The Effect of Short Sleep Duration and Exercise on Glycaemic Control in Healthy Adults

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Abstract

Short sleep durations are common amongst adults across the world. They have been linked to metabolic disorders, and, more specifically, impaired glycaemic control. Acute and chronic physical activity are known to have beneficial effects on metabolic health, and therefore may be able to attenuate the link between short sleep and impaired glycaemic control. The purpose of this thesis was to explore the potential for exercise to alter glycaemic control in short sleep durations. Specifically, the aims were threefold: (a) to understand the impaired glycaemic response to glucose intake over consecutive nights of sleep restriction (Chapter 4); (b) to investigate if exercise could alleviate the sleep restriction induced impairment in insulin sensitivity (Chapter 5); and (c) to determine if these factors act in a similar manner when transferred to the free-living environment (Chapter 6). The findings in Chapter 4 demonstrated that the number of nights of sleep restriction did not appear to affect the impairment in glycaemic control. These findings informed the study in Chapter 5, which used a randomised cross-over design to explore the potential for acute exercise to attenuate the impairment in glucose regulation after a single night of sleep restriction. The findings suggest that sprint interval exercise may be beneficial for the late postprandial period after sleep restriction, as demonstrated by a reduced insulin area under the curve. However, when examined in the free-living environment (Chapter 6), habitual short sleep duration did not show any evidence of impairing markers of glycaemic control when confounding factors such as sex, diet, and body composition were taken into consideration. Collectively, the studies in this thesis confirm that short-term short sleep impairs glucose regulation and suggest that exercise may be beneficial for glucose regulation after short sleep in the acute setting, but findings may be contradictory in chronic settings. Further study is warranted to establish the effects of different exercise modalities on glucose regulation after sleep restriction and to fully understand the link between habitual

sleep duration, physical activity, and glycaemic control. However, a session of sprint interval exercise could be recommended to individuals after acute sleep restriction to alleviate the impairment in insulin sensitivity.

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List of Abbreviations

Akt – protein kinase B (PKB)

AUC – area under the curve

BMI – body mass index

CE – control plus exercise

CON – control

DXA – dual energy x-ray absorptiometry

ECG – electrocardiography

EEG – electroencephalography

EMG – electromyography

EOG – electrooculography

FM – fat mass

HbA1c – glycated haemoglobin

HDL – high density lipoprotein

HOMA – homeostatic model of assessment

IFG – impaired fasting glucose

IGT – impaired glucose tolerance

IRS-1 – insulin receptor substrate-1

IVGTT – intravenous glucose tolerance test

LASS – less physically active short sleepers

LDL – low density lipoprotein

LS – longer sleepers

MASS – more physically active short sleepers

MEQ – morningness-eveningness questionnaire

MET – metabolic equivalent

MVPA – moderate-to-vigorous physical activity

N1 – stage 1 sleep

N2 – stage 2 sleep

N3 – stage 3 sleep

NEFA – non-esterified fatty acids

NREM – non-rapid eye movement sleep

OGTT – oral glucose tolerance test

PARQ – physical activity readiness questionnaire

PI3K – phosphoinositide 3-kinase

PPO – peak power output

PSG – polysomnography

PSQI – Pittsburgh Sleep Quality Index

REM – rapid eye movement sleep

SD – standard deviation

SDS-CL-17 – Sleep Disorders Symptom Checklist-17

SE – sleep efficiency

SEM – standard error of mean

SOL – sleep onset latency

SR – sleep restriction

SRE – sleep restriction plus exercise

SWS – slow-wave sleep

T2D – type 2 diabetes

TST – total sleep time

VAT – visceral adipose tissue

VE – minute ventilation

WASO – wake after sleep onset

WHO – World Health Organisation

Preface

Peer-reviewed publications arising from this thesis:

Sweeney, E.L., Peart, D.J., Kyza, I., Harkes, T., Ellis, J.G. and Walshe, I.H. (2020). Acute Sprint Interval Exercise Alters Impaired Insulin Profiles Induced by a Single Night of Sleep Restriction. *International Journal of Sport Nutrition and Exercise Metabolism.* 30 (2), 139-144. doi: 10.1123/ijsnem.2019-0235

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Sweeney, E.L., Peart, D.J., Kyza, I., Harkes, T., Ellis, J.G. and Walshe, I.H. (2019) Can acute exercise alleviate the impairment in glucose regulation after sleep restriction in healthy humans: A randomised crossover study. *Physiology.*

Sweeney, E.L., Peart, D.J., Ellis., J.G. and Walshe, I.H. (2018) The impairment in insulin sensitivity after sleep restriction does not increase with more nights of sleep restriction. *Sleep and Circadian Rhythms From Mechanism To Function.*

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Author Declaration

I declare that the work contained in this thesis has not been submitted for any other award and that it is all my own work. I also confirm that this work fully acknowledges opinions, ideas and contributions from the work of others.

Ethical clearance for the research presented in this thesis was granted by the Faculty of Health and Life Sciences Research Ethics Committee, Northumbria University.

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Chapter 1 – Introduction

Insufficient sleep is thought to be becoming increasingly common and has recently been branded a ‘public health problem’ by the US Centers for Disease Control and Prevention (Hafner et al., 2017). The National Sleep Foundation recommends a sleep duration of 7 to 9 hours for adults aged 18-64 years old (Hirshkowitz et al., 2015). However, the Great British Bedtime Report stated that despite this recommendation, 74% of individuals sleep less than 7 hours each night (The Sleep Council, 2017). The same report also identified a recent increase in the number of individuals sleeping for less than 5 hours each night, rising from 7% in 2013 to 12% in 2017. Comparable findings have been demonstrated across the world, with data from the US showing an increased prevalence of short sleep from the 1980s to the 2000s (Luckhaupt et al., 2010), and data from the National Health Interview Survey in the United States demonstrating a decrease of approximately 10-15 minutes in sleep duration from 1985 to 2012 (Ford et al., 2015). Further analysis of this data shows more pronounced decreases in sleep duration from 2013-2017 (Sheehan et al., 2019). However, conflicting evidence exists. Some research suggests that the incidence of short sleep (less than six hours) only decreased in specific populations between 1975 and 2006, such as full-time workers (Knutson et al., 2010), or only in specific countries (Bin et al., 2013). Findings from the American Time Use Survey suggest an increase in sleep durations from 2003 to 2016 (Basner and Dinges, 2018). However, this increase was small, averaging an increase of just under one and a half minutes each year, and it could be speculated that this may not be a significant amount to improve health outcomes.

Shorter sleep durations tend to be the result of a later bedtime, rather than an earlier wake time, as demonstrated by research which collected sleep schedules of individuals across the world using a smartphone application (Walch et al., 2016). Time use surveys have found work time to be the most common factor associated to sleep time (Basner et al., 2014). Those who work more, or spend more time commuting or travelling for other purposes tend to have shorter sleep times than those who have shorter work hours or travel less. Other reasons for short sleep durations could be due to socialising and participating in leisure time activities such as watching television (Basner et al., 2014).

