in subjects who remained awake and lying down in a rested position, glucose levels fell on average by 0.5 – 1 mmol/L [19]. Thus the maintenance of glucose levels in nocturnal sleep must be modulated by mechanisms that differ to that in the wake and rested state [18]. In the 24 hour glucose profile in healthy adults, glucose levels rise at around the time of sleep onset, suggesting a state of glucose intolerance [1]. Plasma glucose levels rise between 20 – 30 % during this part of the sleep period, with maximal levels reaching around the middle of the sleep period. This impaired state of 'glucose intolerance' begins to improve during the later part of the sleep period when plasma glucose levels begin to drop and do so progressively toward normal morning values. This variation is thought to be under the control of separate mechanisms; one in early sleep and the second coming into play in the later part of the sleep period. In the first part of the sleep period, as determined from constant glucose infusion studies [12, 20], the rise in plasma glucose is chiefly attributed to a decrease in glucose utilization. It has been estimated that there is approximately a 67% reduction in glucose utilization by the brain [21] and a 33% reduction in peripheral glucose metabolism which could be due to a combination of reduced muscle tone and the rapid anti-insulin effects of the sleep onset GH pulse [22]. This rise in plasma glucose levels is followed by a greater than 50% increase in

insulin secretion. In the second part of the sleep period glucose levels begin to decrease which could be a result of this increase in insulin secretion and/or a reflection of delayed effect of low evening cortisol levels during the evening and early part of the night [23]. Glucose uptake has been found to be greater in the REM and wakeful stages versus NREM sleep [24, 25]. Glucose levels are affected by alterations in sleep as demonstrated by Van Cauter et al [12] in the sleep restricted conditions the glucose response to the ingestion of a carbohydrate rich breakfast, is higher than that of the sleep recovery condition despite a similar insulin secretary response. An approximate 0.8 mmol/L difference in peak glucose levels in response to the high carbohydrate breakfast were observed in the sleep restricted condition. Interestingly the overall rate of glucose disposal in the sleep restricted condition after the high carbohydrate breakfast was 40% slower than that observed in the sleep recovery condition [11]. Thus, if an individual consistently exposes themselves to sleep deprivation they could be increasing their risk of becoming glucose intolerant and potentially increase their risk of T2DM.

1.1.5 Age and sleep

Alterations in sleep take place throughout the normal aging processes with associated neuroendocrine changes. Interestingly these alterations in sleep are apparent in early adulthood; a reduction in SWS (NREM stages 3 and 4) has been observed in early adulthood (30-40 years). This is followed later in adulthood by reductions in REM stages throughout the sleep period [1]. An increase in the number of awakenings and their duration also occurs in later adulthood [1]. These factors result in an overall reduction in sleep quality with concomitant disturbances in endocrine function. Van Cauter et al, 2000

carried out an analysis of sleep and plasma GH and cortisol levels in 149 healthy men between the ages of 18 – 83 years. The data were collected and combined from a number of studies conducted between 1985 and 1999 at 4 different laboratories. The 149 subjects did not suffer any sleep complaints/disorders or have a history of endocrine or psychiatric abnormalities. The group observed major decrease in the levels of GH release from early adulthood to midlife [26]. Furthermore, they found that the reduction in SWS and not age *per se* is associated with the reduction in GH secretion in middle and late life [26]. The clinical significance of decreased SWS has not been fully elucidated. However this concomitant reduction in GH is associated with an increase in adipose tissue, a reduction in muscle mass and strength and a reduction in the exercise capacity [27, 28]. These groups also observed an association with aging and an increase in evening cortisol levels again occurring in mid- and later-life [27, 28]. It has been suggested that modest elevations in evening cortisol levels could facilitate the development of both central and peripheral disturbances that are associated with the glucocorticoid axis, including memory deficits, insulin resistance and sleep fragmentation [29, 30]. The inability to achieve or maintain the quiescence of the corticotropic axis in aging is thought to be a reflection of the loss of REM sleep in old age [26]. These endocrine alterations together with others that are not discussed here, suggest that a number of the metabolic and hormonal hallmarks of aging partly reflect this deterioration in sleep quality [1].Thus prolonged partial sleep deprivation which is common in today's society, could be having a large negative impact on normal metabolism and endocrine function, in otherwise healthy adults. Therefore the sleep disturbed or deprived may represent populations at risk of endocrine and metabolic dysfunction.

In the next section of this chapter I will discuss two such disorders of the metabolism – the Metabolic Syndrome and Type 2 Diabetes. I will then discuss inflammation, a common characteristic and risk factor for these conditions, and then the disorder Obstructive Sleep Apnoea which is the model that we have chosen to investigate the effects of sleep deprivation on glycaemic control.

1.2 The metabolic syndrome and obesity

The metabolic syndrome (MetS) is a common metabolic disorder with a prevalence of between 22 – 39% in developed countries [31]. It is comprised of a number of associated cardiovascular risk factors that increase the risk of future development of cardiovascular disease (CVD) and Type 2 Diabetes (T2DM) [32]. The components of the MetS include; insulin resistance, glucose intolerance, dyslipidemia, hypertension and abdominal obesity (Table 3). The Metabolic Syndrome was first proposed by Dr Gerald Reaven in 1988 and thus was first termed 'Reaven's Syndrome'[33]. Over the last twenty years, as it has become more widely accepted , a number of alternative terms have emerged to describe this disorder namely syndrome X [33], the insulin resistance syndrome [34], and the deadly quartet [35]. There are a number of different definitions for MetS, from a number of different organisations, with the thresholds and ranking order for each component differing as a result of the importance each organisation considered each to contribute to cardiovascular outcomes. Thus, the prevalence varies depending on the definition used [36-39]. Generally speaking if a subject presents with three or more of the individual components then they are classified as having MetS. Here I will discuss the different definitions (Table 4) and the clinical significance of identifying people with MetS.

Component	Description
Visceral Obesity	The accumulation of adipose tissue within the abdominal cavity surrounding many of the vital organs.
Dyslipidemia	A condition of lipid metabolism dysfunction. Manifestations of dyslipidemia include raised plasma triglycerides, raised plasma LDL, raised apolipoprotein B and low plasma HDLC concentrations. This combination is atherogenic and a major risk factor for coronary heart disease (CHD).
Hypertension	A state of chronic increased arterial blood pressure. The desirable range for blood pressure is <130/< 80 mmHg. Hypertension is generally defined as a blood pressure of >140/90 mmHg. However pressures between 120/80 mmHg to 139/89 mmHg are now considered as pre-hypertension. It is associated with dyslipidemia [40] and insulin resistance [41]. HTN is positively and independently associated with CHD and CVD [42].
Insulin resistance	A condition of subnormal response to insulin resulting in a reduction in the cellular uptake of glucose giving rise to a state of hyperglycemia and hyperinsulinaemia.

Table 3: Components of the Metabolic Syndrome

1.2.1 Clinical diagnosis

The World Health Organisation (WHO) produced a working definition of the MetS in 1999 [39, 43]. This definition requires a blood sample so that glucose intolerance can be defined. This is a disadvantage because it is costly and currently not undertaken regularly in routine clinical practice. On the other hand it may provide more power in the prediction of T2DM by comparison with the other definitions [32]. The definition produced by the third report of the National Cholesterol Education Program (NCEP ATP III) in 2002 [44] emphasised waist circumference as an important criteria. The thresholds for each of the components here are less stringent than those usually required in the identification of categorical risk factors [32] due to the cumulative cardiovascular risk that multiple marginal risk factors induce. The International Diabetes Federation (IDF) released a definition for the MetS in 2005 [39]. This definition has separate thresholds for visceral obesity based on ethnic

origin. This is based on the relative risks of metabolic dysfunction for each ethnic group. The criteria for Europids and South Asians only are presented in table 4.

WHO Criteria [45]
The presence of Insulin Resistances as defined by any of the following: • Type 2 Diabetes • Impaired fasting glucose • Impaired glucose tolerance • Or those with a normal fasting glucose level, glucose uptake below the lowest quartile for background population under investigation under hyperinsulinemic- euglycemic conditions AND any two of the following: • Antihypertensive medication and/or high blood pressure (≥140 mmHg/ ≥90 mmHg) • Plasma triglycerides ≥1.7 mmol/L • HDL cholesterol ≤ 0.9 mmol/L (male) and ≤1.0 mmol/L (female) • BMI ≥30 kg/m2 and/or waist: hip ratio ≥0.9 (male) and ≥0.85 (female) • Urinary albumin excretion rate >20 μg/min or albumin:creatine ratio ≥30 mg/g
NCEPT-ATP III [44]
The presence of any three of the following: • Waist circumference >102 cm (male) and >88 cm (female) • Plasma triglycerides ≥1.7 mmol/L • HDL cholesterol <1.0 mmol/L (male) and <1.3 mmol/L (female) • Blood pressure ≥130/≥85 mmHg • Fasting glucose ≥6.1 mmol/L
IDF criteria [46]
The presence of obesity as defined by a waist circumference of: • >94cm and >90cm (males Europids and South Asian) • >80cm (females Europid and South Asian) AND any two of the following: • Plasma triglycerides ≥1.7 mmol/L or specific treatment for this lipid abnormality • HDL cholesterol <1.03 mmol/L (male) and <1.29 mmol/L (female) or specific treatment for this lipid abnormality • Blood pressure ≥130/≥85 mmHg or specific treatment for previously diagnosed hypertension • Fasting glucose ≥5.6 mmol/L or previously diagnosed T2DM

Table 4: Definitions for the classification of MetS

The NCEP and IDF definitions are more widely used both in clinical practice and research due to them being easier in the context of their ease of use in a clinical setting.

CVD has been identified as the primary clinical outcome of the MetS by all of these organisations, however a large body of those with this disorder have insulin resistance and are thus at increased risk of T2DM [32]. Once an individual presents with T2DM their risk of CVD sharply increases. Furthermore having the MetS is associated with various other clinical conditions namely polycystic ovary syndrome, fatty liver disease, gallstones, asthma, sleep disturbances (specifically OSA) and some forms of cancer [32]. The pathophysiology of MetS is not completely clear. However, it has been largely attributed to insulin resistance with excessive flux of fatty acids [47]. MetS can be managed with either lifestyle changes and/or pharmacotherapy to reduce or even eradicate with a common aim to prevent the development of T2DM or CVD [48-50]. Therefore the identification and treatment of MetS has a large clinical significance for the prevention of T2DM and CVD.

1.2.2 Aetiology of MetS

Obesity

Obesity is considered as the main driving force for the development of the MetS [51], due to its association with insulin resistance and sub-clinical inflammation. Although components of the MetS can exist in non-obese subjects, in obese subjects they usually co-exist [51]. Obesity is a broad term and today the terms subcutaneous (peripheral) and visceral (abdominal, central) obesity are preferable, the latter having a greater clinical significance, given its pathological role in disease. Jean Vague in the late 1940s first highlighted the difference between visceral and subcutaneous obesity (android and gynoid obesity, respectively) and suggested that an upper (visceral) body fat distribution was a

significant factor in determining the adverse health consequences of obesity and its metabolic and cardiovascular abnormalities [52]. Computed tomography is the gold standard technique for the measurement of body fat compartments and thus the visceral, subcutaneous and intramuscular fat compartments have been well defined [51]. The classic method used for measuring obesity in a clinical setting has been body mass index (BMI), determined by body weight in kg divided by height in meters squared (kg/m^2). BMI has been categorised into normal, overweight and obese ranges (18.5-25 kg/m^2, 26-30 kg/m^2 and $\geq$ 30 kg/m^2, respectively) in the white European population [53]. However, BMI does not describe the distribution of adipose tissue which is clinically more significant than total body fat [54-57]. Indeed large amounts of visceral fat have been observed in those individuals who are within the 'normal category' for BMI and are thus at increased cardiovascular risk. Furthermore, waist circumference is directly related to all cause mortality when adjusted for BMI [58]. Therefore measuring visceral obesity offers a more sensitive approach to identifying those at risk of the MetS and thus T2DM and CVD.

Obesity and insulin resistance

Obesity is characterised by an increase in adipose tissue. It has been long recognised that obese humans are hyperinsulinaemic and insulin resistant [59]. The link between obesity and metabolic dysfunction is due to the adverse effects of the excess adipose tissue on systemic responsiveness to insulin, which impairs insulin's role in carbohydrate and lipid metabolism resulting in a compensatory hyperinsulinaemic state [60]. It is now known that adipocytes are not just a depot for the storage of fat, but in fact have dual metabolic and

immuno-modulatory function. This is thought to contribute to a level of sub-clinical inflammation that is commonly associated with the aetiology of MetS, T2DM and CVD [61-63]. The inflammatory properties of the adipocytes and impact on metabolism are discussed later in this chapter. Here I will discuss how obesity exerts its detrimental effects on glucose metabolism via excess plasma fatty acid concentration.

The development of obesity often involves a high-fat, high calorific diet and sedentary life-style which results in increased insulin secretion in an attempt to counteract the increased level of circulating glucose and fatty acids [64]. Insulin is the major anabolic hormone of the body; its main physiological actions include acute metabolic action and longer-term effects on growth and development (Table 5)

Stimulatory Effects	***Inhibitory Effects***
• Glucose uptake by muscle and adipose tissue • Glycogen synthesis • Lipid synthesis • Amino acid transport • Protein synthesis • Gene transcription & DNA synthesis (proteins involved in protein and lipid synthesis) • Ion transport (K^+) • Phosphorylation dependent cascades	• Glycogen breakdown • Lipolysis • Protein degradation • Apoptotic cell death

Table 5: The main physiological actions of insulin

The tight regulation of glucose metabolism via insulin action is key to our survival. The hallmark of insulin resistance is hyperglycaemia which poses risks for the development of T2DM and cardiovascular disease. Increased plasma free fatty acids (FFA), evident in the

obese individual, have been reported to independently predict progression to T2DM in Caucasians and Pima Indians in prospective epidemiological studies [65, 66] . The high level of circulating FFA observed in obesity, inhibits insulin signalling and induces metabolic insulin resistance systemically [67]. Plasma FFA concentration is dependent upon the balance between their uptake and their release into the blood stream. Their uptake occurs predominately by their esterification in adipose tissue, the liver and their oxidation in the muscle, heart and other tissues [68]. Their release occurs via the intravascular lipolysis of triglyceride rich lipoproteins and lipolysis of triglyceride stores in adipose tissues [68]. Insulin is the main hormone involved in both these processes. However, in conditions of energy excess, as with the obese individual, a state of insulin resistance emerges as the cells struggle to adapt to a chronic positive energy balance. We see that high plasma FFA concentrations initially result in an increase in triglyceride storage in adipose tissues via insulin action. This occurs up to the point where the adipocyte cannot continue to store this excess energy and thus lipolysis begins to take place with associated insulin resistance. This in turn results in a flux of FFA from the adipose tissue to non-adipose tissues, increasing the extra-adipocytic triglyceride stores. This excess of energy again results in the non-adipose tissues becoming insulin resistant in a bid to prevent further build-up of cellular energy stores (Figure 2). The resultant effect as evident in the MetS, is systemic insulin resistance [67]. Thus increased levels of circulating FFA are thought to play a key role in the development of glucose intolerance in obese individuals and progression to overt T2DM. However, the exact mechanism/s by which elevated plasma FFA concentration interferes with insulin signalling has not been fully elucidated and is beyond the scope of this PhD.

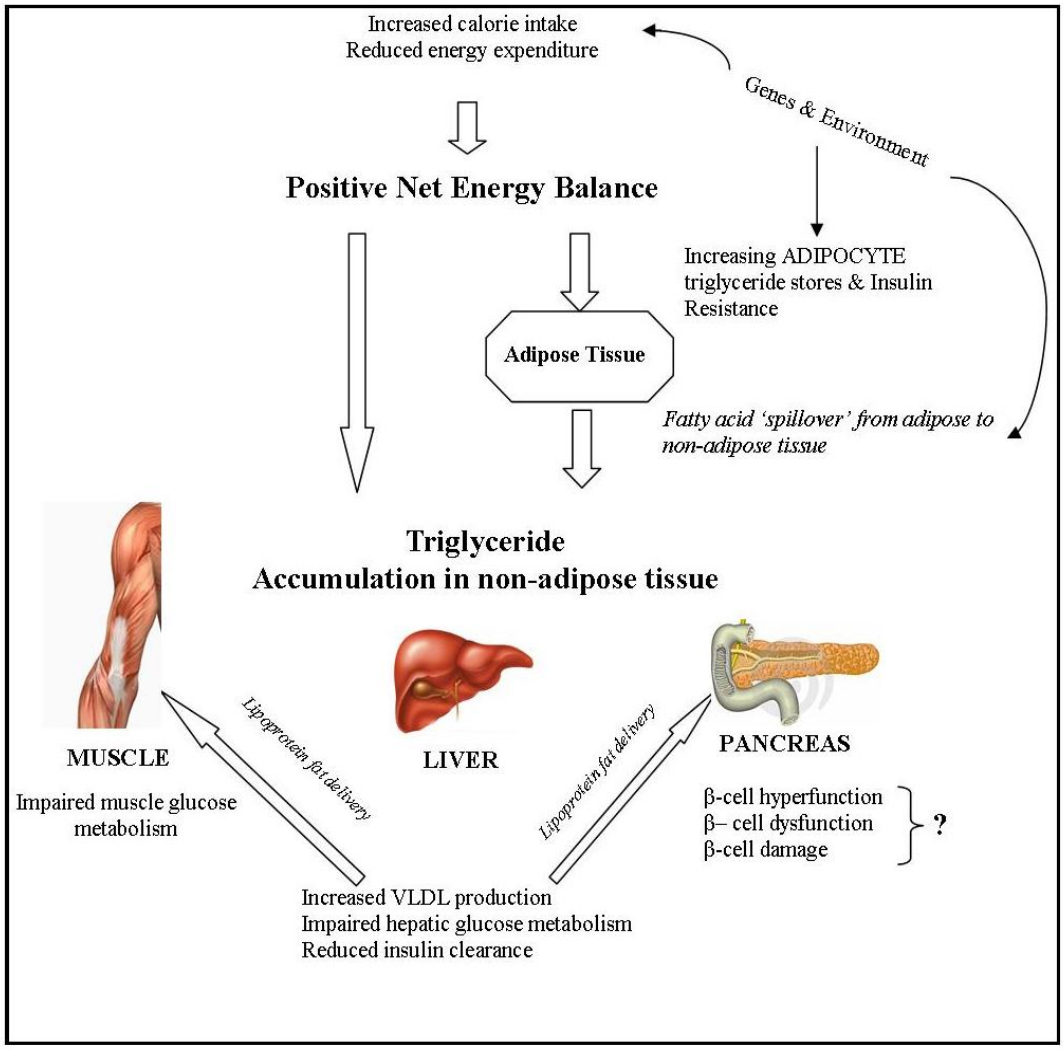


Figure 2: The effects of excess energy. A positive net energy balance resulting from increased caloric intake and sedentary lifestyle, in conjunction with environmental influences and genetic make-up, give rise to accumulation of TGs predominantly in adipose tissue. Excessive accumulation of TGs in adipose tissue results in an insulin resistant state within the adipose tissue and a shift towards TG storage in non-adipose tissue. The glucolipotoxic effect in non-adipose tissue potentially results in systemic insulin resistance and β-cell dysfunction. Adapted from Figure 3 Lewis G et al [68].

1.2.3 Natural history and CVD outcomes of MetS

MetS is a progressive disorder [69] when left untreated or where treatment is unsuccessful. The worsening state of each component increases the risk of developing T2DM and CVD (Figure 3).

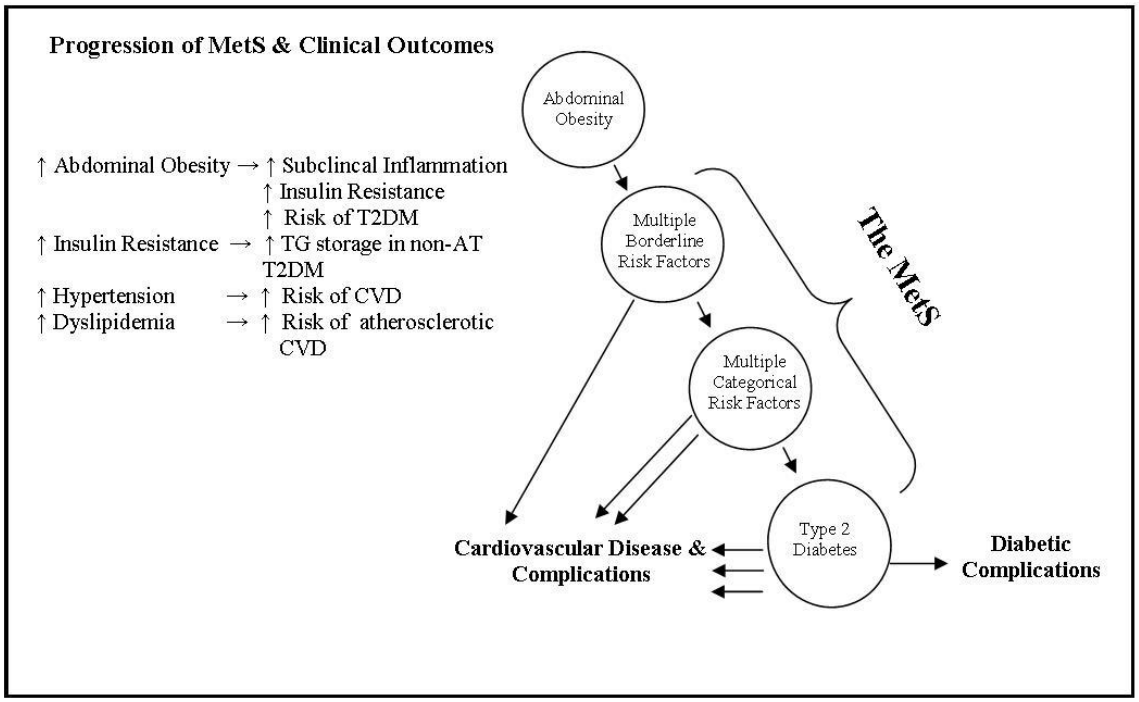


Figure 3: Progression of MetS and the potential clinical outcomes (AT = Adipose tissue) adapted from Figure 1 Grundy SM et al [69].

This is supported by a recent community-based longitudinal study which assessed the risk of T2DM and CVD, in subjects with or without MetS stratified by BMI [70], 2902 subjects had baseline data collected from 1989 - 1992 and were followed for up to 11 years. Subjects were categorised into groups according to their BMI; normal (BMI<25 kg/m^2), overweight (BMI 25-29.9 kg/m^2) or obese (BMI>30 kg/m^2). These groups were further categorised according to the presence or absence of MetS giving rise to six groups. Incident T2DM and CVD were measured throughout the study period. At a mean of 7 years follow-up, 4.9% (141) subjects had developed T2DM. Obesity and presence of MetS accounted for 44.1% of the risk of incident T2DM in this population. At a mean of 11-years follow-up, 8.7% (252) subjects had experienced their first CVD event. Again obesity and the presence

of MetS accounted for a large proportion of the risk of incident T2DM (44.1%) and incident CVD (12.5%) [70]. Those subjects who were overweight or obese without MetS were not at increased risk of developing T2DM. It was reported that those with MetS regardless of BMI category were at a 4 to 11 fold increased risk of incident T2DM in comparison to normal weight non-MetS subjects ($p<0.001$). Similarly those with MetS regardless of BMI category were at increased risk of incident CVD by 1.6- to 2.0 fold compared to those of normal weight non-MetS subjects ($p<0.001$). Results from Wilson et al [71] further support these findings. In this cohort study 25493 middle-aged adults were followed for 8 years and incident CVD, CHD and T2DM were measured. The prevalence of MetS at baseline was 21.4% (95% CI 19.3% - 24.0%). At the 8 year follow-up the prevalence had increased to 33.9% adjusted for baseline age, which represents a 56% increase from baseline. At 8-year follow-up 107 subjects presented with CHD, 174 presented with CVD and 178 presented with T2DM. The group analysed the relative risk of each of these conditions in males and females separately and adjusted for age. They found that males with MetS were at 2.54 times more likely to develop CHD ($p<0.0001$), 2.88 times more likely to develop CVD ($p<0.0001$) and 6.92 times more likely to develop T2DM ($p<0.0001$). The likelihood of females developing CHD and CVD in those with MetS was lower than males with MetS. However, the likelihood of developing T2DM was similar for both males and females with MetS (RR 6.90, $p<0.0001$) [71]. This group concluded that up to 30% of incident CVD in men and approximately 50% of incident T2DM over this 8 year follow-up can be attributed to the presence of MetS [71].

Furthermore, the risk of CVD is augmented further in people with both MetS and T2DM. Bonora et al [72] investigated the cardiovascular risk associated with the presence of MetS in 946 people with established T2DM (~ 9 years). This group measured the prevalence of MetS and presence of CVD at baseline and then incident CVD at 4.5 year follow-up. At baseline the prevalence of MetS was significantly high at 92.3%. The prevalence of CVD was also high at baseline with 31.7% coding positive for CVD [72]. When the patients were stratified by the number of MetS components present at baseline (none, one, two, three or four), the prevalence of CVD increased significantly across the categories (14.3, 18.2, 31.6, 34.1 and 32.6%, respectively, $p<0.05$). The overall prevalence of CVD was significantly higher in those patients with the MetS compared to those without (32.9% vs. 17.8%, $p=0.005$). At 4.5 years 111 had died, of which 6.2% were due to CVD. In those with MetS, cardiovascular mortality was significantly increased (6.8% vs. 1.7%, $p=0.06$). This group emphasize the fact that those patients with isolated T2DM did not develop CVD during the follow-up period. Finally, the incidence of CVD was higher in those with the MetS than in those without (19.9% vs. 3.9%, $p<0.001$). Therefore the identification and correct treatment for this metabolic disorder is essential for preventing or stalling the progression to overt T2DM or CVD. I am now going to briefly discuss the management options for MetS.

1.2.4 Management of MetS

The principal goals for identifying and treating MetS are prevention of T2DM and CVD. The first line in management is the identification of MetS followed by risk assessment with respect to CVD/CHD and T2DM. From this the clinician can tailor the treatment plan for the patient in question. The key point is that the treatment plan is aggressive and multifaceted in order to address all the underlying metabolic abnormalities and coexistent

risk factors simultaneously [73]. Therapeutic lifestyle change (TLC) is the approach adopted by the NCEP ATP III guidelines [74] and includes dietary modification, weight loss, increased physical activity and smoking cessation. In addition any coexistent risk factors also need to be managed such as hypertension and dyslipidemia.

The NCEP ATP III has created a nutrient composition guideline for the diet modification element of the TLC, with the objective of reducing body weight by 7-10%. This is essential as the benefits of weight loss have been highlighted in a recent systematic review by Poobalan A et al [75]. This group report that long term weight loss results in a reduction in total cholesterol and triglycerides with increased HDL-cholesterol. Reductions in plasma glucose levels, blood pressure and insulin resistance was additionally reported [75]. Regular exercise has been shown to improve a number of the risk factors associated with the MetS [76], with the recommended daily minimum exercise being 30 minutes of moderate intensity daily [77]. If no weight loss has been observed after three months (7-10% of baseline body weight) then there is an option for drug treatment in conjunction with continuing diet and exercise changes.

Target	Goal
The **LDL-C** is the main target of therapy for all high-risk patients: CHD and CHD risk equivalent Multiple (2+) risk factors 0-1 risk factor	 <2.6mmol/L <3.4mmol/L <4.2mmol/L
Weight control	- 10% from baseline
Physical activity	30-45 minutes/day for 3-5 days/week
Treatment of hypertension	<140/90 mmHg
Treatment of other frequently encountered lipid abnormalities (high triglycerides and/or low HDL-C values) Non-HDL-C in patients with triglycerides ≥ 2.26mmol/L to ≤ 5.6mmol/L	 High CHD risk <3.4mmol/L Intermediate CHD risk <4.2mmol/L Low CHD risk <4.9mmol/L

Table 6: The NCEP ATP III guidelines for the management of the MetS [74]

Table 6 summarises the NCEP ATP III guidelines for the management of the MetS. The drug therapies that can be used in conjunction with the TLC to achieve some of these targets are beyond the scope of this PhD and therefore are not discussed here.

1.3 Type 2 Diabetes Mellitus

Diabetes mellitus covers an array of disease from the acute and often explosive onset of Type 1 Diabetes (T1DM) to the asymptomatic subjects where Type 2 Diabetes (T2DM) is discovered secondary to another medical incident e.g. hospital admission for heart surgery. These disorders can be collectively termed ‘disorders of glycaemia’ [78], the salient feature of which is abnormal blood glucose control. However, their aetiology differ somewhat and is the basis for their separate classifications (Table 7) and subsequent treatment methods.

Type 1 (β- cell destruction, usually leading to absolute insulin deficiency) • Autoimmune • Idiopathic
Type 2 (may range from predominately insulin resistance with relative insulin deficiency to a predominately secretory defect with or without insulin resistance)
Other specific types • Genetic defects of β-cell function (i.e. Chromosome 20, HNF-4α, MODY1) • Genetic defects in insulin action (i.e. Leprechaunism) • Diseases of the exocrine pancreas (i.e. Pancreatitis) • Endocrinopathies (i.e. Acromegaly) • Drug- or chemical-induced (i.e. Glucocorticoids) • Infections (i.e. Congenital rubella) • Uncommon forms of immune-mediated diabetes (i.e. anti-insulin receptor antibodies) • Other genetic syndromes sometimes associated with diabetes (i.e.Prader-Willi syndrome)
Gestational diabetes[†]

Table 7: Aetiological classification of disorders of glycaemia*. Adapted from table 1 [78]

* As additional sub types are discovered it is anticipated that they will be reclassified with their own specific category. [†] Includes former categories of gestational impaired glucose tolerance and gestational diabetes.

1.3.1 Diagnosis of T2DM

The American Diabetes Association (ADA) in 1997 [79] and the WHO in 1999 [45] have produced working guidelines for the diagnosis of T2DM. The National Diabetes Data Group (NDDG) collaborated with the ADA and published a report for the classification and diagnosis of diabetes mellitus [80]. According to the WHO the gold standard technique used for the diagnosis of T2DM is the Oral Glucose Tolerance Test (OGTT). An OGTT requires a fasting blood sample followed by the consumption of 75g of glucose. The individual is advised to refrain from any physical activity and consumption of anything other than water for 120 minutes. At 120 minutes another blood sample is taken (the post-load/challenge sample) and the levels of glucose are determined from both blood samples. Both the WHO and ADA guidelines state that the diagnosis of diabetes should never be made on the basis of a single abnormal glucose value in an asymptomatic individual, and

that in such cases repeat testing must be undertaken for the verification of the diagnosis. This is not required when the individual presents with symptoms and abnormal blood glucose levels. The diagnostic blood glucose values recommended by the WHO and the collaboration of NDDG and the ADA are given in Tables 8 and 9 respectively.

	Venous plasma glucose concentration (mmol/l)
Diabetes	
Fasting **or**	≥ 7.0
2hr post 75gm glucose load	≥ 11.1
IGT	
Fasting (if measured) **and**	< 7.0
2hr post 75gm glucose load	≥ 7.8 and ≤ 11.1
IFG	
Fasting **and**	≥ 5.6 and < 7.0
2hr post 75gm glucose load (if measured)	<7.8

Table 8: Diagnostic blood glucose values for diabetes mellitus and other categories of hyperglycaemia [45]

Individual presents with:

Classic Symptoms* of diabetes plus casual† plasma glucose concentration ≥ 11.1 mmol/l. **or**

Fasting‡ plasma glucose ≥ 7.0 mmol/l

or

2 hour postload plasma glucose ≥ 11.1 mmol/l during an OGTT**.

In the absence of unequivocal hyperglycemia with acute metabolic decompensation, these criteria should be confirmed by repeat testing on a different day. The third measure (OGTT) is not recommended for routine.

Table 9: ADA criteria for the diagnosis of diabetes mellitus adapted from Committee report [80]. *Casual is defined as any time of day without regard to time since last meal. †Classic symptoms of diabetes include polyuria, polydipsia, and unexplained weight loss, ‡Fasting is defined as no caloric intake for at least 8 h, **The test should be performed using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water

Both guidelines recognise those individuals that present with a level of impaired glucose regulation (IGR). This is sometimes referred to as pre-diabetes or non-diabetic hyperglycaemia and includes a state of impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT) [81]. The identification of this subnormal category is important for the prevention of T2DM and CVD [82] as it identifies a target group.

1.3.2 Prevalence of T2DM

Type 2 Diabetes Mellitus (T2DM) is the commonest type of diabetes affecting between 5-7% of the world's population [1] (Figure 4). This form of diabetes accounts for ~ 85-95% of all cases worldwide [83]. T2DM has two main pathophysiological defects, namely impaired insulin secretion and insulin resistance. This type of diabetes mellitus was previously termed late-onset diabetes or Non Insulin Dependent Diabetes Mellitus (NIDDM) as it was most commonly seen in the elder population. However, in the current climate of obesity today this disorder is evident in young adults and there are even cases of T2DM in children [84].

AT A GLANCE

	2007	2025
Total world population (billions)	6.6	7.9
Adult population (age 20-79, billions)	4.1	5.2
WORLD DIABETES AND IGT (20-79 age group)		
Diabetes		
Comparative prevalence (%)	6.0	7.3
Number of people with diabetes (millions)	246	380
IGT		
Comparative prevalence (%)	7.5	8.0
Number of people with IGT (millions)	308	418

SOURCE: *DIABETES ATLAS THIRD EDITION*, © INTERNATIONAL DIABETES FEDERATION, 2006

Figure 4: Prevalence and future estimated prevalence of T2DM and IGT from Diabetes Atlas 3rd Edition [85]

The prevalence of T2DM is increasing (Figure 4) which is largely attributed to the increasing levels of obesity seen worldwide, specifically in Westernised countries [86]. There is a large variation in the prevalence of T2DM across the world and more specifically in persons of the same ethnic origin who reside in different countries i.e. native country versus recent residence in Westernised countries [87] and in those that have migrated from rural to urban areas within their native country [88]. This phenomena is well described in a recent review by Misra et al [89]. For example Rotimi et al [90] reported a marked gradient in the risk of T2DM amongst the genetically related populations of Nigerians, Jamaicans and US blacks (age adjusted prevalence of T2DM in those aged 25-74 years were 1%, 12% and 13% respectively). It is well documented that persons of South Asian origin are at increased risk of obesity, T2DM and CVD. Specifically, in South Asians who have migrated to the UK or other such westernized countries, it is estimated that the prevalence of T2DM is approximately four times higher than in South Asia [87, 91]. Together this

indicates that there are both environmental and genetic components contributing to this disease (as discussed below).

1.3.3 Pathogenesis of T2DM

The development of T2DM, much like MetS, is progressive. Initially the individual has normal glucose tolerance then a level of insulin resistance develops leading to a compensatory state of hyperinsulinaemia. Insulin production by the β –cells is increased in a bid to cope with the hyperglycaemic state that arises from this reduced response to insulin. This state of hyperinsulinaemia and normal glucose tolerance can be maintained up to a point, but as the level of insulin resistance increases and concomitant defect in insulin secretion presents, we see progression from normal glucose tolerance into impaired glucose tolerance and then finally to overt T2DM [78]. This defect in insulin secretion is a result of the β–cell's inability to maintain this continuously high level of insulin secretion in response to the glucose challenge.

Normal Glucose Homeostasis

In humans the primary source of metabolic energy is glucose and thus the maintenance of glucose homeostasis is key to our survival. Insulin is vital for the utilization of glucose as a source of energy. All cells of the body contain glucose transporters (GLUT) which mediate glucose uptake by all cells/tissues in the body. There are four different types of glucose transporter. These GLUT transporters are not evenly distributed across the different cell types, and do not have the same affinity for glucose (Table 10).

Glucose Transporter	Location and primary function
GLUT1	Most tissues. Involved in basal and non-insulin mediated glucose uptake in many cells
GLUT2	Liver and Pancreatic β-cells. In the latter it is a perquisite with glucokinase for glucose sensing
GLUT3	Brain Involved in non-insulin mediated uptake of glucose in the brain
GLUT4	Skeletal muscle, Adipose tissue and Heart. Responsible for insulin-stimulated uptake in these cells

Table 10: Type, Location and primary function of the four glucose transporters in humans

Blood glucose levels are tightly controlled in normo-glycaemic individuals and it is the anabolic hormone Insulin that maintains the balance between glucose utilisation, storage as glycogen (glycogenesis) and its synthesis (gluconeogenesis). The physiological actions of insulin have been described in table 1.

Synthesis of Insulin

Insulin is synthesised and secreted by the β-cells, located in the Islets of Langerhans. The Islets of Langerhans are clusters of neuroendocrine cells found within the endocrine unit of the pancreas and constitute ~ 1-2% of its mass. Insulin is a protein consisting of 51 amino acids, and is cleaved from its precursor proinsulin, a precursor of the polypeptide preproinsulin. Preproinsulin is synthesised in the rough endoplasmic reticulum of the β-cells. It is then transported to the Golgi apparatus where its signal peptide is removed giving rise to pro-insulin. Once here it is subsequently folded into its native structure consisting of three domains the A, B and C peptides, which are locked together via two disulphide bonds. Specific protease activity then takes place to cleave the centre third of the

molecule, giving rise to the dissociation of the C peptide. The resulting molecule is mature insulin, consisting now of the A-peptide disulphide bonded via its carboxy-terminal to the amino terminal of the B-peptide. The mature insulin and C-peptide are then packaged into secretary granules that are transported to the surface of the β-cells. Once the cell has been appropriately stimulated exocytosis takes place i.e. granular and plasma membrane fusion, releasing insulin and the C-peptide into the blood stream. The biological activity of C-peptide has recently been the subject of much interest, with one study suggesting that it could be playing an active role in atherogenesis in diabetic patients [92]. However, this controversial area will not be discussed here.

Insulin Secretion

The prime stimulus of insulin secretion is blood glucose levels. It is essential to note that a number of gastrointestinal hormones also stimulate insulin secretion i.e. GLP-1, GIP, gastrin and secretin, in addition to other products of digestion such as amino acids, fatty acids and ketone bodies [93]. β-cells are sensitive to absolute glucose concentration and also the rate of change of blood glucose [93]. There is a steady basal release of insulin in addition to the response to change in blood glucose levels [93]. Glucose induced insulin secretion is biphasic (Figure 5).

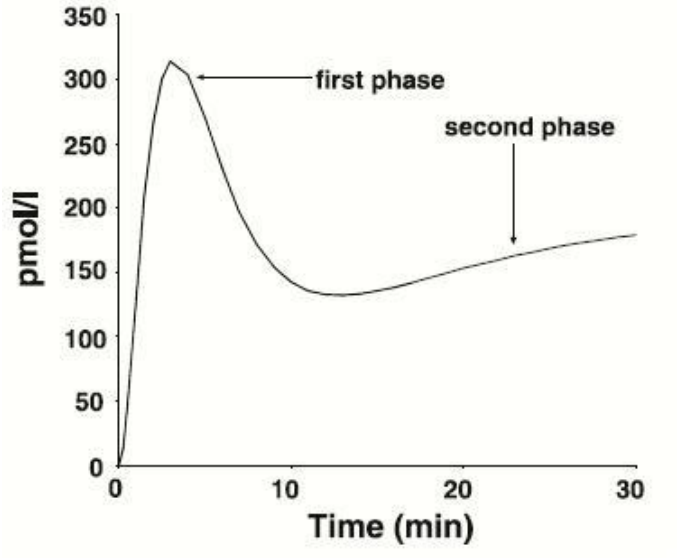


Figure 5: The biphasic response of insulin secretion to glucose (Adapted from Caumo A et al [94])
y axis = insulin concentration (pmol/l)

The first phase, the acute short phase, involves the release of readily available granules i.e. there is a pool of secretory granules present at the plasma membrane of the β-cells. This is then followed by a second sustained phase. In order for the second phase of insulin secretion to occur glucose must first be metabolised; this is permitted because initial glucose uptake takes place in the β-cells via GLUT2 transporters (also found in the liver). This glucose transporter is closely associated with the enzyme glucokinase. Glucokinase is the essential glucose sensor in these cells and has kinase activity. This enzyme phosphorylates glucose to produce glucose-1-phosphate (G-1-P). Within the mitochondrion of the cell G-1-P is subjected to glycolysis to yield ATP. The increase in ATP production within the mitochondrion results in an increase in the ATP:ADP ratio. This gives rise to the closure of ATP-sensitive K^+ channels on the plasma membrane, and thus depolarisation of the plasma membrane. This depolarisation causes the voltage sensitive Ca^{2+} channels to open, followed by an influx of Ca^{2+} into the cytoplasm which then triggers granule translocation. The granules are translocated to the plasma membrane and exocytosis takes place resulting in the second phase of insulin (and C-peptide) secretion into the blood stream.

Insulin can directly enter the blood stream, as the islets are highly vascularised, allowing it to be transported around the body and cause effects in various tissues. It is directly infused via the portal vein into the liver. At the liver insulin exerts its profound metabolic effects via binding with its receptor, the insulin receptor (found in all cells). The transmembrane protein component of this receptor (β-subunits) has tyrosine kinase activity, allowing for the autophosphorylation of its intracellular domains and target proteins within the cytoplasm and thus activation of the downstream signalling cascade (Figure 6). Once the insulin receptors are occupied with insulin and have thus been activated they aggregate into clusters and are subsequently internalised by the surrounding membrane, which invaginates to form an endosome. This process allows for the recycling of the receptor back to the surface and for the degradation of insulin by lysosomes.

In the majority of non-hepatic tissues, insulin increases glucose uptake via increasing the number of GLUTs (which are in a continuous state of turnover). This is viable due to a pool of preformed transporters localised in the cytoplasm – it is their recruitment to the plasma membrane that insulin is responsible for. Exercise has a similar effect in that it increases the recruitment of these transporters to the plasma membrane thus providing the working muscles with energy, hence the importance of an active lifestyle.

Figure 6 is a schematic diagram summarising the insulin signalling pathways. It is clear that glucose homeostasis is a complex physiological process and to determine exactly where there are defects that give rise to T2DM is an even more complex subject that has yet to be fully established. Any number of the events in the cascade, that lead to normal glucose

levels, could be interrupted and it is likely that a number of disruptions present in a single patient. Furthermore these disruptions are unlikely to be the same for two individuals. The cause of hyperglycaemia could be a result of for example abnormalities in the structure of the insulin receptor, or of the insulin molecule itself, or to poor recycling of the insulin receptor, or problems at the glucose transporter or indeed problems further downstream in the insulin signalling cascade. In addition there are the problems faced by a state of prolonged hyperglycaemia which ultimately results in β-cell dysfunction and thus diminished insulin secretion.

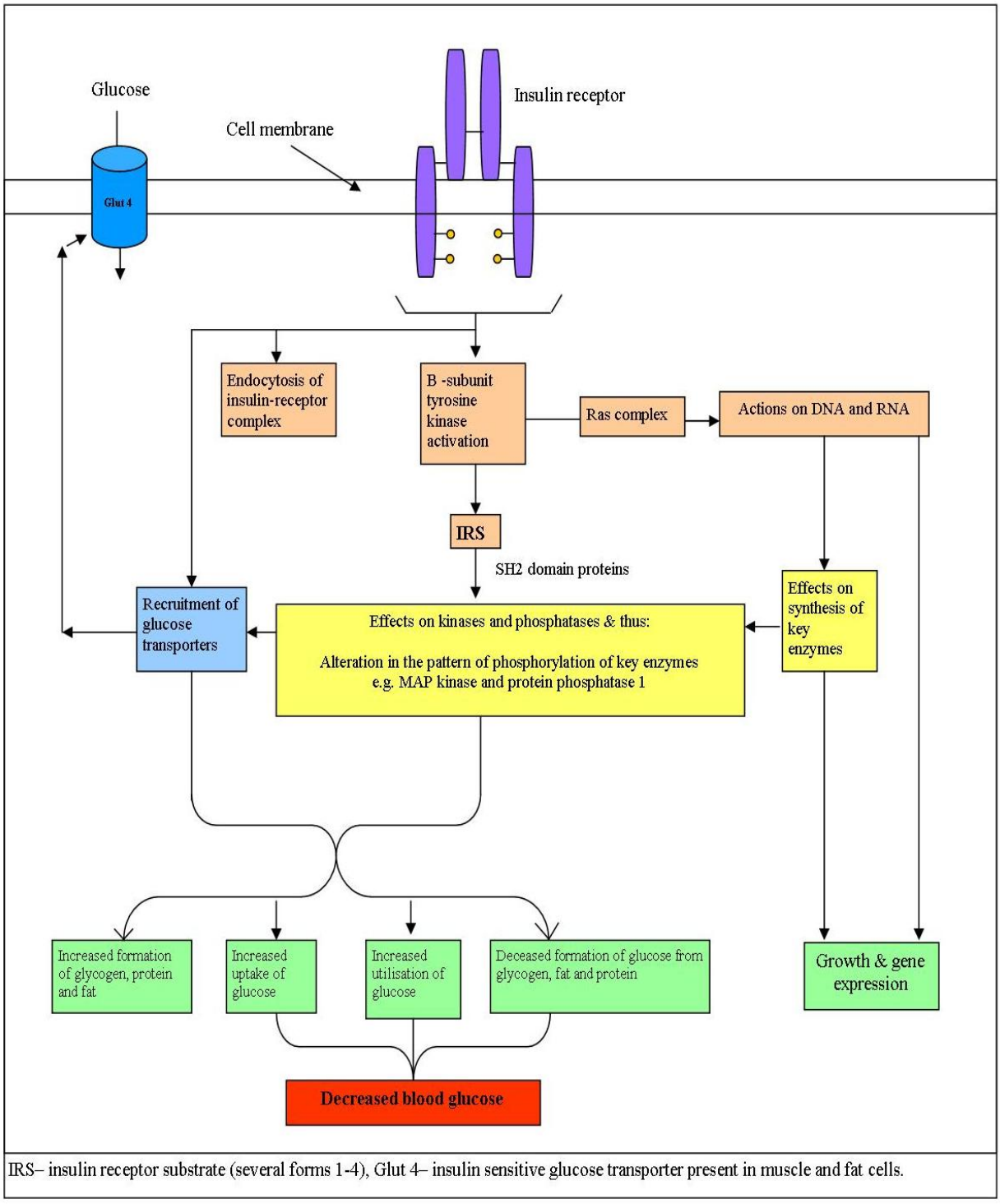


Figure 6: Insulin signalling pathways adapted from [93]. Ras = Regulator of Apoptotic signalling GTPase protein, MAP Kinase = Mitogen activated protein kinase

1.3.4 Aetiology of T2DM

T2DM is a heterogeneous disease. Establishing the exact mechanisms that are involved in its development are and have been difficult to decipher. It is well recognised that a combination of environmental and genetic factors are implicated in the development of this disease including obesity, sedentary lifestyle, smoking, low socioeconomic status, family history of diabetes mellitus and ethnicity. The MetS and obesity, as discussed previously, are main players in the development of T2DM. It is well recognised that T2DM is characterised by a level of sub-clinical inflammation (discussed below) and it is postulated that obesity associated inflammation is a key mechanism in the development of T2DM [61].

A positive family history of diabetes mellitus confers a risk for T2DM. The offspring of a diabetic parent has been reported to have a 40% chance of developing diabetes compared to a population risk of 7%; additionally if both parents have diabetes this risk is increased to 70% [95]. A number of candidate genes have been identified as possible genetic determinants of T2DM, which is thought to be a polygenic disorder. A recent review by Froguel and colleagues [96] covers this topic in detail. The identification of T2DM genes is currently a very active area in research and offers possible future gene therapy options for either the prevention or treatment of this disease.

1.3.5 Complications of T2DM

Chronic hyperglycaemia results in tissue complications in T2DM patients (Table 11) due to damage to these microvascular systems. It is the duration and degree of hyperglycaemia that dictates the severity of the microvascular/macrovascular complications evident in the T2DM population [83]. There are a number of other independent risk factors for the complications associated with T2DM which include hypertension, dyslipidaemia and smoking [97] (Figure 7). Therefore, the management of T2DM aims to tightly control blood glucose levels to prevent the development of these unfortunate and painful complications. I am going to briefly discuss the management options of this complex metabolic abnormality.

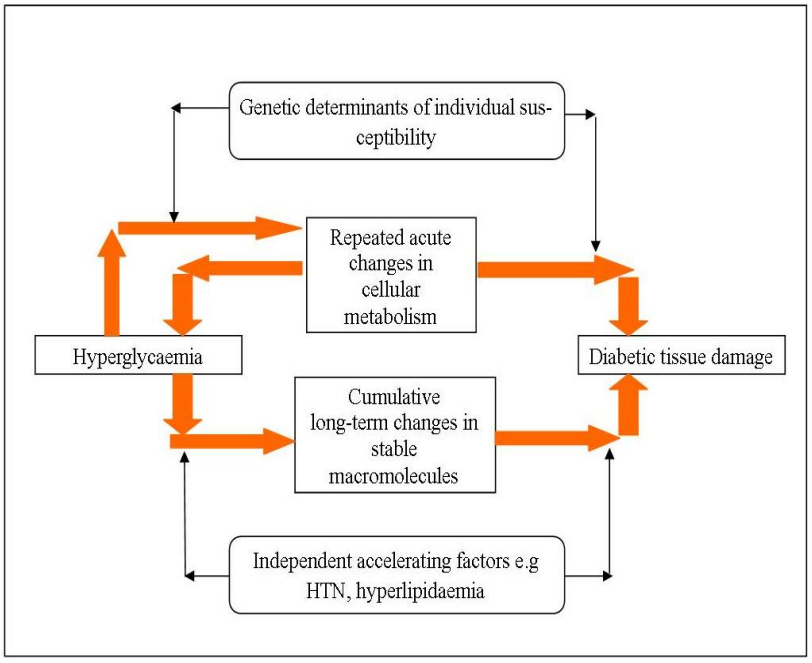


Figure 7: Scheme of mechanisms by which hyperglycaemia and independent risk factors may interact to cause diabetic complications [83]

Complication	Description
Retinopathy	Due to microangiopathy affecting retinal precapillary arterioles, capillaries and venules. Damage is caused both by microvascular leakage due to break down of the inner blood-retinal barrier and microvascular occlusion
Nephropathy	Due to angiopathy of capillaries in the kidney glomeruli. It is a progressive kidney disease (5 stages) and commonly asymptomatic until it is advanced. Microalbuminunria and Proteinurea are manifestations of this disease. Oedema and breathlessness present at the advanced stages. Blood pressure also increases as the disease progresses
Neuropathy	Constitute a diverse group of conditions which can be split into two main groups; those that progress (diffuse Polyneuropathy: symmetrical sensory and autonomic neuropathy) and those that have a relatively acute onset and the patient can recover in 6-8months (mononeuropathies (proximal motor neuropathy, radiculopathies, cranial nerve palsies) and acute painful neuropathies).
Dermopathy	Due to a type of vascular inflammation affecting the small blood vessels of the skin. Presents as reddish brown oval spots commonly on the shins or thighs. These patches often disappear of their accord after a few years
Diabetic foot	Neuropathy and ischaemia are the primary disorders that underlie this condition. There are three main categories of foot problem: neuropathic with intact circulation or ischaemic with or without neuropathy or critically, ischaemic, requiring urgent attention. Ulceration and sepsis are common problems. However gangrene and amputation can be avoided if the lesion is treated and managed early.
Sexual problems	Includes erectile dysfunction, ejaculatory failure and reduced libido in men. Menstrual irregularities and infection during pregnancy in women. Causes are multifactorial.

Table 11: Microvascular complications associated with Diabetes Mellitus

1.3.6 Management of T2DM

The prevalence of MetS in those with T2DM is very high for example a recent study reported the prevalence to be 72.3% in 5928 patients with T2DM [98]. The management of T2DM is almost an extension to that of MetS, with increased physical activity and weight loss again the major aims of treatment. Weight loss, as discussed previously, is associated with reductions in total cholesterol, triglycerides, plasma glucose levels, blood pressure and

insulin resistance [75, 99]. There is a stepwise approach to the management of T2DM [100]:

1. Lifestyle modifications i.e. diet and exercise
2. Oral hypoglycaemic agents i.e. metformin & sulphonylureas
3. Insulin therapy or imerging therapyies such as the GLP-1 analogues or DPP 4 inhibitors

Progression through these steps is dependent upon how the patient responds to the treatment plan. The aim is to lower blood glucose levels and achieve glycaemic control, which in turn reduces the risk of microvascular and macrovascular complications. It is important to note that T2DM is primarily a self-managed condition [100] even though the patient and healthcare professional work closely to produce a treatment plan and will modify this plan depending on whether the patient reaches their targets. In addition each patient would be assessed for other potential comorbidities including dyslipidemia and hypertension. These comorbidities would be treated vigorously in conjunction with the treatment plan for the diabetes with the aim again to reduce the risk of CVD.

The first step of treatment is dietary modification and encouraging the individual to engage in regular physical activity, as is recommended for the MetS, with a target weight loss of between 5-10% [100]. However, diet and exercise will only be successful in achieving glycaemic control in approximately 10-20% of subjects [83]. When diet and exercise alone

fails oral hypoglycaemic agents are introduced - of which there are five types currently available (Table 12). The combination of diet, exercise and an oral hypoglycaemic agent usually reduces HbA1c levels by 0.5-2% [101]. The dosage of these agents can first be altered if no further glycaemic control has been gained from their use. However when the patient reaches maximum dosage without any improvement the next step is to try a combination of these oral agents (two agents). If when using this approach glycaemic control is not maintained then insulin therapy or gliptin/incretin therapy can be introduced; this is quite common in T2DM [102].

Class of compound	Drug name	Mechanism of Action
Biguanide	Metformin	Decrease hepatic glucose production
Sulphonylureas (SUs) and Non-SU insulin secretagogues	Glimepiride, Gliclazide, Glipizide, Glyburide, Tolbutamide, Chlorpropamide, Repaglinide, Nateglinide	Increase pancreatic insulin secretion
Thiazolidinediones (TZDs)	Rosiglitazone, pioglitazone	Increase insulin sensitivity
A-Glucosidase Inhibitors (AGIs)	Acarbose, migitol	Delay digestion and carbohydrate absorption in small bowel
Gliptins	Sitagliptin, Vildagliptin	Inhibit dipeptidyl peptidase -4 (DPP-4) thus raising level of GLP-1 which in turn increases the release of insulin
Incretins	Exentide, liriglutide	GLP-1 analogs – longer half life than GLP-1. Also known as GLP-1 mimetics

Table 12: Oral agents for the treatment of T2DM (with exception to the incretins which are injectable agents)

1.3.7 Cardiovascular Outcomes of T2DM

Subjects with T2DM commonly present with risk factors of CVD including hypertension, dyslipidaemia, visceral obesity and a sub-clinical level of inflammation. It is therefore not surprising that T2DM confers a two to three fold increase in the risk of CVD [103]. It has been reported that 70 - 75% of all deaths in people with diabetes can be attributed to CV complications [104]. Fox et al [105] assessed the effect of diabetes duration on incident CHD morbidity and mortality from data from the original and offspring cohorts of the Framingham Heart Study; 588 subjects who were diagnosed with diabetes between the ages of 30 - 74 years were included. This group estimated the hazard ratio of incident CHD events and mortality over a 12 year follow-up period. The mean duration of diabetes was 7.8 years. They report that the duration of T2DM is significantly and positively related to the risk of CHD mortality and that for each decade of duration of diabetes, the 10 year risk of CHD death was 86% greater [105]. Interestingly, studies have shown that the protective effect of female gender for CVD is silenced in the presence of T2DM and in some cases women with T2DM have been reported to be at a higher risk of CVD than men with T2DM [106, 107]. Juutilainen et al have described T2DM to be a coronary heart disease equivalent [107]. This group assessed the CHD mortality in T2DM subjects without prior evidence of CHD in comparison to non-T2DM subjects with prior myocardial infarction (MI) or without any prior evidence of CHD. Baseline data was collected between 1982-1984 on a random sample of 1373 non-T2DM subjects and 1059 T2DM subjects. Follow-up data were collected until 2001 with a median follow-up period of 17.5 years. They found that the incidence of CHD death was 3.2 per 1000 person years in non-T2DM without prior MI, 26.8 in non-T2DM with prior MI, 26.0 in T2DM subjects without prior MI and 71.4 in

T2DM subjects with prior MI. Furthermore, the incidence of CHD death in T2DM subjects without prior MI versus non-T2DM subjects with prior MI was relatively higher in women than in men. The incidence of CHD death per 1000 person-years was 26.9 in T2DM men without prior MI, 34.1 in non-T2DM with prior MI, 25.2 in T2DM women without prior MI, and 10.8 in non-T2DM women with prior MI [107]. They concluded that diabetes without prior MI and prior MI without diabetes indicates similar risk for CHD death in men and women. However, this increased CVD/CHD risk in females with T2DM compared to males is an area of much debate with evidence both for and against this argument [108] [109, 110].