In the UK, over 1.5 million working hours are lost each year due to either absence or a decline in performance associated with insufficient sleep (Hafner et al., 2017). This is estimated to cost the economy around £40 billion. Thus, there is a need to explore how to combat the consequences of sleep deprivation and attempt to reduce the economic burden.

Parallel to the decrease in sleep duration, the prevalence of type 2 diabetes and insulin resistance has increased, with more than double the number of prevalent cases of type 2 diabetes being recorded in 2013 compared to 2000 (Sharma et al., 2016). With the ever-decreasing sleep durations it has been suggested that insufficient sleep is contributing to the increased incidence of type 2 diabetes (Gangwisch, 2009). Indeed, sleep loss has been associated with many of the leading causes of death in the UK, such as cardiovascular disease, diabetes, and hypertension in both males and females (Luyster et al., 2012). The cost of insufficient sleep may therefore be even higher than £40 billion when the cost of treating these health issues are also taken into consideration.

Whilst the link between sleep and glucose metabolism is becomingly increasingly recognised, many questions still remain surrounding this association, including if the

effect of sleep restriction on metabolic function is cumulative, and if it is possible to attenuate the impairment in metabolic function which results from sleep restriction.

Exercise is a commonly used tool to improve metabolic health. Acute exercise can markedly improve insulin sensitivity for up to two days (Bird & Hawley, 2017). Additionally, regular physical activity can lower the risk of developing metabolic disorders such as type 2 diabetes (Warburton et al., 2006), with adults who achieve more physical activity showing superior insulin sensitivity than those who are less active (Balkau et al., 2008). Thus, exercise could be suggested as a potential intervention to benefit glucose regulation after short sleep. However, studies investigating exercise and glucose regulation in situations of acute or habitual short sleep are limited, therefore further research in this area is warranted.

Accordingly, the overall aim of this thesis is to present studies to address the gaps in current knowledge surrounding the relationship between sleep duration and glycaemic control, and the effect exercise has on this relationship.

Chapter 2 - Literature Review

2.1 Introduction

This review of the literature will present an overview of sleep physiology and glycaemic control and highlight the potential for exercise to alter this relationship. Specifically, it will first outline the structure of sleep, provide a critique on methods to measure sleep, and give an overview of the interaction between sleep and lifestyle factors such as physical activity and diet. An outline of glucose homeostasis and measurement of glucose regulation will then be presented and key literature exploring sleep duration and glycaemic control will be reviewed. Finally, the potential for exercise and physical activity to benefit metabolic function will be discussed.

2.2 Sleep physiology

Sleep is a reversible behavioural state in which an individual is disengaged and unresponsive to the surrounding environment. The regulation of sleep can be described by a two-process framework consisting of process S - a sleep-dependent process – and process C – a sleep-independent process (Borbely et al., 2016). Process S refers to the homeostatic drive to sleep and increases directly with time spent awake. In particular, this homeostatic mechanism is responsible for the regulation of slow-wave sleep (Dijk, 2009). The biological systems driving process S remain largely unknown, however adenosine accumulation is emerging as a likely contributor (Landolt, 2008). Process C - the circadian rhythm - is controlled by a central circadian oscillator known as the superchiasmatic nucleus, and is influenced by zeitgebers including light and dark cycles, food intake, and physical activity (Lewis et al., 2018; Stephan, 2002; Wever et al., 1983). This process regulates the daily rhythms of physiological functions such as glucose metabolism, as well as

promoting sleepiness and wakefulness. The circadian system influences core body temperature and melatonin, which are key factors in the initiation of sleep. An increase in melatonin combined with a drop in body temperature and increased homeostatic pressure promotes sleep (Luyster et al., 2012).

2.2.1 Sleep structure

Sleep typically consists of non-rapid eye movement (NREM) sleep followed by rapid eye movement (REM) sleep which occurs in cycles lasting approximately 90 minutes. NREM sleep is further divided into stage 1 (N1) and 2 (N2), sometimes referred to as light sleep, and stage 3 (N3), commonly known as slow-wave sleep (SWS) or deep sleep (Fuller et al., 2006). In healthy adults, stage two sleep usually dominates, forming about 45-55% of total sleep time, followed by slow wave sleep (15-25%) and REM sleep (20-25%), then finally stage 1 sleep which makes up the remainder (Carskadon & Dement, 2011). Typically, the percentage of slow wave sleep (SWS) within a cycle is inversely related to the number of sleep cycles, with latter sleep cycles containing less SWS than those in the first part of the sleep period. In contrast, there is usually a direct relationship between the number of sleep cycles and percentage of REM, N1 and N2 sleep (Sharma and Kavuru, 2010).

Each sleep stage has a unique set of characteristics, allowing the different stages to be distinguished using polysomnography (PSG). REM sleep is very similar to wakefulness with respect to brain wave activity. As the name suggests, there is eye movement during this stage, as well as irregular breathing and surges in cardiac function. Memory consolidation occurs during REM sleep, with information stored in short term memory being processed and moved to long-term memory (Boyce et al., 2017). Stage 1 sleep acts as a transition between wakefulness and sleep, and is characterised by the disappearance of alpha waves and the appearance of theta waves. Muscle activity begins to decrease during this stage. Brain wave activity

slows and eye movement ceases as an individual transitions into stage 2 sleep. Stage 2 sleep can be recognised by the presence of sleep spindles and K-complexes, which are bursts of brain activity and high amplitude waves, respectively. The spindles in stage 2 sleep are thought to contribute to memory consolidation (Gais et al., 2002). The appearance of delta waves signifies the beginning of stage 3 sleep, during which restorative processes such as tissue growth and repair take place (Fuller et al., 2006).

Sleep structure may be altered under conditions of sleep restriction (Webb and Agnew Jr., 1965). It has been shown that delaying bed time by 1 h and advancing wake time by 2 h results in a decrease in stage 2 sleep and increased REM sleep after 3 nights (Elmenhorst et al., 2008). Similar findings were noted when sleep was restricted to 0000 to 0300 for 4 nights, with decreased stage 2 sleep, however stage 3 and 4 sleep increased in this study (Wu et al., 2010). This is in agreement with previous findings as reviewed by Carskadon and Dement (2011) who state that slow wave sleep is usually favoured following a period of sleep loss, with REM sleep recovery only occurring after slow wave sleep debt has been repaid. In general, this suggests that individuals will show higher proportions of SWS when they are more sleep deprived. The changes in sleep architecture during sleep loss may provide a useful method to indicate whether individuals in a sleep restriction study are truly sleep restricted.