1.3.8 Ethnic Comparison for Risk of Metabolic Dysfunction

It is well recognised that people of South Asian origin have an increased risk of developing the MetS, T2DM and CVD. Globally the highest burden of cardiovascular disease has been reported to come from the South Asian countries [111]. The development of these conditions particularly, within the Westernised South Asian population and when comparing native South Asians who reside in rural areas versus urban areas, occurs at an earlier age and lower body weight [112, 113]. A graded increase in metabolic dysfunction and cardiovascular disease has been observed in a study comparing native South Asian males from villages, urban slums and urban middle-class residences [114]. The key determinant of this increased risk is thought to be the increased visceral fat mass in the South Asian population and intrauterine programming, as discussed in a recent review [115].

1.4 Inflammation

The innate immune system is the body's rapid and first line of defence against potentially harmful substances [116]. The cells involved in recognising these invading foreign substances have a number of receptors that are made up of broad-specificity proteins. These are known as pattern-recognition receptors (PRR) and are not specific to any particular antigen, but recognise conserved structures and molecules that are characteristic of harmful agents [117]. Activation of the PRR gives rise to the activation of nuclear factor-κB (NF-κB) signalling pathways which in turn induce immune response genes [61]. Thus the acute-phase response is activated; this is designed to limit and neutralise injury in order to restore homeostasis. The inflammatory cytokines are produced in macrophages and monocytes as well as other cells in the body [118] and are the key players in inflammation and the acute phase response [61] (Figure 8).

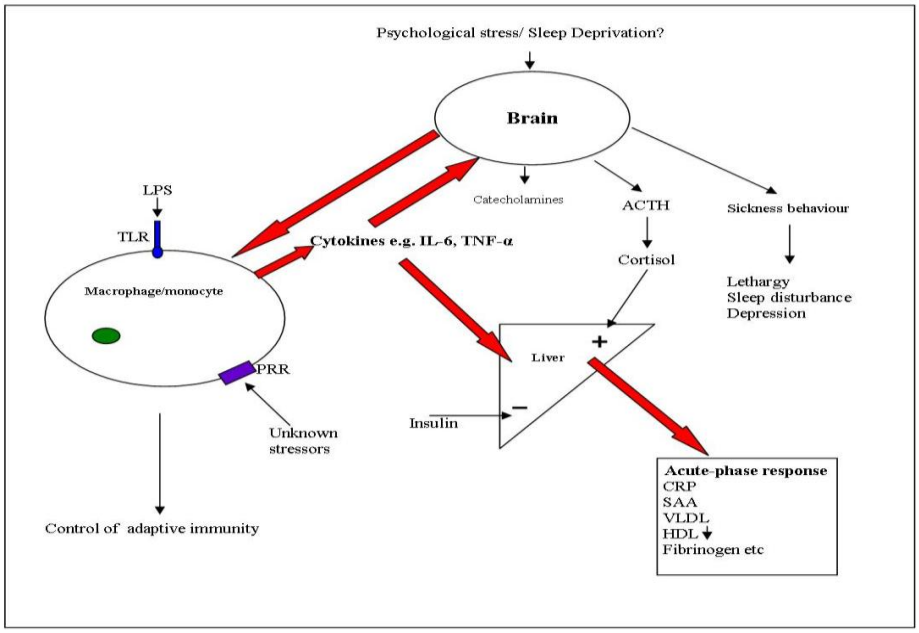


Figure 8: Components of the innate immune system adapted from Pickup JC, 2004 [61] . CRP- C-Reactive Protein, SAA- Serum Amyloid A, VLDL- Very Low Density Lipoprotein, HDL-High Density Lipoprotein. LPS-lipopolysaccharide , TLR-Toll-like receptor, IL-6- Interleukin-6, TNF-a-Tumour necrosis factor

Interleukin-6 (IL-6) and tumour necrosis factor-alpha (TNF-α) are among many of these cytokines which act on the liver to stimulate the production of the acute-phase proteins. Acute phase proteins namely include C-reactive protein (CRP), complement, serum amyloid A, heptaglobin and fibrinogen. These proteins have a number of functions which in this context contribute to host defence, healing and adaptation to insult [116]. The inflammatory cytokines are not limited to stimulating hepatic cells but also act on pancreatic β-cells, adipose tissue and on the neuroendcrine system to stimulate the secretion of corticotropin–releasing hormone, ACTH and therefore cortisol [116]. The acute phase response can be localised or systemic in nature depending on the antigen in question. However, chronic activation of this system can be detrimental and has been observed in a number of chronic diseases including atherosclerosis, arthritis, cancer and more recently T2DM [61]. A number of factors could give rise to the chronic activation of the acute phase response including over nutrition and thus obesity, age, chronic sleep deprivation, smoking, genetic predisposition and neonatal programming (Figure 9).

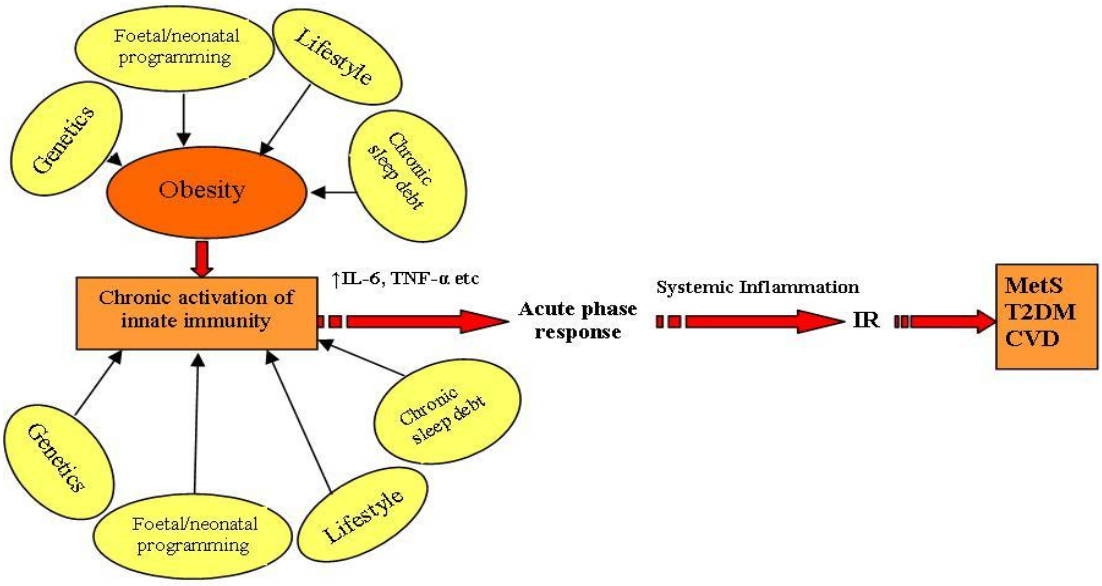


Figure 9: Chronic activation of innate immunity and metabolic dysfunction. Adapted from Pickup JC, 2004 [61] IR = Insulin resistance

The evidence for the association between inflammation in MetS, T2DM and CVD is discussed below.

1.4.1 Obesity, inflammation and insulin resistance

The development of insulin resistance is associated with increasing adipose tissue mass specifically the visceral depots. The adipocyte is described not only as a storage cell but as a secretory cell [119]. It is well recognised that the adipocytes express and secrete a large number of molecules and that are dual metabolic and immuno-modulatory in function [78] in addition to its triglyceride storage capabilities (Figure 10).

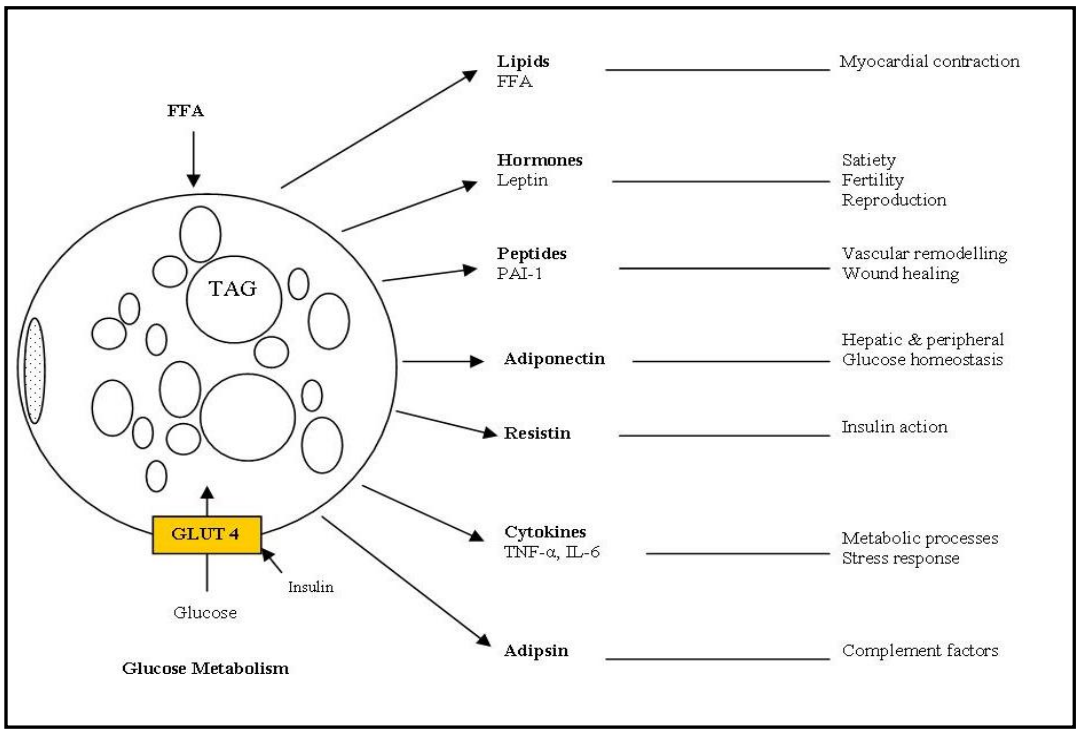


Figure 10: Functions of the adipocyte [78]. TAG- Triacylglycerides, FFA-Free Fatty Acids, PAI-1 – Plasminogen activator inhibitor-1

Adipocytes can influence, by secretion of such molecules, local adipocyte biology and systemic metabolism including that of the brain, liver, muscle, β-cells, gonads, lymphoid organs, and systematic vasculature [119]. Secretion of cytokines, or adipokines, is of major interest when looking at the relationship between obesity and insulin resistance. It is the dysregulation of these adipokines, in obesity, that are implicated in development of metabolic disorders such as MetS and T2DM. The exact mechanism by which excess adipose tissue gives rise to over expression/chronic activation of adipokines has yet to be fully established. This phenomenon cannot be simply explained by the increased number of adipocyte cells in obesity because then every obese individual would develop insulin resistance, MetS or/and T2DM. However the distribution of fat mass has been implicated in

this relationship between obesity and insulin resistance. Park et al [120] took two groups of obese individuals - a subcutaneous obese and a visceral obese group. They compared with the effect of weight reduction on insulin sensitivity. They reported that there were greater beneficial effects on parameters of the metabolic syndrome i.e. fasting plasma glucose and insulin levels and triglycerides in the visceral fat reduction group compared the subcutaneous fat reduction group [120]. This could be attributed to the close proximity of this excess fat mass to vital organs such as the liver and pancreas. This is an important and ongoing area in research. I am now going to briefly discuss a number of these adipokines with respect to their function and evidence for their potential involvement in the development of insulin resistance in the obese individual.

1.4.2 Biological markers of inflammation

Interleukin-6 (IL-6)

IL-6 is a pro-inflammatory circulatory cytokine that is secreted by a large number of cells types including macrophages, lymphocytes and the adipocyte [121]. It is a pleiotropic cytokine whose functions range from increasing basal glucose uptake, inhibition of glycogen synthesis and altering insulin sensitivity in target tissues. Other functions include stimulating the release of adhesion molecules by the endothelium and increasing the hepatic release of fibrinogen [121]. IL-6 and Tumour Necrosis Factor-α (TNF- α) can regulate the hepatic synthesis of CRP and the hepatic acute phase response [121]. In turn TNF-α induces the expression and secretion of IL-6 [122]. The role of IL-6 in the development of insulin resistance is supported by an abundance of epidemiological and genetic studies. IL-6 production and systemic concentrations positively correlate with

increasing adiposity in healthy men and women [123, 124]. It has been reported that a third of total circulating IL-6 concentrations originate from adipose tissue [121]. IL-6 levels significantly decrease with weight loss with concomitant improvement in insulin sensitivity [125]. Elevated levels of IL-6 predict the development of T2DM [126]. Although the mechanisms by which IL-6 induces insulin resistance have not been fully established, however, IL-6 has been shown to induce partial resistance in insulin-dependant glucose uptake [127]. IL-6 secretion has been found in part to be regulated by catecholamines via β -adrenergic receptors [128], and therefore when a subject is under psychological and physiological stress then levels of IL-6 can increase. IL-6 additionally stimulates the hypothalamo-pituitary-adrenal (HPA) axis [129]. IL-6 levels are found to be elevated in conditions that are resultant of hyperactivity of the HPA and sympatho-adrenomedullary axes. For example abdominal obesity, hypertension, insulin resistance and dyslipidemia [130]. Inflammatory cytokines have been shown to be involved in the regulation of sleep in both animals and humans [131]. A number of studies have reported increased levels of IL-6 and TNF-α in the sleep deprived state [132, 133].

Tumour Necrosis Factor-Alpha (TNF-α)

TNF-α is a pro-inflammatory cytokine, secreted by adipose tissue. However, unlike IL-6 this cytokine exerts its effect via auto- and paracrine mechanisms [123]. TNF-α has been implicated in the pathogenesis of MetS and T2DM. Elevated production of TNF-α in adipose tissue has been observed in both animal and human models of obesity and insulin resistance [134]. TNF-α mRNA has been shown to be significantly correlated with BMI, percentage body fat [135] and levels are negatively correlated with weight loss. Elevated

circulatory levels of TNF-α have been reported in T2DM subjects [136], the MetS [137] and subjects with obstructive sleep apnoea [138]. TNF-α has multiple functions within adipose tissue, in addition to its role in the acute phase response. These include down regulation of the expression of the GLUT 4 transporter [139], inhibition of lipoprotein lipase activity (LPL) [135] and of lipolysis [140]. It is clear that TNF-α plays a key role in the regulation of adipose tissue metabolism. Although the mechanisms by which TNF-α influences insulin sensitivity has not been fully established however there are a number of working hypotheses. I will discuss the following two:

1. *GLUT 4 down regulation*

The down regulation of GLUT 4 expression reduces the cell's ability to uptake glucose in response to insulin. Although this is localised to adipose tissue, efficient glucose uptake in adipose tissue is required for normal whole body glucose regulation. This is demonstrated by GLUT4 adipose-tissue-specific knockout mice studies where significant insulin resistance is observed [141]. In addition, T2DM was reversed in T2DM mice lacking skeletal muscle GLUT4 by inducing the over expression of GLUT4 in adipose tissue [142].

2. *Interference with Insulin Receptor Substrate -1 (IRS-1)*

The insulin receptor has intracellular tyrosine kinase activity and this tyrosine kinase activity is an absolute requirement for the biological activities of insulin. However, TNF-α has the ability to disrupt down stream insulin signalling cascades by interfering with this

tyrosine kinase activity at the level of the phosphorylation of insulin receptor substrate (IRS) molecules [101]. This disruption occurs by two different mechanisms, the first involves TNF-α stimulation of the Suppressor Of Cytokine signalling proteins (SOCS), and the second by the phosphorylation of the serine at residue 307 on IRS-1. Both result in the prevention of IRS1 multiple phosphorylation activity and thus downstream signalling, therefore giving rise to the partial/total loss of insulin's physiological effects.

The SOCS proteins suppress cytokine signalling, however TNF-α can induce these proteins. This is important given a recent finding that this family of proteins have been shown to be additionally involved in the negative regulation of insulin signalling [143]. SOCS-3, -1, -2 and -6 can interact with both the phosphorylated insulin receptor and IGF-1 receptor, to ultimately down regulate their downstream signalling capabilities [143-145]. Additionally, it has been reported that SOCS-3 can bind to the major binding residue of IRS1, (phosphorylated Tyrosine 972) [146]. All three mechanism can be induced by TNF-α and thus prevent the downstream signalling from the insulin receptor.

C-reactive protein (CRP)

CRP is an acute phase reactant and a well recognised sensitive marker of inflammation in humans [147, 148]. It is produced by hepatic cells stimulated by cytokines such as TNF-α and IL-6 [121]. CRP is implicated in the pathogenesis of the MetS [149], T2DM [150] and CVD [151]. To date it is unclear whether CRP is merely a marker of inflammation or is actively involved in the pathogenesis of these diseases [152]. However, CRP levels

correlate with several components of the metabolic syndrome that include fasting insulin levels, microalbuminuria, and impaired fibrinolysis [151]. Ridker et al [149] report a significant positive relationship between levels of CRP and an increasing number of MetS components. Yet the metabolic syndrome and serum CRP levels remain independent predictors of new cardiovascular events [151]. Plasma CRP levels are positively correlated with measures of obesity and more specifically with measures of central obesity in South Asians versus Europeans [153]. Additionally, it has emerged that is has a number of direct effects on the vessel wall i.e. induction of adhesion molecule expression in human endothelial cells [152], this and basic research into inflammatory mechanisms of both T2DM and vascular dysfunction provide strong evidence that insulin resistance and atherosclerosis share a common inflammatory basis [149].

Adiponectin

Adiponectin is a hormone like protein synthesised exclusively by the adipocyte and is the dominant secretory product of these cells [154]. This hormone like protein has been reported to have anti-atherogenic and anti-inflammatory properties [155-157] in addition to insulin sensitising effects [158, 159]. Females generally have higher circulating levels [160]. Concentrations of Adiponectin are inversely associated with central and whole body adiposity and hyperlipidaemia independently of BMI [160, 161]. Normal circulatory levels return after weight loss. It has recently been reported to be a powerful marker of diabetes risk in high risk subjects. Mather et al [154] investigated baseline and intervention-associated change in adiponectin levels from subjects participating in the Diabetes Prevention Program (DPP) [154]. DPP is a multicenter randomised clinical trial

investigating the effects of intensive lifestyle changes or metformin versus placebo on the rate of developing T2DM, in at risk subjects. They found that at baseline a 3.0 μg/ml higher level of adiponectin corresponded to a 20 - 40% lower rate of progression to diabetes and that the relationship between adiponectin and diabetes conversion was not different across the three treatment groups (p=0.13). At one year significant increases in adiponectin were observed in all treatment groups after adjustment for baseline adiponectin, weight, age, sex and ethnicity. They report that a ~ 1μg/ml increase in adiponectin was associated with a 16% reduction in the rate of progression to diabetes [154]. In addition hypoadiponectinemia has been reported to be highly associated with coronary artery disease (CAD) [156]. Kumada et al [156] investigated whether hypoadiponectinemia was independently associated with the prevalence of CAD in men. They found that male patients with hypoadiponectinemia (<4.0μg/ml) had a 2-fold increase in CAD prevalence. This relationship was independent of typical CAD risk factors i.e. smoking, hypertension, BMI and diabetes mellitus [156]. The exact mechanisms by which adiponectin exerts its protective role are not fully understood. However, one proposed mechanism has arisen from the reported negative association this adipokine has with the pro-inflammatory cytokines such as TNF-α [162] and CRP [163]. Thus the inhibition of these inflammatory molecules by adiponectin could offer one explanation.

Leptin

Leptin was discovered in 1994 and is a product of the obese (*ob)* gene [164]. It is an adipocyte derived hormone with numerous functions including satiety, energy expenditure, and neuroendocrine function [165]. Leptin is secreted in response to food intake and in turn

inhibits the synthesis of neuropeptide Y, which acts to stimulate appetite. The leptin receptor is not limited to just the hypothalamus and receptor isoforms have been found in skeletal muscle, white adipose tissue and the liver [166-168]. An abundance of animal studies have implicated leptin in the pathogenesis of insulin resistance and diabetes mellitus. *ob/ob* or *db/db* mice strains deficient in leptin or the leptin receptor suffer severe insulin resistance, hyperinsulinaemia, obesity and diabetes mellitus [169]. When leptin is replaced, glucose and insulin levels decrease within hours of administration and this occurs before any changes in food intake or body weight are observed [170]. Leptin is secreted in proportion to adipocyte mass in humans [171] this indicates that obese individuals may exhibit a state of leptin resistance, thus exogenous leptin administration is unlikely to have a major effect as is observed with animal models [119]. In addition obesity due to leptin or leptin receptor mutations in humans is rare [172]. It is important to note however that although this prevalence is low it cannot take away from the body of evidence that places leptin as an important regulator of energy balance in humans. Indeed humans with only one functional leptin gene have significantly lower serum leptin concentrations than controls and also an increased prevalence of obesity, thought to be a result of this hypoleptinaemic state [173]. Hence hypoleptinaemic obese subjects may benefit from therapeutically administered leptin. Heymsfield et al [174] investigated what effect treatment with exogenous leptin had on weight loss in both lean and obese adults. This was a randomized, double-blind, placebo-controlled, multicenter, escalating dose cohort trial with 54 lean and 73 obese men and women. Varying amounts of recombinant human leptin were self administered every morning for 4 weeks in all subjects and for a further 20 weeks in the obese group. Those receiving the highest dosage of leptin had a significant and progressive reduction in body weight (7.1kg) without any changes in glycaemic control. However the

placebo group was also associated with weight loss (1.3kg) and the obese group had been prescribed a diet that reduced their daily energy intake by 500-kcal/day from the amount needed to maintain a stable weight. Therefore, weight loss cannot solely be attributed to the administration of leptin. Interestingly in the highest dosage group serum leptin levels reached values ~30 to 40-fold higher than the placebo and baseline values [174]. This suggests that for humans the amount of leptin required to overcome the leptin resistant state needs to be somewhat higher than first anticipated. The mechanisms involved in the development of leptin resistance are still not fully understood to date. However, given that leptin exerts much of its effects on metabolism and satiety via actions within the ventrobasal hypothalamus [119], it has been suggested that leptin resistance may in part be due to limited entry of leptin into the central nervous system, as leptin transport into the brain appears to be a saturable carrier-mediated process [175]. Therefore the development of a leptin analog which more readily enters the CNS may provide a better therapeutic approach to leptin resistance.

Resistin

Resistin is a recently discovered adipocyte-secreted polypeptide and is a member of a newly discovered family of cysteine-rich secretory proteins termed the resistin-like molecules [176]. It was discovered when investigators were searching for genes that are induced during adipocyte differentiation, but down regulated in the mature adipocyte when exposed to thiazolidinedione [176]. Thiazolidinediones (TZD) are anti-diabetic drugs that regulate gene transcription by binding to peroxisome proliferator activated receptor gamma (PPAR-γ). PPAR-γ is a nuclear hormone receptor found at its highest levels in adipocytes.

Once resistin is secreted it was found to inhibit 3T3-L1 adipogenesis - 3T3-L1 is a type of fibroblast at the preadipocyte stage of cellular differentiation [164]. Resistin has been found in rat and mouse serum [176] and has been implicated in obesity associated insulin resistance. For example levels of resistin have been found to be elevated in both genetic (*ob/ob* and *db/db*) and diet-induced mouse obesity and insulin resistance models [176]. Additionally when resistin is administered into normal mice, glucose tolerance and insulin action were impaired [164]. However evidence for the role of resistin in insulin resistance is conflicting in both animal and human models. There is some evidence supporting a link between plasma levels of resistin, BMI and adiposity with insulin resistance and cardiovascular risk in humans [177-179]. However a number of studies have reported that there is no association between plasma or serum levels of resistin and obesity associated insulin resistance or T2DM [180-182]. It is essential to note that human and mouse resistin is only 59% structurally similar and therefore direct comparisons between human and rodent models in the function of resistin must be made with caution [176]. The role of resistin in the pathophysiology of insulin resistance is thus a continuing topic of debate and further investigation today.

1.4.3 Dyslipidemia

Triglycerides and cholesterol are insoluble and therefore have to be transported throughout the body by carriers known as lipoproteins. Lipoproteins are a biochemical assembly of proteins, lipids, fatty acids and steroids. There are many different classes of lipoprotein in the body depending upon their role and composition. Table 13 gives the roles of the lipoproteins that circulate in the blood carrying various fats.

Chylomicrons	Very low-density lipoprotein (VLDL)	Intermediate density lipoprotein (IDL)	Low Density Lipoprotein (LDL)	High Density Lipoprotein (HDL)
Carriers of TAGs from the intestine to the liver and to adipose tissue (TAG=Triacylglycerides)	Carriers of newly synthesized TAG from the liver to the adipose tissue	Intermediate between VLDL&LDL cannot usually be detected in the blood stream	Carriers of cholesterol from the liver into cells around the body i.e. deposit cholesterol	Carries cholesterol away from the body's tissues & back to the liver (reverse cholesterol transport)

Table 13: Lipoproteins in the circulation

Dyslipidemia is characterised by an abnormal lipid profile namely decreased levels of HDL-C and elevated levels of the LDL-C, VLDL and chylomicrons. Dyslipidemia is associated with T2DM, MetS, atherosclerosis and increased risk of CVD [78]. An increased level of LDL-C is a risk factor for CVD because this lipoprotein is the major cholesterol carrier, carrying ~ 60-80% of the body's cholesterol [97]. Cholesterol when received by LDL-C particles can be used in cells to build new cells, for the biosynthesis of hormones, and some is returned to the liver by HDL-C, in normal metabolic situations. However when there is an excess of cholesterol, circulating LDL-C particles will actively deposit it at arterial walls - a process involved in the development of plaque formation and therefore atherogenic. Low circulating levels of HDL-C is a risk factor for CVD because this lipoprotein is involved in picking up cholesterol from peripheral tissue and transporting it back to the liver (reverse cholesterol transport). This process essential in the prevention of atherosclerosis and CVD i.e. cholesterol accumulation within atherosclerotic plaques occurs when cholesterol influx into the arterial wall (from LDL-C) exceeds cholesterol efflux [183]; therefore these particles need to be in balance for normal cholesterol homeostasis. In the treatment of MetS, T2DM and CVD the identification of dyslipidemia is an important factor for reducing further complications. This condition can be treated with

either statins or fibrates. High circulating LDL-C levels are also implicated in the production of oxidative stress which I will now discuss, given there is evidence that this process is a further risk factor for metabolic dysfunction.

1.4.4 Oxidative Stress

Isoprostanes

Oxidative stress is a product of an imbalance between the exposure to reactive oxygen species (ROS) and antioxidant defences [184]. ROS are atoms or molecules with unpaired electrons in their outer orbit rendering them highly prone to chemical reactions [185]. ROS such as the superoxide anion (O_2^-), hydroxyl radical (OH^-) and peroxynitrite ($ONOO^-$) can be destructive to cells principally via the oxidative degradation of lipids within cell membranes (lipid peroxidation) [185]. Oxidative stress has been recognised in the pathogenesis of a number of acute and chronic diseases including cancer, CVD, lung disease and is implicated in the normal aging process [184]. A number of recent reviews have implicated oxidative stress in the pathogenesis of insulin resistance [186-188] with postprandial hyperglycaemia playing a major role in this cellular imbalance. In the context of overfeeding and a sedentary lifestyle the muscle and adipose tissue cells are subject to a substrate-induced increase in citric acid cycle activity [189]. This results in an excess of the end products- mitochondrial NADH (mNADH) and ROS. The cell has two mechanisms for protecting against the excess of ROS, either by increasing the level of ROS removal compounds (antioxidants) or by reducing their formation. This latter mechanism involves the inhibition of insulin stimulated uptake (i.e. down regulation of GLUT 4 translocation to the plasma membrane) and thus the entrance of energetic substrates into the mitochondria

[186]. However, although there is a reduction in ROS formation, these muscle and adipose tissue cells are now in an insulin resistant state giving rise ultimately to systemic hyperglycaemia further increasing the risk of systemic insulin resistance.

LDL-C particles are another source of ROS. Therefore in the dyslipidemic state there is increased risk of oxidative stress. It is very common for glucose intolerant individuals to present with dyslipidemia given the lifestyle that is characteristic of a glucose intolerant population – high energy intake and low levels of physical activity. Therefore, the level of oxidative stress is likely to be higher than in a non-glucose intolerant population. This is because the ROS are potentially in continuous production as the oxidative stress cascade is being 'fed' by both LDL-C particles and end products of the CTA cycle as the body tries to cope with this high nutrient environment.

The most robust approach to measuring oxidative stress *in vivo* is via the quantification of plasma and urinary F_2-Isoprostanes. 8-Iso-protaglandin $F_{2\alpha}$ (8-Iso$PG_2\alpha$) is one of 15 isomers of the F_2-isoprostane series [190]. Isoprostanes are produced *in vivo* independently of cyclooxygenase (COX) enzymes, the major source coming from free-radical induced peroxidation of arachidonic acid [190]. Hence they are termed prostaglandin like substances [184].

8-Iso$PG_2\alpha$ has a number of physiological properties which include: vaso- and bronchoconstriction, platelet aggregation, induction of DNA synthesis in endothelial cells and enhancement of granulocyte activity and adhesion in endothelial cells [190]. 8-Iso$PG_2\alpha$ levels have been shown to be elevated in conditions such as obesity [191], hypercholesterolemia [192], T2DM [193] and CVD i.e. atherosclerosis [194].

Therefore, there is a strong case for the role of inflammation and oxidative stress in the pathogenesis of metabolic dysfunction and related conditions including T2DM. Obesity is the main driving force of these processes. However, sleep deprivation has recently been identified as a possible player in obesity associated insulin resistance and thus in the development of MetS and T2DM. More specifically, obesity related sleep disorders such as Obstructive Sleep Apnoea (OSA) are of major concern given the individual is simultaneously exposed to metabolic/endocrine derangements associated with obesity and those that are resultant from chronic sleep deprivation due to the sleep disorder itself. OSA is a prevalent sleep disorder which renders the individual at increased risk of T2DM and CVD. I will discuss this in the next section.

1.5 Obstructive Sleep Apnoea (OSA)

Sleep deprivation can be the result of voluntary sleep curtailment, induced sleep curtailment or arise from the numerous sleep disorders recognised today including OSA, periodic leg movement syndrome and insomnia. Identifying and treating such sleep disorders provides a means in which to investigate the metabolic effects of sleep

deprivation. Here I will discuss OSA, which falls into the sleep disordered breathing (SDB) category of sleep disorders, and discuss the evidence that associates this sleep disorder with metabolic dysfunction.

SDB describes a group of disorders characterised by abnormal breathing patterns during sleep and affects between 20- 30% of the general adult population [195, 196]. SDB encompasses a number of obstructive sleep disorders with simple snoring at one end of the spectrum and severe obstructive sleep apnoea (OSA) at the other. OSA is characterised by the recurrent collapse of the upper airway and cessation (apnoea) or reduction of airflow (hypopnoea) leading to arousal from sleep in order to reinitiate breathing. These recurrent arousals result in sleep fragmentation and consequently sleep deprivation. However, the individual is unaware of these recurrent arousals from sleep which is one reason that this condition goes undetected. Visceral obesity, snoring, gasping for breathing and daytime hypersomnolence are characteristics of this sleep disorder.

1.5.1 Prevalence of OSA

Obstructive sleep apnoea is a significant medical problem that affects ~ 4% of middle aged adult males and ~2% of adult females [197]. However, the condition frequently remains undiagnosed in the primary care setting. The prevalence of OSA is greatly increased in subjects with MetS or T2DM (as discussed below). This has been attributed to the salient feature of these conditions, namely obesity. The prevalence of obesity is increasing

dramatically worldwide and therefore the incidence of OSA will increase as with the other obesity associated diseases.

1.5.2 Diagnosis of OSA

At present full overnight PSG studies (Section 1.1.1) are not typically used for the diagnosis of OSA. Instead limited overnight studies are undertaken, which can now be conducted in the comfort of the patient's own home. These limited studies can measure thoraco and abdominal movements in order to identify abnormal breathing patterns (e.g. apnoeas) in addition to airflow and oxygen saturation levels.

The apnoea-hypopnoea index (AHI) is the primary parameter used for the diagnosis of OSA. AHI is the average number of times a subject has an apnoeic or hypopnoeic event per hour of sleep. The AHI is used to determine the severity of OSA: AHI < 5 (non-OSA), 5-15 (mild OSA), 15-30 (moderate OSA) and > 30 (severe OSA) [198]. In order for an episode of cessation of breathing to be classified as apnoea it must last ≥ 10 seconds, hypopnoea is defined as a >50% decrease in breathing from baseline that lasts ≥ 10 seconds. If the patient has an AHI ≥ 5 then they are diagnosed with OSA [198]. Oxygen saturation levels will drop as a result of these events thus O_2 desaturations are another means for identifying OSA. Pulse oximetry can be used alone or in conjunction with the ambulatory sleep kit for the diagnosis of OSA. The table below (Table 14) gives the American Sleep Disorders Association criteria for the diagnosis of OSA.

*A diagnosis of OSA is made when a subject fulfils criteria A **and either** B **or** C*
A : Excessive daytime sleepiness that cannot be explained by other causes
B: Two or more of the following symptoms that cannot be explained by other causes • Choking/gasping for breath during sleep • Recurrent arousals from sleep • Awakening un-refreshed from sleep • Daytime fatigue • Impaired concentration
C: Results from overnight monitoring • ≥ 5 AHI **or** • Reduction in airflow <50% that is associated with a >4% O2 desaturation or an arousal lasting ≥ 10seconds

Table 14: American Sleep Disorders Association criteria for the diagnosis of OSA (adapted from [198])

1.5.3 Aetiology of OSA

The aetiology of OSA is not well understood. However there are a number of well established risk factors for this disorder:

Abnormal upper airway anatomy - A number of studies have shown that patients with OSA have a smaller upper airway compared to normal subjects [199-201]. In non-obese subjects with OSA anatomical abnormalities of the upper airway are more common than in the obese OSA subjects who have increased upper airway soft tissue structures [201]. It is thought that this reduction in size is secondary to enlargements in surrounding the soft tissue and to reductions in, or changes to, the craniofacial structures [202, 203]

Thick short neck - Several reports have emphasised that a short thick neck is a risk factor for OSA. A neck circumference of >16 inches (46.0 cm) in women and >17 inches (43.2 cm) in men correlates with an increased risk for the disorder [201, 204] and increasing neck size positively correlates with increasing severity of the disorder [205].

Obesity

Gender - The prevalence of OSA is greater in men than women [197]. This could be attributed to the fact that upper airway size has been reported to be smaller in men [206]. In addition neck size has been reported to be smaller in women [207] and is it therefore plausible that the size of upper airway soft tissue structures in women are also smaller [1]. Visceral obesity is associated with OSA [208] and therefore the gender differences in fat distribution may also be a contributing factor with visceral obesity being more common in men.

1.5.4 Treating OSA

Overweight or obese people that are diagnosed with OSA are encouraged to lose weight [209]. Additionally if they smoke they are advised to stop due to the epidemiological evidence linking smoking with OSA [210]. However, smoking cessation is associated with weight gain which could further aggravate OSA and therefore should be advised with caution. Patients are also advised not to take sleeping tablets or sedatives and to refrain from drinking alcohol in the evenings. These factors all decrease upper airway patencey, thus increasing the likelihood of apnoeic events [209]. However, the majority of OSA patients will require further treatment. The gold standard treatment for OSA is Continuous Positive Airway Pressure (CPAP). Other options include oral appliances and surgery either for weight loss (gastro-intestinal) or for restructuring of the upper airway in cases where anatomical abnormalities manifest or patients are CPAP intolerant such as mandibulo-maxillary osteotomy and uvulopalataopharyngoplasty [209].

CPAP therapy

CPAP was first developed in 1981 by Sullivan [211]. This machine applies a continuous stream of air that is titrated to a pressure that prevents collapse of the upper airway [209]. Raising the pressure in the upper airway forces it to stay open and thus prevents/reduces the occurrence of apnoeas and hyponoeas. As a result the patient has an uninterrupted and refreshing nights sleep. This treatment in conjunction with patient compliance effectively restores the patient's quality and quantity of sleep and thus quality of life [212]. The CPAP machine consists of a pump, tubing and a nasal mask, of which there are many designs available for the patient to try (Figure 11). A number of CPAP machines now have the capacity to automatically titrate and a number also record patient compliance i.e. the number of nights they use CPAP and the number of hours per night it is used.

Figure 11: CPAP machine and examples of masks available

There a number of side effects of CPAP therapy, which in some cases results in CPAP intolerance and therefore an alternative method of treatment is sought. Such side effects include [209]:

- Claustrophobia - some patients cannot accept the concept of wearing a mask and are anxious whilst wearing the mask and therefore cannot sleep
- Uncomfortable masks - however there are a large number of different designs available now some of which do not require any extra head gear (model in Figure 11) and therefore this problem is usually resolved via a process of trial and error
- Nasal Symptoms – stiffness, rhinitis and drying of the nasal cavity. This is usually attributed to leaks in the mask system and again can be resolved by using a different mask or adjusting the existing one
- Abdominal bloating - a rare side effect

Fortunately the positive effects of CPAP therapy outweigh the side effects. CPAP therapy has been reported in a recent Cochrane systematic review to improve parameters of OSA including AHI, O_2 desaturations, blood pressure, measures of daytime sleepiness, cognitive function and quality of life [213]. Compliance with CPAP therapy is of utmost importance if the patient is to benefit from this treatment. It has been reported that there is a clear relationship between CPAP effectiveness and usage per night. However, the optimum threshold of hours per night depends on the outcome being investigated [214]. For example when assessing the effect of CPAP therapy on subjective daytime sleepiness, the threshold at which increased usage per night is unlikely to provide any further improvements has been reported to be 4 hours/night [214]. By comparison, when investigating the effects on

objective daytime sleepiness the threshold is increased to 6 hours/night [214]. The effects of CPAP on parameters of glycaemic control are discussed in detail in Chapter 3. OSA not only results in chronic sleep deprivation, but also puts a large amount of strain on the cardiovascular system as the subject repeatedly stops breathing throughout the sleep phase. I will now discuss evidence that links this obesity related sleep disorder with CVD, MetS and insulin resistance.

1.5.5 Cardiovascular outcomes of OSA

A large number of studies have indicated OSA as a risk factor for CVD, CHD [215] and more specifically for hypertension (HTN) [216, 217]. Peker et al [218] investigated the incidence of CVD in 182 middle-aged men. 60 subjects were diagnosed with OSA and 122 were non-OSA. Data was collected at baseline (when all were free of HTN, and other CVD, T2DM and pulmonary disease conditions) and at 7 year follow-up. The incidence of CVD was significantly higher in the OSA group versus the non-OSA group (36.7% *vs.* 6.6%, $p< 0.001$). This group report that OSA at baseline was a significant independent predictor of incident CVD (OR 4.9; (95% CI: 1.8-13.6), $p<0.001$). They additionally reported that effective treatment of OSA was associated with a significant risk reduction for incident CVD (OR 0.1; (95% CI: 0.0-0.7), $p<0.001$) after adjustment for baseline age and systolic blood pressure [218].

1.5.6 Association of OSA with insulin resistance and inflammation

The key risk factor of OSA is visceral obesity, as is true for MetS, T2DM and CVD. Subsequently there has been major interest in the possible link between OSA and insulin resistance. Interestingly, fasting insulin and insulin resistance have been found to increase with increasing severity of OSA [219]. Ip et al [219] investigated the relationship between OSA and insulin resistance, using the HOMA-IR method, in 270 subjects which they stratified based on the severity of OSA (Table 15).

AHI stratum					
	Group 1 **<5**	**Group 2** **≥ 5 to <15**	**Group 3** **≥ 15 to <30**	**Group 4** **≥ 30**	***p***
n	85	59	48	78	
AHI	2.3 (1.6)	9.3 (2.8)	20.6 (4.4)	50.2 (13.7)	**<0.001**
BMI (kg/m^2)	24.4 (3.5)	26.9 (4.1)	28.4 (4.6)	29.5 (4.8)	**<0.001**
Neck circumference (cm)	35.7 (3.4)	37.6 (3.8)	39.3 (3.1)	40.7 (3.7)	**<0.001**
Waist circumference (cm)	83.3 (10.1)	89.6 (10.1)	95.2 (9.6)	99.5 (11.9)	**<0.001**
Glucose (mmol/l)	5.3 (1.4)	5.3 (0.8)	5.4 (0.7)	5.6 (0.7)	0.166
Insulin (µIU/ml)	6.8 (4.2)	9.0 (12.7)	9.1 (6.7)	15.6 (34.3)	**<0.001**
HOMA-IR	1.6 (1.1)	2.2 (3.1)	2.3 (2.0)	4.0 (8.4)	**<0.001**
Systolic BP (mm Hg)	123.1 (13.9)	127.4 (17.5)	127.3 (13.5)	130.8 (14.0)	**0.023**
Diastolic BP (mm Hg)	70.6 (10.8)	74.8 (14.3)	78.8 (13.3)	78.8 (12.1)	**<0.001**

Table 15: AHI and Insulin Resistance adapted from Ip et al [219]. Results reported as means (SD)

Each additional apnoea or hypopnoea per hour was associated with an increased fasting insulin and HOMA-IR by 0.5%. A recent systematic review by Punjabi et al [220] concluded that there is an independent association between SDB and impaired glucose homeostasis, and that potential mediators of this association include (amongst others) the

release of pro-inflammatory cytokines [220]. One study included in this systematic review was conducted by Vgontzas et al [62]. This group investigated three areas proposed to be associated with OSA, two of which were whether OSA contributes to changes in circulating cytokine and leptin levels and whether it is a risk factor for insulin resistance independent of obesity. Thirty-six subjects were included in this study; 14 males with OSA, 11 obese controls and 11 non-obese controls. The levels of TNF-α, IL-6 and leptin were highest in the OSA group and progressively lower in the obese and non-obese controls (Table 16).

	OSA (n=14)	Obese controls (n=11)	Non-Obese controls (n=12)	*p*
TNF-α (mean)	3.12 ± 0.18	2.82 ± 0.11	2.66 ± 0.17	0.04
IL-6 (mean)	3.03 ± 0.38	2.30 ± 0.39	1.49 ± 0.22	0.005
Leptin (mean)	27.06 ± 3.18	16.51 ± 2.69	7.97 ± 2.14	<0.001

Table 16: Inflammation and OSA. Adapted from Vgontzas et al [62]. Data presented as mean ± SD

They additionally found that fasting blood glucose levels were significantly higher in the OSA group compared to the obese controls (5.9± 0.23 *vs*. 4.7± 0.24, $p<0.01$). Mean plasma insulin levels were also significantly higher in the OSA group versus the obese controls (25.7± 4.2 *vs*. 14.6± 2.5, $p<0.05$). This data suggest a clear association between OSA and inflammation which could be the linking factor between OSA and insulin resistance. Furthermore, this could aid in understanding the interrelationship between OSA, MetS and T2DM as discussed below.

1.5.7 Association of OSA with MetS

People with OSA have a higher incidence of cardiovascular morbidity and mortality [221]. OSA is independently associated with insulin resistance [62] and subjects with OSA commonly present with hypertension [217] and dyslipidaemia [222]. Therefore, it is not surprising that the prevalence of OSA is greater in those with MetS compared to the general population, given the CVD risk factors that comprise MetS as reported by Coughlin et al [208]. The likelihood of having the MetS has been reported to be 9.1 times higher in people with OSA [208]. OSA is also independently associated with increased blood pressure, increased fasting insulin levels, increased triglyceride concentrations, increased cholesterol:HDL ratio and decreased HDL cholesterol levels [208]. OSA has recently been described as a manifestation of the MetS [223]**.** Given that OSA is independently associated with MetS, and the cardiovascular risk factors that comprise it, it is plausible that OSA could increase the risk of developing T2DM and/or CVD in this high risk population.

1.5.8 Association of OSA with T2DM

The prevalence of glucose intolerance or T2DM in subjects with OSA is higher than that of the general population. Meslier et al [224] screened for OSA, using overnight PSG, in 595 male patients with suspected OSA. Patients also had a 2 hour OGTT to identify glucose tolerance status. This group found that the prevalence of IGT and T2DM was greater in those who were subsequently diagnosed with OSA compared to non-apnoeic snorers

(50.1% vs. 27.8%). This is further supported by Reichmuth et al [225] who reported that the likelihood of a severe OSA patient (AHI ≥ 15) having T2DM was 2.3 times higher compared to patients with AHI <5 (p=0.005). The prevalence of OSA is also reported to be higher in patients with T2DM. West et al reported a prevalence of 23% in male subjects with T2DM [226]. Although there is a clear relationship between OSA and T2DM the direction of the 'cause and effect' has not been established. Whether OSA predisposes to T2DM or vice versa is still a question of debate. However, the concept that one exacerbates the other via detrimental effects on the sympathetic nervous system is a plausible candidate for their association. The sympathetic nervous system is activated by the recurrent arousals from sleep that OSA patients suffer, which could contribute to insulin resistance and/or sympathetic mediated impairments in β–cell function [227]. Additionally autonomic dysfunction, a common complication of T2DM has been reported to contribute to abnormalities in respiratory function. This in turn could be implicated in the development of symptomatic OSA [228]. The identification and management of people with T2DM and OSA and vise versa is a practice that should become common place within primary and secondary care. The ongoing research into the mechanisms that underlie the association between these two conditions is essential given the obesity epidemic that we are currently facing. We are inevitably going to see the prevalence of both increase as the level of obesity rises world wide.

1.6 Summary and hypotheses

The salient features of MetS, T2DM and SDB namely central obesity, insulin resistance, increased CVD risk and the key role that sub-clinical inflammation is seemingly playing in

the pathogenesis of these conditions, suggests that they are in some way interrelated. When we also consider the impact that sleep curtailment and poor sleep quality have on normal functioning of the metabolic and endocrine systems, in addition to the fact that these three conditions are related to one another independently of obesity, then it is apparent that sleep deprivation is a plausible candidate that links them via its negative effect on the inflammatory and the acute phase reaction. We have designed and undertaken two studies to further investigate this possible link.

'The sleep and inflammation study' is a sub-study of the screening arm of the ADDITION study (discussed in Chapter 3) in which subjective measures of sleep quality, daytime sleepiness and SDB were used to assess these parameters of sleep across a spectrum of glucose tolerance. Fasting blood samples were collected for the analysis of known biological markers of inflammation and oxidative stress, which were also assessed across this spectrum of glucose tolerance and parameters of sleep. Having access to the anthropometric data collected in the main body of the screening arm of the ADDITION study, we were additionally able to investigate the association of MetS with inflammation and glucose intolerance in the context of sleep quality.

A systematic review was conducted to collate the existing evidence and determine what impact CPAP therapy has on glycaemic control. A broad literature search was undertaken and the literature systematically reviewed. Unfortunately, due to large study heterogeneity a meta-analysis could not be undertaken. After identifying the limitations of the studies

reviewed, we designed a pilot study with a more robust study design to investigate the effects of sleep restoration on glycaemic control and inflammation in persons with established T2DM and newly diagnosed OSA – The Leicester Sleep and Sugar Study. This study included a control group and we were able to measure CPAP compliance objectively. Three measures of glycaemic control were measured: HbA1c, fasting glucose and 72 hour continuous blood glucose monitoring. This study also included a longer follow-up period than has previously been reported.

Hypotheses:

The Sleep and Inflammation Study

- The prevalence of sleep disorders increases with increase glucose intolerance i.e. highest incidence in T2DM & lowest in normo-glycaemic persons
- That glycaemic control decreases as sleep quality decreases
- The level of sub-clinical inflammation increases with decreased sleep quality and increased sleep disturbance
- South Asian (SA) population display a greater level of inflammation in the normo-glycaemic and prediabetic states
- That prevalence of sleep disorders is greater in the SA population

The Leicester Sleep and Sugar Study

- That glycaemic control improves with improved sleep quality (post CPAP therapy)

- Adipokines and acute phase reactants are positively correlated with the severity of OSA thus increasing sleep deprivation
- CPAP compliance and thus sleep restoration results in a decrease in the levels of these factors.

Chapter Two

The Sleep and Inflammation Study

2.1 Introduction

Sleep curtailment studies in humans have reported increased circulating levels of biological markers of inflammation in the sleep deprived state, with these levels returning to baseline once the sleep debt has been repaid [229]. This suggests that chronic sleep deprivation could be a mechanism which causes the chronic activation of the innate immune response thus giving rise to a state of systemic low grade inflammation. Additionally, similar sleep curtailment studies have reported changes in glycaemic control in the sleep deprived state which again return to baseline levels once the sleep debt has been repaid [11]. Together these studies implicate poor sleep in the pathogenesis of insulin resistant states. In the previous chapter I discussed evidence for an association between OSA, MetS and T2DM. The aim of this study was to further explore these observations and possible relationships. I will describe the study design, final study population, our findings and then discuss these results with respect to the current literature.

2.2 Study Design

The 'sleep and inflammation study' is exploratory by design. It is a sub-study of the screening arm of the Anglo-Danish-Dutch study of Intensive Treatment In peOple with screeN detected diabetes in primary care (ADDITION) study. The ADDITION study is a Europe-wide screening and treatment programme for type 2 diabetes [230]. An extensive assessment, including an oral glucose tolerance test (OGTT), was performed on all participants of the screening arm of the ADDITION study, in addition to the collection of biomedical data. Participants consenting to this sub-study were asked to complete three validated sleep questionnaires and to provide extra fasting blood samples and a urine sample for the analysis of biological markers of inflammation.

The aim of this study was to investigate the level of inflammation across a spectrum of sleep quality and glucose tolerance in a multi-ethnic population. Access to the ADDITION database providing anthropometric and blood chemistry measures permitted the investigation of the possible relationship between sleep and the MetS.

2.2.1 Aims and Hypotheses

Aims:

1. Determine the prevalence of sleep disturbance in a population based multi-ethnic sample of adults aged between 40-75 years
2. Determine if cardiovascular markers are higher in people with sleep disturbances (as classified by the Epworth Sleepiness Scale and sleep assessment questionnaires)
3. Determine if markers of inflammation are raised in people with sleep disturbances
4. Determine if markers of inflammation are raised in people with SDB
5. Determine if markers of inflammation are elevated in South Asians compared to Caucasians with SDB
6. Determine if cardiovascular markers are elevated in people with SDB and if SDB is independently associated with MetS
7. Determine if cardiovascular markers are significantly elevated in South Asians compared to Caucasians with SDB
8. Determine if inflammation is associated with SDB independently of glucose tolerance

9. Determine if each sleep questionnaire can simultaneously discriminate between those with a sleep abnormality and either one of the following conditions:
    - Glucose intolerance
    - The MetS
    - A Framingham cardiovascular risk score >20%

Hypotheses**;**

- The prevalence of sleep disorders/poor sleep quality will be greater in the South Asian versus the Caucasian population
- The prevalence of sleep disorders/poor sleep quality will be greater in those with the MetS
- The level of inflammatory markers will be higher in those with sleep disorders/poor sleep quality
- The level of inflammatory markers will be higher in those who are glucose intolerant
- The level of inflammatory markers will be higher in South Asians with sleep disorders/poor sleep quality compared to Caucasians with sleep disorders/poor sleep quality
- The prevalence of glucose intolerance will be higher in those with sleep disorders/poor sleep quality

2.2.2 Subjects

Subjects aged between 40-75 yrs who participated in the screening arm of the ADDITION study and consented to this sub-study were included. Participants who provided fasting blood samples were matched by age, gender, ethnicity and BMI prior to quantification of biological markers of inflammation.

2.2.3 Ethics and ethical considerations

Approval for this sub-study was granted by the Leicestershire, Northamptonshire and Rutland Research Ethics Committee. Ethical considerations for this sub-study included providing interpreters for non-English speakers or people with low literacy skills. One aspect of this study was a comparison of specific variables between two ethnic groups namely South Asians and Caucasians. However, subjects of 'other' ethnicity were not prevented from participating in this study although their data were not included in the final analysis.

2.2.4 Power calculation

We calculated that 800 samples were required to detect a significant difference ($p<0.05$) in levels of inflammatory biomarkers (IL-6 and adiponectin) between a glucose tolerant and a glucose intolerant group (Appendix II). The main comparisons of interest from this study are between glucose intolerance and normoglycaemia and between South Asians and Caucasians. Therefore, the samples were matched for glycamic status, ethnicity in addition to age and BMI with the view

to obtain groups with similar characteristics. The planned matched sample breakdown is shown in Figure 12.

2.2.5 Sample / data collection

Patients participating in the ADDITION study provided informed written consent to give an extra two fasting venous blood samples (15 mls) using the Monovette system and a urine sample for the purpose of this research.

A 9 ml sample was collected in a labelled serum separator (SST) tube. A 6 ml sample was collected in a labelled lithium Heparin tube. The serum tube was inverted gently 5 times and left to stand upright at room temperature for between 30-60 minutes in order for clotting to take place. The tube was then centrifuged at 4000 rpm at 4°C for 10 minutes. The supernatant was then aliquoted in 0.5 ml amounts into four 2 ml labelled Sarstedt tubes with brown caps. These serum samples were then stored at -80°C until clinical analysis was undertaken. The lithium heparin tube was inverted gently 5 times and centrifuged within 30 minutes of collection at 3000 rpm at 4°C for 10 minutes. The supernatant was then aliquotted in 0.5 ml amounts into two 0.5 ml labelled Sarstedt tubes with yellow caps. These plasma samples were then stored at -80°C until clinical analysis was undertaken. A urine sample was also provided and aliquotted in a 5 ml Sarstedt sterile tube labelled and stored at -20°C until clinical analysis was undertaken.