2.2.2 Measuring Sleep

The differences in brain wave activity and bodily movements enables determination of sleep structure by polysomnography (PSG). Considered the ‘gold-standard’ method of measuring sleep duration and architecture, this multi-parametric test consists of measurements of brain wave activity by electroencephalography (EEG), eye movements by electrooculography (EOG), muscle activity by electromyography

(EMG), heart rhythm by electrocardiography (ECG), breathing rate and airflow, and oxygen saturation (Marino et al., 2013). Polysomnography provides detailed feedback about sleep and, in addition to diagnosing some sleep disorders, can be useful to gain information on sleep architecture in sleep studies. Output variables from polysomnography include total sleep time (TST – the total time spent asleep), sleep latency (SL – the duration from “lights out” to the onset of sleep), REM latency (the duration from “lights out” to the onset of REM sleep), wake after sleep onset (WASO – the duration of wakefulness that occurs after initial onset of sleep), sleep efficiency (SE – the percentage of time in bed spent asleep), and duration and percentage of each sleep stage (NREM and REM). However, much of the time polysomnography must be conducted in a sleep laboratory, meaning individuals must stay in an unfamiliar environment which may affect sleep (Tamaki et al., 2005). Portable PSG equipment may be used in the home environment, however still requires set up by an experienced researcher which may not always be feasible. For use in sleep studies it is therefore advantageous to include a familiarisation night which allows individuals to become accustomed to sleeping in the laboratory and wearing the polysomnographic equipment, accounting for the first night effect or reverse first night effect. The necessary addition of this night may be viewed as a disadvantage to using polysomnography to measure sleep due to the additional cost and inconvenience. Other potential limitations include the specialist knowledge required to set up the equipment and the time and additional cost of analysis of the recordings.

Consequently, in situations where polysomnography is unfeasible, such as sleep studies conducted in the free-living environment or with larger populations, sleep may be measured using actigraphy. Actigraphy monitors are usually watch-shaped devices most commonly worn on the wrist. The recording of movement, temperature and light exposure enables measurement of sleep and activity phases. Although

unable to provide specific information regarding sleep architecture, actigraphy is a valid tool for determining total sleep duration, WASO, SOL, and sleep efficiency (Marino et al., 2013). Wrist actigraphy has been compared with polysomnography in multiple studies and has shown high correlations (above 90% agreement) between measures for distinguishing between sleep and wakefulness in healthy adults (Ancoli-Israel et al., 2003).

Another common method of measuring sleep variables is through the use of sleep logs. Sleep logs are arguably inferior to other methods due to their subjective nature, and correlate poorly with objective measures such as actigraphy and PSG in a range of populations (Ancoli-Israel et al., 2003). Comparison of PSG to subjective sleep measures in the Sleep Heart Health Study showed participants overestimated their total sleep duration by around one hour (Silva et al., 2007). However, in situations where PSG is not feasible, sleep logs may be combined with actigraphy. Often when actigraphy is being used wearers are asked to press a button on the watch to provide a marker of when they are intending to sleep and when they wake. This marker allows distinction between daytime stillness and actual sleep. If this button is not pressed, a sleep diary can provide a useful alternative to determine bed and wake time (Lauderdale et al., 2008b).

2.2.3 Sleep restriction studies

Whilst there is an abundance of knowledge on what happens during sleep, there are still many unanswered questions surrounding why sleep occurs, and the amount of sleep which is optimal for health. To understand more about the purpose of sleep, studies of partial or total sleep deprivation are often conducted to explore the physiological consequences of insufficient sleep. Partial sleep deprivation (sleep restriction) may be more ecologically valid than total deprivation, reflecting sleep durations experienced regularly by individuals due to lifestyle demands such as

work. Studies which utilise multiple consecutive days of sleep restriction may be of particular importance as these can highlight not only the consequences of short sleep, but whether there is an accumulation of these consequences (Banks & Dinges, 2007). Yet, at present there are few studies that have examined whether or not there is a difference between one night and multiple nights of sleep restriction on either physiological or psychological outcomes, so it would be beneficial to determine this to inform future research study protocols.

2.3 Sleep, physical activity and diet

2.3.1 The influence of physical activity and diet on sleep

Sleep may be influenced by lifestyle habits such as physical activity and dietary intake. A recent meta-analysis concluded a small beneficial effect of acute exercise, defined as a single session of exercise, on sleep duration, SOL, SE, SWS time, WASO, and stage 1 sleep time (Kredlow et al., 2015). In the same meta-analysis similar findings were demonstrated for regular exercise, defined as multiple sessions of exercise. Furthermore, three sessions of aerobic exercise each week have been shown to increase sleep duration by around 45 minutes despite no significant changes in time in bed (Baron et al., 2013).

It is possible that the type of exercise may influence the benefit on sleep outcomes. Chronic resistance exercise has beneficial effects on sleep quality, but studies examining the effect of acute exercise on sleep are lacking so it is unclear whether findings would be similar (Kovacevic et al., 2018). The review by Kovacevic et al. (2018) also demonstrates a relationship between intensity and frequency of physical activity and sleep quality, with higher intensity and more frequent bouts producing superior effects on multiple aspects of sleep quality including perceived sleep

quality, SOL, sleep efficiency, and WASO. In contrast, an earlier review (Kredlow et al., 2015) reported no influence of intensity of acute exercise on changes in sleep variables, however, the review reported comparable results regarding duration of acute exercise bouts.

Another aspect of lifestyle which may influence sleep is dietary intake. Several studies have investigated whether diet impacts sleep duration or other aspects of sleep. Specifically, total energy, fat, and fibre intake have all been commonly found to have associations with sleep duration (Dashti et al., 2015). Early experimental studies examining carbohydrate intake on sleep demonstrated decreased SWS and increased REM sleep with a high carbohydrate, low fat diet compared to a normal or high fat, low carbohydrate diet (Phillips et al., 1975; Porter & Horne, 1981). Decreases in sleep onset latency have also been observed after high carbohydrate diets (Lindseth et al., 2013). In the same study individuals had around 3 less wake episodes during sleep when consuming a high protein diet in which 56% of total energy came from protein compared to a control diet where protein made up 15% of total energy. Changes in sleep quality and quantity arising from high protein and carbohydrate diets may be due to increases in tryptophan, serotonin and melatonin. These sleep-promoting substances have been shown to increase with carbohydrate and protein intake (Wurtman et al., 2003), providing a potential link between dietary composition and sleep.

2.3.2 The influence of sleep on physical activity and diet

The relationships between physical activity and sleep, and diet and sleep, appear to be reciprocal. Cross-sectional studies have demonstrated lower levels of physical activity in individuals with shorter sleep durations and poorer sleep quality (Stefan et al., 2018; Štefan et al., 2018). Intervention studies have mimicked these findings. Healthy males showed a decrease in physical activity in the free-living environment

after one night of 4.25 hours of sleep compared to one night of 8.25 hours of sleep, with a 13% decrease in total activity and a lower proportion of time spent doing high intensity activity (Schmid et al., 2009). Additionally, longer sleep onset latencies are predictive of shorter exercise durations the following day (Baron et al., 2013).