All venous blood samples collected as part of the ADDITION study were analysed in the pathology laboratories at the Leicester Royal Infirmary. All clinical analysis of the additional serum, plasma and urine samples that were collected in this sub-study were conducted at the Unilever Corporate Research Laboratory, Colworth Science Park, Bedford by myself and Duncan Talbot of Unilever.

Quantitative analysis of biological markers of inflammation

Quantitation of high density lipoprotein (HDL) cholesterol was performed using the Ultra HDL assay (UHDL) and serum cholesterol using the cholesterol enzymatic assay. Serum triglyceride was measured using the Triglyceride Glycerol Phosphate Oxidase assay. All of the above are products of Abbott Clinical Chemistry and each assay was performed on the ARCHITECH c Systems ^{TM} / AEROSET systems. Quantitation of serum glycohemoglobin (HbA1c) was performed using High Performance Liquid Chromatography (HPLC) on the automated glycohaemoglobin HLC-723G analyzer (Tosoh Bioscience Ltd, UK) and plasma glucose was measured using the hexokinase method. These assays were undertaken in the pathology laboratories within University Hospitals Leicester and repeat testing carried out if the coefficient of variance was $\geq$ 20%. Quantitative analysis of serum for human CRP, ApoA1 and ApoB was carried out on the ABX Pentra clinical chemistry analyser using a latex-enhanced immunoturbidimetric assay. Quantitative analysis of serum for human TNF-α and IL-6 was carried out using the Quantikine High Sensitivity ELISA for human TNF-α /TNFSF1A kit and for human IL-6 kit, respectively. These products are manufactured by R&D Systems. This assay

uses an amplified, quantitative sandwich enzyme immunoassay technique and was performed according to the manufacturer's instructions. The quantitative analysis of serum for leptin and resistin was carried out using the Mediagnost Enyme-Immunoassay ELISA kit for human leptin and human Resistin, respectively. This assay also uses the quantitative sandwich enzyme immunoassay technique with two monoclonal antibodies. The quantitative analysis of serum for human adiponectin was undertaken using the AutoDELFIA 1235 automatic immunoassay system. This assay is based on the binding assay method, although a fluorescent (Eu^{3+}) endpoint is used rather a colourimetric signal. The quantitative analysis of serum for insulin was carried out using the AutoDelfia time-resolved fluoroimmunoassay for insulin, a commercial product of Perkin Elmer. This assay is based on the direct sandwich technique, again with a fluorometric endpoint, and under taken on the PerkinElmer AutoDELFIA 1235 automatic immunoassay system. The quantitative analysis of plasma for human 8-Iso Prostaglandin $F_{2\alpha}$ (8-Iso-PG $F_{2\alpha}$) was undertaken using an in-house assay on the AutoDELFIA 1235 automatic immunoassay system. The assay is a competitive fluorescent immunoassay.

Sleep questionnaires, demographic and biomedical data

Each participant also completed three validated sleep questionnaires. This included the Sleep Assessment Questionnaire (SAQ), the Berlin Sleep Questionnaire (BSQ) and the Epworth Sleepiness Scale Questionnaire (ESS) see Section 1.1.2 for descriptions and Appendix I for a copies of questionnaires. The questionnaires were labelled with the same unique ID number as used for the corresponding stored serum and plasma samples. The ESS and BSQ questionnaires

were sent to the company ABACUS, Luton for data entry. The data were double entered and the ESS scored by blinded persons and sent electronically to Leicester. The BSQ data were sent to Unilever where a scoring program had been written by Joanne Dick (Unilever). The SAQ data was entered via a web-base program designed by "The Sleep Disorders Clinic of The Centre for Sleep and Chronobiology", Toronto, Canada. This data was then sent back to Leicester electronically.

The demographic and biomedical data from the ADDITION study were entered by an independent data clerk. The data included waist circumference, hip measurement, percentage body fat, height, weight, diastolic and systolic blood pressure (average of two readings), total cholesterol, LDL-C levels, HDL-C levels, triglyceride levels, fasting blood glucose levels and post-challenge glucose levels. All measures were conducted using standard operating procedures. The four data sheets (i.e. 3 sleep questionnaire results and the ADDITION data) were then amalgamated in Excel and imported into SPSS version 14 with the clinical analysis results for statistical analysis. Each questionnaire was doubled entered as was the data entered in the ADDITION database. The reliability of the final data base was assessed by double entering a randomly selected proportion (10%) of each data set. All datasets were password protected.

2.2.6 Statistical analysis

All statistical analyses were conducted using SPSS for Windows Version 14 (SPSS; Chicago; IL). Unless otherwise stated, data are either presented as mean ± SD, or in the case of categorical

data, as number and percentage in each category. Independent T-test, χ^2 and Fisher's Exact Tests were performed for comparisons between groups. One way analysis of covariance (ANCOVA) was used to determine differences in inflammatory and cardiovascular markers between the stated groups. For each model in these analyses the mean and 95% confidence intervals are presented. Receiver operating curves (ROC) were performed to determine the sensitivity and specificity of each sleep questionnaire's ability in predicting three conditions (glucose intolerance, the metabolic syndrome and >20% CVD risk). The area under the curve is presented.

2.3 Final study population

A total of 1529 fasting blood samples were collected for this study. An algorithm was used to match each person with diabetes and prediabetes to a normal participant. The matching was stratified by age and BMI for each of the following four groups: Caucasian males, Caucasian females, South Asian males and South Asian females. The initial goal was to obtain equal samples sizes for South Asian and Caucasians with respect to glycaemic status and gender from the samples collected. The study was powered for a total sample size of 800 participants. Figure 12 shows the breakdown of this matching. Each sleep questionnaire was expected to accompany each fasting blood sample (obtained for purpose of profiling markers of inflammation) that was collected. However, a number of those not consenting to the fasting blood samples completed the sleep questionnaires and a number of those consenting to fasting blood samples did not complete the sleep questionnaires. The data collected at the end of this study period is summarised in Figures 13 and 14. Subsequently the final analysis was split into four main groups (Table 17)

- Questionnaire data plus ADDITION data

- Questionnaire data and ADDITION data plus inflammatory profiles
- Questionnaire data plus inflammatory profiles
- Inflammatory profiles and ADDITION data

the flow diagram in Figure 14 details the questionnaire data and fasting bloods that were collected at the end of the study period.

Core Data	**Questionnaire Data**	**ADDITION Data**	**Inflammatory Profiling**
Age	Berlin Sleep Q'aire	Systolic BP	IL-6
Gender	Epworth Sleepiness Scale	Diastolic BP	TNF-α
Ethnicity	Sleep Assessment Q'aire	Total cholesterol	CRP
BMI		LDL-C	Leptin
		HDL-C	Resistin
		Triglycerides	Adiponectin
		HbA1c	Isoprostane (PGF)
		Fasting glucose	Insulin
		120 min glucose	
		Waist circumference	
		Smoking status	
		Medication	

Table 17: Data type collected for sleep and inflammation study

Inflammatory profiling

Due to a combination of low level consent and a low prevalence of prediabetes and T2DM, a total of 576 blood samples were matched for the profiling of inflammatory biomarkers (Figure 13). The descriptives of this cohort are presented in Table 18.

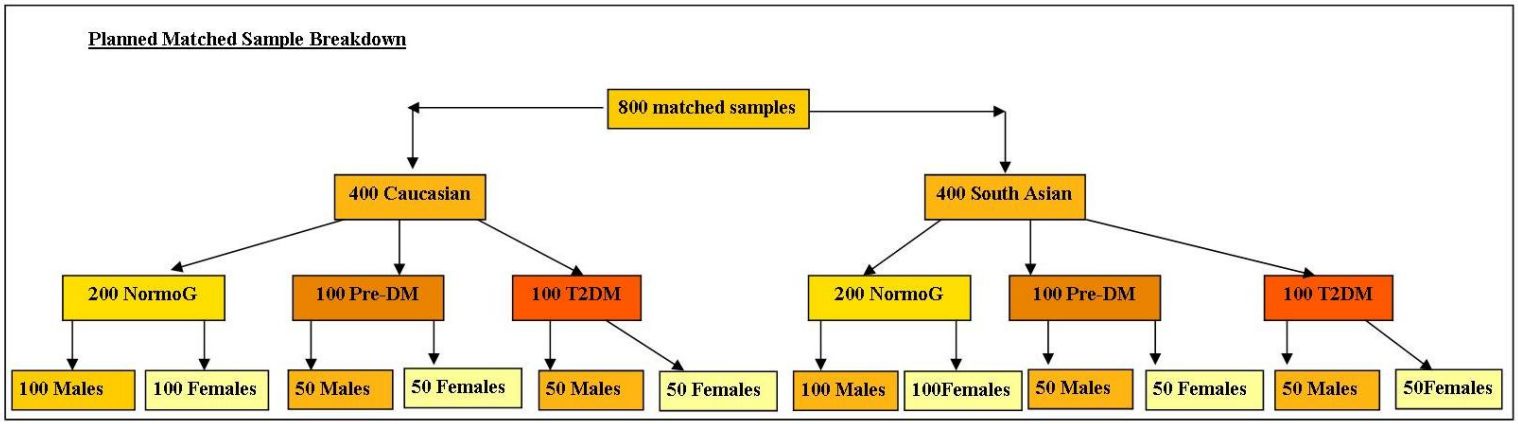


Figure 12: Planned matched sample breakdown for inflammatory profiling

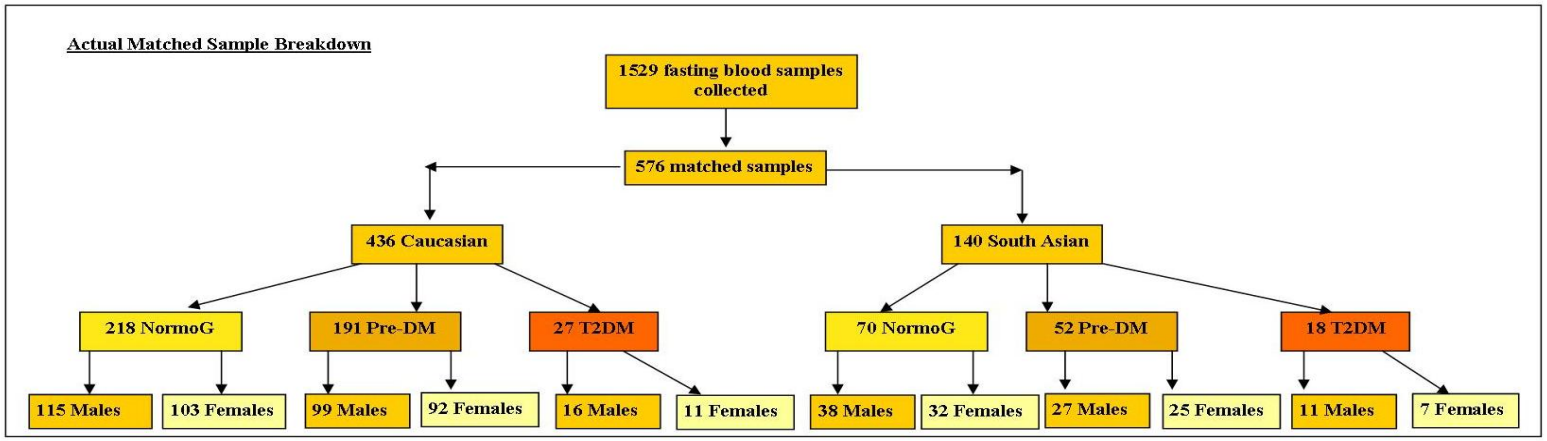


Figure 13: Actual matched sample breakdown for inflammatory profiling

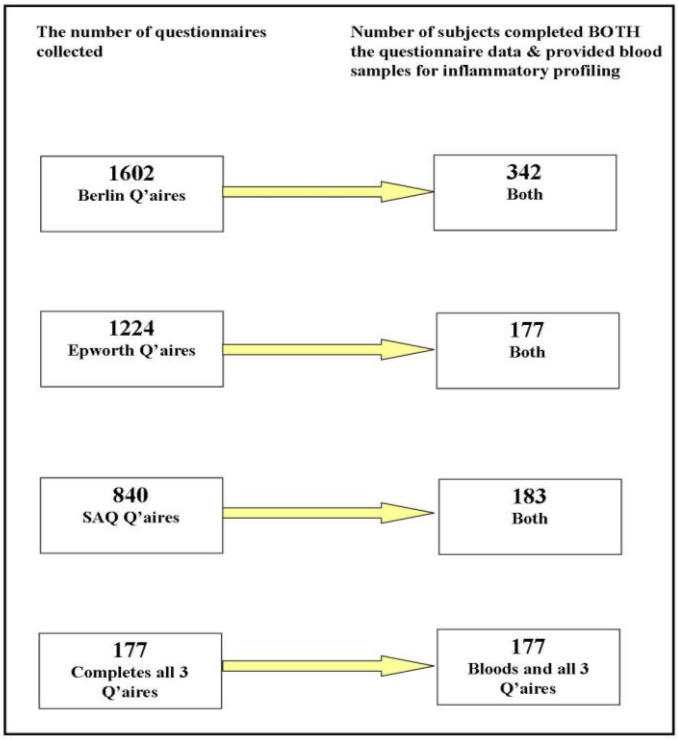


Figure 14: Questionnaire and blood samples collected for inflammatory profiling

2.4 Results

2.4.1 Matched sample characteristics for inflammatory profiling

Table 18 displays the characteristics for those participants that provided fasting blood samples for the analysis of biological markers of inflammation. The Caucasian group have been compared with the South Asian group for age, gender and waist circumference.

	Caucasian (n=435)	South Asian (n=140)	p
Age*	63.64 ± 8.22	57.08 ± 8.88	**<0.0001**
Gender (male)†	229 (52.6%)	76 (54.3%)	0.81
Waist Circumference*	98.66 ± 14.57	96.01 ± 12.37	**0.038**

Table 18: Ethnic comparison of subject characteristics for inflammatory profiling

Mean ± SD reported unless otherwise stated. * t-test, † χ^2 test

2.4.2 The prevalence of sleep complaints as classified by the three questionnaires

The prevalence of those at high risk of SDB was 28.8%, with a significantly higher proportion of males scoring within the high risk category. There was a significant difference in waist circumference between those who scored at high risk compared to those who score low risk of SDB (102.10 ± 11.89 vs. 91.23 ± 12.34, p<0.0001). The prevalence of EDS was 17.4% with no significant difference in age, gender, ethnicity or waist circumference between those with EDS and those without. The prevalence of sleep disturbances as scored by the SAQ was 58.5% again with no significant differences in sample characteristics between those with a sleep disturbance and those without. One hundred and seventy-seven subjects completed all three sleep questionnaires and Figure 15 shows which subjects scored positive/negative for sleep complaints in none, 1, 2 or all three of the questionnaires. The results from these questionnaires are very different per subject in that all three results coincide only 26.5% of the time. In 79 cases (44.6%) only one questionnaire scores positive for sleep disruption the majority of which are classified by the SAQ (63 cases, 79.8%). There were only 10 cases (12.6%) where scores from the BSQ were positive and the other two questionnaires were negative. The ESS was similar where

only 6 cases (7.6%) scored positive where the other two scored negative. This could be a result of the broader scope of the SAQ and therefore it is more likely to identify positive cases for 'sleep disturbance' because it covers a number of sleep disorders compared to the BSQ which is designed only to identify subjects with SDB.

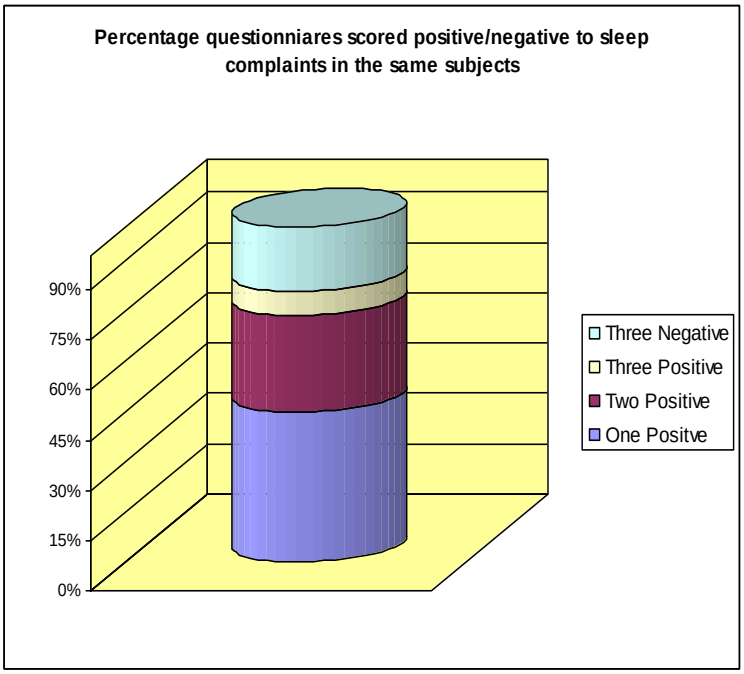


Figure 15: Overview of results from all three sleep questionnaires when they were completed by the same subjects

2.4.3 Cardiovascular marker comparison in people with sleep complaints as classified by the ESS and SAQ

T-tests were performed to investigate if there were any differences in the cardiovascular risk factors between those with excessive daytime sleepiness (EDS) and those without as classified by the ESS (Table 19) and in those with and without sleep disturbance as classified by the SAQ (Table 20).

Cardiovascular Marker	No-EDS (Mean ± SD) n = 1011	EDS (Mean ± SD) n =213	p
Systolic BP (mmHg)	136.28 ± 19.4	135.79 ± 21.21	0.79
Diastolic BP (mmHg)	85.08 ± 10.64	83.92 ± 11.43	0.19
Weight (kg)	77.83 ± 15.11	79.62 ± 16.14	0.07
% Body Fat	33.46 ± 8.72	32.96 ± 8.56	0.22
BMI (kg/m^2)	27.95 ± 4.79	28.43 ± 5.25	0.22
Total cholesterol (mmol/L)	5.57 ± 1.16	5.37 ± 1.02	**0.02**
LDL -C (mmol/L)	3.56 ± 0.91	3.43 ± 0.89	0.73
HDL –C (mmol/L)	1.39 ± 0.47	1.34 ± 0.45	0.19
Triglycerides (mmol/L)	1.44 ± 0.86	1.41 ± 0.81	0.76
HbA1c (%)	5.66 ± 0.46	5.65 ± 0.43	0.92
Fasting Glucose (mmol/L)	5.12 ± 0.54	5.06 ± 0.49	0.18
120min Glucose (mmol/L)	5.64 ± 1.75	5.75 ± 1.81	0.48

Table 19: Cardiovascular marker comparison in those with and without EDS as classified by the ESS. T-test performed results displayed as mean ± SD

There were no significant differences in the levels of the cardiovascular markers between those with EDS and those without in the complete cohort (n= 1224) except for total cholesterol levels (Table 19). Those with EDS had significantly lower levels of total cholesterol however this did not remain significant after adjusting for age, gender and ethnicity.

Cardiovascular Marker	No-Sleep Disturbance (Mean ± SD) n= 349	Sleep Disturbance (Mean ± SD) n =491	p
Systolic BP (mmHg)	135.88 ± 18.63	134.84 ± 18.9	0.47
Diastolic BP (mmHg)	84.80 ± 9.95	84.34 ± 10.37	0.55
Weight (kg)	78.15 ± 15.16	78.62 ± 15.13	0.66
% Body Fat	32.85 ± 7.97	33.39 ± 8.83	0.36
BMI (kg/m^2)	27.85 ± 4.81	28.24 ± 4.87	0.26
Total cholesterol (mmol/L)	5.49 ± 1.03	5.54 ± 1.07	0.47
LDL -C (mmol/L)	3.54 ± 0.86	3.55 ± 0.91	0.94
HDL -C (mmol/L)	1.36 ± 0.46	1.38 ± 0.46	0.65
Triglycerides (mmol/L)	1.35 ± 0.70	1.47 ± 0.99	0.06
HbA1c (%)	5.65 ± 0.46	5.64 ± 0.49	0.73
Fasting Glucose (mmol/L)	5.12 ± 0.58	5.11 ± 0.64	0.85
120min Glucose (mmol/L)	5.61 ± 1.81	5.61 ± 1.82	0.95

Table 20: Cardiovascular marker comparison in those with and without sleep disturbance as classified by the SAQ. T-test performed results displayed as mean ± SD

The levels of cardiovascular markers in those with sleep disturbance compared to those without a sleep disturbance were not significantly different.

2.2.4 Inflammatory profile comparison in people with sleep complaints as classified by the ESS and SAQ

T-tests were performed to investigate whether there were any differences in the levels of inflammatory biomarkers and insulin in those with and without sleep complaints as classified by the ESS (Table 21 and 22) and the SAQ (Table 23 and 24). Inflammatory

biomarkers were tested after transforming to log 10, the anti-log of the means and standard deviations are displayed.

	Non-EDS (n=145)	EDS (n=32)	p
Age*	63.06 ± 8.14	62.13 ± 8.15	0.56
Gender (male)[†]	67 (46.2%)	18 (56.3%)	0.40
Ethnicity (Caucasian) [‡]	127 (87.6%)	29 (90.6%)	0.77
Waist Circumference*	98.67± 12.52	99.10 ± 12.49	0.86

Table 21: ESS inflammatory profile cohort characteristics . Mean ± SD reported unless otherwise stated. t-test, [†] χ^2 test [‡] Fishers Exact Test

Inflammatory Biomarker	No-EDS (Mean ± SD)	EDS (Mean ± SD)	p
TNF-α log10	1.73 ± 1.70	1.44 ± 1.56	0.56
IL-6 log10	1.99 ± 1.99	1.59 ± 2.24	0.34
Leptin log10	17.15 ± 2.43	14.42 ± 2.29	0.30
Resistin log10	4.73 ± 1.53	4.66 ± 1.26	0.87
Adiponectin log10	19.39 ± 1.79	16.67 ± 1.72	0.17
CRP log10	1.91 ± 4.06	1.59 ± 4.66	0.55
PGF log 10	2.55 ± 3.01	3.15 ± 3.36	0.38
Insulin log10	7.20 ± 1.79	6.68 ± 3.24	0.73

Table 22: Inflammatory profile comparison of those with and without EDS as classified by ESS. T-test performed on the log10 of inflammatory biomarkers, the results displayed are the anti-logged mean ± SD

	No-Sleep Disturbance (n=72)	Sleep Disturbance (n=111)	p
Age*	60.58 ± 8.96	60.26 ± 9.43	0.32
Gender (male)[†]	41 (56.9%)	65 (58.6%)	0.95
Ethnicity (Caucasian) [†]	57 (79.2%)	94 (84.7%)	0.45
Waist Circumference*	98.13 ± 12.01	98.17 ± 10.56	0.98

Table 23: SAQ inflammatory profile cohort characteristics. Mean ± SD reported unless otherwise stated. * t-test, [†] χ^2 test

Inflammatory Biomarker	No-Sleep Disturbance (Mean ± SD)	Sleep Disturbance (Mean ± SD)	p
TNF-α log10	1.85 ± 1.96	1.79 ± 1.76	0.76
IL-6 log10	1.82 ± 2.13	2.06 ± 2.03	0.27
Leptin log10	11.09 ± 2.53	13.58 ± 2.55	0.85
Resistin log10	4.94 ± 1.56	4.96 ± 1.54	0.96
Adiponectin log10	16.95 ± 1.84	16.48 ± 1.71	0.75
CRP log10	1.62 ± 3.59	2.05 ± 3.61	0.24
PGF log 10	2.39 ± 3.36	2.21 ± 2.35	0.61
Insulin log10	7.22 ± 1.79	7.09 ± 1.72	0.84

Table 24: Inflammatory profile comparison of those with and without sleep disturbance as classified by the SAQ. T-test performed on the log10 of inflammatory biomarkers, the results displayed are the anti-logged mean ± SD

There were no significant differences in the levels of biological markers of inflammation between those with EDS or a sleep disturbance compared to those without EDS or a sleep disturbance.

2.4.5 Inflammatory profile comparison in people with and without SDB as classified by the BSQ

The characteristics of those who consented to fasting bloods and completed the Berlin Sleep Questionnaire (BSQ), n=342 are given in Table 25. Table 26 displays the use of medication between those with SDB compared to those without and is reported as the frequencies for the use of medication. One way analysis of covariance (ANCOVA) was used to determine if there were any differences in the levels of biological markers of inflammation between those with SDB compared to those without SDB. The unadjusted

and adjusted results are reported in Table 27. All biomarkers are continuous log transformed variables. Reported estimated means and 95% confidence intervals are the back-transformed values.

	Non-SDB n = 215 (62.9%)	**SDB n = 127 (37.1%)**	***p***
Age*	62.10 ± 9.17	61.08 ± 8.07	0.28
Male Gender†	106(49.3%)	73 (57.8%)	0.18
Waist Circumference*	94.65 ± 12.17	104.06 ± 10.89	**<0.0001**

Table 25: BSQ inflammatory profile cohort characteristics. * t-test, † χ^2 test

Type of Medication	**Non-SDB**	**SDB**	**p**
Ace Inhibitors†	10 (5.2%)	20 (17.2%)	**0.001**
α-Blockers†	4 (2.1%)	3 (2.6%)	1.00
Angiotensin-II Receptor antagonist†	3 (1.6%)	9 (7.8%)	**0.01**
β-Blocker*	17 (8.9%)	19 (16.4%)	0.07
Calcium Channel Blockers*	15 (7.8%)	16 (13.8%)	0.14
Diuretics/Thiazides*	21 (10.9%)	19 (16.4%)	0.23
Aspirin*	26 (13.5%)	20 (17.2%)	0.47
Lipid Lowering Statin*	25 (13.0%)	22 (19.0%)	0.21
Lipid Lowering Fibrate†	0 (0.0%)	1 (0.9%)	0.38
Steroids†	1 (0.5%)	4 (3.4%)	0.07
Thyroid/Anti-Thyroid*	10 (5.2%)	13 (11.2%)	0.09
Current smokers	24 (11.2%)	19 (15.0%)	0.40

Table 26: BSQ cohort use of medication and smoking . † Fishers Exact Test, * χ^2 test

The levels of IL-6, leptin and CRP were found be significantly higher in those who scored at high risk of SDB independent of age, gender, ethnicity, smoking status and use of medication. Additionally levels of adiponectin were found to be significantly lower in those at high risk of SDB after adjustment for the same covariates. However, the association between low-grade inflammation and high risk of SDB was lost after further adjustment for waist circumference (Table 27).

One-way analysis of covariance (ANCOVA).

Biomarker	Model 1			Model 2			Model 3			Model 4		
	Non-SDB Mean (95% CI)†	**SDB** Mean (95% CI) †	*p*	**Non-SDB** Mean (95%CI)†	**SDB** Mean (95% CI) †	*p*	**Non-SDB** Mean (95% CI)†	**SDB** Mean (95% CI) †	*p*	**Non-SDB** Mean (95% CI)†	**SDB** Mean (95% CI) †	*p*
TNF-α (pg/ml)	1.70 (1.58,1.84)	1.78 (1.59,1.96)	0.49	1.70 (1.58,1.83)	1.79 (1.62,1.97)	0.44	1.66 (1.53,1.79)	1.81 (1.63,1.99)	0.22	1.63 (1.50,1.77)	1.85 (1.66,2.06)	0.08
IL-6 (pg/ml)	1.79 (1.62,1.96)	2.03 (1.79,2.31)	0.10	1.77 (1.61,1.94)	2.05 (1.82,2.32)	0.06	1.75 (1.6,1.93)	2.09 (1.8,2.4)	**0.04**	1.80 (1.62,1.99)	1.99 (1.74,2.27)	0.28
Leptin (mg/ml)	13.52 (11.99,15.28)	18.53 (15.80,21.67)	**0.002**	13.06 (11.94,14.29)	19.72 (17.54,22.13)	**<0.0001**	13.18 (12.1,14.5)	19.19 (16.9,21.7)	**<0.0001**	14.52 (13.4,15.4)	15.81 (14.2,17.6)	0.24
Resistin (ng/ml)	4.79 (4.52, 5.07)	5.01 (4.65, 5.41)	0.35	4.81 (4.52, 5.08)	5.00 (4.63,5.39)	0.38	4.79 (4.49,5.10)	5.12 (4.71,5.55)	0.23	4.76 (4.49,5.07)	4.98 (4.59,5.39)	0.42
Adiponectin (µg/ml)	19.28 (17.82, 2.08)	16.37 (14.79,18.16)	**0.01**	18.88 (17.66,20.18)	16.94 (15.52,18.49)	**0.055**	19.77 (18.4,21.2)	16.98 (15.5,18.6)	**0.013**	19.41 (18.0,20.9)	17.26 (15.7,19.0)	0.07
CRP (mg/ml)	1.44 (1.20, 1.72)	2.39 (1.89, 3.01)	**0.001**	1.42 (1.19, 1.69)	2.44 (1.94, 3.07)	**<0.0001**	1.44 (1.19,1.73)	2.54 (1.99,3.24)	**<0.0001**	1.59 (1.32,1.92)	2.16 (1.69,2.77)	0.54
PGF (ng/ml)	2.28 (2.11, 2.51)	2.21 (1.97, 2.48)	0.67	2.28 (2.11, 2.51)	2.21 (1.97, 2.48)	0.67	2.27 (2.07,2.49)	2.17 (1.92,2.44)	0.54	2.23 (2.03,2.46)	2.22 (1.95,2.52)	0.93

Table 27: Inflammatory profile comparison of those with and without SDB as classified by the BSQ.

Model 1 = Unadjusted,
Model 2 = Adjusted for age, gender and ethnicity,
Model 3 = Adjusted for age, gender, ethnicity, smoking status and medication,
Model 4 = Adjusted for age, gender, ethnicity, smoking status, medication and waist circumference

2.4.6 An ethnic comparison of inflammatory profiles in those classified with SDB by the BSQ

Subjects who were classified with SDB by the BSQ were categorised according to their ethnic origin and a comparison between South Asians and Caucasians made. Table 28 displays the characteristics of this cohort (n = 127). There were again no significant differences in the use of medication or prevalence of smoking in this subset (Appendix III). One way analysis of covariance (ANCOVA) was used to determine if there were any differences in the levels of biological markers of inflammation between South Asians and Caucasians the unadjusted and adjusted results are reported in Table 29. All biomarkers are continuous log-transformed variables. Reported estimated means and 95% confidence intervals are the back-transformed values.

	Caucasian n=104 (82%)	**South Asian n=23 (18%)**	***p***
Age*	61.84 ± 7.59	57.65 ± 9.41	0.56
Male Gender†	61 (59.6%)	12 (52.2%)	0.74
Waist Circumference*	104.15 ± 11.21	103.62 ± 9.39	0.84

Table 28: Ethnic comparison of those classified with SDB by the BSQ characteristics.
* t-test, † χ^2 test

Levels of TNF-α were significantly higher in the South Asian SDB group independent of age and gender however this association was lost after further adjustment for smoking status and use of medication. Levels of leptin were observed to be higher in the South Asian SDB group however this association only approached statistical significance. However, the level of Isoprostanes were found to be significantly higher in the Caucasian SDB group independent of age, gender, smoking status, use of medication and waist circumference.

One-way analyses of covariance (ANCOVA)

Biomarker	Model 1			Model 2			Model 3			Model 4		
	Caucasian Mean (95% CI)†	**South Asian** Mean (95% CI) †	*p*	**Caucasian** Mean (95% CI)†	**South Asian** Mean (95% CI) †	*p*	**Caucasian** Mean (95% CI)†	**SouthAsian** Mean (95% CI) †	*p*	**Caucasian** Mean (95% CI)†	**South Asian** Mean (95% CI) †	*p*
TNF-α (pg/ml)	1.68 (1.51,1.90)	2.24 (1.78,2.88)	**0.036**	1.69 (1.51, 1.90)	2.24 (1.75, 2.88)	**0.045**	1.67 (1.51,1.89)	2.15 (1.69,2.72)	0.08	1.69 (1.51,1.88)	2.19 (1.71,2.81)	0.06
IL-6 (pg/ml)	1.97 (1.74,2.24)	2.32 (1.79,2.98)	0.264	1.99 (1.76, 2.24)	2.25 (1.74, 2.92)	0.383	2.02 (1.79,2.28)	2.25 (1.74,2.91	0.47	2.02 (1.81,2.28)	2.31 (1.77,2.99)	0.38
Leptin (mg/ml)	17.34 (14.5,20.8)	24.89 (17.1,36.4)	0.090	17.62 (15.6,19.9)	23.23 (17.9,30.1)	0.062	17.91 (15.7,20.4)	22.54 (17.1,29.9)	0.15	17.70 (15.9,19.6)	22.80 (18.1,28.8)	**0.055**
Resistin (ng/ml)	4.86 (4.45,5.32)	5.75 (4.75,6.97)	0.118	4.84 (4.42, 5.29)	5.87 (4.83, 7.14)	0.079	5.05 (4.58,5.57)	5.69 (4.63,6.98)	0.31	5.02 (4.61,5.47)	5.16 (4.28,6.25)	0.79
Adiponectin (µg/ml)	16.67 (14.0,18.5)	15.17 (12.1,19.0)	0.460	16.52 (15.0,18.2)	15.77 (12.9, 9.3)	0.683	16.90 (15.3,18.6)	15.63 (12.8,19.1)	0.51	16.79 (15.3,18.4)	15.88 (12.9,19.5)	0.64
CRP (mg/ml)	2.45 (1.96,3.08)	2.11 (1.31,3.41)	0.579	2.50 (2.00, 3.12)	1.94 (1.20, 3.12)	0.342	2.59 (2.04,3.29)	2.17 (1.31,3.59)	0.54	2.61 (2.07,3.27)	2.06 (1.23,3.43)	0.42
PGF (ng/ml)	2.35 (2.08,2.66)	1.55 (1.19,2.01)	**0.005**	2.33 (2.06, 2.64)	1.61 (1.24, 2.09)	**0.013**	2.29 (2.03,2.60)	1.55 (1.19,2.02)	**0.011**	2.30 (2.03,2.61)	1.45 (1.10,1.91)	**0.004**

Table 29: Ethnic comparison of inflammatory profiles in those classified with SDB by the BSQ

Model 1 = Unadjusted,
Model 2 = Adjusted for age, gender,
Model 3 = Adjusted for age, gender, smoking status and medication,
Model 4 = Adjusted for age, gender, smoking status, medication and waist circumference.

2.4.7 Cardiovascular marker comparison between those with SDB and those without and association with MetS

An independent t-test was performed to determine if cardiovascular makers are higher those with SDB compared to those without in the cohort who completed the BSQ (n=1604).

Variable	Non-SDB n = 1140 (71.2%)	SDB n = 462 (28.8%)	p
Systolic BP (mmHg)	135.61±61	137±16	NS
Diastolic BP (mmHg)	84.1±10.57	87.2±13	**p<0.0001**
Weight (kg)	73.99±13.8	87.2±15.6	**p<0.0001**
Waist Circumference (cm)	91.1±12.3	102.04±11.97	**p<0.0001**
Body fat (%)	32.15±8.27	36.1±8.9	**p<0.0001**
BMI (kg/m^{2})	26.7±4.33	31.14±4.97	**p<0.0001**
HDL-C (mmol/l)	1.46±0.48	1.31±0.44	**p<0.0001**
Triglycerides (mmol/l)	1.31± 0.74	1.66± 1.0	**p<0.0001**
HbA1c (%)	5.6±0.45	5.72±0.49	**p<0.0001**
Fasting Glucose (mmol/l)	5.1±0.53	5.3±0.69	**p<0.0001**
2 hour Glucose (mmol/l)	5.69±1.8	6.23±2.1	**p<0.0001**

Table 30: Measures of cardiovascular markers between those with and without SDB classified by the BSQ. Results displayed as mean± SD

Following this analysis we wanted to determine if the metabolic syndrome was independently associated with SDB, given that the MetS is comprised of a cluster of cardiovascular risk factors. The MetS was classified by the NCEP-ATP III guidelines [74].

Overall 164 (10.2%) subjects were excluded due to missing data required for determining of MetS using this criterion.

Age, gender and ethnicity did not significantly differ between those included in analyses and those that were not. However, those not included in analyses had higher waist circumference (98 ± 12.3 cm vs. 93.8 ± 13 cm, p<0.001 respectively) and had a higher prevalence of SDB (43.6% vs. 27.3%, p<0.001). There was no significant difference in the prevalence of SDB between the South Asian and Caucasian people not included in these analyses (50% and 42% respectively, p=0.28). Table 31 displays the characteristics of the 1438 participants that were included in the final analyses.

	No-MetS n=1165 (81%)	**MetS** n=273 (19%)	**p**
Age*	58.18 ± 10.67	59.31 ± 9.98	0.97
Gender (male)†	531 (45.3%)	139 (50.2%)	0.17
Ethnicity (Caucasian)†	957 (81.4%)	223 (80.5%)	0.81
Waist circumference*	91.34 ± 11.99	104.91 ± 12.08	**<0.0001**
SDB†	155 (13.3%)	94 (34.5%)	**<0.001**

Table 31: The MetS and SDB sample characteristics. *t-test, †χ^2 test. Results displayed as mean ± SD and percentages

Logistic regression was performed to determine whether SDB was independently associated with the MetS (Table 32). The presence of hypertension was determined using the thresholds described in the NCEP ATP III criteria [74]. Age, gender, ethnicity,

hypertension and waist circumference were included as covariates as they represent a group of risk factors for both SDB and the MetS.

	OR of MetS	95% CI	p
SDB unadjusted	3.4	2.6 to 4.5	**p<0.0001**
SDB adjusted for age and gender	3.5	2.7 to 4.6	**p<0.0001**
SDB adjusted for age, gender, ethnicity and HTN	3.9	2.5 to 4.6	**p<0.0001**
SDB adjusted for age, gender, ethnicity, HTN and WC	1.7	1.17 to 2.4	**p=0.005**

Table 32: The likelihood of MetS in the presence of SDB. Results from logistic regression displayed as odd ratio (OR) and 95% confidence intervals. HTN= hypertension

In the unadjusted model an individual with SDB was associated with increased odds of MetS (OR 3.4, 95% CI 2.6 to 4.5, p<0.0001). This remained significant after adjusting for age and gender (OR 3.5, 95% CI 2.7 to 4.6, p<0.0001) and ethnicity. The adjustment for hypertension together with age, gender and ethnicity increased the odds of having MetS in those with SDB (OR 3.9, 95% CI 2.5 to 4.6, p<0.0001). Further adjustment for waist circumference attenuated the relationship but the association remained significant (OR 1.7, 95% CI 1.17 to 2.4, p=0.005). Therefore SDB appears to be an independent risk factor for MetS.

2.4.8 Ethnic comparison of cardiovascular markers in those with SDB as classified by BSQ

This data set includes all people classified with SDB (n= 438). Table 33 shows the demographics of this population. There were no significant differences in the use of medication or prevalence of smoking between the two ethnic groups (Appendix III).

	Caucasian n=365 (83.3%)	**South Asian n=73 (16.7%)**	***p***
Age*	59.42 ± 8.59	51.08 ± 10.26	**<0.0001**
Male Gender†	192 (52.6%)	34 (46.6%)	0.42
Waist Circumference*	101.85 ± 12.11	102.93 ± 11.44	0.48

Table 33: Ethnic comparison of sample characteristics in those classified with SDB by the BSQ. * t-test, † χ^2 test

One way analyses of covariance (ANCOVA) was used to determine if there were any differences in cardiovascular (CV) markers between South Asians and Caucasians with SDB after adjusting for age, gender, smoking status, medication and waist circumference (Table 34). Total cholesterol was found to be higher in the Caucasian SDB group independent of age, gender, smoking status and use of medication. However this association was lost after further adjustment for waist circumference. HbA1c and post-challenge glucose levels (120 minutes) were found to be independently higher in the South Asian SDB group suggesting increased glucose intolerance in this ethnic group at high risk of SDB.

One way analysis of covariance (ANCOVA)

CV marker	Model 1			Model 2			Model 3			Model 4		
	Caucasian Mean (95% CI)†	**South Asian** Mean (95% CI) †	*p*	**Caucasian** Mean (95% CI)†	**South Asian** Mean (95% CI) †	*p*	**Caucasian** Mean (95% CI)†	**South Asian** Mean (95% CI) †	*p*	**Caucasian** Mean (95% CI)†	**South Asian** Mean (95% CI) †	*p*
Systolic BP (mmHg)	138.29 (135.9,140.6)	130.82 (125.7,135.9)	**0.009**	137.9 (137.2,139.6)	135.30 (130.2,140.4)	0.47	137.34 (135.0,139.7)	134.67 (129.4,139.9)	0.38	137.65 (135.3,139.7)	134.63 (129.3,139.9)	0.32
Diastolic BP (mmHg)	87.47 (85.9,89.0)	85.12 (81.8,89.2)	0.37	87.68 (86.1,89.2)	85.03 (81.5,88.6)	0.19	87.71 (86.1,89.4)	84.51 (80.8,88.3)	0.14	87.65 (85.9,89.3)	84.63 (80.8,88.4)	0.16
Tot-Chol (mmol/l)	5.53 (5.42,5.65)	5.25 (4.98,5.53)	0.07	5.54 (5.42,5.66)	5.21 (4.92,5.50)	**0.04**	5.55 (5.44,5.67)	5.23 (4.96,5.51)	**0.04**	5.56 (5.44,5.67)	5.28 (5.00,5.56)	0.08
LDL –C (mmol/l)	3.49 (3.39,3.59)	3.32 (3.09,3.56)	0.19	3.50 (3.39,3.60)	3.29 (3.04,3.54)	0.13	3.51 (3.41,3.60)	3.35 (3.11,3.58)	0.23	3.51 (3.41,3.61)	3.38 (3.14,3.62)	0.23
HDL-C (mmol/l)	1.32 (1.28,1.37)	1.21 (1.10,1.31)	0.05	1.32 (1.27,1.36)	1.22 (1.11,1.33)	0.12	1.32 (1.27,1.36)	1.22 (1.11,1.33)	0.13	1.32 (1.27,1.37)	1.23 (1.11,1.34)	0.14
Triglycerides (mmol/l)	1.68 (1.58,1.79)	1.61 (1.37,1.85)	0.58	1.69 (1.59,1.79)	1.59 (1.33,1.85)	0.47	1.68 (1.58,1.79)	1.52 (1.289,1.79)	0.25	1.68 (1.57,1.78)	1.54 (1.27,1.77)	0.35
HbA1c (%)	5.67 (5.62,5.72)	5.96 (5.84,6.08)	**<0.001**	5.66 (5.61,5.71)	6.03 (5.91,6.15)	**<0.001**	5.65 (5.60,5.69)	5.97 (5.86,6.08)	**<0.001**	5.66 (5.61,5.71)	5.94 (5.83,6.06)	**<0.001**
Fasting glucose (mmol/)	5.30 (5.23,5.37)	5.37 (5.19,5.53)	0.48	5.29 (5.22,5.36)	5.44 (5.27,5.62)	0.12	5.26 (5.19,5.33)	5.40 (5.23,5.58)	0.14	5.27 (5.19,5.34)	5.36 (5.19,5.53)	0.34
120 Glucose (mmol/l)	6.20 (5.98,6.42)	6.57 (6.06,7.08)	0.19	6.14 (5.92,6.36)	6.82 (6.29,7.35)	**0.022**	5.99 (5.77,6.20)	6.66 (6.14,7.18)	**0.022**	5.98 (5.77,6.19)	6.57 (6.06,7.09)	**0.04**

Table 34: Ethnic comparison of cardiovascular markers in those classified with SDB by BSQ
Model 1 = Unadjusted,
Model 2 = Adjusted for age, gender ,
Model 3 = Adjusted for age, gender, smoking status and medication,
Model 4 = Adjusted for age, gender, smoking status, medication and Waist circumference

2.4.9 Are markers of inflammation associated with SDB independently of glucose intolerance?

Sample includes all those who consented to fasting bloods and completed the BSQ (n= 342). The cohort was categorised according to whether they were glucose intolerant, which included both prediabetes and T2DM, and those who had normal glucose tolerance.

	Non-SDB n=215 (62.9%)	**SDB n=127 (37.1%)**	**p**
Glucose Intolerance*	86 (40.0%)	52 (40.9%)	0.95

Table 35: The prevalence of glucose intolerance in those with and without SDB. * χ^2 test

One way analysis of covariance (ANCOVA) was used to determine if there were any differences in inflammatory biomarkers between those with SDB and those without after adjusting for age, gender, smoking status, medication and glucose intolerance (Table 36). All biomarkers are continuous log transformed variables. Reported estimated means and 95% confidence intervals are the back-transformed values. Levels of IL-6, leptin and CRP were found to be significantly higher in those who scored high risk for SDB independent of age, gender, ethnicity, smoking status, use of medication and glucose intolerance. Additionally levels of adiponectin were found to be significantly lower in this group after adjustment for the same covariates. The previous association between low-grade inflammation and SDB was lost after further adjustment for waist circumference.

One way analysis of covariance (ANCOVA)

Biomarker	Model 1			Model 2			Model 3			Model 4		
	Non-SDB Mean (95% CI)†	**SDB** Mean (95% CI) †	*p*	**Non-SDB** Mean (95%CI)†	**SDB** Mean (95% CI) †	*p*	**Non-SDB** Mean (95% CI)†	**SDB** Mean (95% CI) †	*p*	**Non-SDB** Mean (95% CI)†	**SDB** Mean (95% CI) †	*p*
TNF-α (pg/ml)	1.702 (1.58,1.84)	1.78 (1.61,1.96)	0.49	1.66 (1.53,1.79)	1.81 (1.63,1.99)	0.22	1.66 (1.53,1.79)	1.81 (1.63,1.99)	0.22	1.63 (1.50,1.77)	1.85 (1.66,2.07)	0.08
IL-6 (pg/ml)	1.79 (1.62,1.96)	2.03 (1.79,2.29)	0.102	1.75 (1.58,1.93)	2.09 (1.84,2.38)	**0.036**	1.75 (1.58,1.93)	2.08 (1.84,2.38)	**0.037**	1.80 (1.63,1.99)	1.99 (1.74,2.28)	0.28
Leptin (mg/ml)	13.52 (11.99,15.28)	18.53 (15.81,21.68)	**0.002**	13.18 (11.97,14.52)	19.19 (16.94,21.73)	**<0.0001**	13.18 (11.99,14.52)	19.19 (16.90,21.73)	**<0.0001**	14.52 (13.39,15.74)	15.81 (14.22,17.58)	0.24
Resistin (ng/ml)	4.79 (4.52,5.07)	5.01 (4.65,5.41)	0.35	4.79 (4.49,5.10)	5.11 (4.71,5.55)	0.23	4.79 (4.49,5.10)	5.11 (4.71,5.56)	0.23	4.76 (4.49,5.07)	4.98 (4.59,5.39)	0.42
Adiponectin (µg/ml)	19.28 (17.82,20.84)	16.37 (14.79,18.16)	**0.014**	19.77 (18.41,21.23)	16.98 (15.49,18.62)	**0.013**	19.77 (18.41,21.18)	16.98 (15.49,18.62)	**0.014**	19.41 (18.03,20.89)	17.26 (15.67,19.01)	0.07
CRP (mg/ml)	1.44 (1.20,1.72)	2.39 (1.89,3.01)	**0.001**	1.44 (1.19,1.73)	2.54 (1.99,3.24)	**<0.0001**	1.44 (1.19,1.74)	2.53 (1.99,3.23)	**<0.0001**	1.59 (1.32,1.92)	2.16 (1.69,2.77)	0.07
PGF (ng/ml)	2.29 (2.09,2.51)	2.18 (1.95,2.45)	0.50	2.28 (2.07,2.49)	2.17 (1.92,2.44)	0.54	2.28 (2.07,2.49)	2.16 (1.92,2.44)	0.51	2.23 (2.03,2.45)	2.22 (1.96,2.51)	0.92

Table 36: Inflammatory profile comparison between those with and those without SDB adjusting for glucose intolerance.
Model 1 = Biomarker and SDB unadjusted,
Model 2 = Biomarker and SDB adjusted for age, gender, ethnicity, smoking status and medication,
Model 3 = Biomarker and SDB adjusted for age, gender, ethnicity, smoking status, medication and glucose intolerance,
Model 4 = Biomarker and SDB adjusted for age, gender, ethnicity, smoking status, medication, glucose intolerance and waist circumference

Following this a one way analysis of covariance was used to determine if inflammation is associated with glucose intolerance independently of age, gender, ethnicity, smoking status use of medication and waist circumference (Table 37). All biomarkers are continuous log transformed variables. The reported estimated means and 95% confidence intervals are the back-transformed values.

Biomarker	**Model 1**			**Model 2**		
	Glucose Tolerant Mean (95% CI)†	**Glucose intolerant** Mean (95% CI)†	*p*	**Glucose Tolerant** Mean (95% CI)†	**Glucose intolerant** Mean (95% CI)†	*p*
TNF-α (pg/ml)	1.73 (1.59,1.86)	1.69 (1.53,1.89)	0.81	1.72 (1.59,1.85)	1.69 (1.23,1.89)	0.87
IL-6 (pg/ml)	1.82 (1.65,1.99)	1.98 (1.73,2.26)	0.309	1.82 (1.66,2.00)	1.97 (1.72,2.24)	0.35
Leptin (mg/ml)	14.72 (13.39,16.18)	16.14 (14.16,18.45)	0.28	14.62 (13.55,15.74)	15.74 (14.19,17.46)	0.26
Resistin (ng/ml)	4.99 (4.69,5.29)	4.75 (4.37,5.18)	0.37	4.91 (4.63,5.19)	4.74 (4.39,5.13)	0.49
Adiponectin (µg/ml)	19.32 (18.03,20.65)	17.46 (15.89,19.23)	0.097	19.19 (17.91,20.51)	17.46 (15.89,19.19)	0.12
CRP (mg/ml)	1.65 (1.38,1.98)	2.08 (1.61,2.68)	0.15	1.67 (1.39,1.99)	2.03 (1.59,2.59)	0.19
PGF (ng/ml)	2.10 (1.93,2.29)	2.51 (2.22,2.84)	**0.024**	2.09 (1.91,2.29)	2.52 (2.23,2.84)	**0.017**

Table 37: Glucose intolerance and inflammatory profiles.
Model 1 = Biomarker and glucose intolerance adjusted for age, gender, ethnicity, smoking status and medication, Model 2 = Biomarker and glucose intolerance adjusted for age, gender, ethnicity, smoking status, medication and waist circumference

Isoprostane levels were found to be significantly higher in the glucose intolerant group compared to the glucose tolerant group. This association between glucose intolerance and inflammation was independent of age, gender ethnicity, smoking status, use of medication and waist circumference.

2.4.10 Sensitivity of sleep questionnaires in their ability to predict metabolic disorders.

Receiver operating characteristic curves (ROC curves) were used in the following analysis. ROC curves are a statistical method used to evaluate the performance of classification schemes - in this case the ability of sleep questionnaires to correctly identify the three conditions (glucose intolerance (prediabetes or T2DM), MetS or people with a CVD risk score of >20% – as determined by the Framingham Risk Score). The output from this test is the ROC, a plot obtained by calculating the sensitivity and specificity of every observed data value and plotting sensitivity against 1- specificity [231]. The area under the curve (AUC) is calculated from the ROC plot. The AUC is a measure of the overall performance of a diagnostic test and is interpreted as the average value of sensitivity for all possible values of specificity [232, 233]. The AUC is a value between 0 and 1, the closer the value is to 1, the better the overall diagnostic performance of the test. The practical lower limit for a diagnostic test is an AUC of 0.5 [231]. Therefore for use as a screening instrument a score of > 0.8 is acceptable.

a) Which questionnaire is more sensitive at discriminating between those who are glucose tolerant and those who are glucose intolerant?

This sample were categorised according to their glucose tolerance status – glucose tolerant compared to glucose intolerant which included both prediabetes and T2DM. The sample characteristics are given below and the ROC analyses is given in Figure 16.

	Glucose Tolerant n=679 (86.7%)	**Glucose Intolerant** n=101 (12.9)	**p**
Age**	56.79 ± 10.57	61.24 ± 9.46	**>0.0001**
Gender (male)*	333 (49.0%)	54 (54.5%)	0.36
Ethnicity (Caucasian)*	561 (83%)	81 (80.2%)	0.58
Waist Circumference**	93.95 ± 12.61	99.38 ± 12.77	**>0.0001**

Table 38: ROC analysis glucose tolerant and intolerant group characteristics: *χ^2 analysis** t-test analysis

Sleep Questionnaire	**Normal as defined by Q'aire**	**Sleep Problem as defined by Q'aire**	
BSQ	569 (72.9%)	211 (27.1%)	
SAQ	330 (42.3%)	450 (57.7%	
ESS	**Normal**	**Borderline**	**Sleep problem**
	657 (84.2%)	86 (11%)	37 (4.7%)

Table 39: ROC analysis breakdown of sleep complaints for the glucose intolerance cohort

b) Which questionnaire is more sensitive at discriminating between those with the metabolic syndrome and those without as classified by the NCEP- ATP-III criteria?

The cohort were scored according to the NCEP- ATP III criteria for the presence of MetS (n= 702) the sample characteristics are given below and the ROC analyses is given in Figure 17.

	Non-MetS n=553 (78.8%)	**MetS** n=149 (21.2%)	p
Age**	56.82 ± 10.80	58.5 ± 9.82	0.09
Gender (male)*	262 (47.5%)	86 (57.7%)	**0.033**
Ethnicity (Caucasian)*	456 (82.9%)	119 (79.9%)	0.46
Waist Circumference**	91.34 ± 11.52	105.04 ± 12.18	**>0.0001**

Table 40: ROC analysis MetS and non-MetS group characteristics. *χ^2 analysis ** t-test analysis

Sleep Questionnaire	**Normal as defined by Q'aire**		**Sleep Problem as defined by Q'aire**
BSQ	521 (74.2%)		181 (25.8%)
SAQ	259 (42.0%)		407 (25.8%)
ESS	**Normal**	**Borderline**	**Sleep problem**
	591 (84.2%)	77(11.0%)	34 (4.8%)

Table 41: ROC analyses breakdown of sleep complaints for the MetS cohort

C) Which questionnaire is more sensitive at discriminating between those with 20% CVD risk and those with less than 20% CVD risk as classified by the Framingham Risk score?

This cohort was classified regarding a 20% CVD risk using the Framingham Risk score (n=629). The descriptive statistics are given in the table below. The Framingham Risk score is a validated multivariable mathematical function that assigns weights to major coronary heart disease (CHD) risk factors [234]. It has been developed by the Framingham Heart Study for predicting the 10 year risk of clinical CHD events [235]. The sample

characteristics and sleep complaints prevalence are given in tables 42 and 43. The ROC analysis is given in Figure 18.

	<20% CVD Risk n=498(79.3%)	**≥20% CVD Risk** n=131 (20.7%)	**p**
Age**	54.66± 10.29	65.87 ± 5.94	**>0.0001**
Gender (male)*	192 (38.6%)	116 (89.2%)	**>0.0001**
Ethnicity (Caucasian) *	397 (80.0%)	118 (91.5%)	**>0.0001**
Waist Circumference**	93.57 ± 13.27	98.28 ±9.90	**0.004**

Table 42: ROC analyses 20% CVD risk group characteristics , *χ ** t-test.

Sleep Questionnaire	**Normal as defined by Q'aire**	**Sleep Problem as defined by Q'aire**	
BSQ	462 (72.3%)	167 (26.63%)	
SAQ	257 (40.9%)	372 (59.1%)	
ESS	**Normal**	**Borderline**	**Sleep problem**
	529 (84.1%)	72 (11.4%)	28 (4.5%)

Table 43: ROC analyses breakdown of sleep complaints for 20% CVD risk cohort * t test, $^{\dagger}\chi^2$.

Overall all three questionnaires had poor discriminatory ability to detect glucose intolerance, the MetS and a CVD risk score of >20% because all AUC's were below 0.8. However, the BSQ was a better tool for correctly identifying those with MetS compared to the Epworth and SAQ although this questionnaire had moderate discriminatory ability with an AUC value of 0.62. Therefore these questionnaires would not prove to be accurate tools in screening for patients with MetS in primary care.

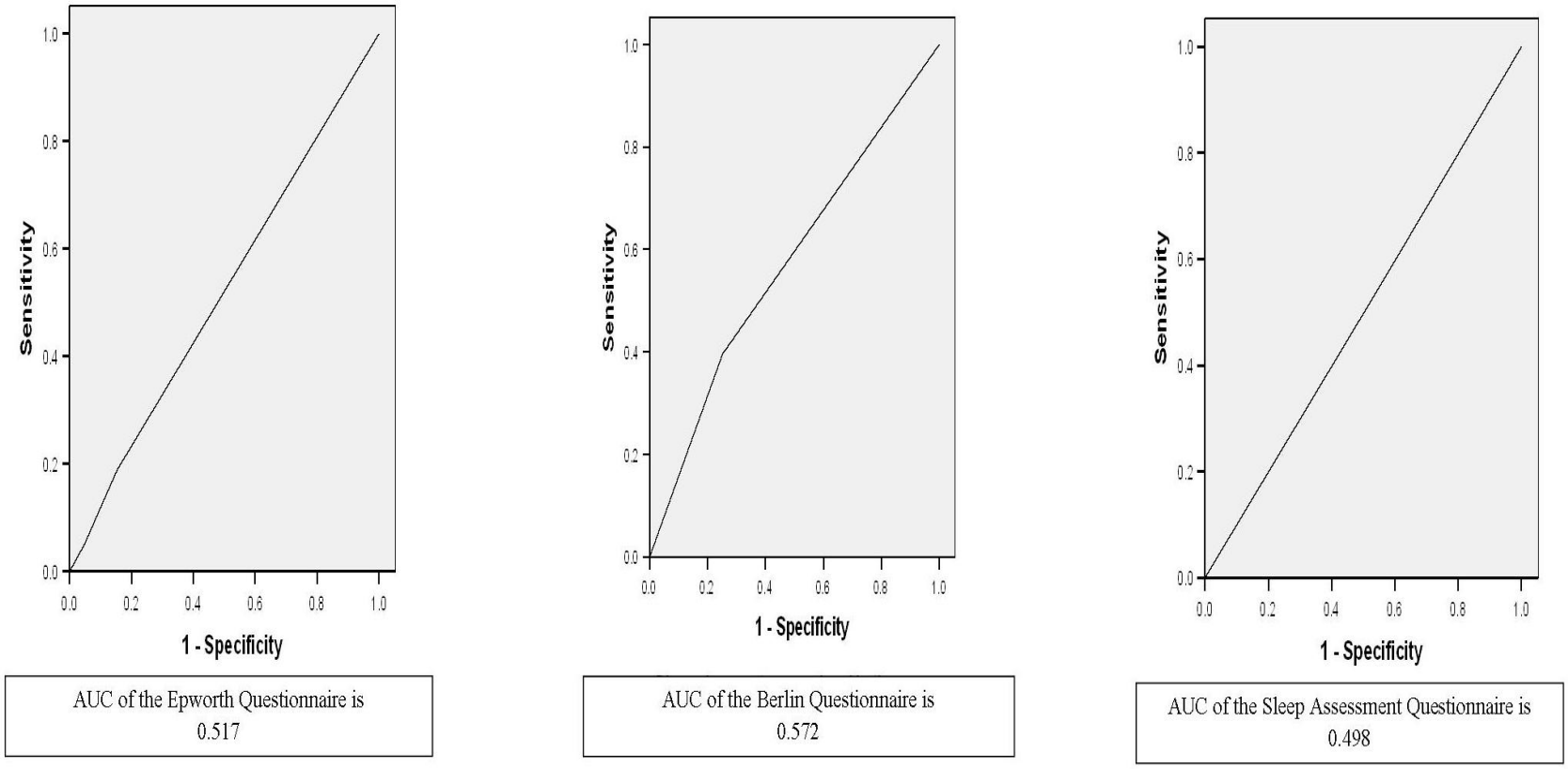


Figure 16: ROC curves for the sensitivity of each questionnaire in predicting glucose intolerance

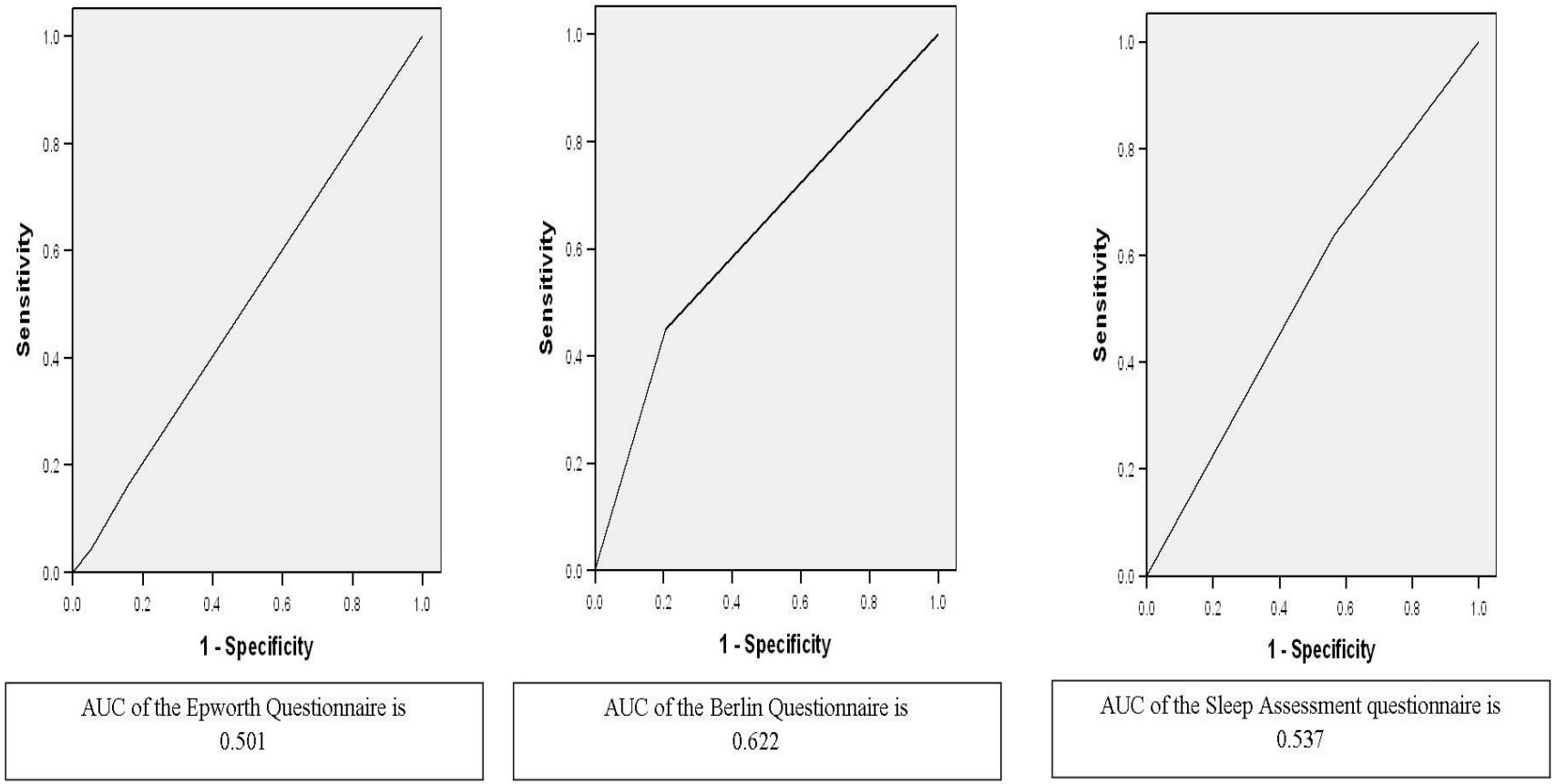
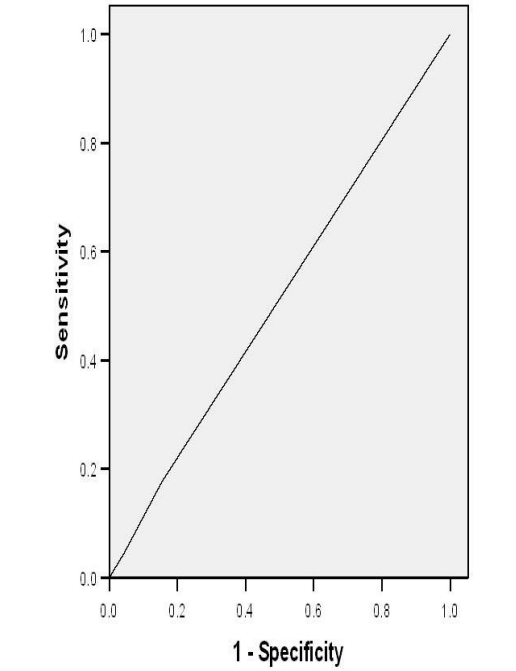
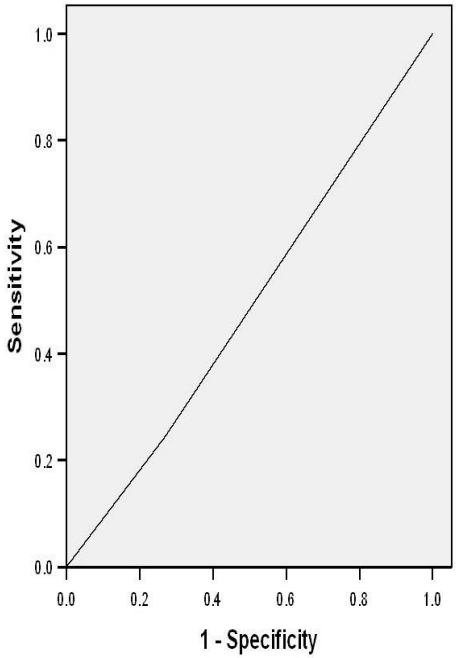


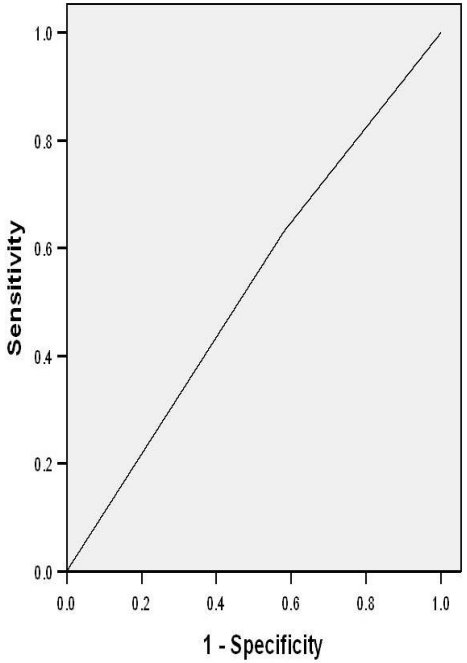
Figure 17: ROC curves for the sensitivity of each questionnaire in predicting MetS



AUC of the Epworth Questionnaire is 0.511



AUC of the Berlin Questionnaire is 0.488



AUC of the Sleep Assessment Questionnaire is 0.525

Figure 18: ROC curves for the sensitivity of each questionnaire in predicting > 20% CVD Risk

2.5 Discussion

The objective of the study was to determine the prevalence of sleep complaints within this cross-sectional study using three different sleep questionnaires. Each questionnaire assesses a different aspect of sleep disruption namely sleep disordered breathing (via BSQ), excessive daytime sleepiness (via ESS) and sleep disturbance (via SAQ). Although the questionnaires have a different emphasis on sleep disruption we would expect that the prevalence identified by each questionnaire would be similar due to the cross over between the questionnaires. For example an individual identified with sleep disordered breathing, using the BSQ, would score positive for sleep disturbance, using the SAQ, and may be captured by the ESS with excessive daytime sleepiness. However, given that individuals presenting with OSA are not all symptomatic i.e. do not suffer with excessive daytime sleepiness, it is plausible for the prevalence of EDS to be lower than that reported for SDB. We observed a large difference in the prevalence of sleep disruption according to these questionnaires, which may reflect the differing levels of specificity and sensitivity between them.

The prevalence of SDB as determined by the BSQ was 28.8% (Section 2.4.2) and is consistent with that reported for the general population (15 – 30%) [197, 236, 237]. The prevalence was significantly higher in males compared to females, which is also consistent with the literature [197]. Those with SDB had significantly higher waist circumferences which was an expected observation given the association between SDB and abdominal

obesity [238]. The prevalence of EDS was lower at 17.4% (Section 2.4.2) with no significant differences between the two groups with respect to age, gender, ethnicity or waist circumference. This was unexpected given that age and obesity are risk factors for excessive daytime sleepiness [239, 240]. However, a large proportion of those with SDB and other sleep problems do not present with EDS when measured subjectively (Figure19). This is thought to arise for a number of reasons which include the individuals regarding their level of daytime sleepiness (or overall energy levels) as normal and rationalising it to be the result of increasing age or other personal stresses. Additionally, it is possible a number of individuals may not report EDS at the sleep clinic to safeguard against the possibility of losing their driving license. Therefore the prevalence of EDS reported here is plausible when we compare it to the two other questionnaires. The most surprising result however came from the SAQ (Section 2.2.4). The prevalence of sleep disturbances was 58.5%, over half of those assessed. However as shown in Figure 19 this questionnaire is likely to capture the largest number of cases given the broad nature of 'sleep disturbance'. For example you would expect it to capture those with SDB, and with EDS and those with other sleep problems like insomnia. There were no significant differences between those with sleep disturbances and those without in terms of age, gender, ethnicity or waist circumference. The high prevalence identified by this questionnaire however questions whether it is a sensitive enough tool for correctly identifying sleep disturbance. This could in part be due to this being the longest of the three questionnaires and possibly the most difficult to complete (only 68.5% of the questionnaires were completed correctly). Furthermore, when looking at the actual overlap of those subjects scoring positive to these questionnaires a positive score for the SAQ is evident in 100% of the 63 cases where > 1 questionnaire scored positive (Figure 20). Only 19% of the time all three questionnaires

scored positive. The high prevalence of SAQ positive scores could also be due to this questionnaire covering a larger number of sleep disturbances and therefore more likely to score positive than the other two questionnaires. Interestingly there were no isolated cases where only the ESS and BSQ scored positive which could be due to a large number of those with SDB not reporting EDS. This is further supported by the total overlap of results of the three questionnaires in only 26.5% cases (Figure 15).

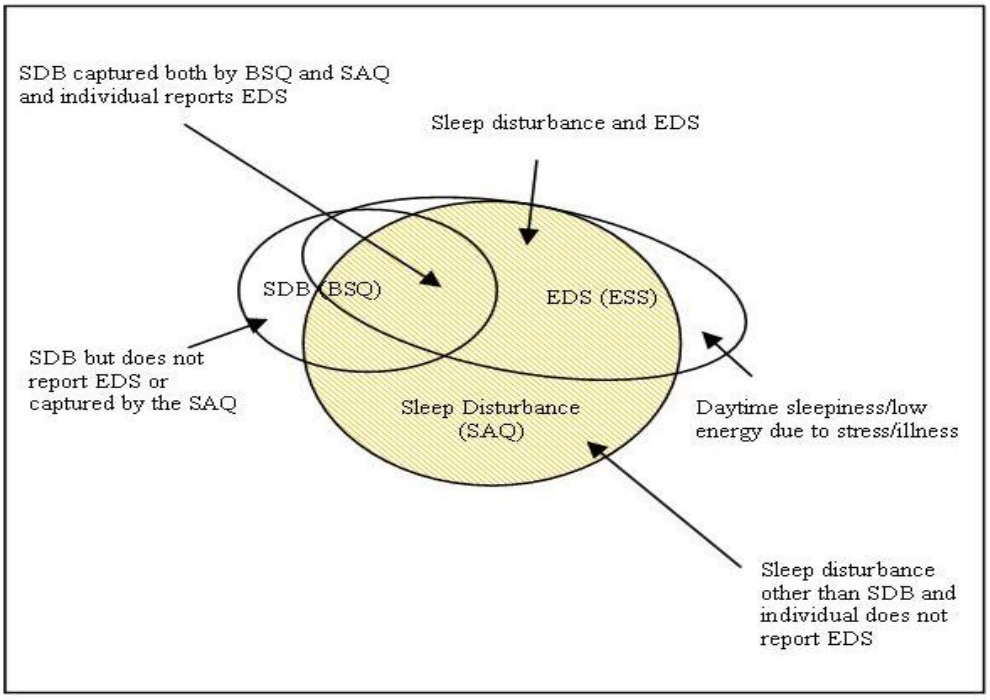


Figure 19: Overlap in those individuals captured by the three sleep questionnaires

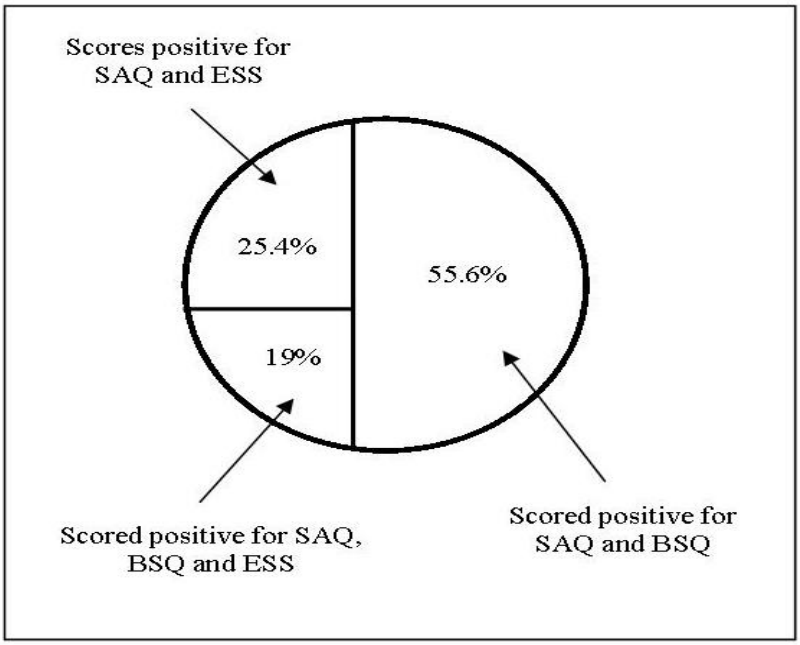


Figure 20: Actual overlap of questionnaires scoring positive for sleep complaint

Cardiovascular markers are clinical measures that are raised in people presenting with overt cardiovascular disease. They can be used to predict the future risk of cardiovascular disease and are therefore referred to as cardiovascular risk factors. In this study the cardiovascular markers include systolic and diastolic blood pressure, total cholesterol, low density cholesterol, high density cholesterol, triglycerides, HbA1c, fasting glucose and postload (OGTT) glucose levels. We looked at cardiovascular markers in those identified with EDS compared to no-EDS and those with a sleep disturbance compared to those with no sleep disturbance.

There were no significant differences between the cardiovascular profiles of those with EDS and those without (Table 19). However, total cholesterol was found to be higher in

those without EDS but this did not remain significant after adjusting for age, gender and ethnicity. We observed no significant differences in cardiovascular profiles between those with a sleep disturbance and those without as defined by the SAQ (Table 20).These findings further support the suggestion that these two questionnaires are relatively non-specific. Furthermore no significant differences between the levels of biological markers of inflammation and either EDS/non-EDS or Sleep Disturbance/no-Sleep Disturbance were observed (Tables 22 and 24).

One way analysis of covariance was used to determine if there were any differences in levels of biological markers of inflammation between those with SDB and those without (Table 27). Levels of interleukin-6 (IL-6) and C-reactive protein (CRP) were found to be significantly higher in those with SDB independent of age, gender, ethnicity, smoking status and use of medication. IL-6 is involved in the secretion of CRP from hepatocytes (Section 1.1.4). Therefore if IL-6 levels are raised then one might expect the level of CRP to be raised also, as we have observed. Those with SDB had significantly higher waist circumference compared to the non-SDB group (Table 25) and the association between SDB and inflammation was lost after further adjustment for waist circumference (Table 27, model 4). Therefore low-grade inflammation in those with SDB is not independent of adiposity. This result was not what we hypothesised, but is plausible given that a common feature of SDB and low-grade inflammation is visceral obesity. IL-6 is a pro-inflammatory cytokine and is not solely secreted by immune cells. It is also secreted by adipocytes and is therefore also a recognised adipokine. Thus with increased adiposity one would expect an increase in the level of IL-6 secretion and thus circulating levels of CRP.

Leptin levels were found to be significantly higher in those with SDB independent of age, gender, ethnicity, smoking status and use of medication (Table 27, model 3). Leptin is a hormone that is also described as an adipokine and therefore with increased adiposity, as is evident in the SDB group, one would expect the levels to be higher. This is suggestive of leptin resistance, given that one of the major roles of leptin in humans is the normal regulation of body weight and energy expenditure [241]. The hyperleptinemia observed in our SDB group can be explained by their significantly higher waist circumference and not by SDB alone, given that the association was lost after further adjustment for this covariate.

Adiponectin is the dominant secretory product of the adipocyte. However, unlike those previously described this adipokine has anti-inflammatory properties and its expression is inversely correlated with adiposity. Levels of adiponectin were found to be significantly lower in those with SDB independent of age, gender, ethnicity, smoking status and use of medication (Table 27, model 3). Hypoadiponectinemia is a common phenomenon in the obese individual as we have observed in our sample. The loss of statistical significance after further adjustment for waist circumference suggests the hypoadiponectinemia observed in those with SDB can be attributed to the increased level of adiposity.

It was hypothesised that SDB is the driving force for a rise in circulating adipokines in those classified with SDB. Thus those with SDB as classified by the BSQ would have a heightened level of inflammatory biomarkers compared to those classified without. However, we found that although levels of inflammatory biomarkers were higher in those

at high risk of SDB, but that adiposity appears to be the driving force for the low-grade inflammation associated with this sleep disorder.

One way analyses of covariance were used to determine if there were any differences in the levels of inflammatory biomarkers between South Asians and Caucasians at high risk of SDB. SDB is a risk factor for CVD which is associated with inflammation, as discussed in a recent review by Berg et al [242]. Additionally the prevalence of CVD is reported to be higher in migrant South Asians compared to Caucasians [243, 244]. Therefore, we hypothesised that South Asians at high risk of SDB would have higher levels of inflammatory biomarkers than Caucasians.

We observed significantly higher levels of TNF-α in the South Asian group (Table 29) independent of age and gender. However, although these levels remained higher, statistical significance was lost after further adjustment for smoking status, medication and waist circumference. Therefore there is no independent association between increased levels of TNF- α in South Asians at high risk of SDB compared to Caucasians. We also observed a trend towards higher levels of IL-6 and resistin in the South Asian group and lower levels of the anti-inflammatory adipokine- adiponectin. However, these between group differences did not reach statistical significance. Leptin levels were found to be significantly higher in the South Asian group and this was independent of age, gender, smoking status, medication and waist circumference. This result suggests an independent association between leptin resistance, South Asian ethnicity and SDB. When we assessed

the levels of cardiovascular markers between these two ethnic groups with SDB we found that HbA1c levels and postprandial glucose levels were also significantly higher in South Asians with SDB independent of age, gender, smoking status, medication and waist circumference (Table 34). Interestingly however, we found that Isoprostane levels were significantly higher in the Caucasian group independent of these same covariates (Table 29), suggesting that Caucasians at high risk of SDB have a higher level of oxidative stress. These results together indicate that the characteristics of people at high risk of SDB may be different between ethnic groups with South Asians characterised by increased level of inflammation and poorer glycaemic control compared to Caucasians. Additionally, Caucasians can be characterised by an increased level of oxidative stress compared to South Asians. This latter finding was unexpected and has not been previously reported in the literature. When we consider the current accepted mechanism linking hyperglycaemia, oxidative stress and inflammation [245], it is unusual that the Caucasian group would display higher levels of oxidative stress when they have a lower level of inflammation and better glycaemic control compared to the South Asian group. These results suggest that there may be differences between ethnic groups and exploring these observations further may help in the understanding of the pathophysiology of SDB.

Independent t-tests were used to determine if there were any differences in the levels of cardiovascular markers in those identified at high risk of SDB using the BSQ (n=1602). The cardiovascular markers of those at high risk of SDB were significantly worse than for those at low risk (Table 30), which is not unexpected given the association between SDB and cardiovascular outcomes (see Section 1.1.5). Furthermore when these markers of

cardiovascular risk were combined to represent a group with or without the MetS (as defined by the NCEP-ATP III criteria), a clear association between the MetS and SDB was observed, with the presence of SDB representing a key risk factor of MetS (Table 32). The odds of MetS was 1.7 times higher in those with SDB, independent of age, gender, ethnicity, presence of hypertension and waist circumference. We additionally found that the prevalence of SDB was significantly greater in those with the MetS (Table 31).

These findings support those of a recent study by Coughlin et al who demonstrated that OSA was independently associated with an increase in the cardiovascular risk factors that comprise the metabolic syndrome and with its overall prevalence [208]. However, we have additionally demonstrated in a multi-ethnic population this association is independent of age, gender, ethnicity, presence of hypertension and waist circumference. This is important as these are common risk factors between these two disorders and a strong relationship between SDB and MetS remains significant after adjustment for these covariates. The prevalence of SDB is high within the adult population and it frequently remains undiagnosed. This is of concern given that the prevalence of SDB will increase with the predicted increase in levels of obesity. Additionally, those with OSA are independently at increased risk of cardiac arrhythmias during sleep [246] and stroke [247]. There is, therefore, a need for the identification of this 'at risk' population. The data presented here shows that the prevalence of MetS is greater in those identified at high risk of SDB, and previous studies have demonstrated that treatment of OSA can improve cardiovascular risk profiles [248, 249]. This data supports the need for the identification and treatment of this population as part of routine care.

One way analyses of covariance was used to determine if inflammation is associated with SDB independently of glucose intolerance (Table 37). The glucose intolerance variable was categorised into three groups – normal, prediabetes and T2DM. We hypothesised that levels of inflammatory biomarkers would be higher in those at high risk of SDB independent of glucose intolerance. That low-grade inflammation apparent in the high risk SDB group is an effect of SDB and not a result of hyperglycaemia [245]. Levels of IL-6, leptin and CRP were found to be significantly higher in the SDB group compared to the non-SDB group independent of age, gender, ethnicity, smoking status, medication and glucose intolerance. However, after further adjustment for waist circumference these differences did not remain significant and therefore can be explained by adiposity. Adiposity, as previously stated, is a salient feature of both glucose intolerance [164], SDB [250] and low-grade inflammation [61] and therefore it is difficult to determine a 'cause and effect' relationship between these three conditions. This study is of an exploratory nature and therefore does not have the capability to determine cause and effect. However, it is clear that adiposity is playing a key role in the observations made within this study.

We found a strong association between increased plasma 8-iso-$PGF_{2\alpha}$ levels and glucose intolerance (Table 37) independent of age, gender, ethnicity, smoking status, medication and waist circumference. This supports the large body of evidence reporting an association between markers of oxidative stress and insulin resistance, IGT and T2DM [191, 251, 252]. Keaney et al [191] investigated the clinical conditions that were associated with systemic

oxidative stress in the Framingham study cohort. The biological marker of oxidative stress that they analysed was urinary 8-epi-$PGF_{2\alpha}$. In their age and sex adjusted univariate analysis they found diabetes ($p<0.0001$) and smoking ($p<0.0001$) to be strongly associated with higher levels of this metabolite. In their multivariate analysis they found a strong independent positive association between fasting glucose levels and urinary 8-epi-$PGF_{2\alpha}$ ($p<0.0001$). When they substituted fasting glucose with diagnosis of T2DM, in this multivariable model, they found the urinary creatinine-indexed 8-epi-$PGF_{2\alpha}$ levels to be 10.8% higher in the T2DM subjects compared to the non-diabetic subjects ($p<0.0001$) [191]. A number of mechanisms have been proposed for the association between oxidative stress and hyperglycaemia with a viscious cycle that results in the glucose intolerant individual (Section 1.4.2). Oxidative stress is thought to play a key role in β-cell dysfunction and therefore glucose intolerance. One mechanism could be the result of the pressure caused by sustained high circulating levels of glucose and FFA, common in overweight sedentary people, where the muscle and adipose tissue subsequently have to protect themselves from the resultant excessive ROS formation by becoming less sensitive to the action of insulin. In addition, the β-cell does not uptake glucose in an insulin-dependent manner, suggesting that in the face of excessive ROS formation (due to hyperglycaemia), the β-cell cannot reduce ROS production by decreasing GLUT4 translocation. Furthermore, the β-cell has low level of antioxidants such as catalase and superoxide dismutase [253], and therefore the alternative protective cellular mechanism of 'mopping up' these free-radicals with antioxitants is not as efficient in the β-cell. The β-cell is therefore exposed to high levels ROS, which are now 'free' to damage the cellular and mitochondrial DNA. The effects of oxidative stress are multifaceted, with the overall result

in the overfed setting being an increased risk of overt T2DM, which is what our results also suggest.

Finally we assessed whether the three different sleep questionnaires had any utility as screening tools for disorders of the metabolism, given the association between sleep deprivation and metabolic dysfunction (Section 2.4.10). This aspect of this study is of high importance given the feasibility of using validated sleep questionnaires to simultaneously identify individuals with sleep problems and either glucose intolerance or at high risk of CVD. To our knowledge no other studies have investigated whether these sleep questionnaires have the ability to correctly identify whether a patient is at risk of anything other than the primary objective of the questionnaire, that is a sleep problem. A questionnaire of high sensitivity and specificity is an economic, quick and effective instrument to use within the clinical setting. We used ROC curve analysis to determine whether the Berlin Sleep Questionnaire, the Epworth Sleepiness Scale and the Sleep Assessment Questionnaire had the ability to correctly identify patients that were glucose intolerant (Figure 16), patients that were classified with the MetS (Figure 17) or patients that had a greater than 20% risk of CVD (Figure 18). Unfortunately each questionnaire failed to correctly classify any of these disease states as each area under the curve was lower than 0.6. The Berlin questionnaire however was a better tool for identifying people with MetS although the AUC at only 0.62 indicates that it is has moderate discriminatory ability for the identification of this condition. Therefore, we can conclude with confidence that none of these questionnaires would be suitable within routine care for simultaneously screening patients for sleep disorders and metabolic dysfunction or cardiovascular risk.

2.5.2 Limitations & Strengths

The Berlin Sleep Questionnaire (BSQ) was used in this study as surrogate for the diagnosis of SDB. The BSQ incorporates questions that have been consistently reported in the literature to successfully predict the presence of SDB [254-259]. It is centred on the main risk factors for this sleep disorder, including snoring, excessive daytime sleepiness, obesity and hypertension. In the clinical setting it is not used independently for the diagnosis of SDB. However it is a validated questionnaire with a sensitivity of 86% and specificity of 77% for identifying significant OSA (defined by a Respiratory Disturbance Index $\geq$ 5)[260], and has been found to be a useful tool in population screening studies for OSA. Sharma et al reported the BSQ capable of identifying subjects at high risk of OSA prior to PSG and that it avoids unnecessary PSG studies for the full diagnosis of OSA [261]. In those subjects that were categorised as 'high risk of SDB' 96.4% (53 out of 55) were subsequently diagnosed with OSA using PSG [261]. We have highlighted significant differences in inflammatory and cardiovascular profiles of those who were found to be at 'high risk' of SDB in an otherwise healthy cohort. Due to limited resources we were unable to take this study further and undertake overnight sleep studies on these participants.

We cannot determine cause and effect with this study. However, the evidence discussed above and the present results do support the theory that SDB is either a precursor for metabolic dysfunction or vice versa. Another limitation of this study is the method used for measuring abdominal obesity. This is a crucial factor that needs to be addressed when

investigating relationships between SDB, low grade inflammation and glucose intolerance, since it is a salient feature in all these conditions and further complicates the establishment of a cause and effect sequence. Although waist circumference is a widely accepted measure of central obesity and is a more powerful measure than BMI [74], it still does not have the power to distinguish between the proportion of intra-abdominal and subcutaneous abdominal fat. Therefore future studies should include techniques that can differentiate between and quantify these two areas of fat deposition using technologies such as computerised tomography and magnetic resonance imaging.

It would have been preferable to have biomarker data for the whole sample as well as a larger South Asian population specifically within the SDB group. However, due to language barriers this was difficult for first generation South Asians who were not sufficiently literate in the English language. An interpreter was employed for non-English speaking patients attending the screening clinics. However, due to time constraints within these clinics and the ratio between interpreter and non-English speaking patients, a large number of questionnaires were not completed. It was not economically feasible to use translated versions of the sleep questionnaires which would be preferable and advisable for future studies. Therefore the South Asian group studied here may not be truly representative of the South Asian population within Leicester.

This study was originally powered for a sample size of 800 (Appendix 2) for the analysis of biological markers of inflammation which we were unfortunately unable to fulfil. This

was in part due to a large proportion of South Asians not providing consent for the extra fasting samples required for this analysis. The matching strategy that we used did not permit us to utilize all 1529 samples that were collected. We hypothesise that the relationships observed in this study would be improved statistically if we had reached our target sample size.

Strengths of this study include that it is the first study investigating levels of biological markers of inflammation and cardiovascular profiles between migrant South Asians and Caucasians with SDB. The overall sample size of 1602 for the BSQ questionnaire and ADDITION data analyses was large and therefore provided a good cross-sectional analysis of a UK multiethnic population. All measurements were conducted using standard operating procedures with fully trained staff within the main body of the ADDITION study. This in addition to the ongoing quality assessment of each dataset throughout the study means that the results presented are reliable. We have collected data on, and adjusted for it in our analysis, a number of confounding variables that have not previously been widely considered in the literature including smoking status, use of medication and glucose tolerance status.

2.5.3 Conclusions

This study supports recently published evidence of an association between SDB (and more specifically OSA), metabolic dysfunction and low-grade inflammation. We have also highlighted that migrant South Asians with SDB have worse cardiovascular and inflammatory profiles than Caucasians with SDB, and that low-grade inflammation could be playing a key role in the greater cardiovascular risk evident in this ethnic group. This is the first study comparing cardiovascular and inflammatory profiles between South Asians and Caucasians with SDB. It further supports evidence that South Asians are at increased cardiovascular risk and that this greater risk appears not to be silenced when comparing South Asians identified with SDB with Caucasians with SDB. In the presence of SDB, as defined by the BSQ, renders the individual at an increased risk of CVD compared to non-SDB. These results support the use of the BSQ, in identifying individuals at high risk of SDB but not for the simultaneous and specific identification of individuals with glucose intolerance, the MetS or high CVD risk. Additionally the screening and management of at risk populations for OSA could have large economic benefits for the health service and improve the quality of life of those at risk in terms of preventing the development of insulin resistant associated diseases. Further ethnic comparison studies in OSA and the effects of treatment on cardiovascular outcomes are required to help elucidate why this ethnic group appears to remain at higher level of cardiovascular risk than their Caucasian counterparts.

Chapter Three

Investigating the Impact of CPAP Therapy on Glycaemic Control

A Systematic Review

3.1 Introduction

Sleep disordered breathing (SDB) describes a group of sleep disorders which share the common feature of abnormal breathing patterns during sleep. This is a spectral disorder with simple snoring at one end to the periodic complete cessation of breath, as with OSA. OSA affects approximately 4% of adult males and 2% of adult females [197] and is characterised by the recurrent collapse of the upper airway during sleep. This results in airway occlusion, giving rise to either a reduction of airflow (hypopnoea), or the cessation of breath (apnoea), usually producing a drop in arterial blood oxygen saturation. The patient has to partially arouse from sleep in order to reinitiate breathing [262]. This can lead to a state of chronic sleep deprivation due to the resultant fragmentation of sleep. The most common symptom of OSA is daytime hypersomnolence, which can have a detrimental effect on quality of life [263]. The severity of OSA is assessed based on the apnoea-hyponoea index (AHI). AHI refers to the average number of these events per hour of sleep (AHI < 5 (within normal limits), 5-15 (mild OSA), 16-30 (moderate OSA) and > 30 (severe OSA) [198]).

OSA has a number of adverse cardiac implications and is an independent risk factor for hypertension [217, 264] and cardiovascular disease [221]. It is independently associated with the MetS [208], insulin resistance [219] and T2DM [224]. The MetS, which comprises a cluster of cardiovascular risk factors, shares salient features with OSA; including central obesity, dyslipidemia [265], hypertension [216, 264] and dysglycaemia [70]. It has recently been postulated that OSA is a manifestation of the MetS [223] and has been described as a "cardio-metabolic" disorder [266]. Punjabi et al, in a recent systematic review, reported an

independent association between Sleep Disordered Breathing (SDB) and impaired glucose homeostasis [220]. This begs the question of whether treatment of OSA could improve glycaemic control in those with established T2DM. The gold standard treatment for OSA is Continuous Positive Airway Pressure (CPAP) therapy. CPAP is delivered by a device that pumps a continuous stream of pressurized air via a close-fitting mask into the nose or the mouth and nose of the patient, thus acting as a 'splint', to prevent the collapse of the upper-airway and provide an uninterrupted night's sleep. This treatment can effectively restore the patient's quality and quantity of sleep and thus quality of life [212]. The reported effects of CPAP therapy on glycaemic control in individuals with OSA are inconsistent. Thus the aim of this systematic review was to further establish from the literature whether CPAP therapy has an impact on glycaemic control in those with OSA.

3.2 Methods

3.2.1 Data sources

A broad literature search was conducted using the MEDLINE (1966 to December wk 4, 2007) and EMBASE (1980 to week 52, 2007) data bases to identify studies published in the English language between the stated dates. Identical search terms were used for both databases; 'Sleep Apnoea Syndromes' or 'Sleep Disordered Breathing' or 'Obstructive Sleep Apnoea' **AND** 'Glucose Intolerance', **OR** 'Glucose Tolerance' **OR** ' Type 2 Diabetes Mellitus' **OR** 'glucose metabolism' **OR** 'Insulin Resistance' **OR** 'Insulin Sensitivity' **OR** 'Insulin Responsiveness'. 'Glucose metabolism' and 'Sleep Disordered Breathing' searches were exploded to include the MeSH terms within the search. The

Cochrane library database (CDSR, DARE, CCTR, HTA and the NHS Economic Evaluation Database) (1991 – Issue 4, 2007) was also searched for any existing systematic reviews for this research question.

3.2.2 Study selection and data extraction

All references were directly imported into Endnote from Medline and Embase, after the removal of any reviews. All duplicate references were removed automatically and the abstracts of the remaining articles were obtained. Following this, all irrelevant abstracts were removed. The full article was then obtained for all remaining abstracts and independently reviewed by two of the authors (Emer Brady, Alison Dunkley), in order to identify those eligible for this review. A standard data extraction form developed and tested for this systematic review was used. The findings of this data extraction phase were compared for consistency and the two independent reviewers then decided which studies were to remain in the review. Studies included for the potential systematic review had all the relevant statistical and study design information entered into an Excel spread sheet.

Studies eligible for inclusion were those that had included a population with OSA and who were not receiving CPAP therapy upon recruitment. Primary or secondary outcome measures included parameters of glycaemic control (fasting blood glucose levels, continuous blood glucose monitoring (CGMS), HbA1c, fasting insulin, insulin resistance or sensitivity or responsiveness) both before and after CPAP therapy. Individual case studies were excluded. The study population comprised adults with or without T2DM and recently

diagnosed (untreated) OSA. The method of glucose intolerance, T2DM or OSA diagnosis was not discriminated against. However, in order to come to a conclusion as to whether glucose tolerance is affected by CPAP therapy, a key inclusion criteria was the measurement of patient compliance. It has been documented that CPAP non-compliance will not have a beneficial affect with respect to day-time sleepiness for the patient and this may have implications for any beneficial effect on glucose intolerance. There is a clear relationship between CPAP effectiveness and usage per night, however, the optimum threshold of hours per night depends on the outcome being investigated [214]. For example, when assessing the effect of CPAP therapy on subjective daytime sleepiness, the threshold at which increased usage per night is unlikely to provide any further improvements has been reported to be 4 hours/night [214]. In comparison, when investigating objective daytime sleepiness this threshold is increased to 6 hours/night [214]. Unfortunately a meta-analysis could not be performed on the data collected due to marked study heterogeneity and large difference in the primary outcome of the studies. Results are therefore presented using a descriptive format to summarise and evaluate the selected studies.

3.3 Results

No existing systematic reviews were identified within the current Cochrane Library. A total of 8096 studies were retrieved (2686 obtained from MEDLINE and 5178 obtained from EMBASE). Three thousand three hundred and twenty-four reviews were removed before all references were imported into Endnote. A total of 4620 references remained after duplicates were removed. Thirty full papers were selected for the review. We used a basic data extraction form for the final selection phase from which 19 studies were selected for

full data extraction, of which 16 studies were relevant to the research question (Figure 21). These 16 studies were then assessed for possible meta-analysis; four studies measured glycaemic control by measuring plasma glucose and insulin levels or a continuous blood glucose monitoring system (CGMS) [248, 267-269]; the remaining twelve studies measured glycaemic control using methods to measure insulin sensitivity/resistance either using the hyperinsulinemic-euglycaemic clamp technique or HOMA-IR method [270-281] (Tables 44 and 45). In total the studies included 325 participants, the overall age range of participants was from 26 to 74 years. The four studies using plasma glucose/CGMS included 60 participants (age ranging from 26 to 59.7 years) and the remaining seven studies using methods of insulin sensitivity included 265 participants (age ranging of from 29 to 74 years). A meta-analysis could not be undertaken with the data collected due to a large degree of study heterogeneity.

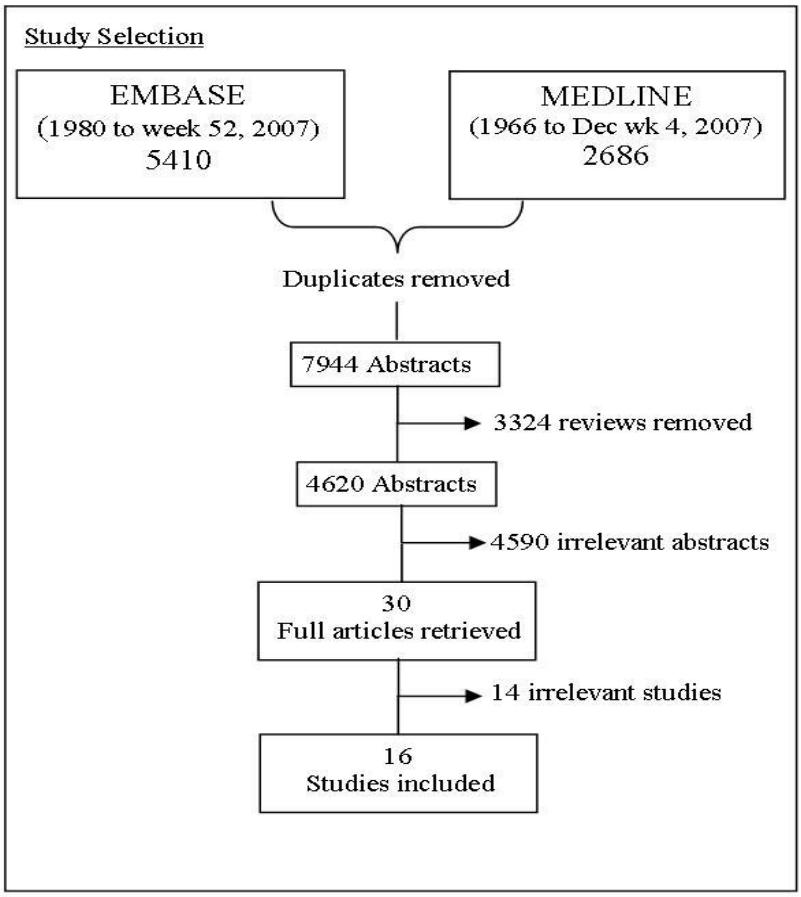


Figure 21: Study selection process for systematic review

3.3.1 Glycaemic control measured via plasma glucose levels and HbA1c

The CGMS uses a glucose sensor that is inserted into the subcutaneous tissue and measures glucose levels at this site continuously over a 72 hour period. It provides an average blood glucose reading every five minutes - thus providing a continuous record of blood glucose levels for the entire 72 hour period. The device allows patients to record events including meals, medication and exercise. When patient data is uploaded, the software provides data on the daily average glucose levels from which fasting and postprandial glucose levels can

be determined. The area under the curve (AUC) can also be determined with some models of this system. The AUC provides information on the amount of time that the subject was within normal plasma glucose levels and the time spent in either a hypo- or hyperglycaemic state.

Glycosylated haemoglobin (HbA1c) is another measure of glycaemic control. HbA1c levels provide a retrospective (3 months) indication of average blood glucose levels [282]. This is based on the fact that the average life span of the red blood cell is between 90-120 days and that glycosylation of haemoglobin is irreversible [282]. HbA1c is the gold standard for measuring chronic glycaemia [283]. International guidelines recommend maintaining HbA1c levels of <6.5% [284] as there is good evidence to show that reduction in HbA1c reduces micro- and macrovascular outcomes [284]. I will now discuss the findings of the 4 studies which measured glycaemic control using these methods which are summarised in Table 44.

Chin K et al [267] measured glycaemic control with fasting glucose and insulin levels and with OGTT in 31 newly diagnosed OSA patients. Twenty-two were treated with CPAP therapy for 6 months, the remaining 9 acted as the control group. In the treatment arm 15 participants were found to have T2DM at baseline. Over the 6 month follow-up period, 9 intervention group subjects lost a significant amount of weight (> 1 kg/m^2). Therefore the sample was split into 3 subgroups: control group (n=9), no body weight reduction (No-BWR, n=13) and body weight reduction group (BWR, n=9), with the latter two groups having received CPAP therapy. At baseline, age, BMI, blood pressure and AHI did not

differ significantly between groups. No significant changes in fasting insulin or glucose levels post-CPAP therapy were observed for the whole treatment group, and data were not reported for the control group. Plasma glucose levels decreased significantly (baseline vs. post-CPAP) during the glucose tolerance test for the BWR subgroup only and a significant reduction in insulin was observed at 180 minutes post-ingestion of 75g glucose. However, exact values are difficult to ascertain from the graphs plotted for this data. This improvement in glycaemic control could be attributed to the significant weight reduction in this group. The level of CPAP compliance was not measured in this study. The authors report that these results support previously published work that the association between insulin resistance and sleep disordered breathing is entirely dependent on body mass [285].

Conversely, Stoohs RA et al [268] found that insulin resistance actually increased significantly post-CPAP therapy. This group studied 5 obese, non-T2DM subjects with severe OSA, and measured glycaemic control using venous sampling of glucose and insulin. The follow-up was 4 weeks after CPAP therapy. Nocturnal venous sampling was additionally performed at 30 minute intervals at baseline and after 8 weeks CPAP therapy. There was no significant change in BMI over the study period. Fasting glucose and insulin had significantly increased by 4 weeks (Table 44). This study did not include a control group and did not report the level of CPAP compliance over the 8 week study period. At 8 weeks results from the nocturnal venous sampling showed a significant increase in plasma glucose and insulin levels, although exact values could not be ascertained from the graphs. It was proposed that these results could be a due to the reappearance of slow wave sleep (SWS) post-CPAP treatment, given that SWS has been shown to be closely correlated with

human growth hormone secretion [286]. Growth hormone in turn has been shown to have anti-insulin activities [287]. Additionally the authors suggested that an increased glucose uptake may have been occurring in the pre-CPAP condition due to the increased work load of respiratory muscles during obstructive apnoeas. This in turn would have the effect of lowering plasma glucose and insulin levels (increased glucose utilization). Thus, in the treatment condition, where respiratory effort has decreased so has the requirement for peripheral glucose utilisation, possibly explaining the reported increased levels of insulin and glucose. They suggest that a very slow resetting of the processes involved in glucose utilisation during sleep in obese subjects could be responsible for the observed results [268] and therefore a longer follow-up period may have yielded different results. However, only five patients were studied and this therefore limits the conclusions reported.

Czupryniak L et al [269] similarly report increased blood glucose levels post-CPAP therapy (Table 44) in 9 obese non-T2DM subjects with OSA. Glycaemic control was measured using the 72 hour CGMS method and they additionally measured fasting plasma glucose and insulin and performed an OGTT pre and post one night of CPAP therapy. Results from the OGTT were reported as not significantly different pre- vs. post treatment; however, the actual data are not reported. Fasting insulin levels increased, but did not reach statistical significance post- compared to pre-CPAP therapy (Table 44). Insulin resistance was measured using the HOMA-IR method and although not statistically significant, there was a trend towards increased insulin resistance from pre- to post-CPAP therapy (Table 44). They reported a 27% increase in mean glucose levels over the 72 hour CGMS recording period (Table 44). However, these values remained within the blood glucose

levels of normo-glycaemic individuals. This group similarly reported that the observed increase in plasma glucose and insulin resistance could be the result of CPAP-induced hormonal changes including increased GH secretion. They stated that these results should be interpreted with caution given the relatively small sample size and short follow-up period. Furthermore the first night of CPAP therapy can elicit a stress response which could account for the rise in plasma glucose levels. In addition the level of CPAP compliance was not measured and therefore it cannot be assumed that all subjects used CPAP throughout the whole study night.

Babu AR et al [248] however observed an improvement in glycaemic control (Table 44). This group measured glycaemic control using HbA1c, fasting plasma glucose levels and 72-hour CGMS in 25 obese subjects with moderate-severe OSA and established T2DM. Data was obtained pre-treatment and 30-90 days post-CPAP. CPAP compliance was also monitored and the final analysis was split into two groups; compliant group (CPAP usage ≥ 4 hours/night, n=12) and non-compliant group (CPAP usage < 4 hours/night, n=12). One subject was removed from the final analysis due to CPAP compliance data being inaccessible. As with the previous studies, there was no control group. A non-significant reduction in fasting glucose levels was observed for the whole sample (Table 44). All one hour post meal glucose levels were generally lower for the whole sample but did not reach statistical significance. However, in the compliant group all one hour post meal glucose levels were significantly reduced for each meal time (Table 44). In the non-compliant group only post breakfast glucose levels were reduced significantly (Table 44). HbA1c was reduced in the whole sample but neither of clinical or statistical significance (Table 44).

The analysis of HbA1c was further investigated by splitting the sample into those with HbA1c >7% (n=17) and those with HbA1c ≤ 7% (n=7) at baseline. A significant reduction in HbA1c was observed for those with HbA1c > 7% at baseline (Table 44). Additionally across the whole sample there was a reduction in the number of glucose values >11 mmol/l as determined by the mean glucose readings by CGMS (Table 44). This team went further still to investigate whether there was an association between change in HbA1c and CPAP duration. They found that within the compliant group there was only a significant correlation between HbA1c reduction and the number of days of CPAP usage (Table 44). This study demonstrated the importance of CPAP compliance in achieving metabolic improvements in people with OSA and T2DM. The authors state that the failure to monitor CPAP compliance in previous studies may have masked a positive treatment effect of this therapy on glucose homeostasis [248].

Study	Sample size	Cohort	Method of analysis	Design	Follow-up	Results
Chin et al [267]	31	OSA BMI: 27.7-32.9Kg/m^2 11 non-T2DM 15 T2DM	Fasting Glucose Fasting Insulin OGTT	Matched controls (n=9) Repeated measures for treatment group reported	> 6 months	Baseline no significant differences in age, BMI, BP or AHI between the two treatment groups. Overall no significant change in fasting glucose or insulin levels (n=31) 13 of the treatment group did not lose weight but 9 of treatment group lost > 1kg/m2 In the weight loss group a significant reduction in glucose levels during the OGTT were observed but not reported
Stoohs et al [268]	5	OSA BMI: 49± 8kg/m^2 Non-T2DM	Fasting Glucose Fasting Insulin 30 min venous sampling overnight (7hrs)	Repeated measures	4 weeks + 8 weeks	No significant change in BMI **4 weeks** – Significant increase in fasting glucose levels 5.99 mmol/l to 7.9mmol/l, $p<0.05$ Significant increase in fasting insulin 28 ± 5 uU/ml to 34 ± 11 uU/ml, ($p<0.0001$. **8 weeks** - Nocturnal plasma glucose levels and insulin significantly increase ($p<0.001$ and $p<0.001$ respectively). Actual values cannot be ascertained from graph.
Czupryniak et al [269]	9	OSA BMI: 34.8± 5.3Kg/m^2 Non-T2DM	Fasting Glucose Fasting Insulin OGTT CGMS	Repeated measures	1 night	No significant change in OGTT results post-CPAP Trend towards reduction in fasting insulin levels: 98.4 ± 51 pM vs. 84.3 ± 43.4 pM. Trend towards increased level of insulin resistance (HOMA-IR): 3.6 ± 2.2 vs. 3.9 ± 2.6 **CGMS** - 27 % increase in mean blood glucose levels recorded 3.5 ± 0.39 mmol/l vs. 4.4 ± 0.61 mmol/l, $p< 0.01$
Babu et al [248]	24	OSA BMI:42.7±8.7Kg/m^2 T2DM	Fasting glucose CGMS HbA1c	Repeated Measures	30-90days	CPAP compliance ≥ 4 hours/ day (n =12), ≤ 4 hours/day (n = 12) Non-Significant decrease in HbA1c in whole group 8.3 ± 2.2% to 7.9 ± 1.8%, $p=0.06$ Subjects with baseline HbA1c > 7% a significant reduction was

						observed: 9.2 ± 2 % to 8.6 ± 1 %, p =0.02 (n = 17). Non-significant reduction in fasting glucose observed for whole group 7.6 mmol/l to 6.8 mmol/l, p>0.05. CPAP compliant group significant reduction in plasma glucose 1 hour postmeal for each meal: Post Breakfast – 11 ± 4.6 to 6.8 ± 1.9 mmol/l, p<0.05 Post lunch – 11.3 ± 4.9 to 7.5 ± 3.1 mmol/l, p< 0.05 Post dinner – 11.4 ± 4.7 to 7.2 ± 2.7 mmol/l, p<0.05 CPAP non-compliant significant reduction post breakfast only – 10.4 ± 2.9 to 7.7 ± 2.7 mol/l, p<0.05 Significant positive correlation between reduction in HbA1c and number of days of CPAP in those that used CPAP > 4 hours/day (r = 0.74, p=0.006). <u>Sub Study</u>: Analysis of those with fasting glucose >11.1mmol/L at baseline a significant decrease in fasting glucose levels observed: 8.2 ± 11 to 4.6 ± 6.5 mmol/l, p = 0.003

Table 44: Summary of studies using the glucose, HbA1c and CGMS method

3.3.2 Glycaemic control measured via insulin sensitivity methods

An alternative way to assess glycaemic control is to measure insulin resistance/insulin sensitivity, also termed insulin responsiveness. This can be carried out using the hyperinsulinemic euglycaemic clamp (clamp method) [288], or by the homeostatic model assessment for insulin resistance (HOMA-IR) [289]. The HOMA-IR method is less accurate than the clamp method, with respect to the requirement for robust primary input data, however, it is still a valid and widely accepted method [290]. It is also clinically more practical as only one fasting plasma sample is required. The clamp method quantifies insulin resistance by measuring the total amount of glucose required to compensate for the increased plasma insulin level without resulting in a hypoglycaemic state. Insulin and a 20% glucose solution are infused through a cannula inserted into a peripheral vein in one forearm and a second intravenous cannula is inserted into the forearm of the other arm for the purpose of sampling. Insulin is continuously infused at a predetermined rate and glucose is infused at a variable rate in order to maintain a level of normo-glycemia. The rate of glucose infusion is determined by the level of blood glucose in the samples taken at predetermined time points - for example every 10 minutes. The rate of glucose infusion during the last 30 minutes of the ~2 hour test determines the level of insulin resistance. If high levels of glucose are required ($\geq$ 7.5mg/min) then the subject is insulin sensitive, however, if low levels are required (< 4 mg/min) this indicates that the subject is insulin resistant. Eleven studies that used these methods to measure glycaemic control were identified (Table 45).