Given that there is a direct relationship between physical activity and sleep efficiency, it is unsurprising that there is an inverse relationship between sleep efficiency and sedentary time (Gubelmann et al., 2018). However, interestingly, there does not appear to be any association between sleep duration and sedentary behaviour (Saleh & Janssen, 2014). This cross-sectional study used 7-day actigraphy data for sleep duration and sedentary time in 1371 adults. There was no difference in sedentary time between sleep duration quintiles.

Experimental short sleep duration causes increases in appetite and total energy and fat intake, despite no changes in energy expenditure (St-Onge et al., 2011). Energy intake was approximately 6% greater during 5 nights of 5 hours sleep compared to 5 nights of 9 hours sleep (Markwald et al., 2013). Furthermore, changes in appetite hormones have been observed in healthy males after 2 nights of sleep restriction to 4 hours, with an 18% decrease in leptin and 28% increase in ghrelin compared to 2 nights of 10 hours sleep (Spiegel et al., 2004). Similarly, altered appetite hormones have been observed after just 1 night of sleep restriction to 4.5 hours compared to a control night of 8.5 hours (Broussard et al., 2016). Twenty-four hour levels of ghrelin were increased approximately 7%, as well as increased post-prandial levels of ghrelin after all meals. Energy intake was increased by an average of 340 kilocalories in the sleep restriction condition, with the extra intake coming from an increased intake of carbohydrates and increased snacking. A decrease of around 200-250 kilocalories per day is thought to be sufficient to elicit a change in bodyweight over the long term (Hall et al., 2017), therefore the increase in energy

intake combined with a decrease in physical activity following short or poor sleep may lead to obesity and metabolic dysfunction.

2.4 Glucose Metabolism

2.4.1 Glucose homeostasis

In order to sustain normal functioning, glucose absorption, uptake and production must be balanced to ensure blood glucose concentrations are kept within the normal range of approximately four to seven mmol/L (Saltiel & Kahn, 2001). The regulation of blood glucose concentration is primarily under the control of two hormones - glucagon and insulin - which operate in a negative feedback manner to maintain levels within the normal range. Figure 2.1 (Roder et al., 2016) provides an overview of how these hormones control the uptake and release of glucose.

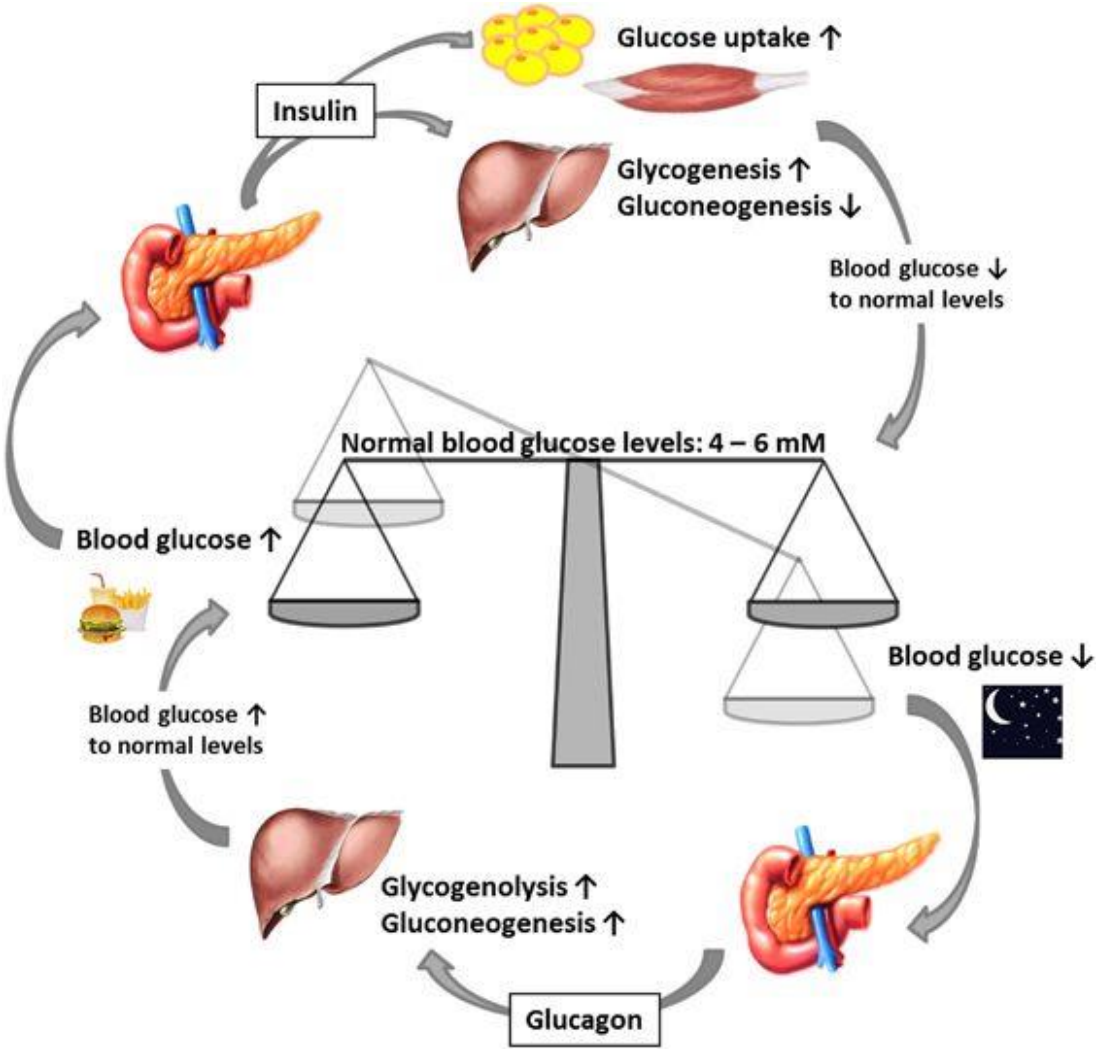


Figure 2.1 Overview of glucose homeostasis from 'Pancreatic regulation of glucose homeostasis.' (Roder et al. 2016). Licensed under CC BY-NC-ND 4.0.

In the post-absorptive state (i.e. during a period of fasting) blood glucose concentration reaches a nadir, with typical values of around 3.0-5.0 mmol/L depending on the length of the fasting period (Gerich, 1993). At this point, glucagon encourages the breakdown of glycogen in the liver (glycogenolysis), inhibits formation of glycogen, and promotes formation of glucose from other substrates such as lactate and amino acids (gluconeogenesis). This combination of glycogen breakdown and glucose production prevents blood glucose concentrations falling below the normal range, known as hypoglycaemia. In the post-absorptive state,

insulin concentrations are low, and most glucose uptake occurs by insulin independent mechanisms according to tissue demand (Gerich, 2000).