Smurra M et al [270] did not observe any improvement in glycaemic control assessed by energy expenditure using indirect calorimetry (Group 1, n=10) and via the clamp method and OGTT (Group 2, n=6). The follow-up was 2 months post-CPAP therapy and patient compliance was measured. Group 1 were CPAP compliant (6.4 ± 0.8 hours/day), with no significant changes observed pre- and post-CPAP therapy for fasting or postprandial plasma glucose levels as determined by OGTT. Results from the indirect calorimetry showed no significant improvement in energy expenditure, glucose oxidation, glucose oxidation per kg, glucose oxidation by free fat mass, or insulin levels. Group 2 were also CPAP compliant but with a slightly lower average use of 5.9 ± 2.5 hours/day. Similarly there was no significant improvement post-CPAP therapy in mean glycaemia, insulin, HbA1c, tissue sensitivity to insulin as determined via the clamp method. Smurra M et al report that their findings do not support previously published data [274, 291], and suggest it is due to study design given that the subjects were not all glucose intolerant and had varying degrees of metabolic dysfunction in Group 1 thus they have limited bias factors regarding glucose metabolism unlike the other studies. Additionally these previous studies did not provide information on dietary intake, change in weight, or levels of physical activity. These parameters are likely to have altered in cases of a long follow-up period (≥ 8 weeks) and therefore if not adjusted for could influence results. It is also important to note that within Group 1 the range of AHI reported suggests that some participants did not in fact have OSA (AHI 31 ± 24). The lowest AHI was 2.4 events/hour in Group 1, which would be classified as a 'normal' reading, given the cut-off point for OSA is 5 events/hour. An AHI of between 5 and 15 is generally classed as mild OSA, and although it would depend on the level of daytime sleepiness that the patient is suffering, it is unlikely that

CPAP therapy would be initiated. This is also the case for Group 2 where the lowest AHI was at the top end of mild OSA (9.23 events/ hour). Therefore, the significance of these results should be interpreted with caution.

Chin k et al found no change in glycaemic control post-CPAP therapy in a second study conducted by this group [271]. They measured glycaemic control using the HOMA-IR and OGTT methods in 40 obese subjects with OSA. They had one night, one month and 6 month follow-up. Data were reported for 34 of these subjects as 6 failed to attend the one month follow up and CPAP compliance was measured. The mean CPAP usage for the cohort was within the compliant range at 1 month (4.3 ± 1.4 hours/day). Fasting blood samples were taken after a 12 hour overnight fast and in the afternoon after a 3 hour fast. The level of glucose intolerance varied within the group with 55% of the sample being glucose intolerant (11 had T2DM and 11 had prediabetes (IFG or IGT)). The insulin resistance index did not change significantly at this follow-up although there was a trend towards improved insulin resistance and a significant reduction in BMI (Table 45) at the 1 month follow-up. The insulin resistance index was not reported for the 6 month post-CPAP therapy. Glycaemic control was a secondary outcome in this study after aminotransferase and leptin analysis. However, this group have suggested that the results from the one night post-CPAP therapy indicate that leptin resistance may improve before insulin resistance [271]. Leptin levels had significantly changed at this time point and there was a trend towards improved insulin resistance at one month. However, neither the insulin resistance index or leptin levels were reported for the 6 month follow-up.

Lindberg, E et al [278] assessed the effect of CPAP therapy on the metabolic profile using the HOMA-IR method, fasting glucose and HbA1c levels. CPAP compliance was measured in their 23 non-T2DM and 5 T2DM over weight/ obese men (29.4 ± 4.24 kg/m^2). The first follow-up was at 3 weeks post-CPAP therapy, when 28 matched controls were also recruited, where all visit 1 measures were undertaken in both groups. The treatment group also had an overnight respiratory study. The two groups also returned for a 6 month follow-up. The treatment group were CPAP compliant and this did not significantly change by the 6 month follow-up. At 3 weeks post-CPAP therapy there was no reduction in HbA1c, fasting blood glucose levels or BMI. However, CPAP therapy was associated with a reduction in fasting insulin levels and in the level of insulin resistance in the treatment compared to the control group (Table 45). At 6 months post CPAP therapy there was a trend towards a reduction in fasting insulin levels compared to baseline values, but this did not reach statistical significance (Table 45). It is important to note that only 11 men returned for the 6 month follow-up in the CPAP group, and this small sample size may account for the lack of a significant change at 6 weeks post therapy. Interestingly all the patients within the CPAP group were asymptomatic of OSA at baseline and had a lower AHI than is routinely required for CPAP initiation (AHI < 10 events/hour).In addition 23 subjects were normo-glycaemic. Despite this, an improvement in insulin resistance was found at 3 weeks post therapy. Insulin like Growth Factor-1 (IGF-1) was also measured and was found to be significantly increased post 3 weeks and 6 months therapy compared to baseline levels (Table 45). The authors suggest that increased IGF-1 could be regarded as another indicator of improved insulin resistance [278].

Coughlin, SR et al [279] conducted a randomised, placebo-controlled, blinded, crossover trial after 6 weeks of CPAP therapy, to assess the metabolic and cardiovascular effects of this therapy. They used the HOMA-IR method on 34 obese non-T2DM males with OSA. After a full overnight polysomnography, with CPAP titration, the patients were randomised to receive either CPAP therapy, or identical sham therapy, for 6 weeks. Baseline data were collected at this point. All patients returned after 6 weeks for a repeat of baseline measures and the treatment groups were subsequently crossed over with a final follow up after a further 6 weeks. Participants were blinded to the treatment allocation and all informed that sham CPAP therapy set at a low pressure (< 1 cmH_2O) may provide some symptomatic benefits. The authors analysed this data in the context of the difference between data at the end of each treatment period instead of the change in individual baseline values. CPAP compliance was measured with a cut off for compliance at 3.5 hours/night of CPAP. Compliance was found to be significantly higher during therapeutic CPAP compared with sham CPAP treatment (Table 45). They observed a reduction in fasting glucose, fasting insulin and HOMA-IR after CPAP therapy that did not reach statistical significance. A subgroup analysis of subjects with an average use of CPAP ≥ 3.5 hours/night (n=23), also showed similar changes in these variables which remained non-significant. These results show a trend towards improved glycaemic control and perhaps the short follow-up period may not have shown the true impact of CPAP therapy on these variables. In addition the participants were non-T2DM individuals and it could be argued that an improvement in glycaemic control would not be expected in an already normo-glycaemic cohort. This group did find that systolic and diastolic blood pressure was reduced significantly with CPAP therapy, and suggest that although this is evidence for a reduction in sympathetic

activation, it may not sufficient to modify the degree of insulin resistance in these participants over this time frame and that it is likely that other mechanisms predominate in driving insulin resistance under these circumstances [279].

Trenell, MI et al [280] used the HOMA-IR method in 29 non-T2DM, obese males with severe OSA. They measured CPAP compliance and had a follow-up at 12 weeks. The sample was split into two groups: the CPAP compliant (≥ 4 hours/night, n=19) and the non-compliant (< 4 hours/night, n=10) group and analysed the data within the two groups. There were no significant differences in baseline characteristics between the two groups with respect to age, waist circumference, fasting glucose, fasting insulin, HOMA-IR, or severity of OSA. After 12 weeks of CPAP therapy there was a trend towards decreased fasting glucose levels and HOMA-IR from baseline in both the compliant and non-compliant groups (Table 45). In complete group analysis the authors observed a correlation between change in HOMA-IR and change in visceral adipose tissue, change in subcutaneous tissue volume and change in leptin levels (Table 45). These associations did not change when the analyses was limited to CPAP compliers only. Additionally they found that the change in leptin and visceral adipose tissue independently predicted changes in HOMA-IR (Table 45) and that change in leptin and subcutaneous adipose tissue also predicted changes in HOMA-IR (Table 45), again with no change when the sample was limited to CPAP compliers only. The authors concluded that change in abdominal adipose tissue is related to an improvement in glycaemic control independently of CPAP use in this cohort [280]. The authors suggest that the lack of a significant reduction in HOMA-IR could be due to the large influence that obesity has in this cohort, in addition to this

population not having T2DM [280]. Although 5 subjects had IFG (n=2 CPAP compliance group and n=3 CPAP non-compliers) and 2 were IGT (n=1 CPAP compliance group n=1 and CPAP non-compliers) the remaining were normo-glycaemic (n=22). As a result large changes in glycaemic control would not be anticipated within this cohort.

Patruno et al [281] evaluated the effects of fixed pressure CPAP compared to autoadjusting CPAP (APAP) on insulin resistance via the HOMA-IR method in 31 obese, non-T2DM patients, with severe OSA. APAP can be a cheaper alternative to CPAP therapy as it reduces the high titration costs associated with CPAP therapy [292], although the machines themselves are more expensive to purchase. APAP automatically detects flow limitation and starting at 4 cmH_2O/breath, it increases the pressure at a rate of 0.2 cmH_2O/breath until 'normal' air flow is achieved. All patients underwent a full overnight PSG for the titration of CPAP. The following morning baseline data were collected and participants were randomised into APAP (n= 15) or CPAP (n=16) treatment arms. CPAP and APAP compliance were measured with a follow-up at 3 months. Those that did not use the machine for $\geq$ 4 hours/night were not included in the final analysis. Baseline characteristics did not differ significantly between the two treatment arms with respect to age, BMI, AHI, HOMA-IR or glucose levels. At 3 months post-therapy there was no significant difference in compliance within the CPAP and APAP treatment arms (Table 45). Both devices delivered a similar level of pressure (Table 45). However, a significant reduction in HOMA-IR values were only observed in the CPAP group (Table 45). The authors speculated that this could be a result of the residual AHI and ODI observed in the APAP group, although these indices are still within what has been previously described as an

acceptable therapeutic range [293]. They suggest that these results could also be due to the presence of sleep fragmentation in the APAP group as it has been reported that APAP is associated with an increased number of arousals during sleep [294]. Nonetheless a significant reduction in the level of insulin resistance was reported for a compliant group of normo-glycaemic, but obese, patients post 3 months CPAP therapy.

Saini J et al [272] investigated plasma insulin and glucose profiles in 8 overweight-obese males with OSA pre- and on the first night of CPAP therapy. It is unclear whether these subjects had diabetes. Blood sampling was performed continuously throughout the two study nights using a peristaltic pump with an indwelling catheter. No significant difference was observed between the non-treatment and treatment nights of the study. Plasma levels of GH and IGF-1 were also investigated in this study and the group report that the observed increased GH secretion during treatment was not correlated to changes in plasma glucose or insulin levels (Table 45). They comment that SWS is involved in the treatment effects on GH secretion - for example CPAP therapy restores SWS and hence an increase in GH secretion was observed, which in turn may have an effect on glycaemic control. However, the short follow-up period (1 night) may not have been a long enough period to see this secondary hormonal effect of CPAP therapy.

Saarelainen S et al [273] used the clamp method in 10 obese, non-T2DM males. CPAP compliance was monitored and participants were followed-up at 3 months. Only 6 of the 10 subjects were CPAP compliant (≥ 4 hours/day) and only these were included in the final

analyses. This group observed a slight, but non-significant, improvement in glucose disposal rate (Table 45). A reduction in the mean fasting insulin levels was observed, but the statistical significance of this was not reported.

Brooks B et al [274] used the clamp method, measured HbA1c, fasting insulin and glucose levels to measure glycaemic control in 10 T2DM, obese subjects with moderate to severe OSA. Participants were followed-up at 4 months. CPAP compliance was measured and one subject was subsequently removed from the analyses. In the 9 remaining subjects a significant improvement of 28% in insulin responsiveness was observed following CPAP therapy (Table 45). This result was not attributed to weight loss as there was no significant change in BMI throughout the study. There was no significant change in fasting plasma insulin, glucose levels or HbA1c. It was reported that this lack of concomitant improvement in glycaemic control could be due to the improvement in insulin responsiveness being relatively modest given the severity of obesity and insulin resistance in these individuals. Additionally, there could be a 'plateau of a dose-response curve between glycaemia and insulin resistance', indicating further improvement of insulin resistance would not improve glycaemic state. Furthermore, 4 months of CPAP therapy may not be a long enough period of time to observe the maximum benefits of CPAP therapy, in addition to the complexities of accurately measuring CPAP compliance.

West, SD et al [295] conducted a randomised controlled trial, using therapeutic and sham CPAP, and used HbA1c levels, the HOMA-IR method and clamp method to assess

glycaemic control. Forty-two obese males with established T2DM were recruited. Baseline measures were collected 10 days before CPAP initiation and then patients were randomised, in a double blind fashion, into either therapeutic (n = 21), or sham CPAP (n = 22) therapy. All participants used the APAP device with the sham machines set to their lowest pressure in addition to the insertion of a flow restricting connector in the machine outlet and 6 extra 4 mm holes inserted into the collar of the main tubing, thus delivering a pressure of < 1 and > 0 cmH_2O [243]. APAP compliance was measured and patients returned after 3 months of therapy for a repeat of baseline measures. There were no significant differences between the therapeutic and sham treatment groups with respect to age, BMI, severity of OSA or HbA1c levels. Participants with poor CPAP compliance were included in the final analysis but 2 patients withdrew from the study. At 3 months follow-up there was a non-significant reduction in HbA1c levels in the therapeutic APAP group, but not in the sham APAP group (Table 45). In addition there was a non-stastistical reduction in HOMA-IR in both groups (Table 45). Clamp was performed in only 33 of the participants, although it is unclear what proportion were from each of the treatment arms. There was no significant difference in insulin concentrations over the last 30 minutes of the clamp between the therapeutic or sham groups at baseline (1405 vs. 1436 pmol/L (p=0.6), respectively) or at 3 months post therapy (1453 vs. 1481 pmol/l (p=0.8), respectively). However, a clear trend in increased insulin levels between baseline and follow-up was seen within each group, but the data were not analysed in this fashion. Data were analysed between groups at baseline and after treatment not pre- and post treatment within the APAP group whilst adjusting for Sham therapy. There was no significant difference in blood glucose levels over the last 20 minutes of the clamp at baseline between the two groups (p

= 0.5) or at 3 month follow-up (p = 0.5). Insulin sensitivity was also measured as the quantity of glucose metabolised (M) per unit of insulin (I). Insulin sensitivity improved in the therapeutic group and decreased in the sham group, however this change did not reach statistical significance (Table 45). Interestingly compliance was measured at >1hr/night of which 5 therapeutic APAP users did not comply and 3 sham CPAP users did not comply. The average use of APAP in the therapeutic group over the 3 months was 3.3 hours/night and 3.5 hours/night in the sham group. When the analysis was repeated including only the APAP compliant patients, the results remained insignificant although the data are not reported. The authors conclude that whilst APAP effectively treated OSA it did not improve glycaemic control or insulin resistance. However, the level of APAP compliance is questionable. In the therapeutic group the mean use of APAP of 3.3 (± 2.6) hrs/night would suggest that a number of subjects were in fact poor compliers and therefore the effect of CPAP therapy on these variables could be largely underestimated. Interestingly these results support those of Patruno V et al [281], who also reported that APAP did not improve glycaemic control or insulin resistance after 3 months of therapy. Both West and Patruno use the Autoset machines, products of Resmed although, unlike West, the participants in Patruno's study did not have T2DM. There is no measure in West's study to suggest that sympathetic activity was decreased. Additionally, the lack of significant improvement in West's study could be, as suggested by Patruno, due to sleep fragmentation that is associated with APAP use [281].

Harsch IA et al [275] used the clamp method to assess glycaemic control in 44 obese, non-T2DM subjects with OSA. Five subjects were found to have IFG at baseline. CPAP

compliance was measured and the follow-up was at 2 days and then at 3 months. The clamp study was performed at 0700 hours after an overnight stay in the fasted state. Apart from 9 subjects, all were CPAP compliant. The non-compliant were not included in the 3 month data analyses. After 2 days of CPAP therapy insulin sensitivity significantly improved for the whole sample (Table 45). At 3 months post-CPAP therapy, there was no further significant improvement in insulin sensitivity from 2 days post-CPAP. However, it did remain significantly improved compared to baseline values (5.75 ± 4.2 μmol/kg/min vs. 7.54 ± 4.84 μmol/kg/min, p=0.0001). When analysis was conducted for non-obese (BMI < 30 kg/m^2) and obese (BMI ≥ 30 kg/m^2) individuals, the results were similar to that of the whole sample. Further exploratory analyses using logistic regression (2 day post-CPAP data) showed that subjects with BMI < 30 kg/m^2 had a 7 - fold increased odds of experiencing an improvement in insulin sensitivity of > 0.58 μmol/kg/min than those with a BMI >30 kg/m^2.

Harsch AI et al [276] conducted a similar study in 9 obese subjects with established T2DM and moderate-severe OSA. The clamp study was performed after a 10 hour overnight fast. CPAP compliance was measured and the follow-up period was post 2 days CPAP and again after 3 months. No significant improvement in glycaemic control was observed after 2 nights of CPAP therapy in this sample. BMI did not change significantly from baseline to 3 months. However, at 3 months post-CPAP therapy, insulin sensitivity significantly improved in all subjects when compared to both baseline and 2 days post therapy (Table 45). The lack of immediate improvement in insulin sensitivity seen in this cohort compared to the previous study is thought to be due to this sample's insulin resistance being more

fixed and genetically determined and therefore more difficult to reverse than for a non-diabetic sample. Additionally improvements in insulin sensitivity may have already taken place with respect to response to medication (4 of the 9 patients were on metformin treatment). However, CPAP therapy improved insulin sensitivity in a non-diabetic and a diabetic population, with the latter requiring a longer duration for improvements to be observed.

Study	Sample size	Cohort	Method of analysis	Design	Follow-up	Results
Smurra et al [270]	16	OSA **Grp 1**: Abnormal metabolism (n=10), BMI: 33 ± 3.8kg/m^2 **Grp 2**: Non-T2DM (n=6) BMI: 26.6 ±3.5kg/m^2	Indirect calorimetry & OGGT (Grp 1) Clamp Study (Grp 2) HbA1c	Matched controls (n=6)	2 months	No significant weight loss observed **Grp 1** - Indirect calorimetry and OGGT post 2 months CPAP compliant 6.4 ± 0.8 hours/day No significant change in post load glucose levels (OGGT) No significant change in energy expenditure, glucose oxidation, insulin levels (calorimetry) **Grp 2** - insulin clamp post 2 months CPAP compliant 5.9 ± 2.5 hours/day No significant change in insulin sensitivity levels (clamp) No significant change in HbA1c levels
Chin et al [271]	40	OSA BMI:31± 4 11 T2DM 11 IFG/IGT	HOMA-IR OGTT	Repeated measures	1 + 6 months	**Post 1 month** CPAP compliant 4.3 ± 1.4 hours/day Significant reduction in BMI 29.2 ± 4.1kg/m2 from 30.1 ± 4.4kg/m2 , p=0.006 No significant change in indexes of insulin resistance – a trend towards improvement from 3.4 ± 2.2 to 2.8 ± 1.4, p=0.24 Insulin resistance index not reported at 6 months
Saini et al [272]	8	OSA BMI:32.7±2.3kg/m2	Venous sampling overnight (8hrs) - aliquoted at 10min intervals	Repeated measures	1 night	No significant change in plasma insulin 28.13 (95% CI: 17.44 – 86.36) vs. 32.76 (95% CI: (17.44 – 65.81) uU/ml, p=0.56 No significant difference in plasma glucose levels 1.11 (95% CI: 0.89-1.60) vs. 1.12(0.95 -1.37) g/L, p = 0.90
Saarelinanen et al [273]	6	OSA BMI:34.4kg/m2 Non-T2DM	Clamp study	Repeated measures	3 months	CPAP compliant 4 – 6.9 hours/day A trend towards increased glucose disposal was observed 3.22 mg/kg/min to 3.99 mg/kg/min A reduction in fasting insulin was observed 21.06 mU/L to 15.9 mU/L but the statistical significance was not reported.
Brooks et al [274]	10	OSA BMI: 42.7±4.3kg/m2	Clamp Study Fasting glucose Fasting insulin	Repeated measures	4 months	No significant weight loss A significant improvement in insulin responsiveness observed - glucose disposal improved

		T2DM	HbA1c			11.4 ± 6.2 vs. 15.1± 4.6umol/kg·min, p<0.05 No change in fasting insulin, fasting glucose levels or HbA1c post CPAP therapy
Harsch et al [275]	31	OSA BMI:32.7± 6.9kg/m2 5 = IFG 26 non-T2DM	Clamp study	Repeated measures	2 days + 3 months	CPAP compliance 5.2 ± 0.91 hours/day No significant changes in BMI or body fat % at 3 months **2 days** - Insulin Sensitivity Index (ISI) was significantly improved after 2days of CPAP ISI 5.75 ± 4.2 to 6.79 ± 4.91 umol/kg/min, p = 0.003 (n=40) **3 months** – No further significant improvement in ISI. But ISI at 3 months compared to baseline was significantly improved: ISI 5.75 ± 4.2 to 7.54 ± 4.84 umol/kg/min, p = 0.001 (n=31) Logistic regression results – Subjects with a BMI < 30 kg/m2 7 times more likely to experience improved insulin sensitivity of > 0.58 umol/kg/min than those with BMI > 30 kg/m2, p = 0.02.
Harsch et al [276]	9	OSA BMI:37.5 ± 5.6kg/m^2 T2DM	Clamp study	Repeated measures	2 days + 3 months	CPAP compliance 5.8 ± 1.2 hours/day No significant change in BMI post 3 months **2 days** - No significant increase in insulin sensitivity **3 months** - Significant increase in insulin sensitivity when compared to baseline ISI 4.38 ± 2.94 to 6.37* umol/kg/min , p= 0.021. And when compared to 2 days post CPAP p=0.015. ISI difference as seen above was markedly greater inpatients with lower BMI than more obese patients **Note**: Statistical analysis re-ran after removing one patient who lost 21kg due to a strict diet. BMI remain unchanged, ISI still insignificant after 2 days and remained significant after 3months (p=0.036) **Influence of BMI** Study group split into 2 groups: BMI ≤ 35 kg/m2 (n=4) and BMI > 35 kg/m2 (n=5): ISI did not differ between groups. After controlling for age, sex, presence or absence of HTN or AND no significant differences in changes observed in ISI. * Upper quartile

West et al [295]	40	OSA Males BMI: 36.7 (26.2-49.2)kg/m^2 T2DM	Clamp HOMA-IR HbA1c	APAP vs. Sham APAP	3 months	APAP compliance 3.3 hours/night sham APAP compliance 3.5 hours/night A trend towards improved insulin sensitivity in the APAP group (+1.7) and a reduction in the sham group (-5.7), p=0.2 Non significant reduction in HbA1c in APAP (- 0.02 %) or in the sham group (+ 0.1%), p=0.7 Non significant reduction in HOMA-IR results in APAP (-1.1) and sham group (-1.1), p=0.3
Lindberg et al [278]	28	OSA Males BMI:29.4 ±4.2kg/m^2 23 non- T2DM 5 T2DM	HOMA-IR Fasting Glucose HbA1c	Matched controls	3 weeks + 6 months	<u>3 weeks post CPAP therapy</u> CPAP compliant 5.2 ± 1.9 hours/ day No significant weight loss No significant change in fasting glucose or HbA1c. Significant reduction in fasting insulin levels in the CPAP group compared to the control group (Δ Fasting Insulin levels -1.8 (-4.1, 0.5)mU/l *vs*. 1.0 (-0.01, 2.0)mU/l, respectively (p= 0.022)). The level of insulin resistance was significantly reduced in the CPAP group compared to the control group (Δ Insulin resistance -0.6 (-1.3, 0.2) vs. 0.5 (0.1, 0.8), respectively, p=0.01. Increase in IGF-1 135 ± 48 vs. 116 ± 32 ng/ml, p=0.03 <u>6 months post CPAP therapy</u> CPAP compliant 5.7 ± 1.9 hours/day Data for change in fasting glucose or HbA1c was not reported trend towards a reduction in fasting insulin levels compared to baseline values (11.9± 13.4mU/l vs. 13.6 ± 11.2 4mU/l, p=0.22) Significant increase in IGF-1 from baseline 138 ± 56.9 vs. 116 ± 32 ng/ml
Coughlin et al [279]	34	OSA Males BMI:36.1 ±7.6kg/m^2 Non-T2DM	HOMA-IR Fasting glucose	CPAP vs. Sham CPAP	6 weeks	CPAP compliance was higher during the therapeutic CPAP vs. sham - 3.9 (0 -7.4) hours/day vs. 2.6 (0 – 7.5) hours/day, p < 0.1 Trend towards a reduction in glycaemic measures in CPAP group versus the sham – Fasting glucose -0.1 (95% CI: 0.3- 0.03) mmol/L Fasting insulin -2.6 (95% CI: -5.9 – 0.8) pmol/L HOMA-IR -0.6 (95% CI: -1.3 – 0.1)
Trenell et al [280]	29	OSA Males BMI:36 ± 8Kg/m^2	HOMA-IR Fasting glucose	Compliant vs. non-	3 months	Non significant trend towards decreased fasting glucose in the compliant (-2%) and non-compliant groups (-4 5). Non-significant trend towards a decrease in insulin resistance in the compliant (-1 %) and

		22 Non-T2DM 5 IFG 2 IGT		complian t CPAP users		non-compliant groups (-19 %). Correlation between change in HOMA-IR and change in visceral adipose tissue, change in subcutaneous tissue volume and change in leptin levels (r= 0.499 (p=0.011), r= 0.520 (p=0.021), and r=0.521 (p=0.008), respectively) The change in leptin and visceral adipose tissue independently predicted changes in HOMA-IR (r= 0.609, p=0.006) and that change in leptin and subcutaneous adipose tissue also predicted changes in HOMA-IR (r=0.726, p=0.001)
Patruno et al [281]	31	OSA BMI:36.5 ± 3.9kg/m^2	HOMA-IR Fasting glucose	CPAP vs. APAP	3 months	CPAP arm were compliant 6 ± 1 hours/day with a titration of 10.8 ± 16 cmH_2O APAP arm were compliant 6.2 ± 0.8 hours/day with a titration of 9.45 ± 1 cmH_2O A significant reduction in HOMA-IR values was observed in the CPAP group (5.2 ± 1.7 to 3.75 ± 1.2, p<0.001) but not the APAP group (4.4 ± 1.8 to 4.2 ± 1.4 (p= >0.05).

Table 45: Summary of studies using HOMA-IR, HbA1c and clamp methods

3.4 Discussion

The objective of this systematic review was to determine from the existing literature whether CPAP therapy, the gold standard treatment for OSA, has an effect on glycaemic control. A meta-analysis could not be undertaken due to the large degree of heterogeneity between studies. A recent Cochrane systematic review of CPAP therapy reported improved parameters of OSA including AHI, O_2 desaturations, blood pressure, measures of daytime sleepiness, cognitive function and quality of life [213]. However, the results of the studies presented here are as contrasting as the differences in the study designs. To draw a conclusive argument for or against an improvement in glycaemic control following CPAP therapy is not possible due to the number of limitations in study designs. Figure 22 outlines the key elements that need to be considered for future studies investigating the impact of CPAP therapy on glycaemic control.

The data presented here are from studies that have been conducted over 14 years (1993 to 2007 [268, 269, 278-281, 295]). There have been considerable developments in CPAP technology over this period, including the development of quieter machines, automated pressure control and a variety of more ergonomic masks. Therefore, CPAP compliance is likely to have improved as a result of less disturbance (for example reduction in the noise produced by the pump), as well as more comfortable pressure algorithms and masks for the patient to choose from. In a number of these studies CPAP compliance was not reported [267-269, 272, 274, 280]. Babu et al [248] reported that CPAP compliance had a significant effect on one hour post-meal glucose levels

compared to a non-compliant group. This is an important parameter that should have been objectively measured in all of these studies.

In addition there is a large amount of variability between what was considered an acceptable level of CPAP compliance. One study classified the acceptable level as ≥ 1 hour/night [295], compared to the majority which classified an average nightly use of CPAP ≥ 4 hours [248, 270, 271, 273, 275, 276, 278, 280, 281] as acceptable. The 4 hour/night cut-off is fairly arbitrary and based on the resolution of significant daytime sleepiness [214]. It may be that cardiovascular risk factors are more related to disorders of cardiovascular stress (high arterial desaturations, raised blood pressure and heart rate) and therefore a higher cut off for CPAP compliance may be required to reduce cardiovascular risk factors than that required for daytime sleepiness. An acceptable level of CPAP use needs further investigation to be established and adhered to in future studies with respect to reducing cardiovascular risk factors, including hyperglycaemia/hyperinsulinaemia. The inclusion of non-compliant patients and/or low compliance cut-off levels in the final analysis could lead to an underestimation of the true beneficial effects of CPAP therapy.

Only 3 of the 16 studies had a true control group [267, 279, 295], while in the remaining studies patients were used as there own controls. When this is considered, along with the small sample sizes in a number of these studies, doubt is raised over their robustness and whether the studies offer enough statistical power to be accurate. Another questionable factor is the difference in the

length of the follow-up periods from pre-CPAP to post-CPAP data collection. Studies ranged from 1 night of CPAP therapy [272], to data collected at 6 months post-CPAP therapy [267].

The recent study by Patruno et al [281] raises another issue with regard to the choice of CPAP machine used. This group found that while the fixed pressure CPAP machine offered beneficial effects in terms of reducing cardiovascular risk factors (blood pressure and glycaemic control) after 3 months of therapy, this was not true of APAP. This is concerning as their data support two other studies reporting that APAP therapy did not reduce systolic or diastolic blood pressure [296, 297], which is an established benefit of the fixed pressure CPAP [298]. West et al [295] also reported no beneficial effect of APAP therapy on either blood pressure or glycaemic control, further supporting the findings in Patruno's study. APAP therapy is becoming more widely used due to its economic advantage in reducing the titration costs that are required for CPAP therapy [293]. Although it is effective in reducing daytime sleepiness and parameters of OSA (AHI/ODI etc) [293, 299] future studies are required to determine its effects on cardiovascular risk factors, such as glycaemic control.

The follow-up period is also an important point to consider, particularly since there is likely to be an adjustment period for the first time CPAP user. A number of patients will require time to get used to wearing a mask throughout the night as well as the noise of the pump. In addition there is also the time required to repay the chronic sleep debt and allow the sleep pattern to normalise. People with OSA are in a state of chronic sleep deprivation upon initialization of CPAP therapy.

The first phase of sleep to be repaid is SWS followed by REM. One study [272] did examine SWS and found that this significantly increased with CPAP therapy. However, no comparison was made to that of the 'normal' subject. Therefore one possible explanation for these conflicting results is that the follow-up periods were not long enough to account for this possible adjustment period and therefore the results may not reflect the true long term benefits of CPAP therapy. Improvements in glycaemia may emerge after prolonged use of CPAP therapy.

Baseline characteristics of the sample populations differed widely between studies, both in terms of glycaemic status (normo-glycaemic to T2DM), level of obesity and severity of OSA. It is plausible that no improvement in glycaemic control would be expected in those subjects that are normo-glycaemic at baseline and therefore the clinical significance of investigating glycaemic control in these subjects is questionable. This is supported by the fact that the majority of improvements were observed in those subjects who had impaired glycaemic control at baseline [248, 271, 274, 276]. The level of glycaemic control in people with T2DM or subjects with prediabetes in conjunction with the method used to determine glycaemic change could potentially have a large impact on the results reported in these studies. Monnier and colleagues [300] recently reported a progressive shift in the respective contributions of fasting and postprandial hyperglycaemia when patients progress from moderate to high hyperglycemia (determined by HbA1c levels). They report that that contribution of postprandial glucose excursions, on overall glycaemic status, are predominant in patients with moderate T2DM (non-insulin treated) in comparison to those with worsening diabetes where the contribution of fasting glucose is higher [300]. Therefore measuring insulin resistance via the HOMA-IR is questionable in patients with

established T2DM given that fasting glucose levels are used for calculating the level of insulin resistance.

Additionally fasting glucose levels were observed to be playing a major role in glycaemic status when HbA1c levels are $\geq$ 8.4% and postprandial hyperglycaemia predominating when HbA1c levels are < 8.4% [300]. Therefore it is important to consider the level of diabetes control in participants before the method of assessing glycaemic control is selected. For example in poorly controlled people with T2DM (HbA1c > 8.4%), fasting glucose levels would be a more accurate measure of glycaemic control as opposed to postprandial glucose levels. Weight loss throughout the study period may be another influencing factor. The results were conflicting as only two of the eleven studies reported significant weight loss throughout the study period [267, 271]. Nonetheless, this is still a significant finding even if changes in glycaemic control are secondary to weight loss as a result of CPAP therapy. It still supports the hypothesis that this treatment improves glycaemic control, if only as a secondary outcome to weight loss. Interestingly, Harsch et al [275] found that insulin sensitivity showed the greatest improvement in the less obese subjects (BMI < 30 kg/m^2) and concluded that the likelihood of observing an improvement in insulin sensitivity is greatest in those who are not obese.

The studies presented here are not truly comparable, due to the large difference in the sample size. However, the data does suggest that CPAP therapy may have an influence on glycaemic control in those subjects that are obese with impaired glycaemic control [248, 271, 274, 276].

Again the crucial factor to consider is the method used to measure glycaemic control. It seems apparent that simple fasting plasma glucose levels do not provide a sensitive enough method for assessing such a complex physiological impairment. For example, two studies reported changes in post-load glucose levels post-CPAP therapy, although no changes were observed in the same participants for fasting blood glucose levels during the OGTT [248, 271]. Results from the clamp studies found significant improvements in insulin sensitivity in three studies [266, 274, 275] with one small study of 6 participants [273] reporting a non-significant improvement in glucose disposal. This suggests that this method of assessing glycaemic control is more sensitive. The lack of change in fasting glucose levels could also be due to a short follow-up period. Improvements in fasting plasma glucose levels may be secondary to improved insulin sensitivity and therefore alterations may only be seen after prolonged CPAP therapy use. Conversely, two studies did report an increase in fasting plasma glucose and insulin levels. However, these studies had a small sample size (5 to 6 participants) and did not report CPAP compliance or have a control group. Thus the true significance of these findings is questionable [268, 269].

The strengths of this systematic review include the wide search strategy performed on multiple databases and the involvement of two independent reviewers for the study selection and data extraction phases. Limitations include our inability to undertake a meta-analysis due to the nature of the studies presented here, and the vast differences in methodology used to measure glycaemic control. Additionally, the primary outcome measure was not glycaemic control in some of the studies.

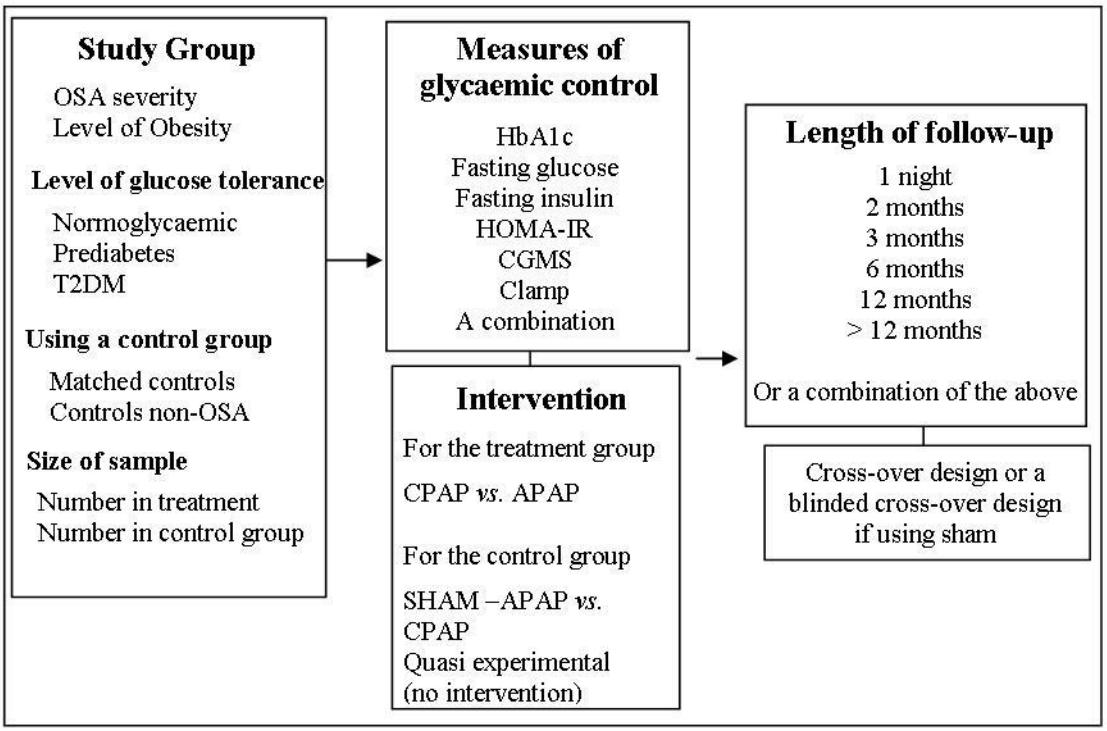


Figure 22: Key considerations in study design for future studies

3.5 Conclusion

In summary, the results presented here are inconclusive with respect to the effect of CPAP therapy on glucose tolerance in subjects with OSA. This can be largely attributed to the differences in each of the study designs. A number of these studies do show that CPAP therapy is beneficial in obese glucose intolerant subjects, and that even though this may be secondary to weight loss, it may still be a significant benefit of this therapy. Therefore the screening and treatment of obese and glucose intolerant individuals for OSA could potentially have large implications for the prevention of progression to T2DM and in reducing CVD risk. However, in order to conclusively answer the question of whether glycaemic control is improved by CPAP therapy in subjects with OSA, further research is required. It is essential that these studies have larger, better defined sample populations (with respect to baseline glycaemic status), and use sensitive, accurate and detailed methods of measuring glycaemic control. For example CGMS data would provide more information about the individual's level of glycaemic control than a fasting glucose results would. It is important that control groups are used to adjust for possible placebo effects. The follow-up periods of such studies should be longer (≥ 3 months) when considering the complex nature of glycaemic control. Fixed pressure CPAP machines should perhaps be used instead of APAP. Finally it is essential that CPAP compliance is measured objectively, to ensure patients are using the machine and to facilitate review and results against actual effective treatment duration.

Chapter Four

The Leicester Sleep and Sugar Study

4.1 Introduction

Obstructive sleep apnoea (OSA) is a sleep disorder characterised by recurrent collapse of the upper airway, resulting in airway occlusion and subsequent asphyxia. The repeated cessation of breath causes the patient to awaken enough to reinitialise breathing, although the patient will not generally fully awake from sleep. This results in the fragmentation of sleep as it may occur hundreds of times per night thus rendering the patient in a state of chronic sleep deprivation. Patients may suffer from excessive daytime sleepiness as a result of these recurrent apnoeas. Additionally, increased sympathetic activity has been observed in OSA patients [301-303], thus increasing the likelihood of insulin resistance and cardiovascular disease [304], due to the association between heightened sympathetic drive and metabolic abnormalities [305]. This disorder affects approximately 4% of adult males and 2% adult females [197] and is associated with T2DM [225], MetS [208] and CVD [221]. This is a population at risk of early mortality, hence the identification and management of this high-risk group is potentially an important aspect of cardiovascular disease prevention.

OSA is also independently associated with insulin resistance [219]. Insulin resistance is a key feature of both the metabolic syndrome and T2DM. Chronic low-grade inflammation is a potential cause for the development of insulin resistance [61]. It is a recognised pathological feature in the development of T2DM and CVD and is evident in subjects with the MetS. Pro-inflammatory cytokines are found to be elevated in the sleep deprived state and it is known that glucose tolerance is directly affected by sleep deprivation [11]. OSA therefore provides a suitable model for investigating such relationships. The ‘gold standard’ treatment for OSA is continuous positive airway

pressure (CPAP) therapy. A CPAP device administers a continuous stream of air that splints open the airway thus preventing airway occlusion. Therefore the effect of restoration of sleep on glucose tolerance and inflammation can be investigated by comparison of the pre-treatment and post-treatment states for CPAP therapy.

The effects of CPAP therapy on glucose tolerance in people with OSA are currently ambiguous, as discussed in chapter 3. A number of studies have reported an improvement in insulin sensitivity post-CPAP therapy [248, 274-276, 278, 281] . However, other studies have reported no beneficial effect of CPAP therapy on glycaemic control in people with OSA [267, 270-272, 277, 279] . These contrasting findings can be largely attributed to differences in study design. Subsequently future intervention studies need to consider the inclusion of:

- Larger sample sizes
- Longer follow-up period (≥ 3 months)
- Objective measures of patient compliance to CPAP therapy
- Sensitive and robust tools for assessing glycaemic control

This pilot study was designed with the aim of addressing some of the issues highlighted in Figure 22. The purpose of this research was to further investigate if sleep restoration via CPAP therapy has a positive effect on glucose tolerance in subjects with T2DM. Moreover, the effect of sleep restoration on the levels of recognised biological markers of inflammation was also investigated.

4.1.2 Aims and hypotheses

The aim of this study was to determine if glycaemic control improves in obese people with established T2DM and moderate to severe OSA, following treatment with CPAP therapy. The following hypotheses were made:

- Glycaemic control (as assessed by both HbA1c and continuous blood glucose monitoring system (CGMS) will improve in people with T2DM treated with CPAP therapy
- In those treated with CPAP therapy:
    1. Levels of inflammation will decrease
    2. Measures of obesity will improve
    3. Systolic and diastolic blood pressure will decrease
    4. Daytime sleepiness and mood will improve

4.2 Study design

4.2.1 Study population

The target sample size for this pilot study was based on the sample size reported in a previous study by Babu et al [248]. Within this study, 25 people with T2DM were treated with CPAP therapy and significant changes in postprandial glucose levels were reported (Chapter 3). A control group was included in the current study as this was a limitation of a number of previous studies (Chapter 3). A sample size was calculated using the data from Babu et al [248] by Joanne Dick, Unilever Statistics Group, Colworth Science Park, Sharnbrook, UK. A sample size of 25 pairs would provide this trial with 80% power to identify a change in HbA1c of 1.3% from baseline at the 5%

significance level (Appendix IV) at 6 months. Therefore, the target was to obtain a complete data set for 50 patients - 25 of whom would be in the treatment group and 25 in the non-treatment group.

Although for a pilot study a sample size based on obtaining a statistically significant difference is not technically required, given that a pilot study is predominantly conducted in order to establish a sample size for the clinical trial that would follow, we decided to include one. The sample size was determined for logistical reasons as apposed to detecting a significant reduction in HbA1c *per se*. For example it was essential to factor in an estimated recruitment time scale in addition to ensuring that financial requirements could be met for this study i.e. glucose sensors, assay kits and the cost of other such consumables. Furthermore, our local ethics committee recommends that a sample size is determined even for small trials such as this in order to assess whether our recruitment strategy is realistic, the relevant funding is in place and that the trial is completed within the estimated time course.

Quasi-experimental study designs lack random assignment and can be viewed as a poor investigative tool because of this. However, randomisation was considered an impractical approach given the lack of standardisation in the methods used for creating 'sham CPAP' inaddition to ethical concerns and the potential danger, for example road accidents, of denying treatment for those identified with moderate-severe OSA. In using a non-equivalent control group we cannot eliminate the possibility of confounding bias and have a limited ability to draw causal inferences. However, to reduce bias we identified and statistically controlled for the potential effects of confounding variables

in the between group analyses (ANCOVA) in addition to conducting repeated measures analyses on both the treatment and non-treatment groups thus strenghing any trends observed in the treatment group.

4.2.2 Inclusion and exclusion criteria

Inclusion criteria

Treatment group

- People with established T2DM (> 3 months)
- Newly diagnosed moderate to severe OSA (AHI ≥ 15 events/hour) at the pre-treatment stage
- BMI ≥ 30 kg/m^2.

Non-treatment group

- People with established T2DM
- BMI ≥ 30 kg/m^2
- Non- OSA (AHI ≤ 5 events/hour).

Exclusion criteria

- People with excessive daytime sleepiness and an AHI ≥ 5 < 15
- People without T2DM.
- Newly diagnosed people with T2DM (< 3 months)
- People previously diagnosed with OSA who were receiving CPAP or had declined treatment previously.
- People with a BMI< 30 kg/m^2.

4.2.3 Recruitment

All patients were identified using the Diabetes Clinic database at University Hospitals of Leicester. Patients were systematically approached according to the date of their next appointment with the Diabetes Clinic. Patients with imminent appointments were approached verbally via their clinician or research nurse. All potential participants approached in this manner received a patient information sheet and invitation letter, in addition to background information from the healthcare professional. Patients without imminent appointments or who failed to attend a clinic were posted an invitation with a patient information sheet, reply slip and pre-paid reply envelope. Patients who indicated an interest in taking part in the study were contacted by telephone and an appointment scheduled. If no response was received from patients after two weeks of the initial mailing then a second identical mailing was sent out. All patients who indicated no interested in participating in the study or who did not respond to the second mailing were removed from the invitation list and were not contacted again with respect to this research project.

4.2.4 Data collection (figure 23 patient flow)

A flow diagram summarising the study design is provided in Figure 23.

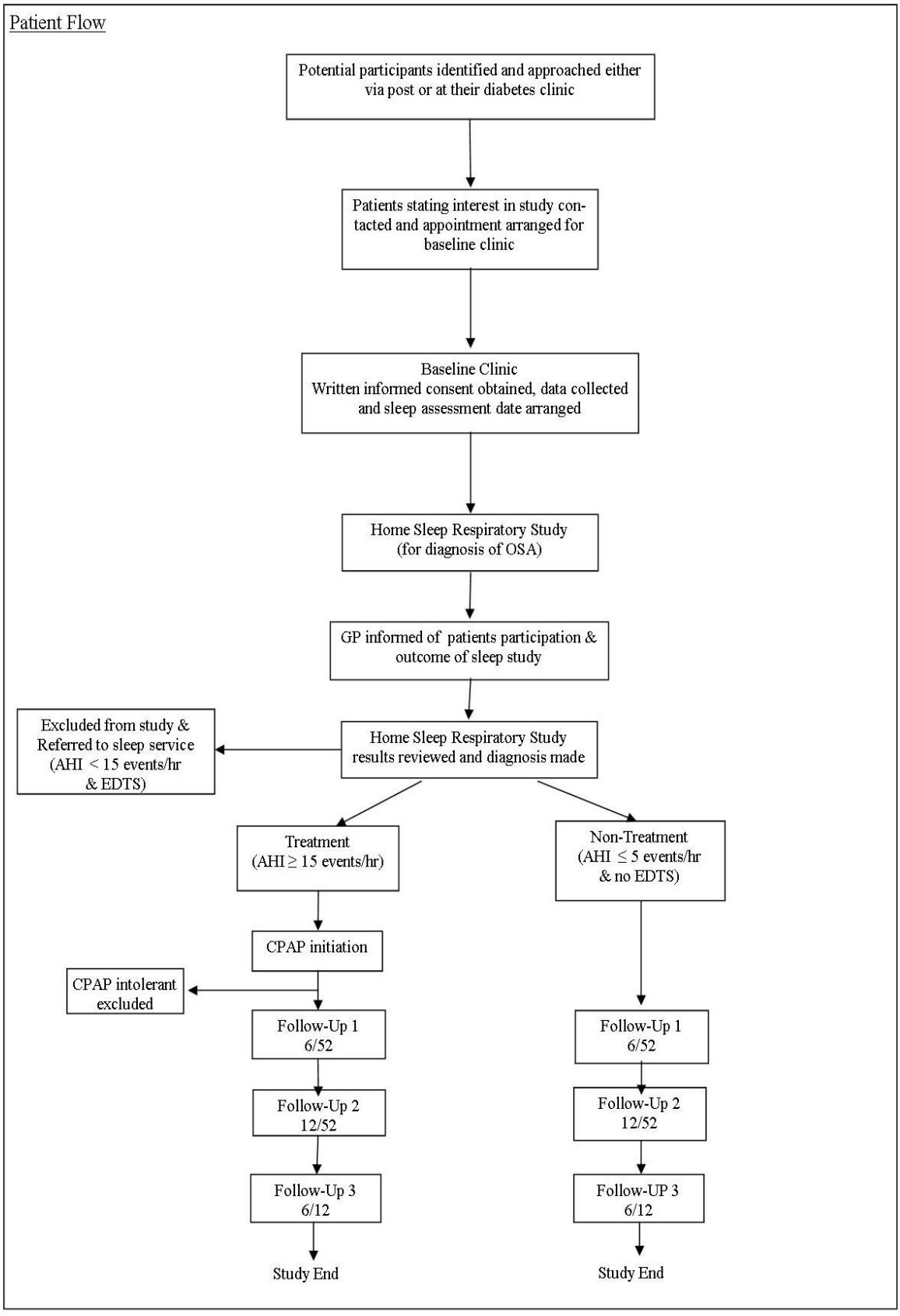


Figure 23: Patient pathway through the Leicester Sleep and Sugar study

At the baseline data collection visit, informed written consent was obtained and participants completed questionnaires and biomedical data and blood samples were collected for the quantification of biological markers of inflammation (Table 46).

Biomedical data	Biomarkers	Questionnaires
HbA1c	TNF-α	Berlin sleep Questionnaire
72 hour continuous blood glucose monitoring (CGMS)	IL-6	Epworth sleepiness scale
Fasting glucose	CRP	Quality of Life (WHOBREF)
Weight	Leptin	Index of Physical activity (IPAQ) short form
BMI	Adiponectin	Hospital Anxiety and depression score (HADS)
% Body fat	Insulin	Health questionnaire –medical and family history and current medication
Waist circumference	Isoprostanes	
Waist-hip ratio		
Neck circumference		
Systolic and diastolic blood pressure		

Table 46: Data collected during LSS

This study was comprised of two phases a screening phase and a treatment phase. In the screening phase patients attended the initial baseline data collection clinic and provided informed written consent. Following this, the recruited participants undertook a home respiratory sleep study for the clinical diagnosis of OSA (Embletta PDS, Embla Broomfield, USA see section 4.2.4). Participants attended the Sleep Laboratory at Leicester General Hospital to be set-up with the Embletta home sleep assessment kit. The results were all assessed by one experienced sleep disorders consultant who was blinded to all other patient data. Participants diagnosed with moderate to severe OSA became part of the treatment group. Those that were diagnosed with mild - non OSA

became part of the non-treatment group. Both groups then took part in the second phase of the study. This required each participant to attend three follow-up clinics (after initiation of CPAP therapy in the intervention group), where all baseline measurements were repeated. All participants' general practitioners were informed that they had been screened for OSA, of the results and whether they were continuing to participate in the study.

All participants diagnosed with OSA or those that required further investigation due to significant excessive daytime sleepiness were registered at and invited to attend the Leicester Sleep Service for a consultation. Prior to CPAP initiation identified patients underwent an unattended in-home monitoring using an auto-titrating CPAP device (Respironics UK, Ltd). The information from the autoset machine allowed the sleep technician to determine the CPAP pressure requirements for each individual. The pressure required differs between subjects, the aim being to achieve a level that maintains upper airway patency, oxyhaemoglobin saturation and sleep continuity in addition to be being comfortable for the patient. A ResMed S8 escape machine (ResMed UK, Ltd) was set at the appropriate CPAP level for each subject and therapy was continued at a fixed pressure.

Once CPAP therapy was initiated, the dates for the follow-up data collection visits were arranged. The follow up visits for both arms of the study took place at 6 weeks, 12 weeks and 6 months. Additionally, compliance to CPAP therapy was recorded objectively at each visit via the compliance meter built into the ResMed S8 escape CPAP machine.

4.2.4 Home Sleep Assessment and diagnosis of OSA

The Home Sleep Respiratory study kit is a product of Embla Systems (Embla Broomfield, USA) – Embletta PDS. It is an ambulatory polygraphic device that is fully compliant with CMS and American Academy of Sleep Medicine recommendations for portable monitoring [306]. Each participant was set-up with this portable kit. Four external sensors were attached between the patient and the device so that a number of sleep parameters could be recorded (Figure 24). These included;

Respiratory effort: determined by two respiratory effort sensors. One placed around the abdominal and one around the thoracic region of the patient which was then connected to the 'proxy' (a five prong port connected to the Embletta).

Snoring and air flow: determined by a nasal cannula connected via a Luer Lock channel on the top of the device to a pressure sensor.

Oxygen saturation levels: determined by the Embletta pulse oximeter. This was placed and secured onto the index finger of the patient with the other end connected to the 'proxy' attachment.

Additionally, the device has internal sensors including a three-dimensional gravitational sensor for measuring body position and movement.

Figure 24: The Embletta pds Home Sleep Respiratory study kit

The participant then returned home to follow their normal evening routine and sleep. The following morning, the kit was returned to the hospital and the data were uploaded onto a password protected computer. The software 'Somnologica for Embletta'(Medcare Flaga, 2004, Iceland) produced a sleep report for each patient. A sleep expert then used this report to clinically diagnose the patients in conjunction with the results from the screening questionnaires (the Berlin Sleep Questionnaire and the Epworth Sleepiness scale, Appendix I).

Diagnosis of OSA was based on the American Sleep Disorders Association criteria (Table 47). Further information on the diagnosis of OSA can be found in Chapter 1 section 1.5.2.

Diagnosis of OSA is made if the Subject meets either A or B and C of the following criteria
A : **Excessive daytime sleepiness that cannot be explained by other causes**
B: At least one of the following symptoms that cannot be explained by other causes • Choking/gasping for breath during sleep • Recurrent arousals from sleep • Awakening un-refreshed from sleep • Daytime fatigue • Impaired concentration
C: Results from overnight monitoring • ≥ 15 AHI **or** • Reduction in airflow < 50 % that is associated with a > 4 % O_2 desaturation or an arousal lasting ≥ 10 seconds

Table 47: American Sleep Disorders Association criteria for the diagnosis of OSA (adapted from [198])

4.2.5 Continuous blood glucose monitoring

At each of the four data collection points, all participants underwent continuous blood glucose monitoring for a period of 72 hours using the MiniMed Continuous Glucose Monitoring System (CGMS; Medtronic Diabetes, Northridge, CA). This device measures interstitial fluid glucose levels every 10 seconds from which the mean over 5 minutes is calculated and then stored. It has a detection range of 2.2-22.0 mmol/l and requires the patient to input capillary blood glucose readings at least 4 times a day for the purposes of calibration of the device. The device probe was inserted into the subcutaneous layer of the abdomen and the patient was shown how to enter their blood glucose readings and provided with written instructions. Each participant received a new capillary blood glucose monitoring system (Ascensia Contour Blood Glucose Monitoring System, Bayer HealthCare LLC, USA) and was asked to use this in place of their normal device. Additionally, those patients that were confident with the CGMS

system were asked to input data including when insulin was administered or any other diabetes related drug, when they engaged in exercise or when they had a meal.

4.2.6 Quantification of biological markers of inflammation

See table 46 for biomarkers quantified and section 2.2.5 for methodology.

4.2.7 Ethics and ethical considerations

Ethical approval was granted for this study by the Leicestershire, Northamptonshire and Rutland Research Ethics Committee. Patients were recruited from the diabetes clinic at the University Hospitals of Leicester (UHL) which provided access to a large number of people with T2DM. People of South Asian origin make up 27.69% of the population within Leicester, with 25.73% being of Indian origin [307]. Leicester has the largest Indian population of any local authority area in England and Wales [307] thus we had access to a large multiethnic population. Patients participated in this study only after providing written informed consent.

All patients diagnosed with OSA who did not wish to continue in the research study continued to receive care for any sleep disorder in the routine manner. In addition, any patient that had an AHI < 15 but were symptomatic of excessive daytime sleepiness were removed from the study and referred to the Sleep Clinic at Leicester for further investigation and treatment where necessary.

A Hospital Anxiety and Depression Scale (HADS) was administered to all participants at each time point of the study. A score of ≥11 indicates a moderate to severe case of depression [308]. Patients who scored ≥11 were contacted and asked if they wished this information to be passed on to their general practitioner in order for them to receive appropriate care. The diabetes care of any patient choosing not to participate in the study was not affected.

4.2.8 Statistical analyses

All statistical analyses were conducted using SPSS for Windows version 16 (SPSS; Chicago; IL). Data are either presented as mean ± SD, or in the case of categorical data, as number and percentage in each category, unless otherwise stated. Independent t-tests were used for between group analyses of study characteristics at baseline. Logistic regression was used to determine if any of the baseline variables were significant predictors of moderate to severe OSA with results reported as odds ratio and 95% confidence intervals. Spearman's Rank Order correlation was used to determine if glycaemic measures were correlated with parameters of OSA. One-way repeated measures analyses of variance was used to determine if there were any significant changes within groups between the four time points of the study (or three time points for CGMS data). These results are reported as means ± SD and change from baseline (baseline result – follow-up result). Each follow-up was compared with the baseline data in these unadjusted models. Following this, one-way analysis of covariance (ANCOVA) was used to determine if there were any significant changes in each variable at 6, 12 or 24 weeks post CPAP therapy between the treatment group and the non-treatment group. Prior to the ANCOVA the change in each variable at each time

point was calculated by subtracting the baseline variable from each of the follow-up variables. There are unadjusted and adjusted (for age, gender, ethnicity and baseline variables) models reported for these analyses.

All CGMS data were exported into Microsoft Excel. The mean proportion (%) of time spent either above, below or within the glycaemic ranges stated below were calculated for each patient over the recording period and adjusted for 24 hours (Figure 25).

- Above target ≥ 10.0 mmol/L
- Within target 4.0 mmol/L - 9.9 mmol/L
- Below target ≤ 3.9 mmol/L

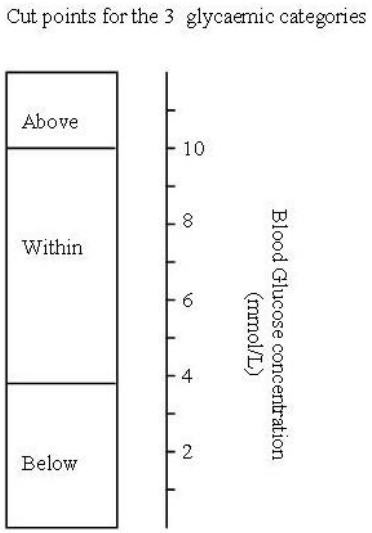


Figure 25: Cut points for 3 glycaemic categories

The number of hyperglycaemic events (≥ 10.0 mmol/l for a duration of ≥ 10 minutes) and hypoglycaemic events (≤ 3.1 mmol/l for a duration of ≥ 10 minutes) adjusted for 24 hours were determined, in addition to the average length of time (minutes) spent in each category. There is limited published work on CGMS data these ranges were chosen based on a recent study conducted by Bode et al [309].

The definition of a hypoglycaemic event is currently a matter of debate within the literature and between clinicians as discussed in a recent review [310]. Plasma blood glucose levels of <3.9 mmol/l are considered hypoglycaemic because at levels lower than 3.9 mmol/l physiological responses are evident including a reduction in endogenous insulin and an increase in pancreatic glucagon secretion [311], hence this threshold was used for the analyses of proportion of time spent below. Furthermore, blood glucose levels above 10 mmol/l are associated with T2DM complications thus the main objective in diabetes management is to keep blood glucose levels below this threshold with the aim to maintain blood glucose levels around 7 mmol/l in such patients (Section 1.3.6).

However, blood glucose levels do drop below 3.9 mmol/l in healthy individuals without any clinically significant outcomes, particularly in women [310] and therefore this definition potentially leads to an over estimation of clinically significant hypoglycaemic events. Blood glucose levels of <3.1 mmol/l result in autonomic (sweating or shaking) and neuroglycopenic symptoms (confusion, incoordination, speech difficulties) [312, 313]. A number of studies have used this definition for determining hypoglycaemic events in people with diabetes mellitus [314-316]. Additionally a more robust biochemical definition has been used within the literature to further avoid defining healthy people as hypoglycaemic [314, 317, 318] with a lower threshold of <2.2 mmol/l.

It is plausible that the first definition used to define a hypoglycaemic event would not only captured hypoglycaemic events but normoglycaemia too and thus influence the

results. In order to rule this out a second analysis was run using the stricter definition for a hypoglycaemic event ≤ 2.2 mmol/l for a duration of at least 20 minutes. Again repeated measures were used for this within group analyses. The mean ± SD is given for each of the time points in addition to the percentage change from baseline.

Furthermore each participants' data file was split according to daytime hours (0600 - 2159) and nighttime hours (2200 - 0559) and the same statistical tests performed to determine if there were any significant changes occurring within these separate time frames. All subsequent data were entered into SPSS and analysed using the same statistical tests described above.

Missing data

Missing data were not entered into the final datasets with no recoding used for cells with missing data. The function '*Exclude cases pairwise*' was used in each of the statistical tests performed to deal with any missing data therefore the cases that have missing data that were required for that specific test were excluded.

4.3 Results

4.3.1 Final study population

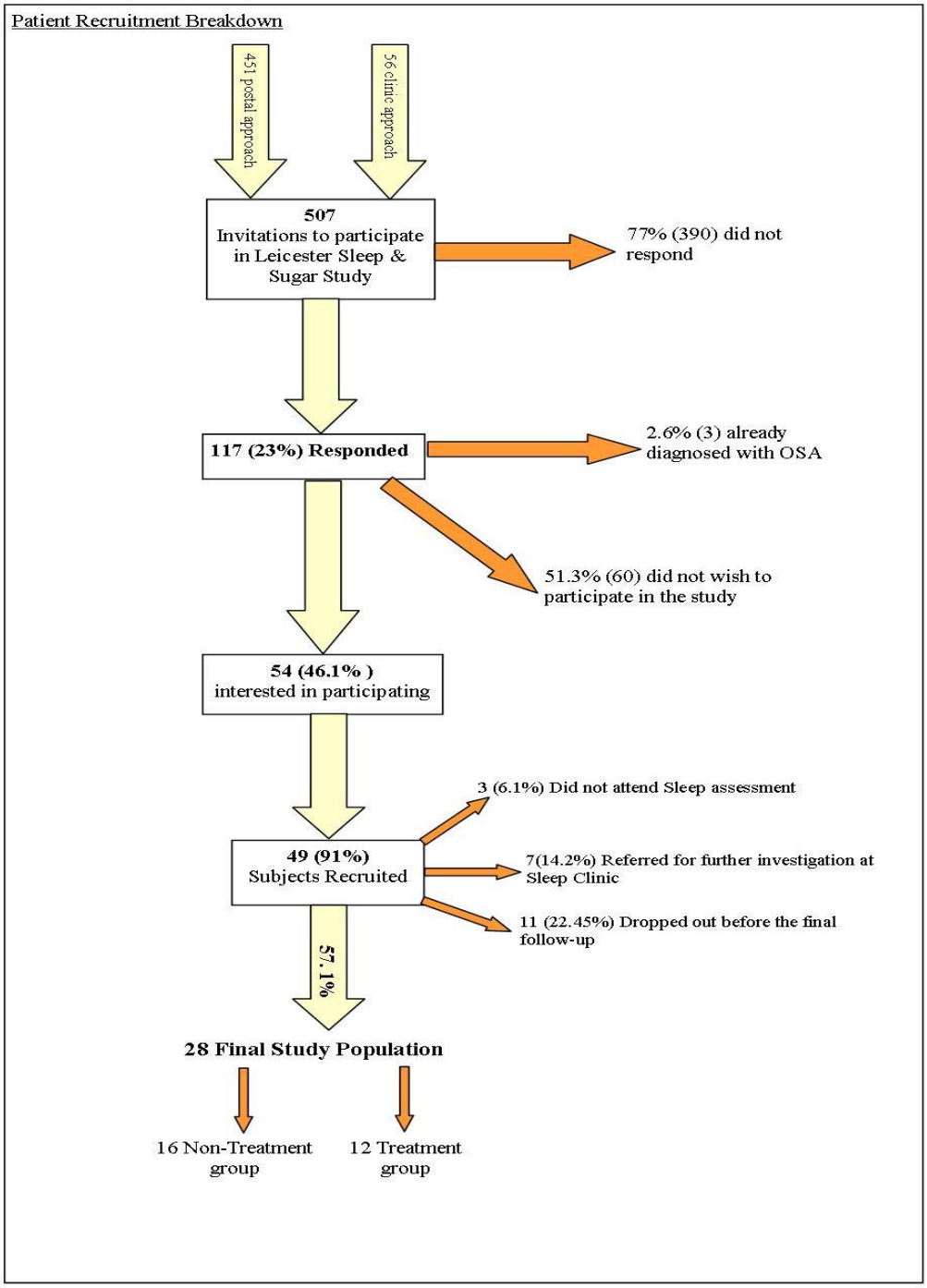


Figure 26: Patient recruitment breakdown

Response rate

Five hundred and seven people with T2DM and a BMI ≥ 30 kg/m^2 from secondary care were invited to participate in this study - 451 were invited by a postal invitation and 56 were approached at their 6 month diabetes review at a clinic by a health care professional.

One hundred and seventeen (23%) people responded to the invitation; 51.3% (60) of respondents did not wish to participate, 2.6% (3) had been previously diagnosed with OSA and were receiving CPAP therapy and were therefore excluded. Forty-six percent (54) of respondents were interested in participating of which 91% were successfully recruited onto the study. Twenty-five of those recruited had been approached within the diabetes clinic and 24 had been invited via the post. The clinic approach was significantly more successful than the postal approach in recruiting patients onto this screening and treatment study (44.6% vs. 5.3%, p<0.0001).

4.3.2 Final study population & baseline characteristics

Forty-nine obese (BMI > 30 kg/m^2) persons with T2DM were recruited onto the Leicester Sleep and Sugar Study between November 2006 and April 2008. The characteristics of this cohort prior to the home sleep respiratory study are displayed in Table 48.

Variable	Mean ± SD or percent
Age	56.9 ± 10.3 (range 35 -77 years)
Gender (Male)	19 (38.8%)
Ethnicity	Caucasian 40 (81.6%)
	South Asian 5 (10.2%)
	Other 4 (8.2%)

Table 48: Leicester Sleep and Sugar study (LSS) whole cohort characteristics (results displayed as either mean ± SD or n (%))

Three (6.1%) of these patients did not return for the home sleep respiratory study and therefore a total of 46 participants were screened for OSA. The prevalence of moderate to severe OSA (AHI >15 events/hour) in this cohort of obese people with T2DM was 43.5% (Table 49). The prevalence of OSA (mild-severe) as defined by greater than or equal to five apnoeic/hyponoeic events per hour was 53.8%.

Diagnosis	Prevalence (n (%))
Moderate to severe OSA	20 (43.5%)
Mild OSA	2 (4.35%)
Non-OSA	19 (41.3%)
Other sleep complaint	7 (15.2%)

Table 49: The prevalence of OSA

Those patients that did not fit the criteria for either OSA or non-OSA (n=7) were removed from the study and referred to the Leicester General Hospital Sleep Laboratory for further assessment, leaving a total of 39 participants in the study. There were no significant differences in age, gender, ethnicity, smoking status or duration of T2DM between the two groups (Table 50). There were no significant differences in the use of medication between the two groups (Table 51). As expected there were significant differences between AHI and oxygen desaturation index (ODI) between those with diagnosed with moderate-severe OSA and those with non-OSA (Table 52). The

difference observed in percentage snore time between the two groups had a trend towards statistical significance.

Variable	Non-OSA (n=19)	OSA (n=20)	p
Age* (years)	56.4 ± 10.2	55.8 ± 11.3	0.87
Gender (male)*	9 (47.4%)	9 (45.0%)	1.00
Ethnicity(Caucasian)†	16 (84.2%)	16 (80.0%)	0.92
Current Smokers†	2 (10.5%)	4 (20.0%)	0.66
Duration T2DM*(years)	7.87 ± 5.45	11.63 ± 6.76	0.10

Table 50:Comparison of characteristics between non-OSA and OSA patients. * t-test, ** Fishers Exact test, †χ^2test

Medication	Non-OSA (n=19)	OSA (n=20)	p
Insulin*	9 (23.1%)	11 (28.2%)	0.25
Oral Anti-Diabetic Agent (OAD) †	15 (38.5%)	17 (43.6%)	1.00
Combination therapy (Insulin + OAD)*	8 (20.5%)	9 (23.1%)	0.41
Anti-hypertensive*	9 (23.1%)	12 (30.8%)	0.13
Lipid lowering*	10 (25.6%)	11 (28.2%)	0.38

Table 51 : Use of medication between OSA and Non-OSA groups. *Results from χ^2 and† Results from Fishers Exact test reported as n (%)

Variable	Non-OSA (n=19)	OSA (n=20)	p
AHI (events/hour)	3.05 ± 2.31	24.52 ± 18.92	**<0.001**
ODI (events/hour)	4.48 ± 3.24	27.85 ± 19.97	**<0.001**
Snore time (% sleep time)	9.69 ± 12.08	48.26 ± 8.40	0.056

Table 52: Breathing parameters associated with OSA a comparison between groups independent t-test, results displayed as mean ± SD

Systolic blood pressure, neck and waist circumference were significantly higher in the OSA group compared to the non-OSA group (p=0.01, p=0.04 and p= 0.05, respectively) (Table 53). Although, none of the other variables were significantly different between groups, diastolic blood pressure, body weight and BMI were higher in the OSA group. Additionally, perception of quality of life and of health and levels of physical activity (determined by the IPAQ questionnaire) were lower in the OSA group compared to the non-OSA group (Table 53).

Variable	**Non-OSA (n=19)**	**OSA (n=20)**	**p**
Systolic BP (mmHg)	130.46 ± 21.63	145.22 ± 12.12	**0.01**
Diastolic BP (mmHg)	83.21 ± 13.43	88.85 ± 10.36	0.15
Weight (kg)	110.23 ± 15.99	121.48 ± 31.147	0.16
Waist Circumference (cm)	118.68 ± 7.83	127.65 ± 17.96	**0.05**
WHR	0.96 ±0.076	0.98 ± 0.12	0.44
BMI (kg/m^2)	39.69 ± 5.90	43.85 ± 8.54	0.085
% Body Fat	37.71 ± 17.41	32.33 ± 19.82	0.37
Neck Circumference (cm)	38.5 ± 10.24	44.04 ± 6.30	**0.04**
ESS	9.21 ± 4.60	11.16 ±4.23	0.18
HADS	6.29 ± 2.5	7.32 ± 3.96	0.37
Perception QoL	3.44 ± 0.81	3.28 ± 1.02	0.62
Perception Health	2.69 ± 1.08	2.61 ± 0.98	0.83
IPAQ	4086.12 ± 5384.53	1493.83 ± 3893.69	0.11

Table 53: Baseline differences in biomedical and questionnaire data between OSA and Non-OSA groups. Results from independent t-tests between group analysis. Results displayed as mean ± SD.

Eleven (29.73%) participants dropped out of the study before the final follow-up measures. Therefore the final study population comprised of 28 obese people with T2DM. There were no significant differences between those patients that dropped out from the study and those that completed the study (Table 54).

Variable	Non-Dropout (n=28)	Dropout (n=11)	p
Age* (years)	56.0 ±10.85	56.27 ± 10.51	0.94
Gender (Male)**	13 (46.4%)	5 (45.5%)	1.00
Ethnicity (Caucasian) †	23 (82.1%)	9 (81.8%)	0.57
Waist circumference* (cm)	123.54 ± 13.15	122.64 ± 18.28	0.87

Table 54: Dropout patient characteristics compared to non-dropout patients.
*t-test, ** Fishers Exact test, †χ^2test

Due to the considerable risks associated with OSA, it was considered unethical to have included a traditional control group in this study. We decided not to use sham CPAP as again it was considered unethical not to treat patients that were diagnosed with OSA. Furthermore, there is no standard method of constructing sham CPAP and therefore the robustness of the study design could have been questioned in addition to financial constraints. Subsequently, for the purpose of answering the research question, the treatment group (those diagnosed with moderate-severe OSA) served as their own controls in the following within group analyses. This type of study design is thus quasi experimental the same statistical tests were carried out for the non-treatment group, thus allowing comparisons to be made. Additionally, between group analyses were used to further explore our findings, however conclusions drawn from these results should be interpreted with caution.

4.3.3 Descriptives of final study population

Variable	Non-Treatment (n=16)	Treatment (n=12)	p
Age* (years)	54.56 ± 11.32	57.50 ± 7.26	0.41
Gender (male)	8 (50.0%)	5 (41.7%)	0.72
Ethnicity(Caucasian)	14 (87.5%)	10 (83.3)	0.48
Current Smokers*	2 (12.5%)	3 (25.0%)	0.69
Duration T2DM (years)*	6.87 ± 4.57	12.80 ± 7.74	**0.024**

Table 55: Final study sample - a comparison the two groups. Results reported for Fishers Exact test and * t-test

Medication	Non-Treatment (n=16)	Treatment (n=12)	p
Insulin	9 (56.3%)	9 (75.0%)	0.68
Oral Anti-Diabetic agents (OAD)	15 (93.8%)	10 (83.3%)	0.56
Combination therapy (Insulin + OAD)*	8 (50.0%)	7 (58.3%)	1.00
Anti-hypertensive therapy	9 (56.3%)	10 (83.3%)	0.24
Lipid lowering therapy	10 (62.5%)	9 (75.0%)	0.69

Table 56: Final study sample - use of medication between the groups. Results from Fishers Exact test reported as n(%). * Results from χ^2

Variable	Non-Treatment (n=16)	Treatment (n=12)	p
AHI (events/hour)	3.40 ± 2.32	25.95 ± 13.80	**<0.001**
ODI (events/hour)	4.78 ± 3.64	30.12 ± 11.97	**<0.001**
Snore time (% sleep time)	8.73 ± 9.57	76.13 ± 100.08	**0.012**

Table 57: Final study sample - a comparison of breathing parameters associated with OSA using independent t-test, results displayed as mean ± SD.

There were no significant differences in age, gender, ethnicity, number of current smokers or use of medication between the treatment and non-treatment groups. However, the duration of T2DM was significantly higher in the OSA group (p=0.0024). As expected the three parameters of OSA were also significantly higher within the treatment group compared to the non-treatment group in the final study population (Table 57).

4.3.4 Baseline results - study measures associated with the presence of OSA

Logistic regression was carried out to determine if any of the baseline variables were associated with moderate to severe OSA (AHI > 15 events/hour) independently of age, gender and ethnicity (Table 58).

Variable	Unadjusted OR (95% CI)	p	Adjusted* OR (95% CI)	p
% Below Target	0.83 (0.69, 0.99)	**0.036**	0.81 (0.66, 0.99)	**0.038**
% Above Target	0.99 (0.97, 1.03)	0.88	1.00 (0.97, 1.03)	0.97
% Within Target	1.02 (0.99, 1.06)	0.26	1.02 (0.98, 1.05)	0.32
HbA1c (%)	0.84 (0.51, 1.39)	0.49	0.62 (0.32, 1.19)	0.15
Systolic BP (mmHg)	1.06 (1.01, 1.11)	**0.022**	1.07 (1.01, 1.13)	**0.017**
Diastolic BP (mmHg)	1.04 (0.99, 1.10)	0.15	1.05 (0.98, 1.11)	0.15
Weight (kg)	1.02 (0.99, 1.05)	0.18	1.03 (0.99, 1.06)	0.11
Waist Circ (cm)	1.05 (0.99, 1.11)	0.07	1.06 (1.00, 1.11)	**0.05**
WHR†	1.29 (0.68, 2.34)	0.44	1.68 (0.70, 4.00)	0.24
BMI (kg/m^2)	1.09 (0.99, 1.19)	0.11	1.09 (0.97, 1.12)	0.09
%Body Fat	0.98 (0.95, 1.02)	0.37	0.98 (0.94, 1.02)	0.26
Neck Circ (cm)	1.14 (0.99, 1.31)	0.062	1.12 (1.02, 1.52)	**0.03**
ESS	1.11 (0.95, 1.29)	0.18	1.11 (0.95, 1.30)	0.18
HADS	1.10 (0.89, 1.35)	0.36	1.12 (0.88, 1.40)	0.36
IPAQ	1.00 (1.00, 1.00)	0.14	1.00 (1.00, 1.00)	0.14
QoL	0.82 (0.37, 1.75)	0.61	0.87 (0.38, 2.00)	0.74
QoH	0.93 (0.47, 1.82)	0.82	0.97 (0.47, 2.00)	0.94
Total Cholesterol (mmol/l)	0.58 (0.19, 1.80)	0.34	0.61 (0.19, 2.61)	0.61
Triglycerides (mmol/l)	0.61 (0.24, 1.55)	0.302	0.44 (0.13, 1.51)	0.19
HDLC (mmol/l)	0.63 (0.011, 37.31)	0.82	0.18 (0.00, 157.47)	0.62
LDL (mmol/l)	0.97 (0.19, 4.78)	0.97	1.30 (0.19, 9.02)	0.79

Table 58: Baseline variables that predict OSA. Results displayed as odds ratio's (OR) and 95% Confidence intervals. * Adjusted for age, gender and ethnicity. † Waist-Hip-Ratio (WHR) is multiplied by 10 for purpose of logistic regression. HADS: Hospital anxiety and depression score. IPAQ: International physical activity questionnaire. WHOQoL (BREF): World Health Organisation Quality of life questionnaire – Breif version. % Below Target = proportion of time blood glucose levels below target ($\leq$ 3.9 mmol/L). % Above Target = proportion of time blood glucose levels above target ($\geq$ 10.0 mmol/L). % Within Target = proportion of time blood glucose levels within target (>3.9 - 9.99mmol/L) as determined from CGMS data

A higher proportion of time spent below target (blood glucose levels ≤ 3.9mmol/L) as recorded by CGMS is associated with a 19% decreased likelihood of OSA. Higher systolic blood pressure is associated with a 7% increased likelihood of OSA. Higher waist circumference is associated with a 6% increased likelihood of OSA. Neck circumference is associated with a 12% increased likelihood of OSA.

4.3.5 Glycaemic control correlates of OSA

Spearmans rank order correlation was used to investigate if HbA1c, proportion of time spent in hypoglycaemic (≤ 3.9 mmol/l), hyperglycaemic (≥ 10.0 mmol/l) or normoglycaemic (4 – 9.9mmol/l) ranges were correlated with the AHI (Table 59) or with ODI (Table 60). ODI is defined as a drop in oxygen saturation levels by 4% which lasts no longer than 120 seconds. During the sleep period any oxygen desaturations of >50% were regarded as artefacts and excluded automatically by the software used to score the sleep study results.

Glycaemic measure	Correlation coefficient (r)	p
HbA1c	0.019	0.92
% hyperglycaemic	0.051	0.802
% Hypoglycaemic	-0.32	0.11
% Normoglycaemic	0.033	0.87

Table 59: Glycaemic measures correlated with AHI**.** Results reported for Spearmans rank order correlation between variables and AHI (n=31)

Glycaemic measure	Correlation coefficient (r)	p
HbA1c	0.067	0.74
% hyperglycaemic	0.204	0.31
% Hypoglycaemic	-0.403	**0.037**
% Normoglycaemic	-0.12	0.59

Table 60: Glycaemic measures correlated with ODI. Results reported for spearmans rank order correlation between variables and ODI (n=31)

The proportion of time spent within the hypoglycaemic range is negatively correlated with ODI – thus the higher the frequency of oxygen desaturation events the less time spent within the hypoglycaemic range.

Spearman's rank order correlation was used to determine if the frequency and duration of hyper- and hypoglycaemic events were correlated with either AHI (Table 61) or ODI (Table 63). Tables 62 and 64 display results for spearmans rank order correlation between hypoglycaemic events and AHI and ODI using the second stricter definition for hypoglycaemia - blood glucose levels ≤ 2.2mmol/l for ≥ 20min.

The frequency of hypoglycaemic events (≤ 3.1mmol/l for ≥ 10 minutes) adjusted for 24 hours and for night-time hours is significantly negatively correlated with the AHI (Table 61). The average length of hyperglycaemic events was also found to be significantly negatively correlated with AHI during the night-time hours (Table 61). In addition the frequency and duration of hypoglycaemic events as defined in this model are both significantly negatively correlated with oxygen desaturation levels as determined by ODI when adjusted for 24 hours and night-time hours (Table 62). The frequency and average length of hypoglycaemic events (≤ 2.2mmol/L for ≥ 20min) adjusted for 24 hours are significantly negatively correlated with the frequency of oxygen desaturation – ODI (Table 64).

Hyperglycaemic event- Blood glucose levels ≥ 10.0mmol/L for ≥ 10min						
	24hours		**Daytime (6000-2159)**		**Night-time (2200-0559)**	
	r	**p**	**r**	**p**	**r**	**p**
No of events	0.017	0.93	-0.13	0.52	-0.034	0.86
Average length	-0.120	0.54	0.010	0.93	-0.45	**0.016**
Hypoglycaemic event- Blood glucose levels ≤ 3.1mmol/L for ≥ 10min						
	24hours		**Daytime (6000-2159)**		**Night-time (2200-0559)**	
	r	**p**	**r**	**p**	**r**	**p**
No of events	-0.49	**0.009**	-0.19	0.33	-0.48	**0.011**
Average length	-0.29	0.13	-0.067	0.74	-0.46	**0.017**

Table 61: Hyper- and hypoglycaemic events and AHI. Results reported for spearman's rank order correlation between (n=31)

Hypoglycaemic event- Blood glucose levels ≤ 2.2mmol/L for ≥ 20min						
	24hours		**Daytime (6000-2159)**		**Night-time (2200-0559)**	
	r	**p**	**r**	**p**	**r**	**p**
No of events	-0.29	0.13	-0.17	0.39	-0.25	0.19
Average length	-0.303	0.12	-0.16	0.42	-0.23	0.25

Table 62: Hypoglyceamic events defined as blood glucose levels ≤ 2.2mmol/L for ≥ 20min and AHI. Results reported for spearman's rank order correlation between AHI and hypoglycaemic events (n=31)

Hyperglycaemic event- Blood glucose levels ≥10.0mmol/L for ≥ 10min						
	24hours		**Daytime (6000-2159)**		**Night-time (2200-0559)**	
	r	**p**	**r**	**p**	**r**	**p**
No of events	-0.04	0.84	-0.062	0.75	-0.14	0.49
Average length	0.065	0.74	0.141	0.47	-0.36	0.062
Hypoglycaemic event- Blood glucose levels ≤ 3.1mmol/L for ≥ 10min						
	24hours		**Daytime (6000-2159)**		**Night-time (2200-0559)**	
	r	**p**	**r**	**p**	**r**	**p**
No of events	-0.601	**0.001**	-0.37	0.098	-0.512	**0.006**
Average length	-0.37	**0.055**	-0.199	0.32	-0.51	**0.007**

Table 63: Hyper- and hypoglycaemic events and ODI. Results reported for spearman's rank order correlation (n=31)

Hypoglycaemic event- Blood glucose levels ≤ 2.2mmol/L for ≥ 20min						
	24hours		**Daytime (6000-2159)**		**Night-time (2200-0559)**	
	r	**p**	**r**	**p**	**r**	**p**
No of events	-0.38	**0.045**	-0.23	0.24	-0.25	0.21
Average length	-0.396	**0.037**	-0.22	0.27	-0.28	0.15

Table 64: Hypoglycaemic events defined as blood glucose levels ≤ 2.2mmol/L for ≥ 20min and ODI. Results reported for spearman's rank order correlation between ODI and hypoglycaemic events (n=31)

4.3.6 CGMS analyses – Investigating the change in proportion of time spent within three different glycaemic ranges

Repeated measures analysis was used to determine if there was any change in the proportion of time spent within the three glycaemic ranges stated below. The sample size for these analyses was small (n = 9) due to the high number of incomplete CGMS datasets that were obtained at baseline, 6 weeks and 6 months. Thus these results need to be interpreted with caution.

There were no significant differences in the use of medication or duration of T2DM between the treatment (n = 5) and non-treatment group (n = 4) for these analyses (Table 65).

Medication	Non-Treatment (n=4)	Treatment (n=5)	P
Insulin	3 (75%)	3 (60%)	0.59
Oral Anti-Diabetic agents (OAD)	3 (75%)	3 (60%)	0.59
Combination therapy (Insulin + OAD)	2 (50%)	2 (40%)	0.64
Anti-hypertensive therapy	3 (75%)	3 (60%)	0.59
Lipid lowering therapy	3 (75%)	3 (60%)	0.59
Duration T2DM (years)*	10.00 ± 6.63	11.20 ± 6.72	0.79

Table 65: Use of medication in subjects who provided complete CGMS data – comparison between treatment and non-treatment groups. Results from Fishers Exact test reported as n (%). * Results from t-test

The proportion of time (%) spent within the following glycaemic ranges was determined within each group and repeated measures analysis undertaken to determine

if there was any change in the time spent within these categories at each time point of the study. All results are adjusted to 24 hours, day-time hours and night-time hours (Table 66 – treatment group, Table 67 – Non-treatment group). Graphical representations of these results are shown in Figures 27 - 32. The criteria for the three categories are:

- Above target ≥ 10.0mmol/l
- Below target ≤ 3.9mmol/l
- Within target 4-9.9mmol/l

In the treatment group at 6 weeks a reduction in the proportion of time spent above target was observed when adjusted for 24 hours (- 60.8 %), daytime (- 52.9 %) and during the night-time (- 71.8 %). This reduction in time spent above target was maintained at 6 months however the size of the reduction was lower at - 29.7 %, - 25.7 %, and 30.7 % respectively (Table 66). The proportion of time spent below target increased at 6 weeks when adjusted for 24 hours (+ 313.6 %), daytime (+ 144.3) with the greatest increase observed during the night-time (+ 17366.7 %). Again these observed increases were maintained at 6 months with a further increase observed when adjusted for 24 hours (+ 347.1 %) and during the night-time (+ 23383.3 %). However, during the daytime the size of the observed increase was smaller than that at 6 weeks (+ 94.8 %). The proportion of time spent within target again had increased at 6 weeks when adjusted for 24 hours (+ 39.2 %), the daytime (+ 33.3 %) and during the night-time (+ 43.2 %). At 6 months the observed increase was still evident but the size reduced at + 11.4 %, + 14.8 % and + 0.56 %, respectively (Table 66).

In the non-treatment group a reduction in the time spent above target at 6 weeks and 6 months when adjusted for 24 hours, daytime and the night-time was observed, however these reductions were a lot smaller than those observed for the treatment group (Table 67). An increase in the proportion of time spent below target was observed at 6 weeks adjusted for 24 hours, daytime and night-time for the non-treatment group and again these reductions were a lot smaller than that observed for the treatment group (Table 67). Interestingly, at 6 months the time spent below target decreased for all the adjusted time frames which is not what was observed for the treatment group (Table 67 and 66, respectively). The proportion of time spent within target actually decreased at 6 weeks when adjusted for 24 hours and during the daytime in the non-treatment group. An increase was however observed during the night-time which again was smaller than observed for the treatment group. However, at 6 months there was an increase in the time spent within target when adjusted for 24 hours, daytime and during the night-time which were slightly higher increases than those observed for the treatment group (Table 67).

24 hours				
Glycaemic range	**Time**	**Mean ± SD**	**% change from baseline**	**p**
Above Target (%)	Baseline	44.97 ± 27.04		
	6 weeks	17.64 ± 17.52	- 60.8%	
	6 Months	31.61 ± 21.11	- 29.7%	0.29
Below Target (%)	Baseline	2.06 ± 3.77		
	6 weeks	8.52 ± 7.31	+ 313.6%	
	6 Months	9.21 ± 10.77	+ 347.1 %	0.11
Within Target (%)	Baseline	53.03 ± 25.47		
	6 weeks	73.84 ± 16.49	+ 39.2 %	
	6 Months	59.07 ± 15.29	+ 11.4%	0.35
Day-time				
Above Target (%)	Baseline	42.59 ± 26.50		
	6 weeks	20.05 ± 18.38	- 52.9%	
	6 Months	31.62 ± 19.86	- 25.7 %	0.43
Below Target (%)	Baseline	3.07 ± 5.68		
	6 weeks	7.50 ± 5.37	+ 144.3%	
	6 Months	5.98 ± 5.75	+ 94.8 %	0.24
Within Target (%)	Baseline	54.34 ± 24.08		
	6 weeks	72.45 ± 15.79	+ 33.3 %	
	6 Months	62.40 ± 16.06	+ 14.8 %	0.46
Night-time				
Above Target (%)	Baseline	46.63 ± 25.91		
	6 weeks	13.17 ± 18.19	- 71.8 %	
	6 Months	32.29 ± 29.52	- 30.7 %	0.13
Below Target (%)	Baseline	0.06 ± 0.14		
	6 weeks	10.48 ± 13.37	+ 17366.7 %	
	6 Months	14.09 ± 19.95	+ 23383.3 %	0.24
Within Target (%)	Baseline	53.31 ± 25.92		
	6 weeks	76.35 ± 18.43	+ 43.2 %	
	6 Months	53.61 ± 24.76	+ 0.56 %	0.33

Table 66: Change in proportion of time spent within the three glycaemic ranges -treatment group (n = 5)

24 hours				
Glycaemic range	**Time**	**Mean ± SD**	**% change from baseline**	**p**
Above Target (%)	Baseline	39.41 ± 14.63		
	6 weeks	36.36 ± 12.13	- 7.7 %	
	6 Months	32.21 ± 17.14	- 18.3 %	0.86
Below Target (%)	Baseline	5.79 ± 6.91		
	6 weeks	13.03 ± 13.91	+ 125.0 %	
	6 Months	1.67 ± 1.85	- 71.2 %	0.083
Within Target (%)	Baseline	54.80 ± 9.98		
	6 weeks	50.60 ± 9.91	- 7.7 %	
	6 Months	66.12 ± 15.44	+ 20.7 %	0.49
Daytime (0600 – 2159 hours)				
Above Target (%)	Baseline	34.84 ± 16.23		
	6 weeks	33.75 ± 9.48	- 3.1 %	
	6 Months	32.58 ± 14.29	- 2.26 %	0.97
Below Target (%)	Baseline	7.41 ± 8.31		
	6 weeks	13.96 ± 18.09	+ 88.4%	
	6 Months	1.74 ± 1.66	- 76.5 %	0.59
Within Target (%)	Baseline	57.75 ± 12.04		
	6 weeks	52.29 ± 15.53	- 9.5 %	
	6 Months	65.68 ± 13.11	+ 13.7 %	0.66
Night-time (2200-0559 hours)				
Above Target (%)	Baseline	46.30 ± 21.54		
	6 weeks	33.87 ± 13.39	- 26.8 %	
	6 Months	22.05 ± 29.44	- 52.4 %	0.39
Below Target (%)	Baseline	3.66 ± 6.04		
	6 weeks	11.79 ± 10.50	+ 222.1 %	
	6 Months	1.85 ± 3.21	+ 49.5 %	0.55
Within Target (%)	Baseline	50.04 ± 15.62		
	6 weeks	54.34 ± 3.39	+ 8.6 %	
	6 Months	76.10 ± 27.71	+ 52.1 %	0.47

Table 67: Change in proportion of time spent within the three glycaemic ranges - non- Treatment group (n = 4)

Figure 27: Treatment Group - Glycaemic Ranges adjusted 24hrs (n=5)

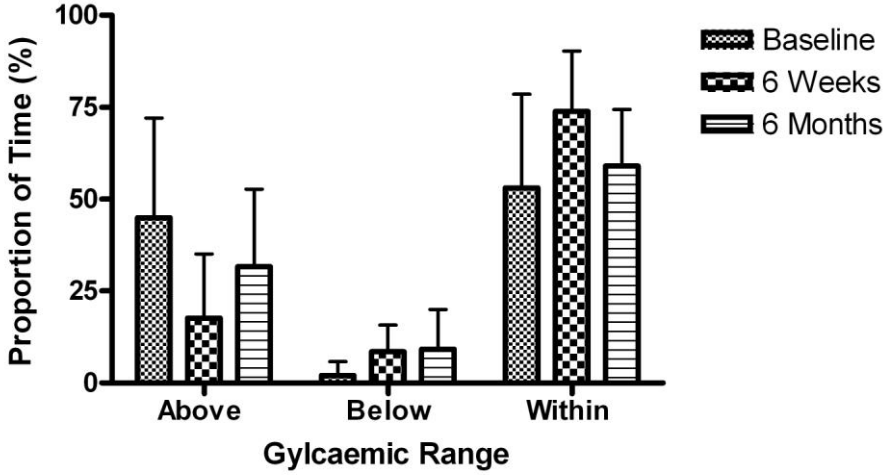


Figure 28: Treatment - Daytime Gycaemic Ranges (n=5)

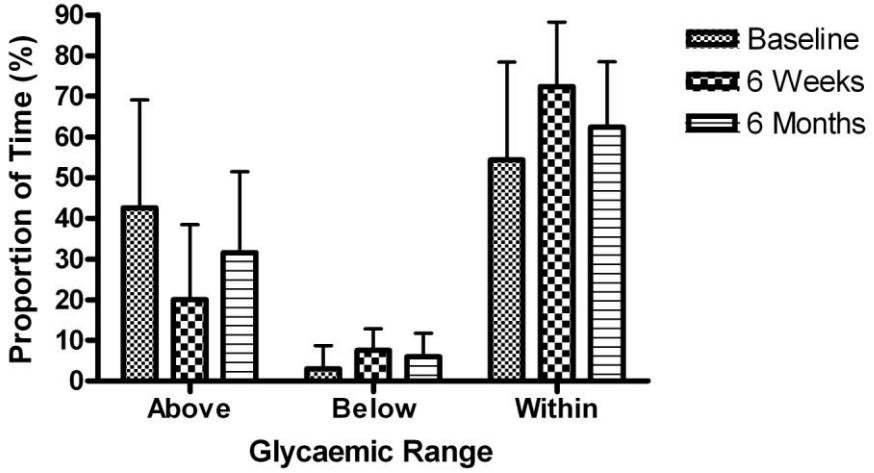


Figure 29:Treatment - Night-time Glycaemic Ranges (n=5)

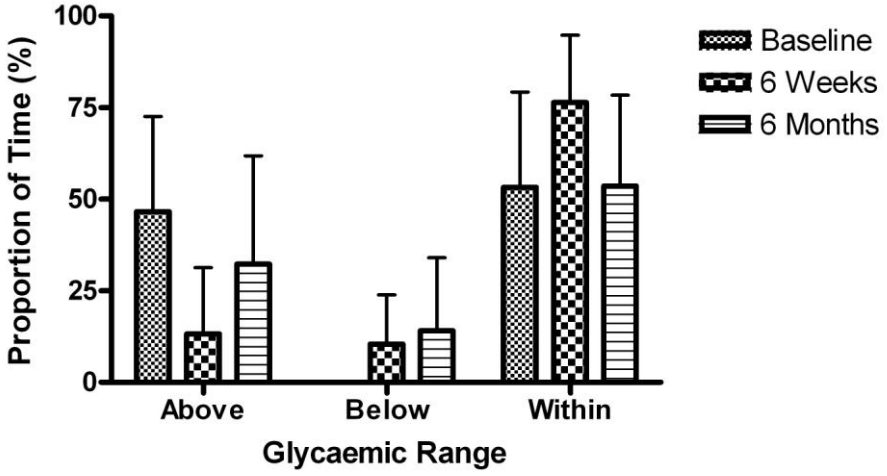


Figure 30: Non-Treatment - Glycaemic Ranges Adjusted 24hrs (n=4)

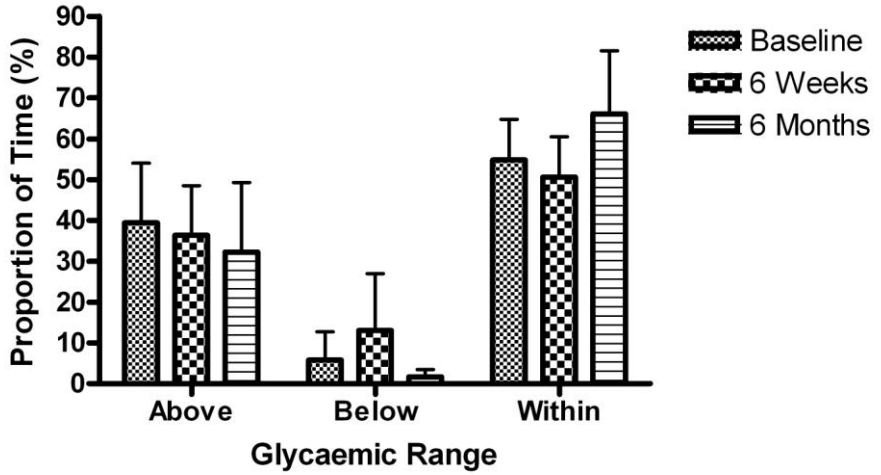


Figure 31: Non-Treatment- Daytime Glycaemic Ranges (n=4)

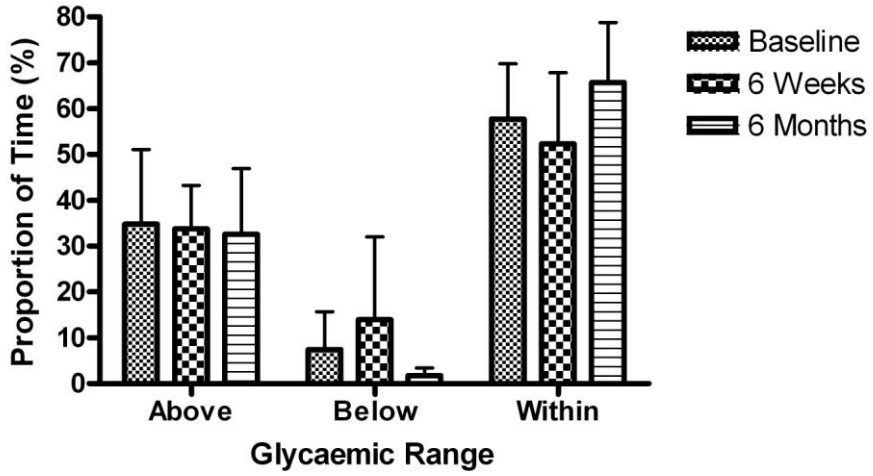
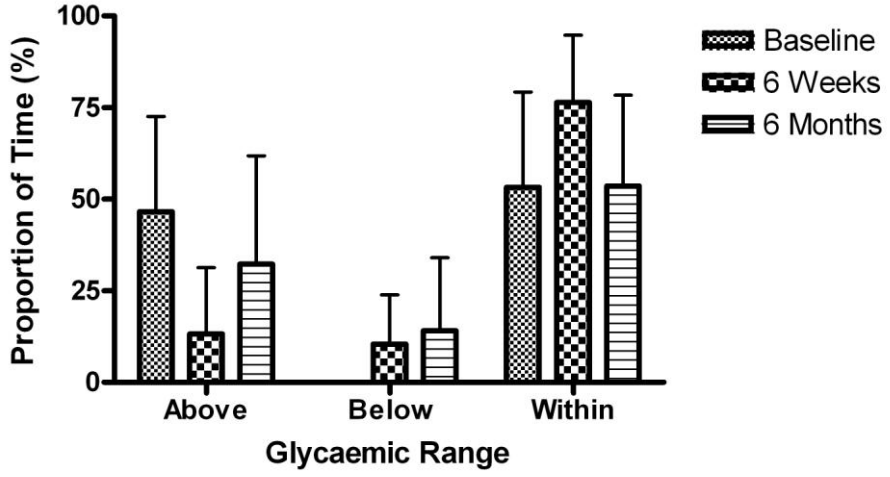


Figure 29:Treatment - Night-time Glycaemic Ranges (n=5)



4.3.7 CGMS analyses continued – Investigating the change in the frequency and duration of hyper- and hypoglycaemic events within groups

Repeated measures analyses were used to determine if the frequency and duration of either hyperglycaemic or hypoglycaemic events changed across the study period within each group (adjusted for 24 hours). These events were defined as:

Hyperglycaemic event ≥ 10.0 mmol/L for a duration of ≥ 10 minutes

Hypoglycaemic event ≤ 3.1 mmol/L for a duration of ≥ 10 minutes

In the treatment group at 6 weeks a reduction in the number of hyperglycaemic events was observed adjusted for 24 hours (- 38.3 %), daytime (- 26.6 %) with the largest reduction observed during the night-time (- 75.9 %). A similar reduction in the average length of these hyperglycaemic events was observed when adjusted for 24 hours, daytime and during the night-time (Table 68). At 6 months however an increase in the number of hyperglycaemic events was observed when adjusted for 24 hours (+ 8.5 %). These events were occurring during the daytime as an increase in hyperglycaemic events was only observed here (+ 21.5 %) and a reduction observed during the nigh-time (- 48.9 %). The average length of these hyperglycaemic events decreased when adjusted for 24 hours and statistically significantly during the daytime ($p = 0.038$). However, although a reduction in the frequency of hyperglycaemic events was observed at 6 months during the night-time the duration of these events actually increased by + 129.9% (Table 68).

In the treatment group an increase in the number of hypoglycaemic events was observed at 6 weeks when adjusted for 24 hours (+ 650.0 %), daytime (+ 206.5 %) and during the night-time (+ 258.0 %). The average length of these hypoglycaemic events decreased when adjusted for both 24 hours and daytime hours but increased during night-time (Table 68). At 6 months the observed increased frequency of hypoglycaemic events was still evident when adjusted for 24 hours, daytime and night-time hours although the increase from baseline was lower than that observed from baseline to 6 weeks (Table 68). There was an increase also in the average length of these hypoglycaemic events when adjusted for 24 hours, daytime and night-time hours (Table 68).

In the non-treatment group (Table 69) there was an increase in the number of hyperglycaemic events at 6 weeks adjusted for 24 hours (+ 10.5 %) and daytime (+ 91.0 %). However, there was a reduction in the average length of these events when adjusted for both 24 hours and daytime hours (Table 69). During the nigh-time there was a small reduction in the number of hyperglycaemic events at 6 weeks (-3.51%) and an increase in the duration of these events (+ 32.7 %). At 6 months an increase in both the number and duration of hyperglycaemic events was observed when adjusted for 24 hours (Table 69). However, there was an increase in both the number and duration of hyperglycaemic events during the daytime and night-time with the average lengths of these events also increased (Table 69). The number of hypoglycaemic events and their duration increased at 6 weeks when adjusted for 24 hours (Table 69). However at 6 months both the frequency and duration of these events decreased when adjusted for 24 hours. During the daytime at 6 weeks and 6 months a reduction in the number of hypoglycaemic events was observed. However at 6 weeks their duration had increased but by 6 months the average length of hypoglycaemic events during the day decreased

(-37.5 %). During the night-time at both 6 weeks and 6 months there was an increase in the number and duration of hypoglycaemic events in the non-treatment group (Table 69).

When looking at change in the frequency and duration of hypoglycaemic events using the stricter definition of ≤ 2.2 mmol/L for a duration of at least 20 minutes the results are different. There is an increase at both 6 weeks and 6 months for both 24 hours and daytime hours, in the treatment group, in both the frequency and duration of these events (Table 70). During the night-time there is no change in the number of hypoglycaemic events at 6 weeks or 6 months using this definition, however there is an increase in the duration of these events at both time points (Table 70). By comparison in the non-treatment group using this definition for hypoglycaemia the results are quite different to the treatment group whilst at 6 weeks there is an increase in the number and duration of hypoglycaemic events when adjusted for 24 hours and during the daytime, during the night-time no change is observed from baseline for any of the variables. Furthermore at 6 months there is no change in the frequency or duration of hypoglycaemic events from baseline when adjusted for 24 hours, daytime or during the nigh-time (Table 71).

Variable	Time	Mean ± SD	% change from baseline	p
24 hours				
No of Hyper events	Baseline	9.40 ± 1.95		
	6 weeks	5.80 ± 4.44	- 38.3 %	
	6 months	10.20 ± 5.85	+ 8.5 %	0.41
Average length of hyper event	Baseline	148.00 ± 93.72		
	6 weeks	73.20 ± 69.12	- 50.5 %	
	6 months	142.80 ± 63.52	- 3.5 %	0.40
No of Hypo events	Baseline	0.40 ± 0.55		
	6 weeks	3.00 ± 3.00	+ 650.0 %	
	6 months	1.60 ± 1.67	+ 300.0 %	0.29
Average length of hypo event	Baseline	26.40 ± 39.08		
	6 weeks	18.00 ± 18.18	- 31.8 %	
	6 months	37.80 ± 45.59	+ 43.2 %	0.69
Daytime (0600 – 2159)				
No of Hyper events	Baseline	7.08 ± 6.72		
	6 weeks	5.20 ± 4.32	- 26.6 %	
	6 months	8.60 ± 5.77	+ 21.5 %	0.57
Average length of hyper event	Baseline	117.60 ± 96.10		
	6 weeks	65.00 ± 52.98	- 44.7 %	
	6 months	106.20 ± 29.84	- 9.7 %	**0.038**
No of Hypo events	Baseline	0.31 ± 0.42		
	6 weeks	0.95 ± 1.68	+ 206.5 %	
	6 months	0.60 ± 0.59	+ 93.5 %	0.52
Average length of hypo event	Baseline	25.80 ± 38.75		
	6 weeks	12.60 ± 17.86	- 51.2 %	
	6 months	26.00 ± 29.88	+ 0.78 %	0.69
Night time (2200 – 0559)				
No of Hyper events	Baseline	5.39 ± 3.09		
	6 weeks	1.30 ± 1.30	- 75.9%	
	6 months	2.75 ± 1.59	- 48.9%	0.19
Average length of hyper event	Baseline	128.00 ± 48.66		
	6 weeks	73.40 ± 97.40	- 42.7 %	
	6 months	293.20 ± 385.6	+ 129.1 %	0.29

No of Hypo events	Baseline	0.00 ± 0.00		
	6 weeks	2.58 ± 2.36	+ 258.0 %	
	6 months	1.00 ± 1.41	+ 100.0 %	0.17
Average length of hypo event	Baseline	0.00 ± 0.00		
	6 weeks	16.00 ± 15.79	+ 1600.0%	
	6 months	28.00 ± 41.38	+ 2800.0%	0.24

Table 68: Change in frequency and duration of glycaemic events – Treatment (n=5)

Variable	Time	Mean ± SD	% change from baseline	p
24 hours				
No of Hyper events	Baseline	9.50 ± 2.52		
	6 weeks	10.50 ± 5.80	+ 10.5 %	
	6 months	5.75 ± 3.20	- 39.5 %	0.61
Average length of hyper event	Baseline	152.50 ± 54.28		
	6 weeks	142.00 ± 46.22	- 6.9 %	
	6 months	169.00 ± 91.71	+ 10.8%	0.86
No of Hypo events	Baseline	2.00 ± 2.45		
	6 weeks	2.25 ± 2.63	+ 12.5 %	
	6 months	0.75 ± 0.96	- 62.5%	0.76
Average length of hypo event	Baseline	16.00 ± 20.54		
	6 weeks	110.00 ± 144.68	+ 587.5%	
	6 months	11.25 ± 14.36	- 29.7 %	0.57
Daytime (0600 – 2159)				
No of Hyper events	Baseline	4.58 ± 1.96		
	6 weeks	8.75 ± 4.92	+ 91.0 %	
	6 months	4.75 ± 2.63	+ 3.7%	0.27
Average length of hyper event	Baseline	118.50 ± 53.76		
	6 weeks	106.00 ± 44.09	- 10.5 %	
	6 months	153.00 ± 86.93	+ 29.1 %	0.79
No of Hypo events	Baseline	1.27 ± 1.47		

	6 weeks	0.92 ± 0.78	- 27.6 %	
	6 months	0.28 ± 0.32	- 77.9 %	0.60
Average length of hypo event	Baseline	16.00 ± 20.54		
	6 weeks	91.00 ± 139.06	+ 468.8 %	
	6 months	10.00 ± 12.25	- 37.5%	0.66
Night time (2200 – 0559)				
No of Hyper events	Baseline	4.45 ± 1.59		
	6 weeks	2.89 ± 1.99	- 35.1 %	
	6 months	2.00 ± 1.00	+ 55.1%	0.19
Average length of hyper event	Baseline	146.00 ± 40.51		
	6 weeks	193.67 ± 64.59	+ 32.7%	
	6 months	158.67 ± 211.60	+ 8.7 %	0.87
No of Hypo events	Baseline	0.00 ± 0.00		
	6 weeks	1.49 ± 1.50	+ 149 %	
	6 months	0.33 ± 0.58	+ 33 %	0.58
Average length of hypo event	Baseline	0.00 ± 0.00		
	6 weeks	41.00 ± 37.03	+ 4100 %	
	6 months	11.67 ± 20.21	+ 1167 %	0.56

Table 69: Change in frequency and duration of glycaemic events – Non - Treatment (n=4)

Change in the frequency and duration of hypoglycaemic events (≤ 2.2 mmol/L for a duration of at least 20 minutes).