When blood glucose concentration rises above normal levels, usually in the post-prandial state, insulin is released by the pancreatic beta cells to return blood glucose to within the normal range. The release of insulin promotes glucose uptake and storage as well as blunting gluconeogenesis and glycogenolysis (Saltiel & Kahn, 2001). The suppression of gluconeogenesis and glycogenolysis by insulin are thought to be more important in regulating blood glucose concentrations in the post-prandial state than its excitatory actions (Sonksen and Sonksen, 2000). In the peripheral tissues, insulin-stimulated glucose uptake occurs predominantly in skeletal muscle tissue (DeFronzo & Tripathy, 2009). When insulin binds to receptors in the skeletal muscle, a signalling cascade known as the insulin signalling pathway (Figure 2.2) is triggered, which ultimately leads to translocation of GLUT-4 molecules to the membrane. Glucose is then taken up into the peripheral tissues by facilitated diffusion and either used immediately or converted to glycogen to be stored in the liver or muscle (Adeva-Andany et al., 2016). Typically, blood glucose peaks around 15-30 minutes post ingestion of a meal or glucose bolus. Similarly, large increases in insulin are observed at this time and serve to counteract the increase in glucose concentration by facilitating glucose disposal. The glucose and insulin profiles in the postprandial state are of particular interest as they can be indicative of hyperglycaemia and insulin resistance - two primary characteristics of type 2 diabetes (American Diabetes Association, 2009).

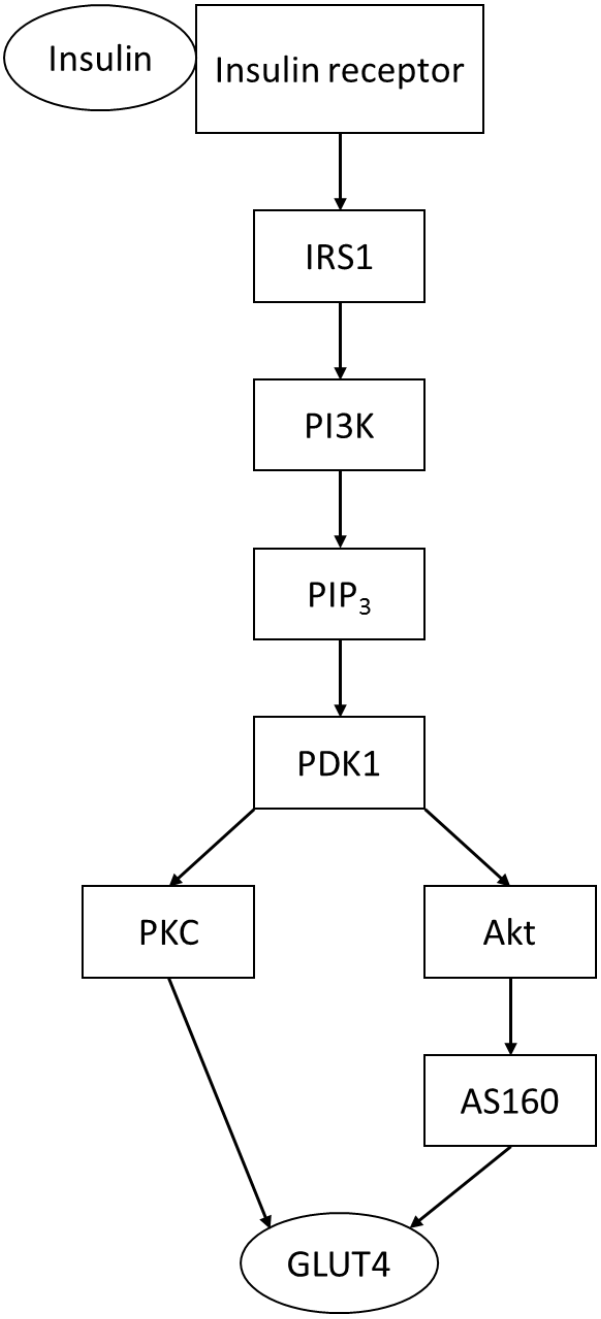


Figure 2.2. Insulin signalling pathway facilitating insulin mediated glucose uptake by translocation of GLUT4.

2.4.2 Impaired glucose metabolism

When blood glucose homeostasis is compromised, blood glucose may increase to concentrations outside the normal range. This can occur either in the fasted state, postprandial state, or both. This is often characterised as decreased insulin sensitivity, which may lead to a period of hyperglycaemia. Chronic hyperglycaemia, and decreased insulin sensitivity can lead to the development of metabolic disorders such as type 2 diabetes mellitus (T2D).

Impaired glucose metabolism can manifest in several ways. Firstly, as impaired fasting glucose, which occurs when blood glucose concentration falls between 6.1 mmol/L and 6.9 mmol/L in the post-absorptive period. Impaired fasting glucose may be present without impaired glucose tolerance (Meyer et al., 2006). Secondly, impaired glucose tolerance, which occurs when there is compromised insulin action in the early and late postprandial phase resulting in a prolonged bout of hyperglycaemia (Meyer et al., 2006). Individuals with impaired glucose tolerance are six times more likely to develop type 2 diabetes compared to individuals free from any metabolic abnormalities (Santaguida et al., 2005). Thirdly, blood glucose concentrations may be increased significantly above normal values in either the fasting or 2 h postprandial state, indicating type 2 diabetes. Reference values for blood glucose concentrations as defined by the World Health Organization (2006) are given in Table 2.1.

Currently, poor diet, obesity and physical inactivity are thought to be the biggest risk factors for developing a metabolic disease related to impaired glucose metabolism, such as type 2 diabetes (Qin et al., 2010; Steyn et al., 2004). However, as will be presented in section 2.5 of this chapter, it is becoming increasingly recognised that sleep may also play an important role in maintaining healthy glucose metabolism.

Table 2.1. WHO classification criteria for normal glucose tolerance, IFG, IGT and T2D.

	Fasting	2 h OGTT	HbA1c
Normal	<6.1 mmol/l	<7.8 mmol/l	20-42 mmol/mol
IFG	6.1 – 6.9 mmol/l		
IGT		7.8 - 11.0 mmol/l	
T2D	≥ 7.0 mmol/l	≥ 11.1 mmol/l	≥ 48 mmol/mol (6.5%)

World Health Organisation cut-off values for normal glucose tolerance, impaired fasting glucose (IFG), impaired glucose tolerance (IGT) and type 2 diabetes (T2D) according to fasting, 2 h oral glucose tolerance test (OGTT) concentrations and glycated haemoglobin concentrations (HbA1c).

2.4.3 Measurement of glycaemic control

The ability to measure markers of glycaemic control is of extreme importance for both clinical practice and research studies. The gold standard method of measurement is the hyperinsulinemic-euglycaemic clamp (DeFronzo et al., 1979). For the clamp technique, insulin concentrations are raised using a continuous insulin infusion. Glucose is then infused at a rate which will maintain euglycaemia. The rate of glucose infusion is indicative of whole body glucose uptake, and therefore reflects tissue insulin sensitivity. This method is a direct measure of insulin resistance and enables quantification of insulin sensitivity under steady-state conditions. However, the clamp technique is complex and expensive, requiring specialist knowledge and frequent blood sampling in addition to a constant insulin infusion over several hours (Singh & Saxena, 2010). Due to the complex nature of the clamp, other methods to estimate insulin resistance and glucose control have been developed, including the intravenous glucose tolerance test (IVGTT), oral glucose tolerance test (OGTT) and several insulin sensitivity indexes such as homeostatic model of assessment (HOMA) and Matsuda index (Singh & Saxena, 2010). Each of these surrogate markers have advantages and disadvantages which must be considered prior to deciding which is most suitable for use. In research studies investigating glucose control and sleep, the commonly used surrogate

markers for measuring glycaemic control are the OGTT, IVGTT, HOMA, and Matsuda index.

The IVGTT (Bergman et al., 1979) involves administration of an intravenous glucose bolus following an overnight fast. Blood samples are obtained at regular intervals and glucose and insulin are measured. Minimal model analysis can then be conducted which enables estimation of insulin sensitivity and glucose effectiveness (Muniyappa et al., 2008). Despite being less labour-intensive than the clamp method, the IVGTT still requires intravenous delivery of a glucose bolus and blood sampling over three hours, making it time consuming.

A less labour-intensive method of measuring glycaemic control is the OGTT. The standard OGTT involves ingestion of 75 grams of glucose dissolved in 300 ml of water following a period of fasting (Muniyappa et al., 2008). Blood is sampled prior to ingestion of the glucose drink and at several timepoints during the following two hours. Glucose and insulin are measured from the blood samples and can give an indication of glucose control and, as outlined in the section above, diabetes risk.

Furthermore, estimates of insulin sensitivity can be derived from the OGTT. HOMA uses fasting glucose and insulin concentrations to estimate insulin sensitivity. It is calculated as:

$$\text{HOMA-IR} = \frac{\text{fasting plasma glucose (mmol/l) x fasting plasma insulin (µIU/ml)}}{22.5}$$

As fasting measurements are used, HOMA primarily reflects hepatic insulin sensitivity (Abdul-Ghani et al., 2007). Alternatively, if an estimate of whole body insulin sensitivity is required, the Matsuda Index may be a more suitable measure (Matsuda & DeFronzo, 1999):

$$\frac{10000}{\sqrt{(\text{fasting insulin (µIU/ml)} \times \text{fasting glucose (mmol/l)}) \times (\text{mean glucose (mmol/l)} \times \text{mean insulin (µIU/ml)})}}$$

The Matsuda Index is highly correlated (r = 0.73) with whole-body glucose disposal during a euglycaemic clamp and uses concentrations over the two hour OGTT, making it a more dynamic measure of insulin sensitivity than HOMA (Matsuda & DeFronzo, 1999; Otten et al., 2014). The Matsuda index is thought to be reflective of skeletal muscle glucose uptake since it uses postprandial measurements (Rynders et al., 2016). However, surrogate measures of insulin sensitivity such as the Matsuda Index show poor correlation with insulin sensitivity in the post-exercise period, underestimating the increase in insulin sensitivity in both moderate and high intensity exercise protocols (Rynders et al., 2016). Therefore, these measures should be used with caution if measured in close proximity to an exercise bout.

2.4.4 Alternative markers of glycaemic control

The measures mentioned previously all provide information on glycaemic control in the acute setting, since outcomes represent glucose tolerance at the specific time the test is conducted. For measurement of chronic glucose control, glycated haemoglobin (HbA1c) is commonly used (American Diabetes Association, 2006). Glycated haemoglobin is a haemoglobin molecule which has formed a covalent bond with glucose. HbA1c represents an average of glucose control over the previous three months, which is the approximate lifespan of the red blood cell (Nathan et al., 2007). Whilst HbA1c may act as a diagnostic criteria for diabetes, it is also useful as a predictor of future diabetes risk (Edelman et al., 2004). Individuals with HbA1c concentrations above 42 mmol/mol (6%) have a five-year risk of 25-50% for developing type 2 diabetes, approximately twenty times higher compared to those with HbA1c concentrations below 31 mmol/mol (5%) (Zhang et al., 2010). As HbA1c is a measure of chronic glycaemic control, it is less influenced by the previous days behaviours.

There are also multiple alternative markers which are associated with HbA1c and reflective of glycaemic control. Adiponectin is a hormone produced by adipose tissue. It contributes to the regulation of glycaemic control by increasing fatty acid oxidation, thus reducing serum fatty acid concentrations and hepatic and skeletal muscle intracellular triglycerides (Diez & Iglesias, 2003). Research has established an inverse association between adiponectin concentrations and risk of developing type 2 diabetes, with a relative risk of 0.72 for each 1-log μg/ml increase in adiponectin (Li et al., 2009). Negative correlations have been demonstrated between adiponectin and HbA1c and adiponectin and fasting glucose across the glucose tolerance spectrum in middle aged adults (Fernandez-Real et al., 2004). In addition, circulating adiponectin concentrations show a direct correlation with IVGTT, OGTT, and clamp derived insulin sensitivity (Cnop et al., 2003; Tschritter et al., 2003).

Lipid profiles may also provide information about glycaemic control status. Higher fasting levels of triglycerides and lower high density lipoprotein (HDL) have been identified as markers of insulin resistance (Laws & Reaven, 1992). Insulin resistance is thought to decrease HDL concentrations by reduced synthesis of ApoA-I, increased hepatic triglyceride lipase (HTGL), and decreased lipoprotein lipase (LPL) (Bjornstad & Eckel, 2018). Increased triglycerides are also thought to occur in insulin resistance due to increased HGTL and decreased LPL, as well as increased hepatic VLDL synthesis (Bjornstad & Eckel, 2018). Furthermore, combining these values to obtain a triglyceride-to-HDL ratio has been established as one of the best indicators to identify insulin resistant individuals (McLaughlin et al., 2003). Cut-points of 1.47 mmol/L for triglyceride concentration and 1.8 (SI units) for triglyceride-to-HDL ratio have been suggested to indicate insulin resistance (McLaughlin et al., 2003). Low density lipoprotein (LDL) and LDL-to-HDL ratio have demonstrated moderate positive correlations with HbA1c, with LDL-to-HDL showing

a sensitivity of 98% and specificity of 56% as a predictive biomarker of glycaemic control (Artha et al., 2019), Therefore, when determining metabolic status of an individual, adiponectin, triglycerides, and cholesterol may all be used as indicators of glycaemic control. This may be of advantage in large studies where glycaemic status is required as these markers can be measured from a single blood sample using relatively simple techniques.