Variable	Time	Mean ± SD	% change from baseline	p
24 hours				
No of Hypo events	Baseline	0.00 ± 0.00		
	6 weeks	0.102 ± 0.23	+ 10.2 %	
	6 months	0.23 ± 0.35	+ 24.0 %	0.29
Average length of hypo event	Baseline	0.00 ± 0.00		
	6 weeks	14.40 ± 32.19	+ 1440.0 %	
	6 months	27.60 ± 48.95	+ 2760.0 %	0.37
Daytime (0600 – 2159)				
No of Hypo events	Baseline	0.00 ± 0.00		
	6 weeks	0.10 ± 0.22	+ 10.0 %	
	6 months	0.08 ± 0.18	+ 8.0 %	0.47
Average length of hypo event	Baseline	0.00 ± 0.00		
	6 weeks	14.40 ± 32.19	+ 1440.0 %	
	6 months	5.00 ± 11.18	+ 500.0 %	0.47
Night time (2200 – 0559)				
No of Hypo events	Baseline	0.00 ± 0.00		
	6 weeks	0.00 ± 0.00	0.0 %	
	6 months	0.13 ± 0.29	+ 13.0%	0.37
Average length of hypo event	Baseline	0.00 ± 0.00		
	6 weeks	0.00 ± 0.00	0.0%	
	6 months	22.60 ± 50.54	+ 2260.0%	0.37

Table 70: Change in frequency and duration of hypoglycaemic events for treatment group, n = 5. Results from repeated measures analyses (Hypo ≤ 2.2 mmol/L ≥ 20 minutes).

Variable	Time	Mean ± SD	% change from baseline	p
24 hours				
No of Hypo events	Baseline	0.00 ± 0.00		
	6 weeks	0.56 ± 0.74	+ 56.0 %	
	6 months	0.00 ± 0.00	0.0 %	0.23
Average length of hypo event	Baseline	0.00 ± 0.00		
	6 weeks	43.00 ± 58.55	+ 4300.0 %	
	6 months	0.00 ± 0.00	0.0 %	0.24
Daytime (0600 – 2159)				
No of Hypo events	Baseline	0.00 ± 0.00		
	6 weeks	0.58 ± 0.76	+ 58.0 %	
	6 months	0.00 ± 0.00	0.0 %	0.23
Average length of hypo event	Baseline	0.00 ± 0.00		
	6 weeks	43.00 ± 58.55	+ 4300.0 %	
	6 months	0.00 ± 0.00	0.0 %	0.24
Night time (2200 – 0559)				
No of Hypo events	Baseline	0.00 ± 0.00		
	6 weeks	0.00 ± 0.00	0.0 %	
	6 months	0.00 ± 0.00	0.0 %	-
Average length of hypo event	Baseline	-		
	6 weeks	-	-	
	6 months	-	-	-

Table 71: Change in frequency and duration of hypoglycaemic events for non-treatment group, n = 4. Results from repeated measures analyses (Hypo $\leq$ 2.2 mmol/L $\geq$ 20 minutes)

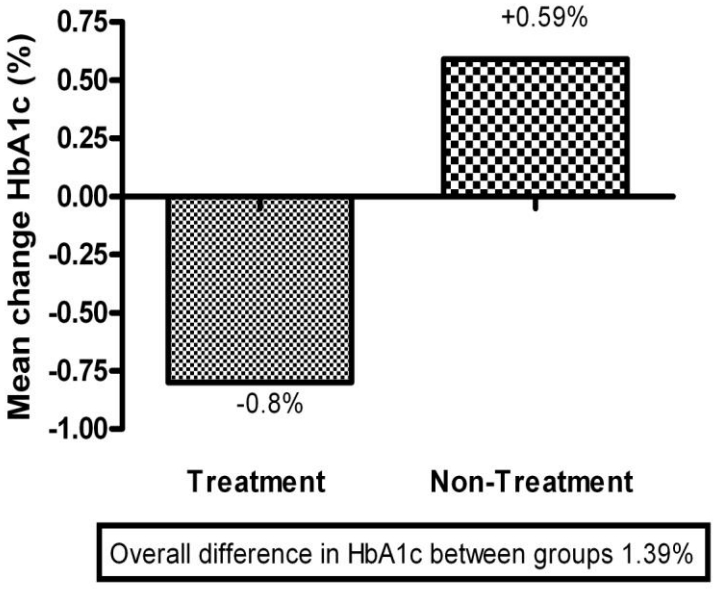
4.3.8 Glycaemic control analyses- investigating the change in glycaemic measures between baseline and the three study time points

Repeated measures analyses were used to determine if there were any significant changes in the levels of fasting HbA1c, glucose or insulin after 6 weeks, 12 weeks or 6

months of CPAP therapy within each group (Tables 72 and 73). The overall change in HbA1c levels in each group is shown graphically in Figure 33.

Initially HbA1c levels increased within the treatment group however, by 6 months levels had decreased by 0.8% although this was not statistically significant. Similarly there was an initial increase in HbA1c levels at 6 weeks within the non-treatment group although this increase was maintained throughout the study period. Fasting glucose and insulin levels also increased at 6 weeks within the treatment group and remained higher than baseline levels at 6 months however the proportion of the increase had markedly reduced compared to the levels reported for 6 weeks. There was an initial reduction in fasting glucose levels within the non-treatment group. At 12 weeks and 6 months the fasting glucose levels within this group increased which is also evident for fasting insulin levels across the time points of this study. Additionally, there were no significant differences in the mean change in these glycaemic measures between the two groups at each of the three time points (Table 74).

Figure 33: Mean change in HbA1c at 6 months



Variable	Time Point	Mean ± SD	Δ Mean from BL	p
HbA1c (%)	Baseline	9.46 ± 2.24		
	6 weeks	10.19 ± 3.19	0.73	
	12 weeks	9.45 ± 1.99	-0.01	
	6 months	8.66 ± 1.97	-0.80	0.19
Fasting Glucose (mmol/l)	Baseline	7.57 ± 2.12		
	6 weeks	9.18 ± 4.92	1.61	
	12 weeks	8.28 ± 3.19	0.71	
	6 months	7.89 ± 3.79	0.32	0.48
Insulin (µIU/ml)	Baseline	18.88 ± 0.55		
	6 weeks	29.56 ± 2.92	10.68	
	12 weeks	22.87 ± 3.41	3.99	
	6 months	20.15 ± 4.40	1.27	0.45

Table 72: Measures of glycaemic control mean changes across study time points – treatment group, n = 10. Results reported from repeated measures analyses

Variable	Time Point	Mean ± SD	Δ Mean from BL	p
HbA1c (%)	Baseline	9.87 ± 2.59		
	6 weeks	10.50 ± 2.82	0.63	
	12 weeks	10.51 ± 3.25	0.64	
	6 months	10.46 ± 3.14	0.59	0.55
Fasting Glucose (mmol/l)	Baseline	8.56 ± 3.45		
	6 weeks	7.72 ± 2.69	-0.84	
	12 weeks	9.75 ± 4.25	1.19	
	6 months	9.48 ± 4.72	0.92	0.56
Insulin (µIU/ml)	Baseline	21.18 ± 1.91		
	6 weeks	21.94 ± 1.65	0.76	
	12 weeks	27.45 ± 1.71	6.27	
	6 months	24.23 ± 1.72	3.05	0.49

Table 73: Measures of glycaemic control mean changes across study time points – non- treatment group, n = 8. Results reported from repeated measures analyses

Variable	6 weeks			12 weeks			6 months		
	Treatment Mean(95%CI)	**Non-Treatment** Mean(95%CI)	**p**	**Treatment** Mean(95%CI)	**Non-Treatment** Mean(95%CI	**p**	**Treatment** Mean(95%CI)	**Non-Treatment** Mean(95%CI	**p**
HbA1c (%)	0.77 (-0.59, 2.13)	0.45 (-0.79, 1.69)	0.72	0.13 (-0.71, 0.97)	0.44 (-0.54, 1.42)	0.62	-0.55 (-1.72, 0.62)	0.67 (-0.33, 1.66)	0.12
FG(mmol/l)	1.33 (-1.24, 3.91)	-1.41 (-3.86, 1.03)	0.13	0.11 (-2.12, 2.33)	2.10 (-0.54, 4.74)	0.25	0.88 (-0.47, 2.23)	0.404 (-0.78, 1.59)	0.59
I (µIU/ml)	1.06 (0.64, 1.77)	1.17 (0.78, 1.76)	0.77	0.82 (0.54, 1.26)	1.29 (0.87, 1.95)	0.14	1.38 (0.69, 2.71)	1.21 (0.68, 2.14)	0.77

Table 74: Between group analyses (ANCOVA) analyses for change in glycaemic measures across study period. Results reported mean (95%) and adjusted for age, gender, ethnicity, smoking status and baseline variable. FG=Fasting glucose, I= Insulin

4.3.9 Biological markers of inflammation analyses - investigating the change in biological markers of inflammation from baseline and the three study time points

Repeated measures analyses were used to determine if there were any significant changes in the levels of biomarkers of inflammation within each group after 6 weeks, 12 weeks or 6 months of CPAP therapy (Tables 75 and 76). There was a significant increase in the levels of IL-6 after 12 weeks of CPAP therapy (mean change + 1.05pg/ml, p=0.047) within the treatment group. However, in both the treatment and non-treatment groups there were no other statistically significant changes in the levels of biological markers of inflammation measured. Furthermore, the mean changes reported for each of the biological markers measured are relatively discrete at each time point and within the two groups.

Following this one-way analyses of covariance were used to determine if there were any significant differences between the two groups in the levels of biological markers of inflammation after 6 weeks, 12 weeks or 6 months of CPAP (Table 77).

There were no statistically significant differences in the mean change in the levels of biological markers of inflammation between the treatment and non-treatment groups at 6-, 12- or 6 months of CPAP therapy.

Variable	Time Point	Mean ± SD	Δ Mean from BL	p
TNF-α (pg/ml)	Baseline	1.88 ± 1.45		
	6 weeks	2.05 ± 1.29	0.17	
	12 weeks	1.97 ± 1.41	0.09	
	6 months	2.09 ± 1.30	0.21	0.94
IL-6 (pg/ml)	Baseline	2.84 ± 1.97		
	6 weeks	2.29 ± 1.94	-0.55	
	12 weeks	3.89 ± 1.42	1.05	
	6 months	2.87 ± 1.65	0.03	**0.047**
CRP (mg/ml)	Baseline	3.45 ± 3.48		
	6 weeks	2.78 ± 3.88	-0.67	
	12 weeks	5.03 ± 1.99	1.58	
	6 months	5.94 ± 3.03	2.49	0.059
Leptin (mg/ml)	Baseline	28.92 ± 2.81		
	6 weeks	29.28 ± 2.59	0.36	
	12 weeks	26.84 ± 2.75	-2.08	
	6 months	27.74 ± 2.67	-1.18	0.56
Resistin (ng/ml)	Baseline	3.86 ± 1.54		
	6 weeks	3.28 ± 1.36	-0.58	
	12 weeks	3.57 ± 1.38	-0.29	
	6 months	4.21 ± 1.24	0.35	0.28
Adiponectin (µg/ml)	Baseline	6.45 ± 2.10		
	6 weeks	5.77 ± 1.53	-0.68	
	12 weeks	5.55 ± 1.58	-0.9	
	6 months	7.41 ± 2.33	0.96	0.24
PGF (ng/ml)	Baseline	1.16 ± 1.88		
	6 weeks	1.19 ± 2.25	0.03	
	12 weeks	1.42 ± 2.17	0.26	
	6 months	1.15 ± 2.12	-0.01	0.24

Table 75: Biological markers of inflammation – changes in levels across study period in the treatment group, n = 6. Results reported from repeated measures analyses

Variable	Time Point	Mean ± SD	Δ Mean from BL	p
TNF-α (pg/ml)	Baseline	2.41 ± 1.32		
	6 weeks	2.44 ± 1.28	0.03	
	12 weeks	2.55 ± 1.31	0.14	
	6 months	2.36 ± 1.28	-0.05	0.58
IL-6 (pg/ml)	Baseline	2.49 ± 1.77		
	6 weeks	2.19 ± 1.87	-0.30	
	12 weeks	2.56 ± 2.68	0.07	
	6 months	2.43 ± 1.97	-0.06	0.52
CRP (mg/ml)	Baseline	5.49 ± 2.61		
	6 weeks	7.29 ± 1.95	1.8	
	12 weeks	2.26 ± 2.27	-3.23	
	6 months	4.57 ± 2.91	-0.92	0.74
Leptin (mg/ml)	Baseline	33.88 ± 2.27		
	6 weeks	31.76 ± 2.39	-2.12	
	12 weeks	33.77 ± 2.16	-0.11	
	6 months	27.08 ± 1.68	-6.8	0.43
Resistin (ng/ml)	Baseline	4.37 ± 1.29		
	6 weeks	4.23 ± 1.29	-0.14	
	12 weeks	4.05 ± 1.45	-0.32	
	6 months	4.38 ± 1.38	0.01	0.69
Adiponectin (µg/ml)	Baseline	4.79 ± 1.59		
	6 weeks	4.35 ± 1.49	-0.44	
	12 weeks	4.58 ± 1.49	-0.21	
	6 months	4.51 ± 1.46	-0.28	0.26
PGF (ng/ml)	Baseline	2.32 ± 2.67		
	6 weeks	2.27 ± 2.73	-0.05	
	12 weeks	2.38 ± 2.60	0.06	
	6 months	2.09 ± 2.97	-0.23	0.64

Table 76: Biological markers of inflammation – changes in levels across study period in the non-treatment group, n = 7. Results reported from repeated measures analyses

Variable	6 weeks			12 weeks			6 months		
	Treatment Mean (95%CI)	**Non-Treatment** Mean (95%CI)	**p**	**Treatment** Mean (95%CI)	**Non-Treatment** Mean (95%CI	**p**	**Treatment** Mean (95%CI)	**Non-Treatment** Mean (95%CI	**p**
TNF-α (pg/ml)	1.00 (0.87, 1.16)	1.02 (0.90, 1.16)	0.84	1.03 (0.89, 1.18)	1.01 (0.89, 1.16)	0.87	0.95 (0.77, 1.18)	1.09 (0.92, 1.30)	0.33
IL-6 (pg/ml)	0.79 (0.59, 1.06)	0.92 (0.72, 1.17)	0.44	1.20 (0.83, 1.74)	1.17 (0.82, 1.65)	0.91	1.06 (0.68, 1.65)	0.93 (0.66, 1.33)	0.64
CRP (mg/ml)	0.73 (0.44, 1.21)	1.28 (0.86, 1.92)	0.096	1.41 (0.85, 2.32)	0.86 (0.52, 1.43)	0.19	1.36 (0.71, 2.62)	0.94 (0.58, 1.51)	0.36
Leptin (ng/ml)	1.00 (0.85, 1.18)	0.97 (0.86, 1.11)	0.81	0.91 (0.75, 1.10)	0.94 (0.78, 1.13)	0.79	1.03 (0.79, 1.33)	0.23 (0.67, 1.03)	0.19
Resistin (ng/ml)	0.86 (0.73, 1.02)	0.97 (0.84, 1.11)	0.31	0.93 (0.77, 1.12)	0.99 (0.84, 1.19)	0.604	0.95 (0.76, 0.85)	0.95 (0.80, 1.14)	0.97
Adiponectin (µg/ml)	1.01 (0.82, 1.25)	0.89 (0.75, 1.05)	0.34	1.03 (0.74, 1.43)	0.93 (0.68, 1.26)	0.64	1.18 (0.88, 1.59)	1.00 (0.78, 1.28)	0.402
PGF (ng/ml)	1.03 (0.68, 1.56)	1.25 (0.89, 1.75)	0.46	1.16 (0.83, 1.63)	0.99 (1.16, 0.71)	0.56	0.95 (0.77, 1.18)	1.36 (0.84, 1.91)	0.15

Table 77: Biological markers of inflammation – a between group analyses (ANCOVA) of change over study period. Results reported as anti-logged mean (95%).Adjusted for age, gender, ethnicity, smoking status and baseline variable

4.3.10 Biomedical analyses: Investigating change in biomedical measures from baseline and the three study time points

Repeated measures analyses were used to determine if there were any significant changes in biomedical measures recorded after 6 weeks, 12 weeks or 6 months of CPAP therapy within each group (Tables 78 and 79). In the treatment group there was an initial reduction in systolic blood pressure which was maintained at 12 weeks but not maintained at 6 months of CPAP- therapy (Table 78). A reduction in diastolic blood pressure, body weight, waist circumference and BMI was observed at each of the study time points within the treatment group. There is an initial reduction in body fat percentage which is not maintained at 12 weeks or 6 months within the treatment group. There is a reduction of 0.58 cm in neck circumference within the treatment group at 6 months although this is also true for the non-treatment group (Table 79). However, these observations did not reach statistical significance. In the non-treatment group a reduction in both body weight and BMI was observed across the study time points. Additionally, at 12 weeks and 6 months there is a reduction in body fat percentage within the non-treatment group which was not observed in the treatment group. However, at 6 months an increase in waist circumference, systolic and diastolic blood pressure is observed within the non-treatment group. Furthermore, results from between group analyses report a significant reduction in waist circumference at 6 months post-therapy in the treatment compared to the non-treatment group.

Following this a one-way analysis of covariance was used to determine if there were any significant differences in the change in biomedical variables after 6 weeks, 12 weeks or 6 months of CPAP (Table 80). There was a significant decrease in waist

circumference in the treatment compared to the non-treatment group at 6 months post-CPAP therapy.

Variable	Time Point	Mean ± SD	Δ Mean from BL	p
Systolic BP (mmHg)	Baseline	145.33 ± 14.39		
	6 weeks	135.08 ± 13.71	-10.25	
	12 weeks	135.17 ± 16.83	-10.16	
	6 months	145.54 ± 24.99	0.21	0.23
Diastolic BP (mmHg)	Baseline	86.08 ± 10.62		
	6 weeks	80.63 ± 9.46	-5.45	
	12 weeks	78.17 ± 8.00	-7.91	
	6 months	83.00 ± 11.29	-3.08	0.072
Body Weight (kg)	Baseline	111.30 ± 23.46		
	6 weeks	110.02 ± 21.89	-1.28	
	12 weeks	110.18 ± 21.88	-1.12	
	6 months	110.12 ± 23.23	-1.18	0.66
Waist Cir (cm)	Baseline	128.25 ± 17.27		
	6 weeks	126.38 ± 16.38	-1.87	
	12 weeks	125.92 ± 14.26	-2.33	
	6 months	125.33 ± 17.77	-2.92	0.096
WHR	Baseline	0.99 ± 0.12		
	6 weeks	0.97 ± 0.093	-0.02	
	12 weeks	0.99 ± 0.098	-	
	6 months	0.99 ± 0.11	-	0.66
BMI (kg/m^2)	Baseline	42.22 ± 7.73		
	6 weeks	41.23 ± 6.58	-0.99	
	12 weeks	41.55 ± 6.77	-0.67	
	6 months	41.37 ± 6.93	-0.85	0.52
Body Fat (%)	Baseline	42.90 ± 5.17		
	6 weeks	38.84 ± 18.92	-4.06	
	12 weeks	43.23 ± 5.66	0.33	
	6 months	42.99 ± 4.81	0.09	0.89
Neck Circ (cm)	Baseline	44.04 ± 6.29		
	6 weeks	44.21 ± 5.25	0.17	
	12 weeks	43.88 ± 4.49	-0.16	
	6 months	43.46 ± 5.23	-0.58	0.87

Table 78: Biomedical measures – change across study period within the treatment group, n = 12

Variable	Time Point	Mean ± SD	Δ Mean from BL	p
Systolic BP (mmHg)	BL	131.91 ± 24.05		
	6 weeks	127.14 ± 21.21	-4.77	
	12 weeks	132.95 ± 24.77	1.04	
	6 months	135.18 ± 14.06	3.27	0.55
Diastolic BP (mmHg)	BL	84.32 ± 14.81		
	6 weeks	84.14 ± 13.21	-0.18	
	12 weeks	84.77 ± 13.53	0.45	
	6 months	84.86 ± 11.12	0.54	0.23
Body Weight (kg)	BL	112.79 ± 18.60		
	6 weeks	109.98 ± 10.97	-2.81	
	12 weeks	109.59 ± 10.76	-3.20	
	6 months	110.02 ± 12.17	-2.77	0.78
Waist Cir (cm)	BL	118.91 ± 6.59		
	6 weeks	120.95 ± 4.61	2.04	
	12 weeks	121.55 ± 9.65	2.64	
	6 months	120.64 ± 6.77	1.73	0.43
WHR	BL	0.99 ± 0.80		
	6 weeks	0.99 ± 0.064	-	
	12 weeks	1.01 ± 0.087	0.02	
	6 months	1.00 ± 0.082	0.01	0.74
BMI (kg/m^2)	BL	40.07 ± 7.31		
	6 weeks	39.09 ± 4.70	-0.98	
	12 weeks	38.91 ± 4.54	-1.16	
	6 months	39.03 ± 4.75	-1.04	0.77
Body Fat (%)	BL	45.15 ± 4.34		
	6 weeks	48.70 ± 2.99	3.55	
	12 weeks	41.60 ± 4.62	-3.55	
	6 months	41.76 ± 4.69	-3.39	0.15
Neck Circ (cm)	BL	41.87 ± 4.36		
	6 weeks	43.58 ± 4.39	1.51	
	12 weeks	43.20 ± 3.88	1.33	
	6 months	41.32 ± 3.87	-0.55	0.031

Table 79: Biomedical measures – change across study period within the treatment group, n = 16

Variable	6 weeks			12 weeks			24 weeks		
	Treatment Mean (95%CI)	**Non-Treatment** Mean (95%CI)	**p**	**Treatment** Mean (95%CI)	**Non-Treatment** Mean (95%CI	p	**Treatment** Mean (95%CI)	**Non-Treatment** Mean (95%CI)	p
Sys BP (mmHg)	-8.61 (-17.62, 0.39)	-4.05 (-12.33, 4.23)	0.47	-7.94 (-21.33, 5.45)	-1.79 (-15.17, 11.59)	0.53	8.32 (-6.32, 22.96)	-1.49 (-12.53, 9.54)	0.29
Dia BP (mmHg)	-5.86 (-11.92, 0.19)	-0.51 (-6.11, 5.09)	0.19	-6.24 (-11.80, -0.68)	-0.39 (-5.95, 5.17)	0.15	-2.90 (-9.48, 3.67)	1.31 (-3.71, 6.33)	0.31
Weight (kg)	-0.43 (-4.62, 3.75)	-3.22 (-7.23, 0.79)	0.34	0.74 (-3.93, 5.41)	-4.93 (-9.59, -0.26)	0.11	-1.12 (-5.89, 3.66)	-2.07 (-5.82, 1.69)	0.76
BMI (kg/m^2)	-0.63 (-2.25, 0.99)	-1.13 (-2.68, 0.42)	0.66	-0.21 (-2.03, 1.61)	-1.66 (-3.48, 0.16)	0.28	-0.76 (-2.49, 0.97)	-0.86 (-2.21, 0.50)	0.93
WHR	-0.009 (-0.040, -0.023)	0.003 (-0.026, 0.032)	0.58	-0.010 (-0.060, 0.041)	0.023 (-0.027, 0.074)	0.36	0.021 (-0.017, 0.058)	0.00 (-0.029, 0.030)	0.39
Waist circ (cm)	-1.03 (-4.78, 2.73)	1.67 (-1.78, 5.12)	0.306	-1.93 (-6.49, 2.63)	1.75 (-2.81, 6.31)	0.30	-3.15 (-5.99, -0.32)	1.69 (-0.45, 3.83)	**0.014**
Body Fat (%)	3.48 (-0.58, 7.55)	0.82 (-3.65, 5.29)	0.38	-0.071 (-2.16, 2.02)	1.19 (-1.29, 3.69)	0.44	-0.95 (-6.14, 4.24)	3.57 (-0.28, 7.42)	0.18
Neck Circ (cm)	0.85 (-0.66, 2.35)	0.37 (-1.02, 1.75)	0.65	0.57 (-1.16, 2.31)	0.66 (-1.07, 2.39)	0.95	0.13 (-2.11, 2.37)	-0.78 (-2.47, 0.92)	0.53

Table 80: Biomedical measures – a between group analyses (ANCOVA) of change over study period. Results displayed as mean and 95% confidence interval. Adjusted for age, gender, ethnicity and baseline variable

4.3.11 Questionnaire data analyses: Investigating the change in questionnaire scores from baseline and the three study time points

Repeated measures analyses were used to determine if there were any changes in the scores of each questionnaire administered at each of the time points in this study within each group (Tables 81 and 82). A significant reduction in daytime sleepiness (ESS questionnaire) is observed in the treatment group at 6 weeks post-CPAP therapy which is maintained at 12 weeks and 6 months. Additionally, a reduction in depression scores (HADS questionnaire) is evident within the treatment group across all three time points of the study. Furthermore, these changes are also reported in the between group analyses (Table 83) with an additional significant reduction in the perception of quality of life observed within the non-treatment group at 6 months.

Variable	Time Point	Mean ± SD	Mean change from baseline	p
ESS	BL	14.33 ± 4.41		
	6 weeks	8.67 ± 5.39	-5.66	
	12 weeks	8.83 ± 3.55	-5.50	
	6 months	9.17 ± 6.31	-5.16	**0.030**
HADS	BL	7.88 ± 4.32		
	6 weeks	6.13 ± 2.85	-1.75	
	12 weeks	5.13 ± 3.09	-2.75	
	6 months	5.38 ± 3.20	-2.50	0.41
PQoL	BL	3.40 ± 0.89		
	6 weeks	4.00 ± 0.71	0.60	
	12 weeks	4.40 ± 0.55	1.00	
	6 months	3.80 ± 0.45	0.40	0.54
PQoH	BL	3.00 ± 1.00		
	6 weeks	3.60 ± 0.89	0.60	
	12 weeks	3.40 ± 1.14	0.40	
	6 months	3.80 ± 0.45	0.80	0.58
IPAQ*	BL	590.67 ± 1021.58		
	6 weeks	1906.89 ± 3222.04	1316.22	
	12 weeks	1957.33 ± 3682.35	1366.66	
	6 months	2815.67 ± 5278.25	2225.00	0.58

Table 81: Questionnaire scores – change across study period in the treatment group, n = 12. Results reported from repeated measures analyses. ESS: Epworth Sleepiness questionnaire, PQoL: Perception of Quality of life, PQoH: Perception of Quality of Health, IPAQ: International Physical Activity Questionnaire.* Results reported as Total Mets-minute/week

Variable	Time Point	Mean ± SD	Mean change from baseline	p
ESS	BL	10.78 ± 3.15		
	6 weeks	10.67 ± 3.32	-0.11	
	12 weeks	10.00 ± 3.43	-0.78	
	6 months	12.00 ± 4.21	1.22	0.64
HADS	BL	7.70 ± 1.77		
	6 weeks	7.50 ± 2.68	-0.20	
	12 weeks	6.70 ± 1.42	-1.00	
	6 months	8.40 ± 4.33	0.70	0.32
PQoL	BL	3.22 ± 0.83		
	6 weeks	3.44 ± 1.13	0.22	
	12 weeks	3.56 ± 0.73	0.34	
	6 months	2.56 ± 1.24	-0.66	**0.012**
PQoH	BL	2.56 ± 1.01		
	6 weeks	2.56 ± 0.88	-	
	12 weeks	3.38 ± 0.71	0.77	
	6 months	2.56 ± 1.24	-	0.25
IPAQ*	BL	2550.27 ± 3345.71		
	6 weeks	1187.09 ± 1226.84	-1363.18	
	12 weeks	2190.55 ± 2103.24	-359.72	
	6 months	2916.73 ± 4807.50	366.46	0.29

Table 82: Questionnaire scores – change across study period in the non-treatment group (n = 16). Results reported from repeated measures analyses. ESS: Epworth Sleepiness questionnaire, PQoL: Perception of Quality of life, PQoH: Perception of Quality of Health, IPAQ: International Physical Activity Questionnaire.* Results reported as Total Mets-minute/week

Variable	6 weeks			12 weeks			24 weeks		
	Treatment Mean (95%CI)	**Non-Treatment** Mean (95%CI)	**p**	**Treatment** Mean (95%CI)	**Non-Treatment** Mean (95%CI)	**p**	**Treatment** Mean (95%CI)	**Non-Treatment** Mean (95%CI)	**p**
ESS	-4.67 (-7.21, -2.12)	-0.86 (-3.17, 1.44)	**0.037**	-4.54 (-7.22, -1.87)	-1.89 (-4.08, 0.320)	**0.016**	-3.23 (-7.37, 0.91)	-0.59 (-3.43, 2.23)	0.300
HADS	-1.88 (-3.51, -0.26)	0.35 (-1.01, 1.70)	**0.045**	-3.24 (-4.77, -1.71)	-0.71 (-2.06, 0.64)	**0.030**	-2.71 (-5.01, -0.41)	0.87 (-1.00, 2.75)	**0.027**
IPAQ*	-822.79 (-3188.60, 1543.02)	-1200.77 (-3251.95, 850.41)	0.81	128.33 (-2639.88, 2896.53)	638.70 (-1409.05, 2686.45)	0.77	2970.34 (-4168.86, 1009.53)	2062.16 (-3261.26, 7385.58)	0.84

Table 83: Questionnaire scores – A between group analyses (ANCOVA) for change across study period. Results reported mean (95%).Adjusted for age, gender, ethnicity, smoking status and baseline variable. ESS: Epworth Sleepiness questionnaire, PQoL: Perception of Quality of life, PQoH: Perception of Quality of Health, IPAQ: International Physical Activity Questionnaire.* Results reported as Total Mets-minute/week

4.3.12 Compliance data

Patient compliance with CPAP was measured objectively by an inbuilt compliance meter and recorded at each of the follow-up clinics. The following tables are a breakdown of compliance for the whole group (Table 84), non-compliers alone (Table 85) and for compliers alone (Table 86). There was no significant change in the use of CPAP compliance across the study period (Table 84).

Time	CPAP usage (Mean ± SD)	Range	p
6 weeks	5.75 ± 1.45	3.00 - 7.75	-
12 weeks	5.74 ± 1.49	2.96 – 7.59	0.94
6 months	5.93 ± 1.77	2.86 – 8.35	0.23

Table 84: CPAP compliance for entire treatment group (mean hours/night), n=12. Results reported for repeated measures analyses

Time	CPAP usage (Mean ± SD)
6 weeks	3.24 ± 0.33
12 weeks	3.05 ± 0.12
6 months	2.97 ± 0.14

Table 85: Non-compliers mean CPAP use per night (mean hours/night), n = 2

Time	CPAP usage (Mean ± SD)	Range
6 weeks	6.25 ± 0.94	5.00 – 7.59
12 weeks	6.28 ± 0.89	5.00 – 7.59
6 months	6.52 ± 1.21	4.43 – 8.35

Table 86: Compliers – mean CPAP use per night (mean hours/night), n=10

4.4 Discussion

This study was comprised of two phases the first involved screening for the sleep disorder OSA, and the second treatment with CPAP therapy, the ‘gold standard’ for OSA. The primary objective was to determine whether CPAP therapy improves glycaemic control in obese people with T2DM. Secondary objectives included determining the prevalence of OSA in this high-risk population, the effects of CPAP therapy on circulating levels of inflammatory biomarkers, measures of adiposity, blood pressure and a number of self-reported psychometric measures.

4.4.1 Control group

Chapter 3 discussed the shortcomings of a number of studies investigating the effects of CPAP therapy on glycaemic control and it was highlighted that the majority of these studies did not include a control group. Although, the non-treatment group is not ideal or may not even be considered a true control group, we did not consider it ethical to conduct a classical randomised control trial. Therefore, for the purpose of answering these research questions the treatment group (moderate-to-severe OSA) served as their own controls with additional between group analyses performed in order to crudely account for potential influence of study effect on these results. This type of study design is known as quasi experimental.

4.4.2 Glycaemic control and CPAP therapy

CGMS data

72 hour continuous blood glucose monitoring (CGMS) was offered to all participants at each time point within this study. However, due a number of reasons not all CGMS data were obtained from all participants. This included refusal to wear the device, malfunctioning of glucose sensors and some participants felt overwhelmed entering capillary blood glucose readings which were required (at least four times a day) to calibrate the monitor. Specifically, at 12 weeks, only three sufficient datasets were obtained thus it was decided that analyses could not be conducted for this time point.

No statistically significant changes were observed with respect to proportion of time spent within the three glycaemic ranges or the frequency and duration of hyper- or hypoglycaemic events after 6 weeks or 6 months of CPAP therapy (Tables 66 - 71). The final sample size for the CGMS data analyses before and after CPAP therapy was considerably smaller than expected (n=9). Therefore, we feel that the non-significant results that are reported here are because the study is underpowered. However, a number of trends have been observed that are consistent throughout the analyses and therefore this part of the study is being treated in terms of a 'proof of concept' study which we feel warrants further exploration with larger sample sizes.

Table 66 displays results from repeated measures analyses in the treatment group for change in the proportion of time spent within the three glycaemic ranges. There is a large reduction (- 60.8%) in the time spent above target at 6 weeks when adjusted for 24

hours. This reduction in hyperglycaemia appears to be occurring predominantly during the night-time (-71.8% vs. -52.9%) and the shift out of hyperglycaemia is split towards both normoglycaemia and hypoglycaemia. There is a greater increase in the time spent below target than that for within target when adjusted for 24 hours, daytime and night-time hours. However, the threshold used to define hypoglycaemia (≤ 3.9mmol/L) may not be accurate in defining *true* hypoglycaemia. Consequentially, the shift from hyperglycaemia to normoglycaemia may be underestimated here [310]. This shift from hyperglycaemia to normo/hypoglycaemia is maintained at 6 months (Table 66). However, the reduction in the proportion of time spent above target is lower than what is observed at 6 weeks. A reduction in the time spent above target was observed in the non-treatment group (Table 67), however, the percentage reduction was much lower than that observed for the treatment group; for example at 6 weeks the reduction in time spent above target was -60.8% in the treatment group versus only -7.7% in the non-treatment group.

These trends are consistent with some of the current literature. For example, Babu and colleagues reported a significant reduction in the number of CGMS readings >11 mmol/L after 30-90 days of CPAP therapy in subjects with T2DM [248]. Conversely, Czupryniak and colleagues [319] reported a significant increase in mean glucose levels over the 72 hour monitoring period. Although this is a sizable and significant increase (27%), the study was performed in non-diabetic patients who remained normoglycaemic throughout (4.4 ± 0.61 mmol/L) [319]. Unfortunately, in the current study we were unable to analyse the CGMS data with respect to fasting or post-prandial glucose levels as reported by Babu et al [248]. We did not ask participants to log food times through

an ‘event’ function present on the CGMS device. However, as previously stated a large number of subjects found it difficult to enter data into the device after leaving the clinic.

The definition of a hypoglycaemic event, as previously discussed, is ambiguous within the literature and therefore two separate criteria were used in determining the frequency and duration of hypoglycaemic events within each group (Tables 68 - 71). Again, from these analyses a reduction in hyperglycaemia is evident in the treatment group at 6 weeks (Table 68) - both a reduction in the frequency and average length of these events is evident. This reduction appears to be predominantly occurring during the night-time. Interestingly, there is a large increase in the frequency and duration of hypoglycaemic events when defined as $\leq$ 3.1 mmol/L for $\geq$ 10 minutes (Table 68) compared to the second more stringent definition of $\leq$ 2.2 mmol/L for $\geq$ 20 minutes (Table 70). Further supporting the previously discussed shift out of hyperglycaemia towards normoglycaemia in obese subjects with OSA treated with CPAP therapy. At 6 months there is a small increase in the frequency of hyperglycaemic events however there is a reduction in the average duration of these events. Interestingly, the increased frequency and duration of hypoglycaemic events is maintained at 6 months in the treatment group (Table 68), the same is not true of the non-treatment group (Table 69).

To our knowledge, no other study has investigated the effects of CPAP therapy on the number and duration of hyper- or hypoglycaemic episodes during daytime or night-time hours in this study population using CGMS. The pattern of the shift from the hyperglycaemic range into the normoglycaemic range is consistent within the treatment group and warrants further investigation with longer follow-up periods and larger

sample sizes. Because it is difficult to deduce whether the non-significant findings are due to CPAP therapy having no effect on these variables or whether it is due to the study being significantly under powered.

HbA1c, fasting glucose and insulin

There were no statistically significant changes in the levels of fasting glucose, insulin or HbA1c after 6, 12 or 24 weeks in the treatment or non-treatment groups (Tables 72 and 73). However, the observed change in HbA1c levels in the treatment group and the difference between the two groups is a clinically important trend. The effects of CPAP therapy on measures of glycaemic control are inconsistent within the literature as discussed in chapter 3. The results reported here can be supported by a number of studies [208, 270, 272-274, 280], but is also contradicted by a number of other studies [248, 267, 278, 320]. Again further investigation is needed in this context with larger sample sizes and longer follow-up periods to further explore these findings.

Although the change in HbA1c at 24 weeks in the treatment group did not reach statistical significance (Table 72), a reduction of 0.80% is considered to be of clinical significance. This suggests that long term CPAP therapy is lowering circulating blood glucose levels. Furthermore, the level of HbA1c in the non-treatment group at 24 weeks had increased by 0.59% (Table 73) and although again this is not statistically significant the overall difference in the change between the two groups is 1.39% (Figure 34). This level has clinical significance for micro/macro- vascular outcomes associated with T2DM and further supports the findings from our CGMS data – a shift out of hyperglycaemia and towards normoglycaemia. West and colleagues observed a small

change in HbA1c levels after three months of APAP therapy (see Chapter 3) in obese subjects with T2DM (HbA1c -0.02, p=0.7) [277], which also did not reach statistical significance. Additionally, Babu and colleagues observed a reduction of 0.4% in 24 obese subjects with T2DM after 30-90 days of CPAP therapy a change that approached statistical significance [248]. However, on splitting their analyses in to two groups based on a baseline HbA1c threshold of either $\leq$ 7% and >7%, those with a baseline level >7% had a significant reduction in HbA1c levels at a magnitude similar to what we report in this study (-0.6%). However, the sample size for these analyses was unexpectedly small (n=10, treatment group and n=8, non-treatment group) and therefore it most likely is underpowered.

4.4.3 Prevalence of OSA

Out of 46 patients who undertook the home sleep respiratory study, the prevalence of moderate to severe OSA was found to be higher (43.5%) than what is reported for the general population (2-4%). Forty-three point five percent of the final sample had an AHI and ODI that was considered high enough for the patient to benefit from treatment with CPAP. Additionally, 15.2% (7) of subjects were found to have other sleep complaints that warranted further investigation at the Leicester Sleep Clinic (Figure 26). These results indicate that obese people with established T2DM are a population with a high incidence of sleep complaints which are currently under-diagnosed. Furthermore, the response to screening was significantly higher in those who were targeted within the clinic by a health care professional (44.6% vs. 5.3%, p<0.0001). There is a large body of evidence linking poor sleep to poor health as discussed in Chapter 1. More specifically, the cardiovascular risks that are independently associated with OSA [215,

218] further highlighting the importance of screening these patients within the clinical setting as part of their routine care.

4.4.4 Risk factors for OSA

There were no significant differences in age, gender, ethnicity or current smoking status between those with moderate-severe OSA and those with non-mild OSA. However, neck circumference and systolic blood pressure were found to be significantly higher in the moderate–severe OSA group at baseline (Table 53). Results from logistic regression analyses investigating which of the baseline variables best predict moderate-severe OSA (AHI ≥ 15) further support these results. In the adjusted model, high systolic blood pressure, waist circumference and neck circumference were found to be independently associated with OSA (Table 58), which is supported by the literature. Neck circumference is strongly and positively correlated with AHI ($r=0.74$, $p<0.001$) [321]. Dixon and colleagues additionally reported a neck circumference of ≥ 43 centimetres as a strong independent predictor of moderate-severe OSA (OR 13.2 (95%CI 2.4, 75), $p=0.004$) furthermore, it was reported to be a better predictor of OSA than both age and BMI [321]. Additionally, presence of OSA has been reported to increase the likelihood of hypertension independent of a number of potential demographic and anthropometric confounders (OR 1.37 (95%CI: 1.03, 1.83), $p=0.005$), [217].

Interestingly, results from CGMS data show a negative relationship between OSA and hypoglycaemia. The proportion of time spent below target i.e. blood glucose levels < 3.9 mmol/L, was identified as an additional independent predictor of OSA. The longer the proportion of time spent below target was associated with a 19% decreased

likelihood of OSA (Table 58). This association has not been previously reported in the literature; however it is consistent with the literature if you translate reduction in hypoglycaemia to increased blood glucose levels. For example increased sympathetic output has been consistently shown to be associated with OSA [304]. Increased sympathetic drive is associated with metabolic changes including increased insulin resistance [304, 305] and therefore it is plausible that a negative association between hypoglycaemia and OSA risk would exist. Furthermore, when investigating correlations between glycaemic measures as determined by CGMS and parameters of OSA determined from the home sleep respiratory study, we found the proportion of time spent below target to be significantly and negatively correlated with ODI (Table 60). Thus, increased frequency of oxygen desaturation is associated with a decrease in the frequency of hypoglycaemic events which can be translated to an increase in hyperglycaemia. This relationship is further supported by the results from Spearman's rank order correlation between AHI, ODI and hypoglycaemic events (Tables 61, 63 and 64). These results are again consistent with the increased sympathetic drive theory – apnoeic events increase sympathetic output resulting in increased circulating levels of blood glucose. However, this is an area that is not well described in the literature and therefore further investigation into the suggested association between AHI/ODI and hypoglycaemia with larger sample sizes are needed to fully establish the mechanisms involved.

4.4.5 Biological markers of inflammation

There were no significant changes in the levels of biological markers of inflammation in either the treatment or non-treatment group at 6 weeks, 12 weeks or 6 months (Tables

75 and 76). Therefore, in this population of established T2DM, subjects with newly diagnosed and previously untreated moderate-severe OSA, CPAP therapy has no significant effect on the levels of these recognized biological markers of inflammation within 6 months. However, this could again be attributed to the small sample size within this study, as they are inconsistent with the existing literature. For example circulating levels of leptin have been reported to decrease after CPAP therapy in obese subjects with newly diagnosed and previously untreated OSA [241, 267]. Yokoe and colleagues reported a significant decrease in the levels of IL-6 and CRP in obese subjects with newly diagnosed and previously untreated OSA without concomitant change in BMI [132]. Arias and colleagues reported significant decrease in circulating levels of TNF-α post-CPAP therapy [322]. A significant increase in circulating levels of adiponectin have been observed in obese subjects with newly diagnosed and previously untreated OSA again without concomitant change in BMI [323]. However, this pilot study by design may well lack the required power to identify any statistical differences in such variables. For example we were unable to analyse every participants' fasting blood samples due to difficulties in obtaining each of the required samples at every time point in the study, in addition to poor follow-up attendance resulting in a small overall sample size for these analyses (treatment n=6, non-treatment n=7). Therefore drawing conclusions of the effects of CPAP therapy on inflammatory cytokines from these results should be done with caution.

4.4.6 Clinical/biomedical variables

There were no statistically significant changes in the clinical variables recorded after 6 weeks, 12 weeks or 6 months in either the treatment or non-treatment group (Tables 78

and 79). Again, this could be a consequence of this pilot study being underpowered. There are some changes evident within the treatment group which suggests that CPAP therapy may impact on these measures. An initial reduction in systolic blood pressure is evident within the treatment group which is not maintained after 6 months. Similarly the reduction in diastolic blood pressure observed in the treatment group at 6 and 12 weeks is maintained but attenuated at 6 months post therapy (Table 78). There were no significant differences in the prevalence of hypertension over the course of the study, as classified by the IDF criteria [86], or in the use of antihypertensive or other medication from that what was reported at baseline (Table 56). Furthermore similar changes were not observed in the non-treatment group (Table 79). This observation of an initial improvement in blood pressure that is not maintained after 6 months of CPAP therapy is supported somewhat by the current literature. A recent Cochrane review [324] reported conflicting findings between the six studies they reviewed and attributed these differences to variations in the baseline mean blood pressures of each study population [324]. However, in a more recent review conducted by Bazzano and colleagues [325], CPAP therapy was reported to have a significant positive effect on both systolic and diastolic blood pressure reduction [325]. A pooled mean change in systolic blood pressure of -2.46 mmHg (95% CI: -4.31, -0.62) and in diastolic blood pressure -1.83 mmHg (-3.05, -0.61) was reported. Increased CPAP compliance is also associated with greater mean reduction in systolic blood pressure [325]. However, the results from a placebo trial not included in either of these reviews reported a significant reduction in daytime blood pressure after one week of CPAP therapy. This was true also for the placebo group and therefore the results could be attributed to a non-specific response (study effect), [326]. Additionally, Hermida and colleagues [327] investigated the effects of CPAP therapy on hypertensive patients with OSA. They observed no

reduction in the incidence of hypertension but did observe a small change in ambulatory blood pressure after four months of CPAP therapy. The change observed was similar to that of the patients who did not receive CPAP therapy and therefore can be attributed to 'ambulatory blood pressure monitoring (ABPM) pressor effect' [327]. However, the results reported by Bazzano and colleagues from their meta-analyses confirm that CPAP therapy does have an overall significant clinical effect on blood pressure [325]. The results observed in this study suggest that CPAP therapy has a short-term clinically significant effect on blood pressure reduction which is not maintained post-six months' of therapy. It is essential to note that only one of the studies included in Bazzano's meta-analyses had a study period up to six months. The average length of the studies included was 7.37 weeks and therefore our observation of no long term clinical benefit of CPAP therapy on daytime blood pressure could be true. Furthermore, long-term studies of the effects of CPAP therapy on blood pressure in both hyper- and normotensive patients need to be conducted. It is essential to note that there were no changes in the medication of any of these subjects between baseline and each follow-up and therefore these results cannot be attributed to the introduction or change in type or dose of antihypertensive medication.

A moderate reduction in body weight is evident within the treatment group after 6 weeks' therapy which is maintained after 6 months' therapy (Table 78). This observation is supported by the similar moderate reduction in BMI within the treatment group at 6 weeks which is maintained at 6 months post therapy. A moderate reduction in neck circumference is also evident within the treatment group after 6 months' of therapy. However, these observations could be attributed to non-specific study effect given reductions in body weight, BMI and body fat percentage are observed in the non-

treatment group at all three study points. These reductions are of a slightly higher magnitude than that observed for the treatment group. A similar reduction in neck circumference is also observed between the two groups (Table 80). However, a reduction in waist circumference of approximately 3 centimetres is evident in the treatment group and not in the non-treatment group where an increase of approximately 2 centimeters is observed. Therefore CPAP therapy can be associated with a reduction in adiposity. This is further supported by the significant difference between the mean change in waist circumference at 6 months post therapy between the treatment and non-treatment groups (Table 80).

There is not a large availability of published work on the effects of CPAP therapy on weight reduction and currently there are discrepancies between what has been published. Redenius and colleagues reported no decrease in BMI in either males or females treated with CPAP for 1 year [328] as a number of other studies have reported [274, 276, 278]. However, they did observe an increase in BMI within the females treated for OSA (0.55 ± 0.3 kg/m^2, $p=0.032$) which is inconsistent with changes in body weight that have been previously reported [267, 329]. Chin and colleagues [267] reported a significant reduction in body weight in 41% (n=9) of their study population post 1 and 6 months post CPAP therapy. Furthermore, within the current study there are discrepancies with respect to the reduction in measures of adiposity as a significant reduction in waist circumference emerged after 6 months' therapy within the treatment group which was not observed within the non-treatment group. This therefore suggests that there is a change in body fat distribution specifically visceral adiposity, and not in overall body weight or body fat percentage in obese individuals with T2DM and newly treated moderate-severe OSA. This is consistent with the further findings of Chin and

colleagues who reported significant reduction in visceral fat accumulation (VFA) and subcutaneous fat accumulation (SFA) (p=0.0003) in their whole study population. However, when they split the analyses into two groups those who had lost weight and those that had not, VFA had decreased significantly in both the groups but a significant reduction in SFA was only observed in the group who had lost weight [267]. Additionally, Trenell and colleagues reported an 8 and 2% decrease in the volume of visceral and subcutaneous adipose tissue, respectively, in 19 obese normoglycaemic subjects with OSA after twelve weeks of CPAP therapy [330]. Therefore, CPAP therapy may not facilitate overall weight loss but could have long-term positive effects on body fat distribution and therefore indirectly reduce the cardiovascular and metabolic risks associated with abdominal obesity.

4.4.7 Questionnaire data

A significant improvement in daytime sleepiness was reported for the treatment group at 6 weeks post therapy which remained significant at 6 months (Table 81). This is consistent with the current literature as it is widely accepted that subjects with moderate-severe OSA who suffer excessive daytime sleepiness will feel more alert and less sleepy during waking hours when CPAP therapy is used for at least four hours a day [324, 331-335]. This is of clinical and social significance for a number of reasons including the association between excessive daytime sleepiness and road accidents. Yamamoto and colleagues reported a reduction from 33% to 0% in the incidence of road accidents in 39 patients with OSA post two years of CPAP therapy [332], an observation that is further supported by work conducted by Cassel and colleagues [335]. This group reported a reduction in the rate of accidents from 0.8 per 100,000km to 0.15

per 100, 000km ($p<0.01$) post 12 months of CPAP therapy in 59 obese individuals with moderate-severe OSA [335].

However, results reported from within group analyses indicate that there were no significant changes in depression scores (HADS), perceived quality of life (PQoL) or health (PQoH) or in physical activity (IPAQ) after 6 weeks, 12 weeks or 6 months of CPAP therapy in the treatment group. A moderate improvement in PQoL is observed in the non-treatment group which could be attributed to non-specific study effect (Table 82). Although, results from between group analyses (Table 83) which are adjusted for age, gender, ethnicity, smoking status and baseline variable indicate that there is a significant improvement in depression and anxiety scores in the treatment compared to the non-treatment group at 6 weeks post therapy which is maintained at 6 months. This observed improvement in mood as determined by the HADS questionnaire is again consistent with the current literature [324, 332, 333]. The exact mechanisms involved in the observed association between OSA, excessive daytime sleepiness and poor mood are not fully understood. However, it is plausible that the level excessive daytime sleepiness experienced by some individuals with OSA prohibits the individual from completing everyday tasks or interacting socially compared to individuals who do not suffer with this condition and therefore has a negative impact on mood.

4.4.8 CPAP compliance

Patient compliance with CPAP was measured objectively by an inbuilt compliance meter and recorded at each of the follow-up clinics. The mean time of CPAP use was greater than 4 hrs per night as is recommended by the sleep laboratory. Furthermore

there were no significant changes in the levels of CPAP use across the study period (Table 84). Assessment of compliance at each of the study time-points showed that 2 patients consistently used CPAP therapy for less than 4 hours per night (Table 85). When these two patients were removed from the analysis, the mean CPAP usage had improved over each of the study time points for the remaining 10 subjects (Table 86). Therefore the results we report here cannot be attributed to poor CPAP compliance. There were no significant changes in the results for change in HbA1c, fasting glucose or insulin after removal of non-compliers. Following this Pearson's product moment was used to determine if there was a correlation between hours of CPAP use and change in HbA1c within the treatment group after 6 months of therapy. A very small non-significant negative correlation was found between use of CPAP therapy (hours/night) and change in HbA1c ($r = -0.14$, $p=0.68$). When the two non-compliers were removed the degree of the negative correlation improved however it remained statistically insignificant ($r = -0.26$, $p=0.51$). This is inconsistent with the literature where Babu et al [248] have reported a significant positive correlation between reduction in HbA1c and the number of days of CPAP in those that used CPAP > 4 hours/day ($r = 0.74$, $p=0.006$).

4.4.9 Study strengths and limitations

One of the major limitations of this pilot study is its small sample size under powered. It is possible that the lack of significant findings may be attributed to the study being underpowered. We calculated that an overall sample size of fifty subjects (25 per group), would provide 80% power to detect significant difference in HbA1c levels of 1.3% change from baseline at a 5% significance level. However, due to the low

response rate and unforeseen large dropout numbers we were unable to fulfill these criteria. Additionally, due to difficulties in obtaining fasting blood samples from a number of subjects, poor follow-up attendance and difficulties with the CGMS devices (as previously discussed) we were unable to obtain complete data for each of the main areas of investigation. Thus this study should be regarded as a 'proof of concept' study.

A number of limitations of CGMS have been reported in the literature [314, 336, 337] namely differences between interstitial blood glucose and intravenous blood glucose levels. The relationship between the two is poorly understood and thought to be variable in the face of rising and decreasing blood glucose levels with circulating insulin concentrations also thought to influence a difference [336]. However, CGMS provides a continuous stream of data unlike data collected through self monitoring of capillary blood via finger stick testing. In addition, the data are more likely to be representative of the patients actual 24 hour glucose profile as they are able to go about their daily routines as normal. Furthermore, CGMS has been found to be better at detecting hypoglycaemic events than self reported measures – finger stick [314, 337], specifically nocturnal hypoglycaemic events [336].

There are many strengths of this study which include the diverse range of variables collected and that the same researcher collected all data from each patient at each of the study time points. Additionally, standardised operating procedures were used throughout the study, and there was no variation in the equipment used for or provided to each subject. The diagnosis of OSA was made by a fully trained clinician and each patient received the routine follow-up care that is standard for those receiving CPAP therapy. We were able to objectively measure CPAP compliance and found that 83.3%

of patients were indeed CPAP compliant and that there were no significant differences in the level of compliance across the study period. The quantification of biomarkers of inflammation was blinded. A further strength in this study is the use of CGMS in this cohort and the emergence of some original and consistent patterns of the effect of CPAP therapy on glycaemic control at 6 weeks and 6 months post therapy.

4.5 Conclusion and future work

In summary, we report that there is a high prevalence of sleep disorders, specifically OSA in obese patients with T2DM. Treatment with CPAP therapy for 6 months significantly reduces excessive daytime sleepiness, improves mood and significantly reduces waist circumference in this patient population. We report that CPAP therapy has a positive short-term effect on lowering blood pressure but that this is not maintained at 6 months. Although results from HbA1c and CGMS data analyses were statistically insignificant, we feel our data suggests that CPAP therapy does have a lowering effect on blood glucose levels and that this study was under powered to reach statistical significance. We found no significant changes in the levels of biological markers of inflammation post-CPAP therapy but again feel that this could be attributed to the study being insufficiently powered. Due to the potential benefits of CPAP therapy on mood, waist circumference and glycaemic control, it is essential that further work is conducted in this field. Specifically in patients with T2DM given the higher prevalence of this sleep disorder within this population. In addition to the longer term potential CPAP has in preventing the development of micro- and macrovascular complications associated with T2DM that result from prolonged states of hyperglycaemia. Future studies need to include larger sample sizes, longer study periods and as suggested previously include food and sleep diaries. Additional studies are

required to further investigate the association between AHI/ODI and hypoglycaemia in untreated OSA patients. Finally, the key finding of this study is that obese patients with established T2DM should be screened for sleep disorders and treated as part of their routine care.

Chapter Five

Final Discussion

5.1 Aims and Objectives

This PhD was centred on investigating how sleep deprivation affects glycaemic control and sub-clinical inflammation. SDB was the primary model used for investigating sleep deprivation. A number of subjective measures were implemented for assessing SDB, excessive daytime sleepiness and general sleep disturbance. Furthermore, OSA was objectively measured in a previously undiagnosed population to evaluate the effects of treatment on glycaemic control and inflammation. Normoglycaemia, prediabetes and overt T2DM were used as models for investigating glycaemic control in the studies conducted for this PhD.