2.5 Sleep and glycaemic control

Glucose control typically exhibits a 24-hour pattern, with peak glucose tolerance occurring in the morning and reaching a nadir in the middle of the night. Studies have shown that sleep plays an important role in maintaining this pattern of glucose control, and in particular the peak glucose tolerance in the morning. Sleep can impact glucose and insulin concentrations independent of circadian effects (Shea et al., 2005). Shea and colleagues demonstrated alterations in glucose during sleep which were not explained by circadian effects, and, similarly, Van Cauter and colleagues (1991) observed changes in glucose during daytime sleep which mimic changes that occur during night time sleep, suggesting alterations can occur independent of circadian patterns. During sleep, brain glucose utilisation is decreased and there is a reduction in systemic glucose uptake (Boyle et al., 1994). Furthermore, plasma lipid profiles show a diurnal pattern, with triglycerides and free fatty acids reaching a nadir during normal sleeping hours (Pan & Hussain, 2007). Taken together, it is apparent that sleep plays an important role in glucose regulation, and thus any alterations to sleep patterns may influence glycaemic control.

It has been proposed that humans have evolved with “thrifty” genes (Neel, 1962). As the brain functions using solely glucose as a fuel source, insulin resistance may

have been beneficial to prevent brain hypoglycaemia in previous years when carbohydrate intake was scarce and diets were predominantly meat- and fish-based (Pijl, 2011). However, in combination with the current Western style diet and environment where high-calorie foods are widely available, these previously advantageous mechanisms to promote higher glucose concentrations may make individuals more susceptible to developing type 2 diabetes.

2.6 Short sleep duration and metabolic dysfunction

Epidemiological evidence has linked chronic short sleep duration to a number of adverse health outcomes, including cardiovascular disease, obesity, and type 2 diabetes (Luyster et al., 2012). Compared to individuals reporting a sleep duration of 7 hours each night, a habitual sleep duration of 6 hours or less each night may double the risk of developing type 2 diabetes in adult males, even when confounding factors such as smoking status and waist circumference are accounted for (Yaggi et al., 2006). Similar findings were demonstrated in a sample of males and females taken from the National Health and Nutrition Survey, with those individuals sleeping less than 5 hours each night having an increased likelihood of having developed type 2 diabetes at follow-up (Ayas et al., 2006). The risk of developing type 2 diabetes in short sleepers has been shown to be similar to that of more known risk factors such as physical inactivity (Anothaisintawee et al., 2016). Additionally, a recent meta-analysis examining the effect of sleep restriction on insulin sensitivity demonstrated an effect size similar to the effect of physical activity on insulin sensitivity (Zhu et al., 2019).

2.6.1 Sleep duration and glucose metabolism

2.6.1.1 Cross sectional evidence – sleep duration and metabolic markers

The increased risk of developing metabolic disorders such as type 2 diabetes in individuals with chronic short sleep durations may be due to altered glucose metabolism. Several cross-sectional studies have demonstrated an association between sleep duration and HbA1c levels (Nakajima et al., 2008; Ohkuma et al., 2013; Twedt et al., 2015). However, the majority of these studies used individuals who already had a metabolic disorder such as type 2 diabetes. A meta-analysis examining sleep duration and glycaemic control in those with type 2 diabetes showed that short sleep was consistently associated with higher HbA1c concentrations (Lee et al., 2017), with a pooled mean difference of 0.23%. Similar findings were reported for long sleep durations, but to a slightly lesser extent. This U-shaped association between sleep duration and HbA1c levels indicates poorer glycaemic control in those with type 2 diabetes who do not achieve the optimal amount of sleep each night. However, due to the cross-sectional design of these studies, it is not possible to determine the causality or direction of the relationship.

Whether a similar association between sleep duration and HbA1c levels exists in healthy non-diabetic individuals, or not, is uncertain. Both Nakajima and colleagues (2008) and Hancox and Landuis (2012) demonstrated a significant negative relationship between HbA1c and sleep duration, with durations of less than seven hours showing higher HbA1c levels. Similarly, Potter et al. (2017) showed a trend for a negative association. On the other hand, a recent study failed to demonstrate a relationship between sleep duration and HbA1c in non-diabetic individuals without sleep disorders (Whitaker et al., 2018), and in a study by Nakajima et al. (2017) the increased odds ratio for poorer HbA1c with short sleep duration was mitigated when other lifestyle factors such as exercise and alcohol and smoking intake were adjusted for. Thus, it is possible that lifestyle factors such as diet and exercise may

contribute to the relationship between sleep duration and HbA1c and should therefore be taken into consideration in any studies examining the relationship between sleep and glycaemic control.

In addition to HbA1c, studies have examined the relationship between sleep duration and serum lipids. Some studies observed no association between serum lipid profiles and sleep duration (Anujuo et al., 2015; Bos et al., 2019), whilst others have found higher triglyceride and lower HDL concentrations in short sleepers (Bjorvatn et al., 2007; Kaneita et al., 2008). Kaneita and colleagues (2008) observed a relative risk of 1.51 for high triglyceride concentrations and 5.85 for low HDL cholesterol in women sleeping less than five hours compared to between six and seven hours each night. A lower risk of high LDL was also demonstrated in males achieving at least eight hours of sleep each night. In contrast Anujuo et al. (2015) demonstrated positive associations between short sleep and total cholesterol and short sleep and low HDL cholesterol, but only in individuals of Moroccan and South-Asian Surinamese ethnic origins. The majority of ethnicity groups did not display any associations between sleep duration and lipid profiles.

There are some limitations in these studies which should be considered. Firstly, in the studies which demonstrated a relationship between sleep duration and HbA1c, sleep duration was self-reported and variable. Self-reported measures may overestimate sleep duration compared with objective measures such as actigraphy (Lauderdale et al., 2008a). Short sleepers may overestimate sleep duration by around one hour, and normal sleepers by approximately half an hour. Subjective measures of sleep should therefore be avoided where possible due to the potential for bias. Furthermore, sleep quality is difficult to ascertain from subjective measures of sleep, therefore it is unclear whether sleep fragmentation may be contributing to the alterations in metabolic markers.