The first study 'The Sleep and Inflammation Study' was cross-sectional and exploratory by design. Individuals were recruited from those attending the screening phase of a multi-centre population based T2DM screening study. This provided access to a population with a spectrum of glucose intolerance and a means to investigate relationships between sub-clinical inflammation, sleep deprivation and glucose intolerance. Furthermore, this multi-centre screening study allowed access to a number of biomedical and anthropometric data thus we were able to investigate cardiovascular profiles and determine any relationships between sleep deprivation, specifically SDB, and the Metabolic Syndrome. A further objective of this study was to determine the prevalence of sleep disruption and of OSA within the two separate populations studied. The three sleep questionnaires used in the first study were assessed for their potential use within a clinical setting for identifying individuals who are either glucose intolerant have the MetS or with >20% 10 year risk of cardiovascular disease.

A systematic review was undertaken to determine, from the existing literature, if continuous positive airway pressure therapy (CPAP), the 'gold standard' treatment for OSA, affects glycaemic control. This review highlighted a large number of inconsistencies and shortcomings in the design of the studies reviewed. Therefore, the 'The Leicester Sleep and Sugar study', aimed at determining the effect of CPAP therapy on glycaemic control, was able to address some of these shortcomings. We additionally sought to determine whether CPAP therapy impacts inflammation and cardiovascular markers such as blood pressure and adiposity. This pilot-intervention study was designed so that the participants acted predominately as their own controls. However, by including a non-treatment group, between group analyses could be undertaken to control for non-specific study effect.

5.2 Findings

5.2.1 The Sleep and Inflammation study

The prevalence of sleep disruption as determined by the three sleep questionnaires used in this study was found to be:

- higher than expected from the **SAQ**
- lower than expected from the **ESS**
- in accordance with the literature for the **BSQ**

The results from between group analyses for each of the questionnaires are of interest. No significant differences in the levels of biological markers of inflammation or cardiovascular markers were observed between the two groups – sleepiness/sleep disturbance as defined by either the ESS or SAQ, respectively. This suggests that these two questionnaires lack the required specificity to accurately identify sleep disturbance.

Subsequently, these two questionnaires may over/under estimate the prevalence of sleepiness/sleep disturbance.

In contrast to the SAQ and ESS questionnaires, significant differences were evident in the levels of biological markers of inflammation and cardiovascular markers between the two groups as defined by the BSQ. However, none of the three questionnaires were sensitive or specific enough to identify the MetS, glucose intolerance or >20% 10 year CVD risk in our receiver operating curve analyses. This was an unexpected result in particular that the BSQ was not sensitive enough to identify those with MetS given the differences observed between those classified at high risk of SDB (SDB group) and those at low risk (non-SDB group). Waist circumference and male prevalence were significantly higher in the SDB group. Levels of CRP, IL-6 and leptin were found to be significantly higher in the SDB group and adiponectin significantly lower independent of age, gender, ethnicity, smoking status and medication. However, with further adjustment for waist circumference these associations were attenuated and were non-significant. Our hypothesis of an independent association between SDB and inflammation was therefore rejected. However, adipose tissue is an active endocrine organ and the predominant source of secretion of IL-6, leptin and adiponectin. Subsequently, adipose tissue is an indirect source for the secretion of CRP whose hepatic secretion is regulated largely by adipokines such as IL-6. Therefore the reported dependent relationship between low-grade inflammation and SDB is plausible due to the common feature of visceral obesity within these two conditions.

However, adiposity does not explain the significant differences observed between South Asians and Caucasians with SDB. South Asians with SDB were found to have higher circulating levels of leptin and, interestingly, significantly lower levels of 8-iso PGF-2α, as a measure of oxidative stress, (independent of the same covariates). Furthermore, South Asians with SDB were found to have poorer glycaemic control (significantly higher HbA1c and postprandial glucose (Table 31)), again independent of the same covariates. These observations have not been previously reported and suggest that the characteristics of people with SDB are different between these two ethnic groups. Caucasians at high risk of SDB have higher levels of oxidative stress and South Asians have a higher level of inflammation in addition to poorer glycaemic control. This suggests that South Asians could be more sensitive to the detrimental effects of this sleep disorder given that oxidative stress is a prerequisite for systemic inflammation [61] and indeed hyperglycaemia [191]. However, further delineation of the mechanisms involved here is needed in addition to investigating ethnic differences in those diagnosed with this sleep disorder. This may aid in understanding why South Asians have an increased risk of CVD within the general population.

The BSQ was found to be an insensitive tool for discriminating between those with MetS and those without (AUC=0.622) however, we did find SDB to be an independent risk factor for MetS (Table 28). Although the BSQ could not be used in the clinical setting for the simultaneous identification of those with SDB and MetS, there is an independent relationship between these two conditions. Furthermore, the prevalence of MetS in those scoring high risk of SDB was high at 19% (Table 31). Therefore, sleep clinic patients attending screening for SDB (more specifically OSA) should also be assessed for metabolic abnormalities and especially those of South Asian origin. This

could potentially have a large impact on reducing progression towards CVD which is associated with both this sleep disorder and the MetS [44].

5.2.2 The Leicester Sleep and Sugar study

The prevalence of moderate-severe OSA is higher in obese people with established T2DM. Therefore, this is a population that would benefit from the inclusion of screening for sleep disorders as part of their routine care. A significant negative correlation was found between features of OSA (AHI/ODI) and lower blood glucose levels. The more frequent apnoeas or oxygen desaturation events the lower the proportion of hypoglycaemia this is consistent with the sympathetic drive theory. Treatment of this sleep disorder with CPAP therapy was found to have a beneficial effect on adiposity, mood and daytime sleepiness. Unfortunately, this pilot study was largely underpowered and therefore the results for the effects of CPAP therapy on glycaemic control did not reach statistical significance. However, the results do suggest that CPAP therapy improves glycaemic control. This was demonstrated by a shift from hyperglycaemia towards normoglycaemia (CGMS data), in addition to a 0.8% reduction in HbA1c levels within the treatment group at 6 months of CPAP therapy. Furthermore, there was an increase in HbA1c levels in the treatment group at 6 months resulting in an overall difference between groups at 6 months of 1.39% thus suggesting that CPAP therapy has a positive clinically significant effect on glycaemic control. The effects of CPAP therapy on blood pressure suggest a transient lowering that is not maintained after 6 months.

These results emphasise that a relationship exists between OSA, blood pressure and poor glycaemic control. However, this study was not designed to identify a causal relationship and currently the mechanisms involved in the association between OSA and metabolic dysfunction have not been fully elucidated. Figure 34 is a diagram displaying the potential mechanisms that link OSA with metabolic dysfunction, high blood pressure and ultimately increased risk of T2DM and CVD.

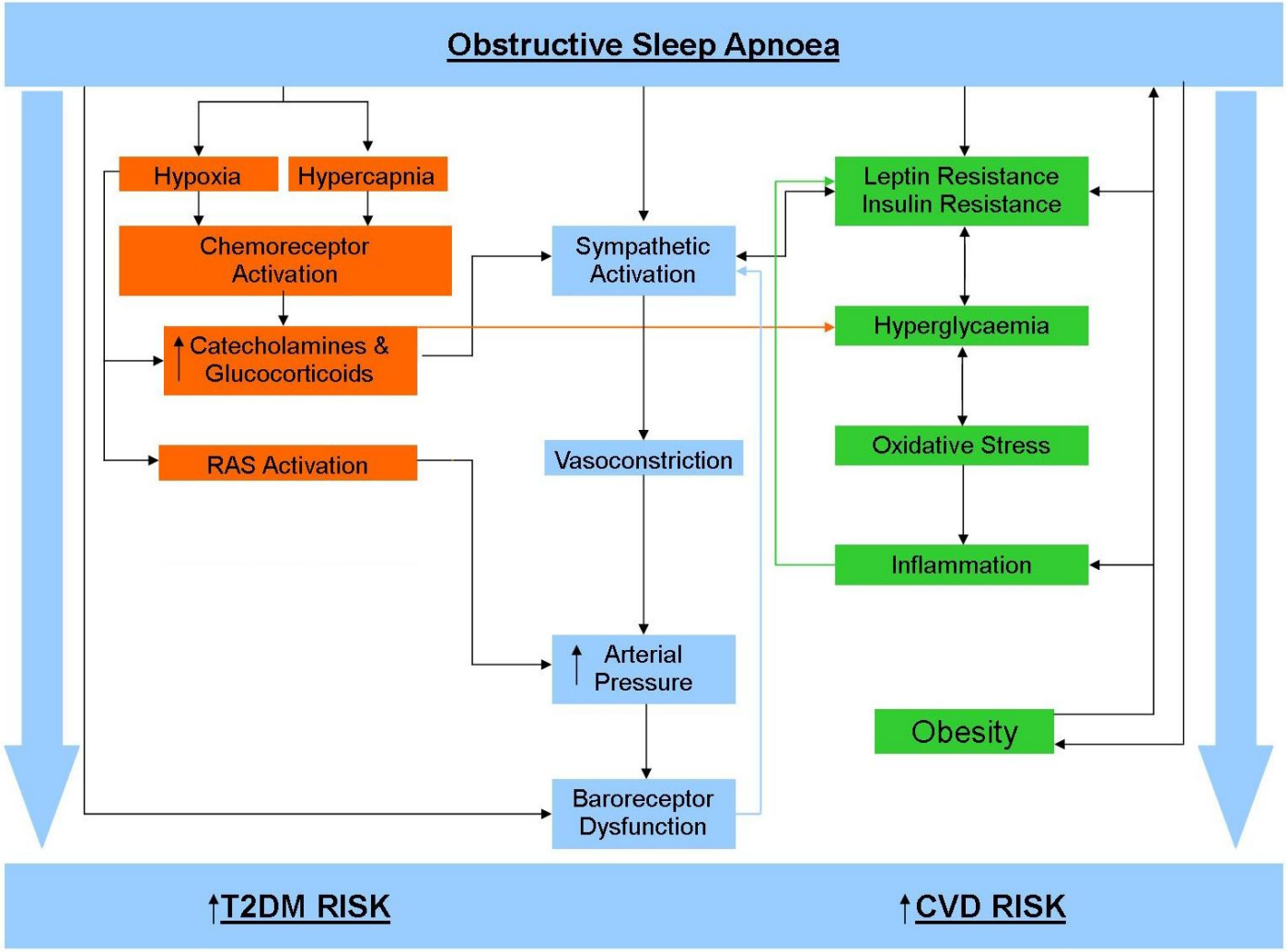


Figure 34: Potential mechanisms linking OSA to metabolic dysfunction

5.3 Potential mechanisms linking OSA to metabolic dysfunction

The physiological effects of OSA are potentially vast given the complex nature of this disorder. Figure 34 was created in an attempt to visually convey the potential mechanisms involved that link OSA to T2DM and CVD.

Transient activation of the sympathetic nervous system is observed in patients with OSA during apnoeic events. However, the precise mechanisms by which *long-term* heightened sympathetic activity arises are not well understood. Individuals with OSA are exposed to repeated hypoxia and hypercapnia due to recurrent apnoeic and hyponoeic events throughout the night. Hypoxia and hypercapnia result in the activation of arterial chemoreceptors, which are located mainly within the carotid and aortic bodies. Chemoreceptor activation increases sympathetic outflow to resistance blood vessels causing vasoconstriction; an effect potentiated during apnoea by withdrawal of the inhibitory influence of ventilation on sympathetic outflow [338].

Hypoxia results in the secretion of catecholamines and glucocorticoids. The exact mechanisms involved here are not fully understood; however this can occur indirectly through chemoreceptor activation [339]. An increase in their secretion can also occur directly due to the physiological stress of the apnoeic events of OSA [339]. Increased circulating catecholamines and glucocorticoids have a number of effects which include sympathetic activation and increased blood glucose levels through stimulation of gluconeogenesis and inhibition of glycogenesis.

The baroreflex is homeostatic mechanism involved in maintaining short-term systemic blood pressure. The baroreceptors are stimulated by rises in blood pressure which leads to a reduction in sympathetic output in order to lower arterial pressure [340, 341]. Baroreceptor dysfunction is observed with OSA and may be attributed to resetting of the baroreceptor set point in consequence of recurrent apnoeic events. Thus in OSA patients with transient increases in sympathetic activity, baroreceptor dysfunction serves to potentiate such responses.

The Renin-Angiotensin-Aldosterone System (RAS) is involved in the long-term maintenance of arterial blood pressure by promoting salt and water retention (Figure 35) [338, 342, 343]. Activation of this system ultimately results in increased systemic blood pressure and increased sympathetic activity. Hypoxia associated with OSA leads to the activation of the RAS system and thus increases arterial pressure.

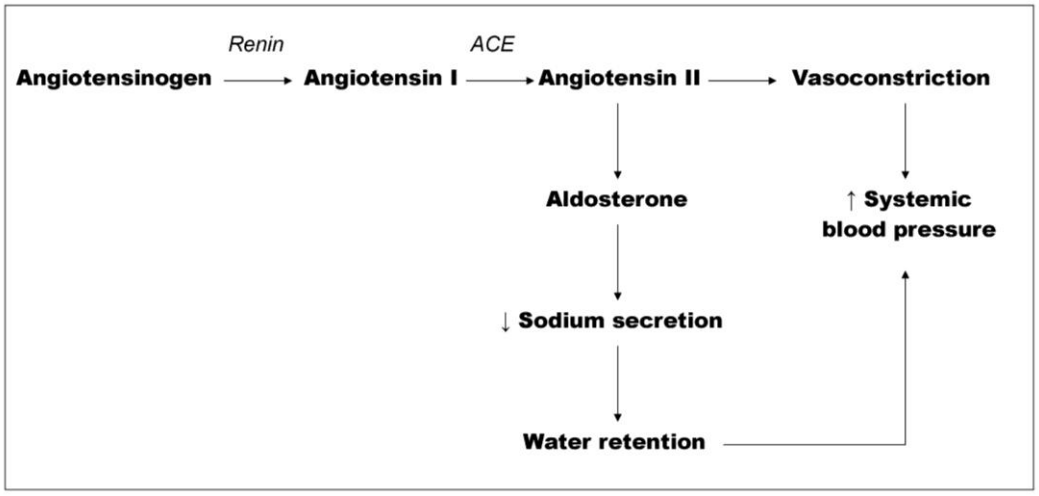


Figure 35: A schematic diagram of the Renin-Angiotensin-Aldosterone System (RAS).
ACE= Angiotensin Converting Enzyme

Importantly, increased sympathetic activity is known to be maintained after the apnoeic event has ceased and even during wakeful hours [342]. Increased sympathetic activity itself has numerous physiological effects which render the subject at increased risk of both CVD and T2DM. It is associated with insulin and leptin resistance. Both leptin and insulin are important in energy homeostasis (Chapter 1) and therefore if the physiological responses to these hormones are reduced an increase in plasma glucose levels is expected. Hyperglycaemia is also likely to occur via other mechanisms in patients with OSA.

Hyperglycaemia is associated with oxidative stress [191, 245] in addition to potentiation of insulin resistance (Section 1.2.2 and 1.4.1). Furthermore, OSA is associated with a sub-clinical inflammation (Section 1.5.6) which could further exacerbate the insulin resistant state of these individuals.

A salient feature of OSA is visceral obesity. However, the causal relationship between these two conditions is ambiguous but it is widely accepted that each condition aggravates the other.

The results from this study identified a significant negative relationship between oxygen desaturation levels and AHI and hypoglycaemia. This could be translated to a positive relationship between oxygen desaturations/AHI and hyperglycaemia. CPAP therapy significantly reduces apnoeic events thus prevents oxygen desaturations in patients with OSA and therefore could reduce the potential rise in circulating blood glucose levels.

The potential mechanisms linking OSA, CVD and metabolic dysfunction (MetS and T2DM) together are vast and complex and are further complicated by the salient features of these disease states - specifically visceral obesity. Therefore deciphering the true direction of cause and effect is a difficult task. However, it is clear that the presence of one of these diseases renders the individual at very high risk of developing another and any combination of these states results in exacerbation of each by the other. Therefore, if an individual is diagnosed with any one of these diseases they should be screened and risk assessed for the others as a matter of routine in order to treat before all three emerge overtly. This could have major benefits not only for the quality and longevity of life for these high risk patients but economically for the health service.

5.4 Strengths and limitations

The major limitation of the studies conducted in this PhD is sample size. In the 'Sleep and Inflammation study' (Chapter 2) a larger South Asian population that is more representative of the general population within Leicester would have been preferable in investigation of biological markers of inflammation and subjective sleep quality/SDB. In the 'Leicester Sleep and Sugar study' a larger treatment group would have been preferable in addition to complete datasets for the CGMS and biomarker analyses.

The Berlin Sleep Questionnaire (BSQ) was used in the 'Sleep and Inflammation study' as a surrogate for the diagnosis of SDB. A home respiratory study would have been a more robust approach in answering the research questions for this study. However, this was neither financially nor logistically feasible at the time this study was conducted.

However, the BSQ is a validated questionnaire and has been consistently reported to successfully predict the presence of SDB in a large number of individuals [254-259].

There are a number of strengths in both studies conducted for this PhD which include the collection of a diverse range of variables; the consistent use of standardised operating procedures. The scoring of sleep questionnaires was blinded and the data were double entered for accuracy**.** Furthermore, the 'Sleep and Inflammation study' is the first to investigate levels of biological markers of inflammation and cardiovascular profiles between migrant South Asians and Caucasians with SDB. The overall sample size of this study was large, at 1602 participants, providing a good cross-sectional analysis of the population within Leicester.

In the 'Leicester Sleep and Sugar study' the same researcher collected all data from each patient at each of the study time points, there was no variation in the equipment used for or provided to each subject. The diagnosis of OSA was made by a fully trained clinician and each patient received the routine follow-up care that is standard for those receiving CPAP therapy. We were also able to objectively measure CPAP compliance.

Ethnicity encompasses self-definition and group identification defined from within [344] with a large cultural component potentially influencing a person's self-identification. Therefore the scientific validity of such categorization may be questionable given the potential heterogeneity of the population within the ethnic group chosen, as described in [345]:

"…one can find a wide variety of genetic background, culture, lifestyle and health related behaviors affected by a place of origin, when the group or its ancestors arrived in the country, religion and current social class"

From an epidemiological stand point using ethnicity as a variable encourages conceptualizing a population along ethnic lines where ethnic boundaries could thus become deemed as natural and perpetual running the risk of 'mass stereotyping'. This could lead to social deprivation, an important covariate in health research, being overseen as a potential causative factor in favour of the ethnic variable that is used. This needs to be considered when interpreting the results reported here given a large proportion of the South Asian people recruited reside within the inner city, which has a low socioeconomic status and was not statistically controlled for. Therefore, translating such findings to the broader UK South Asian population or indeed just within Leicestershire County should be done so with caution. The debate around defining ethnic groups and using 'ethnicity' as a variable in health research goes beyond the scope of this PhD, however it is an important issue that should not be overlooked.

Finally, although a meta-analysis could not be undertaken for the systematic review (Chapter 3), a wide search strategy was performed on multiple databases and two independent reviewers were used for the study selection and data extraction phases.

5.5 Future Work

This field of research – sleep, inflammation and insulin resistance is still in its infancy. The intricate neuroendocrine mechanisms involved and existence of comorbities make it a complex area in which to determine not only independent relationships but the direction of cause and effect. Furthermore, this patient population can be difficult to work with due to illness, language barriers and in obtaining biological samples (i.e. blood). However, the potential benefits of such research are huge with respect to the patients' quality of life and to the health service. Future research is required to further establish the findings reported here and those highlighted within the literature using both animal and human models. It is essential, as emphasised throughout this thesis, that future studies include large sample sizes, robust measures of glycaemic control and are longitudinal in design. Furthermore, future intervention studies need to utilise well-defined control groups and again have long follow-up periods (>3 months). Ultimately, such studies could aid in the prevention of progression from obesity related sleep disorders to T2DM and CVD.

Investigation into the psychosocial barriers preventing patient compliance with CPAP therapy is also an area that deserves much attention. Determining what factors influence patient compliance provides the patient and the healthcare professional with a platform in which to build strategies to overcome them and thus get the best out of this therapy. Sleep duration is a parameter that has been explored in a number of cross sectional and lifestyle intertervention studies with long (>10 hours/day) and short (≤6 hours/day) sleep time being associated with metabolic dysfunction and increased mortality. Exploring the determinants of habitual sleep duration and the meachanisms involved in

its assication with metabolic dysfuntion is crucial in providing a basis in which to educate the general population on good sleep hygiene and thus potentially reduce the incidence of metabolic abnormalities such as T2DM.

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Appendix I

SAQ

CENTRE FOR SLEEP AND CHRONOBIOLOGY THE SLEEP ASSESSMENT QUESTIONNAIRE©

Patient Name:						Male		
						Female		
Today's Date:								
	Day	Month	Year	Height:		inches	Weight:		lbs.
Date of Birth:				or		cm	or		kg

PLEASE ANSWER EACH QUESTION BY CHECKING THE **ONE ANSWER** THAT FITS BEST

Over the past **month**, how often have you experienced the following……..

	Never	**Rarely**	**Some times**	**Often**	**Always**	**Don't Know**
1. Difficulty falling asleep?						
2. Sleeping for less than 5 hours?						
3. Sleeping more than 9 hours?						
4. Repeated awakenings during your sleep?						
5. Loud snoring?						
6. Interruptions to your breathing during sleep?						
7. Restlessness during your sleep (e.g. move your legs or kick)?						
8. Nightmares or waking up						

frightened or crying out loud?						
9. Waking up before you want to (i.e., getting less sleep than you need)?						
10. Waking up NOT feeling refreshed or thoroughly rested?						
11. Waking up with aches or pains or stiffness?						
12. Falling asleep while sitting (e.g., reading, watching t.v.)?						
13. Falling asleep while doing something (e.g., driving, talking to people)?						
14. Working shifts?						
15. Working night shifts?						
16. Irregular bed time and/or wakeup time during work or weekdays?						
17. Taking medication for sleep or nervousness?						

For further information on the Sleep Assessment Questionnaire© contact Dr. Harvey Moldofsky, Sleep Disorders Clinic, Centre for Sleep and Chronobiology, 340 College Street, Suite 580, Toronto, Ontario, Canada, MST 3A9. Phone (416) 603-9531, FAX (416) 603-2388, website: www.sleepmed.to

Berlin *Questionnaire*

Sleep Evaluation in Primary Care

1. Complete the following:

Height: ____ Weight: _____ Age: ____ Gender: M F

category 1

2. **Do you snore?**
   - ☐ Yes
   - ☐ No
   - ☐ Don't know

If you snore:

3. **Your snoring is:**
   - ☐ Slightly louder than breathing
   - ☐ As loud as talking
   - ☐ Louder than talking
   - ☐ Very loud. Can be heard in adjacent rooms

4. **How often do you snore?**
   - ☐ Nearly every day.
   - ☐ 3-4 times a week
   - ☐ 1-2 times a week
   - ☐ 1-2 times a month
   - ☐ never or nearly never

5. **Has your snoring ever bothered other people?**
   - ☐ Yes
   - ☐ No

6. **Has anyone noticed that you quit breathing during your sleep?**
   - ☐ Nearly every day
   - ☐ 3-4 times a week
   - ☐ 1-2 times a week
   - ☐ 1-2 times a month
   - ☐ never or nearly never

category 2

7. **How often do you feel tired or fatigued after your sleep?**
   - ☐ Nearly every day
   - ☐ 3-4 times a week
   - ☐ 1-2 times a week
   - ☐ Never or nearly never

8. **During your waketime, do you feel tired, fatigued, or not up to par?**
   - ☐ Nearly every day
   - ☐ 3-4 times a week
   - ☐ 1-2 times a week
   - ☐ 1-2 times a month
   - ☐ never or nearly never

9. **Have you ever nodded off or fallen asleep while driving a vehicle?**
   - ☐ Yes
   - ☐ No

10. **If yes, how often does it occur?**
    - ☐ Nearly every day
    - ☐ 3-4 times a week
    - ☐ 1-2 times a week
    - ☐ 1-2 times a month
    - ☐ never or nearly never

11. **Do you have high blood pressure?**
    - Yes

BMI = _____

Scoring Questions: Any answer within a box outline is a positive response

Scoring Categories:

Category 1 is positive with 2 or more positive responsesto questions 2-6
Category 2 is positive with 2 more positive responses to questions 7-9
Category 3 is positive with 1 positive response &/or a BMI > 30

Final Result: 2 or more positive categories indicate a high likelihood of sleep disordered breathing

The Epworth Sleepiness Scale

How likely are you to doze off or fall asleep in the situations described below, in contrast to just feeling tired? This refers to your usual way of life in recent times. Even if you haven't done some of these things recently try to work out how they would have affected you.

Use the following scale to circle the most appropriate number for each situation :- **0**= would **never** doze, 1=**Slight** chance of dozing, **2**= **Moderate** chance of dozing, **3**= **High** chance of dozing

Situation	**Chance of Dozing**			
Sitting and reading	0	1	2	3
Watching TV	0	1	2	3
Sitting, inactive in a public place(e.g. a theatre or a meeting)	0	1	2	3
As a passenger in a car for an hour without a break	0	1	2	3
Lying down to rest in the afternoon when circumstances permit	0	1	2	3
Sitting quietly after lunch without alcohol	0	1	2	3
In a car, while stopped for a few minutes in the traffic	0	1	2	3
Total Score	☐			

Appendix II

Power calculations for relationships between GTT group and biomarkers

Joanne Dick, Unilever Colworth

Introduction

This document is concerned with estimating the sample sizes required to detect differences between GTT groups for a selection of biomarkers. The study is exploratory and therefore a large number of end-points are being examined. A few of these biomarkers have been selected for power calculations.

Summary statistics upon which the power calculations are based have been taken from the published literature. Although there are many papers describing studies that examine the selected biomarkers, the data is often collected from different populations, age ranges, ethnic groups etc. and is therefore not directly applicable. The power calculations are based on the most appropriate data found following a literature search.

The main comparisons of interest from the study are between pre-diabetics and normals and between Asians and Caucasians. There is little information in the literature to help with the latter comparison, so the power calculations are carried out for the pre-diabetics (or diabetics, depending on information available) vs the normals in various populations.

Calculations

The calculations for each of the selected biomarkers are examined in more detail below. All the calculations have been carried out assuming 80% power and a 5% significance level. The calculations have been carried out assuming equal numbers in both groups, so the total number of patients, as presented in the graphs, needs dividing by two in order to get the number per group.

The calculations have been carried out assuming the difference between the normals and pre-diabetics (or diabetics) will be examined using Analysis of Variance (ANOVA). The calculations have been carried out using Proc Glmpower in SAS v9.1.3. A number of covariates, such as age and BMI are likely to be included in the final analysis. The power calculations assume that two additional covariates would be included in the analysis, however in reality this number may be more. The calculations require an estimate of the correlation between the biomarker of interest and all the covariates. As we have no real indication of what this might be, the calculations have been performed for a range of correlations.

Adipontectin.

The data used for adiponectin has been taken from a study on Japanese Americans living in Hawaii by Nakanishi et al. The individuals were diagnosed as having normal glucose tolerance, impaired glucose tolerance, or diabetes. Various biomarkers were examined in the study, and the relationships between these biomarkers and the GTT group were examined[1].

The information quoted in the paper is in the form of mean +/- standard error, as summarised in the table below:

	NGT (N=167)	IGT (N=70)	DM (N=22)
Adiponectin (μg/ml)	11.82 +/- 0.97	9.11 +/- 1.12	7.44 +/- 1.53

It was stated in the paper that the data were not normally distributed. Therefore in practice a transformation of the data to normality may be required, but for the purposes of the power calculations we shall assume that the data are normally distributed.

Figure 1 is a plot of the total number of patients against the mean difference of adiponectin detectable between the NGT group and the DM group.

Figure 2 is a plot of the total number of patients against the mean difference of adiponectin detectable between the NGT group and the IGT group.

Figure 1

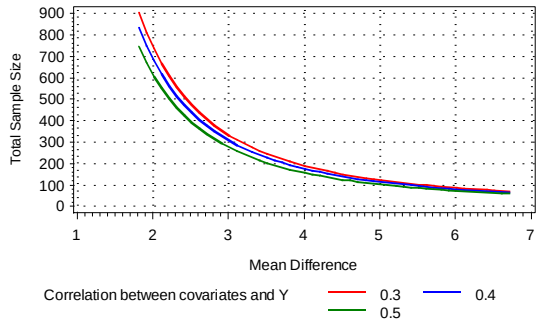
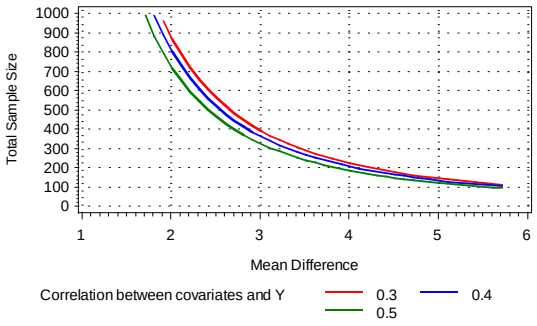


Figure 2:



From figure 1 it can be noted that to detect a difference of 4 μg/ml between the NGT and DM groups a total sample size in the region of 180 would be required, which is 90 in each group.

Apolipoprotein A1 and Apolipoprotein B

The data for the calculations for the apolipoproteins is taken from the paper by Pickup et al regarding three groups of Caucasians aged 35-64. The three groups consist of a control group, and 2 groups with non-insulin-dependent diabetes (NIDDM) that have different definitions of metabolic syndrome x. The information given in the paper is in the form of mean +/- standard deviation and is summarised in the table below:

	NIDDM Syndrome X-positive (n=19)	NIDDM Syndrome X-negative (n=25)	Non-diabetic (n=25)
Apo A1 (mg/dl)	1.11 +/- 0.13	1.24 +/- 0.2	1.47 +/- 0.3
Apo B (mg/dl)	1.17 +/- 0.25	0.99 +/- 0.17	1.12 +/- 0.23

For the purposes of the power calculations the results from the positive and negative syndrome groups for the NIDDMs are combined, assuming a normal distribution, to give estimates of the mean and standard deviation for an overall diabetic group. There is no information in the paper to contradict the assumption of normality.

Figure 3 is a plot of the total number of patients against the mean difference detectable of Apo A1 between the diabetic group and the non-diabetic group.

Figure 4 is a plot of the total number of patients against the mean difference detectable of Apo B between the diabetic group and the non-diabetic group.

Figure 3:

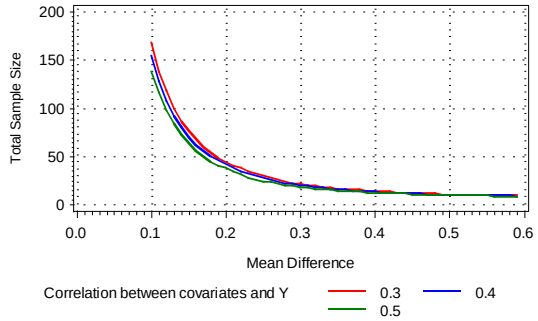
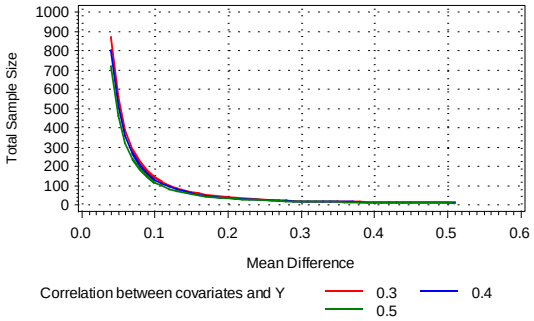


Figure 4:



IL-6

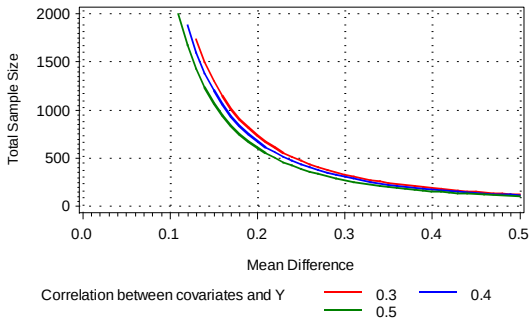
Data for the IL-6 calculations are taken from a study carried out in Japan as described by Moriwaki et al. The study consists of 151 diabetic patients and 80 controls, all of which were less than 65 years old. The information quoted in the paper was in the form of mean +/- standard error, and is summarised in the table below:

	NGT (N=80)	T2DM(N=151)
IL-6 (pg/ml)	0.65 +/- 0.08	0.73 +/- 0.10

There is no information in the paper to contradict the assumption of normality of the IL-6 observations. It should again be noted that this calculation in no way takes account of the proposed comparison between Asians and Caucasians.

Figure 5 is a plot of the total number of patients against the mean difference in IL-6 detectable between the NGT and T2DM group.

Figure 5:



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Appendix III

Additional results tables

Data set includes only those classified with SDB, who additionally provided fasting blood samples, for this analyses (n = 127). Showing their use of each medication within.

Type of Medication	Caucasian	South Asian	p
Ace Inhibitors†	16 (17.2%)	4 (17.4%)	1.00
α-Blockers†	3 (3.2%)	0 (0%)	1.00
Angiotensin-II Receptor Antagonist†	7 (7.5%)	2 (8.7%)	1.00
β-Blockers†	16 (17.2%)	3 (13.0%)	0.76
Calcium Channel Blockers†	13 (14.0%)	3 (13.0%)	1.00
Diuretics/Thiazides†	16 (17.2%)	3 (13.0%)	1.00
Aspirin†	17 (18.3%)	3 (13.0%)	0.76
Lipid Lowering Statins†	17 (18.3%)	5 (21.7%)	0.77
Lipid Lowering Fibrates†	1 (1.1%)	0 (0%)	1.00
Steroids†	3 (3.2%)	1 (4.3%)	1.00
Thyroid/Anti-Thyroid†	8 (8.6%)	5 (21.7)	0.13
Current smokers†	16 (15.4%)	3 (13.0%)	1.00

Use of Medication in those classified with SDB by the BSQ (n=127) † Fishers Exact Test

This data set includes all people classified with SDB (n= 438) showing their use of each medication.

Type of Medication	Caucasian	South Asian	P value
Ace Inhibitors*	44 (12.8%)	5 (6.9%)	0.23
α-Blockers†	8 (2.3%)	1 (1.4%)	1.00
Angiotensin-II Receptor Antagonist*	28 (8.1%)	5 (6.9%)	0.92
β-Blockers*	51 (14.8%)	6 (8.3%)	0.21
Calcium Channel Blockers*	38 (11.0%)	6 (8.3%)	0.64
Diuretics/Thiazides*	68 (19.7%)	8 (11.1%)	0.12
Aspirin*	52 (15.1%)	6 (8.3%)	0.19
Lipid Lowering Statin*	61 (17.7%)	11 (15.3%)	0.75
Lipid Lowering Fibrate†	1 (0.3%)	0 (0%)	1.00
Steroids†	22 (6.4%)	3 (4.2%)	0.59
Thyroid/Anti-Thyroid*	23 (6.7%)	8 (11.1%)	0.29

Current smokers*	68 (19.8%)	10 (13.9%)	0.32

Use of Medication in those classified with SDB by the BSQ (n=127) † Fishers Exact Test

IPAQ: Further sub-analyses

Investigating change in the amount (minutes/week) of moderate exercise, vigorous exercise and walking from baseline in the non-treatment and treatment groups. The data were non-normally distributed thus the non-parametric test Wilkcoxon was used for paired samples.

	Baseline Mean ± SD	6 weeks Mean ± SD	p
Treatment group			
Moderate (minutes/week)	190.0 ± 421.9	453.3 ± 695.5	0.29
Vigorous (minutes/week)	430.0 ± 617.1	25.0 ± 61.2	0.14
Walking (minutes/week)	305.0 ± 578.7	163.33 ± 182.5	0.72
Non-Treatment			
Moderate (minutes/week)	190.9 ± 470	240.0 ± 547.9	0.50
Vigorous (minutes/week)	144.6 ± 324.5	10.9 ± 36.18	0.27
Walking (minutes/week)	441.8 ± 426.8	385.7 ± 361.1	0..48

	Baseline Mean ± SD	12 weeks Mean ± SD	p
Treatment group			
Moderate (minutes/week)	18.0 ± 26.83	156.0 ± 316.0	0.29
Vigorous (minutes/week)	228.0 ± 412.2	222.0 ± 311.5	0.85
Walking (minutes/week)	72.0 ± 107.3	313.0 ± 500.5	0.35
Non-Treatment			
Moderate (minutes/week)	150.0 ± 474.3	61.0 ± 115.5	0.50
Vigorous (minutes/week)	159.0 ± 338.3	139.0 ± 249.0	0.92
Walking (minutes/week)	458.0 ± 446.4	255.5 ± 378.4	**0.035**

	Baseline Mean ± SD	12 weeks Mean ± SD	p
Treatment group			
Moderate (minutes/week)	222.0 ± 463.6	372.0 ± 578.1	0.11
Vigorous (minutes/week)	492.0 ± 668.7	198.0 ± 364.7	0.29
Walking (minutes/week)	318.0 ± 646.1	786.0 ± 1022.7	0.11
Non-Treatment			
Moderate (minutes/week)	190.9 ± 470.0	43.64 ± 110.9	0.29
Vigorous (minutes/week)	144.6 ± 324.5	141.8 ± 288.1	0.68
Walking (minutes/week)	441.8 ± 426.8	505.1 ± 509.8	0.78

There were no significant changes in the amount of moderate or vigorous exercises or walking from baseline to 6 weeks, 12 weeks or 6 months after CPAP therapy in the treatment or non-treatment groups.

Appendix IV

Power calculations for Leicester Sleep and Sugar Study

Joanne Dick, Unilever, Colworth

Introduction

The aim of the pilot study is to investigate the effects of restoration of sleep on glycemic control. Power calculations are very difficult for pilot studies, because by the nature of a pilot study, there is little information as to what may happen when the study is run.

Currently, the design of the pilot study has three groups, a control group and two groups that receive the treatment (an immediate and staggered start group). In this instance, there are results from a published study available for use in the power calculations[1]. The results from this enable the sample size required to detect a difference in HbA_{1c} from baseline to 3 months to be calculated, within the group that receives the sleep restoration treatment.

It should be borne in mind that the comparison from baseline to 3 months is just one possible comparison within the pilot study. There is no information regarding comparison of the treatment groups, or comparisons at other time points within the groups.

Data

It was stated in the paper that the data could not be transformed to normality, so a non-parametric test was used to analyse the data. For the purpose of the power calculations we can only assume the data will be normally distributed as we only have the data in the form of means and standard deviations. However, in reality since the measure of HbA_{1c} is a percentage it is unlikely the data will be normally distributed, and either a transformation or non-parametric test will be required to analyse the data.

The information taken from the Babu et al paper is summarised below:

	HbA_{1c} levels at baseline Mean +/- SD	HbA_{1c} levels at 3 months Mean +/- SD
All subjects	8.3% +/- 2.2%	7.9% +/- 1.8%
Patients with HbA_{1c} levels > 7% at baseline	9.2% +/- 2.0%	8.6% +/- 1.8%

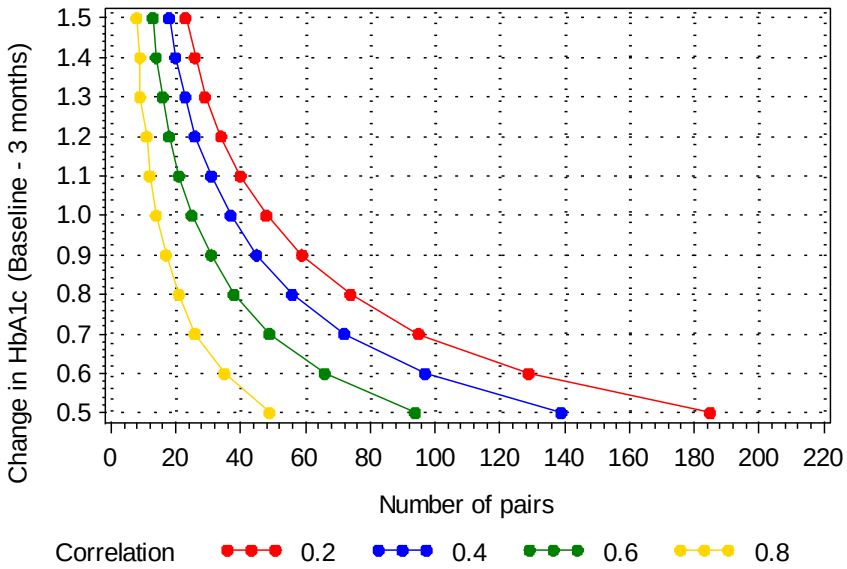
Calculations

The calculations have been carried out as if the difference between baseline and 3 months will be examined using a paired t-test. The calculations are performed using Proc Power in SAS v9.1.3. A paired t-test requires the assumption of normally distributed data, which as mentioned above may not be a realistic assumption. Additionally it should be noted that there are likely to be other factors incorporated into the final analysis, such as age. The inclusion of such factors is not taken into account in these calculations. The calculations are based upon assuming the power is 80%, which is commonly assumed to provide a good trial, and a significance level of 5%.

Figures 1 and 2 are plots of the change in HbA_{1c} levels against the number of pairs needed. Figure 1 shows the results for all subjects, and Figure 2 shows the results for the patients that entered the study with HbA_{1c} levels > 7%.

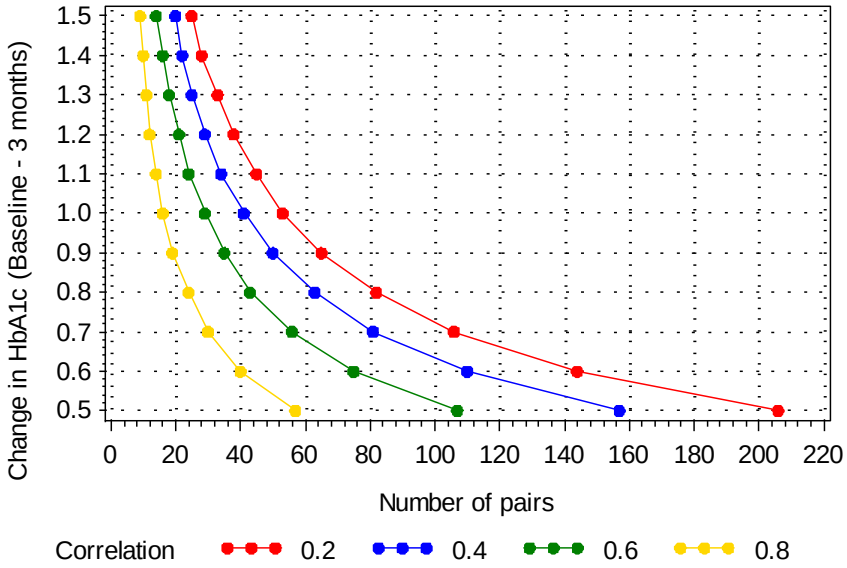
The change in HbA_{1c} levels are the decrease from baseline, so a value of 1 in figure 1 would indicate that the HbA_{1c} level had gone from 8.3% at baseline to 7.3% after 3 months. The number of pairs is the number of subjects for which there is complete baseline and 3 month information. The correlation refers to the correlation between the baseline and 3 month results. Since this correlation is unknown, the results have been plotted for a range, starting with a weak correlation of 0.2 up to a strong correlation of 0.8. It can be noted from the graphs that the stronger the correlation assumed, the smaller number of pairs required.

Figure 1: Sample sizes based on data from all subjects



If, for example, the correlation is 0.4, the standard deviations of the baseline and 3 month groups are 2.2% and 1.8% respectively, and the sample size is 25 pairs then the trial will have roughly 80% power to identify a change of 1.3% from baseline at the 5% significance level.

Figure 2: Sample sizes based on data from subjects with HbA1c > 7% at baseline



Reference:

1) BABU AR, HERDEGEN J, FOGELFELD L, SHOTT S, MAZZONE T. Type 2 Diabetes, Glycemic Control, and Continuous Positive Airway Pressure in Obstructive Sleep Apnea.

Archives of Internal Medicine. 165 (4): 447-52, 2005 Feb 28

Appendix V – Conference Abstracts

The Metabolic Syndrome and sleep disordered breathing
Emer Brady, Dr K Khunti, Duncan Talbot, Jo Dick, Dr C Hanning, Dr P Watson1, Dr A Hall, Prof. M Davies

Poster Presentation Diabetes UK Annual Professional Conference, 2007

Aim: Patients with Metabolic Syndrome (MS) are at increased risk of developing Type 2 diabetes (T2DM) and cardiovascular disease. MS is independently associated with Sleep Disordered Breathing (SDB), which affects 30% of the population and is independently associated with insulin resistance. We wanted to investigate whether the risk of SDB is associated with the components of the MS.{ **Method**: Participants were taken from a large prospective primary care screening study, which screens for T2DM. Demographic and biomedical data were additionally recorded. 1337 completed the validated sleep questionnaire 'The Berlin Questionnaire'. **Results**: The prevalence of SDB risk in this population was 28%. An independent t-Test was conducted. We found that the risk of SDB had a statistically significant association with fasting and postprandial blood glucose levels ($p<0.00$, $p<0.000$ respectively), HbA1c ($p<0.01$), triglyceride levels ($p<0.000$), HDLC ($p<0.000$), diastolic blood pressure ($p<0.000$) and waist circumference ($p<0.000$). **Conclusion**: This data indicates that the risk of SDB is associated with components of the MS. Therefore screening for and treating SDB within primary care could be beneficial in the prevention of cardiovascular disease and impaired glucose tolerance that is associated with the MS

Prevalence of sleep disordered breathing and cardiovascular risk factors in a multiethnic community population
Emer Brady, Dr K Khunti, Duncan Talbot, Jo Dick, Dr C Hanning, Dr P Watson1, Dr A Hall, Prof. M Davies

Poster Presentation Diabetes UK Annual Professional Conference, 2007

Aim: To determine the prevalence of sleep disordered breathing (SDB) in a multi-ethnic community population. A further aim was to determine the association of SDB with cardiovascular risk factors. **Methods**: We screened 1337 people aged 40-75 years using a 75g OGTT in a mixed ethnic population (18.2% South Asian). Individuals attended after an overnight fast and were given a 75gm oral glucose tolerance test (OGTT). Patients had anthropometric measurements and bloods for cardiovascular risk. Participants completed the validated 'Berlin Sleep Questionnaire'. **Results**: The prevalence of SDB was 28%. The prevalence in men was significantly higher (32%vs 26% in women, $p= 0.05$). There was no difference in prevalence of SDB between South Asians and White Europeans. SDB was associated with significantly higher fasting glucose (4.02mmol/l vs. 3.51, $p=0.000$) and postprandial blood glucose levels (6.1 mmol/l vs. 5.6, $p=0.000$)), HbA1c (5.7% vs. 5.6 % $p=0.01$), triglyceride levels (1.67 vs. 1.34 $p=0.000$), HDLC (1.26 vs. 1.43, $p=0.000$) and diastolic blood pressure (88.3 vs. 84.2 $p=0.000$). **Conclusion**: Screening for SDB within primary care could be beneficial to identify individuals at increased risk of T2DM and CVD.

Investigating the Levels of Biological Markers of Inflammation in a Non-Sleep Disordered Breathing versus a Sleep Disordered Breathing Population

EM Brady, MJ Davies , AP Hall , D Talbot ,J Dick C Hanning PJ Watson, D Webb, N Taub, K Khunti

Poster presentation Diabetes UK Annual Professional Conference 2008 - short listed for the Basic science award

Aim: To investigate the levels of inflammation and prevalence of glucose intolerance in a population with Sleep Disordered Breathing (SDB) versus a non-SDB population identified by the Berlin sleep Questionnaire (BSQ). **Methods**: Participants were taken from a large prospective primary care T2DM screening study. 342 people aged 40-75 years completed the BSQ, biomedical and demographic data were additionally collected. **Results**: The prevalence of SDB was 37%. The prevalence of glucose intolerance was not significantly different between groups. There was no significant difference in the prevalence of SDB between ethnic groups. BMI, % Body Fat, Waist circumference and Diastolic BP were higher in the SDB group (31.5 vs. 27.8kg/m2 ($p<0.001$), 36.6 vs. 32.9% ($p<0.001$), 104.1 vs. 94.65cm vs. ($p<0.001$), 88 vs. 85.4mmHg ($p=0.04$) respectively). The levels of CRP, IL-6 and leptin were higher in the SDB (4.0 vs. 3.5mg/ml ($p=0.001$), 2.5 vs. 2.4pg/ml ($p=0.03$), 27.8 vs. 19.9ng/ml, ($p=0.005$) respectively). The levels of adiponectin were lower in the SDB (18.9 vs. 22.8ug/ml ($p=0.007$) respectively). Fasting glucose, insulin and triglyceride levels were higher in the SDB group (5.5 vs. 5.4mmol/l ($p=0.029$), 10.6 vs. 7.7ulU/ml ($p<0.001$), 1.7 vs. 1.4mmol/l ($p=0.012$) respectively). After adjusting for BMI adiponectin remained significantly different between groups and the difference in Leptin levels between groups approached significance ($p=0.055$). **Conclusion**: These results suggest that identifying those at risk of SDB via a simple validated questionnaire concomitantly identifies those at increased CVD risk which cannot be explained by BMI, a major risk factor for SDB and T2DM.

Sleep disordered breathing is independently associated with the metabolic syndrome in a multiethnic population: An inflammatory link? Oral DUK 2008

EM Brady, MJ Davies , AP Hall , D Talbot J Dick C Hanning PJ Watson D Webb, N Taub, K Khunti

Oral presentation Diabetes UK Annual Professional Conference 2008

Objective: To determine the prevalence of sleep disordered breathing (SDB) in a multi-ethnic population and compare the cardiovascular risk profile in those with SDB between ethnic groups. Additionally determine if SDB is independently associated with the metabolic syndrome (MetS), high sensitive C-reactive protein (hsCRP) and adiponectin.**Methods**: 1602 participants within a UK multi-ethnic population underwent an oral glucose tolerance test, anthropometric measurements were recorded and the Berlin Sleep Questionnaire completed. MetS was classified according to NCEP-ATP III criteria.**Results**: The prevalence of SDB was 28.9% and did not differ between the two ethnic groups. South Asians had a higher body fat percentage (38.4 ± 10% vs. 35.6 ± 9%, $p=0.016$), HbA1c (6.0 ± 0.65% vs. 5.7 ± 0.44%, $p=0.001$) and lower HDLC (1.2 ± 0.2mmol/l vs. 1.32 ± 0.5mmol/l, $p=0.002$). Caucasians were older (59.6 ± 8.6yrs vs. 51 ± 10.3yrs, $p<0.001$) and had higher systolic blood pressure (138.4 ± 19.4mmHg vs. 130.8 ± 19.4mmHg, $p<0.001$). The prevalence of the MetS was 19%. SDB was associated with MetS after adjusting for age, gender, ethnicity and waist circumference (OR: 1.9, 95% CI: 1.4-2.7, $p<0.0001$); hsCRP (upper vs. lower quartile) was associated with SDB after adjusting for age, gender, ethnicity, waist circumference and MetS (OR: 3.4, 95% CI: 1.52-7.6, $p=0.003$). This association was not observed for adiponectin. **Conclusion:** SDB may mediate its role in MetS via the inflammatory pathway. Routine screening for SDB within primary and secondary care may have a role in the in prevention of cardiovascular disease and type 2 diabetes.

Are urinary isoprostane levels higher in glucose intolerant South Asians compared to Caucasians?

EM Brady, MJ Davies , AP Hall , D Talbot, J Dick ,PJ Watson D Webb, K Khunti

Oral Presentation Diabetes UK Annual Professional Conference, 2009 Short listed for the Nick Hales Young Investigator Award

Aim: To compare levels of oxidative stress in South Asians and Caucasians with prediabetes or T2DM. **Method**: Participants were recruited from a large prospective primary care, T2DM screening study. 214 glucose intolerant (prediabetes and T2DM) participants provided fasting blood samples, a urine sample and demographic data. One way analyses of covariance were used to determine any difference in the levels of the 8-isoprostane-F2- alpha urinary metabolite - 2,3-Dinor-8-Iso Prostaglandin-F1-alpha, a marker of oxidative stress, between these two ethnic groups.**Results**: 144 (67.3%) were Caucasian and 70 (32.7%) were South Asian, with no significant differences in gender (p=0.38), waist circumference (p=0.84)) or prevalence of smoking (p=0.64) between groups . However, the South Asian group were significantly younger than the Caucasian group (55.89 plus/minus SD 4.4 vs. 63.22 plus/minus SD 8.50, p<0.005). The levels of 2,3-Dinor-8-Iso Prostaglandin F1-alpha were significantly higher in the South Asian group (mean 11.02nM mMcreat-1 (95% CI:8.99,13.49) vs. 7.78 (6.85,8.83), respectively, p=0.007) after adjustment for age, gender, smoking status, waist circumference and HbA1c. **Conclusion**: We have shown that glucose intolerant South Asians exhibit a higher level of systemic oxidative stress compared to Caucasians. Oxidative stress is thought to precede endothelial dysfunction and play a key role in beta-cell dysfunction in glucose intolerant people. Therefore, these results may help the understanding of the higher progression rates in South Asians from glucose intolerance to overt T2DM.

Ethnic differences in glycaemic control, inflammation and oxidative stress in subjects at high risk of Sleep Disordered Breathing.

Brady EM, Davies MJ, Talbot D, Dadd T, Hall AP, Khunti K

Poster Presentation Diabetes UK Annual Professional Conference , 2009

Aim: To compare levels of markers of inflammation, oxidative stress and HbA1c between South Asians and Caucasians at high risk of Sleep Disordered Breathing (SDB).**Method**: Participants were recruited from a large prospective primary care, T2DM screening study. 342 completed the validated sleep questionnaire 'The Berlin Questionnaire', provided fasting blood samples and demographic data. 127 (37.1%) were classified as being at high risk of SDB and used in these analyses. One way analyses of covariance were used to determine any differences in these three parameters between the two ethnic groups. **Results**: 104 (82%) were Caucasian and 23 (18%) were South Asian, with no significant differences in age (p=0.56), gender (p=0.74) or waist circumference (p=0.84) between groups. Levels of Leptin and HbA1c were significantly higher in the South Asians compared to Caucasians after adjustment for age, gender, smoking status, medication and waist circumference (mean 22.80ngml^{-1} (95% CI: 18.1,28.8) vs. 17.70 (15.9, 19.6), p=0.05, respectively and 6.06% (5.83, 6.26) vs. 5.76 (5.66, 5.85), p=0.019, respectively). The Caucasian group had significantly higher levels of 8-iso-$PGF_{2\alpha}$ after adjustment for the same covariates (2.30ngm^{-1}l (2.03, 2.61) vs. 1.45 (1.10,1.91), p=0.004).**Conclusion:** The characteristics of people at high risk of SDB are different between ethnic groups. South Asians can be characterized by increased inflammation and poorer glycaemic control compared to Caucasians. Additionally, Caucasians at high risk of SDB can be described by an increased level of oxidative stress compared to South Asians. These results suggest that there are differences in the pathophysiology of SDB between these two ethnic groups.