The different methods employed to determine sleep duration may partly explain the inconsistent findings between studies. Sleep was measured by actigraphy in the study by Whitaker and colleagues (2018), in contrast to others that used subjective measures (Hancox & Landhuis, 2012; Nakajima et al., 2008; Nakajima et al., 2017; Ohkuma et al., 2013). Additionally, although some studies attempted to account for confounding factors, body mass index was often used to indicate body composition. Body mass index does not distinguish between fat tissue and lean tissue, and is therefore thought to have limitations when used as the only measure of body composition. Impaired glucose metabolism tends to be associated with fat mass (Bower et al., 2017), so using a method which can identify fat mass, such as DXA scanning, would improve these studies. Therefore, additional research in this area using objective and gold-standard methods is warranted as presence of an association may have implications for predicting future risk of developing a metabolic disorder.

2.6.1.2 Experimental evidence – sleep duration and metabolic outcomes

There is a plethora of studies which have used experimental sleep restriction to examine the impact of reduced sleep duration on glucose metabolism. A variety of sleep restriction protocols have been used, ranging from a single night of 4 hours sleep (Donga et al., 2010) to 14 nights of 5.5 hours sleep each night (Nedeltcheva et al., 2009). Likewise, several different methods have been used to measure glucose metabolism. Some studies utilised a hyperinsulinemic-euglycaemic clamp (Buxton et al., 2010; Donga et al., 2010; Rao et al., 2015), which enables a detailed insight into glucose metabolism as mentioned above. Others employed IVGTTs (Broussard et al., 2012; Buxton et al., 2010; Nedeltcheva et al., 2009; Spiegel et al., 1999) or less complex methods such as an OGTT or a mixed macronutrient meal (Eckel et al., 2015; Schmid et al., 2011; Wang et al., 2016). Although less detail can be obtained using the OGTT or mixed meal method, these are arguably more

ecologically valid. Despite the differing methodologies, these studies are consistent in their conclusion that sleep restriction is detrimental to glucose metabolism.

Two weeks of sleep restriction by 3 hours each night resulted in an approximately 10% increase in 2 h glucose values during an OGTT, and around a 20% decrease in insulin sensitivity (Nedeltcheva et al., 2009). Additionally, after only a single night of sleep restriction to four hours, endogenous glucose production was increased and glucose disposal was decreased during a hyperinsulinemic-euglycaemic clamp, and whole body insulin sensitivity was reduced by around 20% (Donga et al., 2010). However, the sleep protocol employed in this study permitted sleep from 0100 to 0500 in the sleep restriction condition, compared to 2300 to 0730 in the control condition. Whilst this keeps midpoint of sleep consistent, it presents the limitation that duration between wake time and the hyperinsulinemic-euglycaemic clamp was different between conditions. This may influence findings, as research has demonstrated an impact of morning circadian misalignment on insulin sensitivity (Eckel et al., 2015).

The evidence presented above clearly demonstrates a role of sleep duration in glucose regulation. So far, however, no research has examined whether the impairment in glucose regulation is related to the number of nights or hours per night of sleep loss. Obtaining this evidence from the current literature would be challenging due to widely varying methodologies regarding measurement of glucose regulation and sleep restriction protocols employed.

2.6.2 Underlying mechanisms

Whilst it is well established that there is a link between both chronic and acute short sleep duration and impaired glucose metabolism, why this occurs remains elusive. Several mechanisms (Figure 2.3) have been proposed in the literature including altered sympathetic activation (Spiegel et al., 2004), decreased brain glucose

utilisation, increased systemic inflammation (Mullington et al., 2010; Vgontzas et al., 2004), altered circadian gene expression (Cedernaes et al., 2015), changes in energy metabolism in skeletal muscle (Vondra et al., 1981) and altered peripheral insulin signalling (Broussard et al., 2012; Rao et al., 2015). Studies examining these potential mechanisms have provided mixed findings, suggesting there may be multiple mechanisms contributing to the impaired glucose regulation after sleep restriction.

Changes in the HPA axis and sympathetic activation have been observed after sleep restriction ranging from a single night to seven nights. Late afternoon and evening cortisol levels were increased after six nights of four hours sleep and seven nights of five hours sleep in healthy males (Buxton et al., 2010; Spiegel et al., 1999). Similarly, a single night of four hours sleep increased cortisol levels by 37% the following evening compared to an eight hour sleep (Leproult et al., 1997). However, during a hyperinsulinemic euglycaemic clamp conducted the morning after a single night of four hours sleep, cortisol levels did not differ from the control condition (Donga et al., 2010). This suggests that the impact of sleep restriction on cortisol levels may be more pronounced in the evening than the morning. Increased levels of cortisol in the evening have been demonstrated to have a larger impact on glucose regulation than elevated morning concentration, with increases in insulin concentrations and an approximately 30% decrease in insulin clearance (Plat et al., 1999). Additionally, limited studies have observed an increase in catecholamines following sleep restriction (Buxton et al., 2010; Irwin et al., 1999). However, the increases in epinephrine and norepinephrine observed by Buxton and colleagues (2010) did not correlate with changes in insulin sensitivity during a hyperinsulinemic euglycaemic clamp, suggesting altered sympathetic activity may not be a main contributor to impaired glucose regulation after sleep restriction.

Increased inflammation has been demonstrated after sleep restriction in some studies (Irwin et al., 2006; Meier-Ewert et al., 2004), but findings were less consistent in others, with only some inflammatory markers increasing (Haack et al., 2007). Several studies also noted a sex-specific change in inflammation, with increased NF-kB occurring only in females in a study by Irwin and colleagues (2008), and increased TNF-alpha in males but not females in another study (Vgontzas et al., 2004). The inconsistent findings suggest that whilst inflammation may play a role in impaired glucose regulation after sleep restriction, it may depend on the protocol and population used, highlighting the possibility that the underlying mechanisms are multi-factorial.

Reduced peripheral insulin sensitivity has been repeatedly demonstrated after sleep restriction (Broussard et al., 2012; Donga et al., 2010; Rao et al., 2015), suggesting that a change in insulin signalling in either skeletal muscle and/or adipose tissue is plausible. Few studies have attempted to directly assess changes in peripheral insulin signalling. Broussard and colleagues (2012) investigated insulin signalling in adipocytes following four nights of sleep restriction to 4.5 hours. Akt phosphorylation was reduced in the sleep restricted condition compared to four nights of control sleep. Similarly, peripheral insulin signalling was investigated in skeletal muscle following two nights of sleep restriction (Sweeney et al., 2017). A decrease in whole body insulin sensitivity was also noted, with skeletal muscle Akt activity tending to be lower after sleep restriction. As outlined previously, Akt plays a key role in the insulin signalling pathway in peripheral tissues, ultimately leading to GLUT4 translocation in skeletal muscle – the primary area of glucose uptake in the postprandial state. Therefore, alterations in the insulin signalling pathway, involving Akt or another step of the pathway, provides a plausible mechanism for the disruption of whole-body glucose regulation after sleep restriction. Future research

should explore whether interventions targeting the insulin signalling pathway can alleviate the impairment in whole body insulin sensitivity after sleep loss.

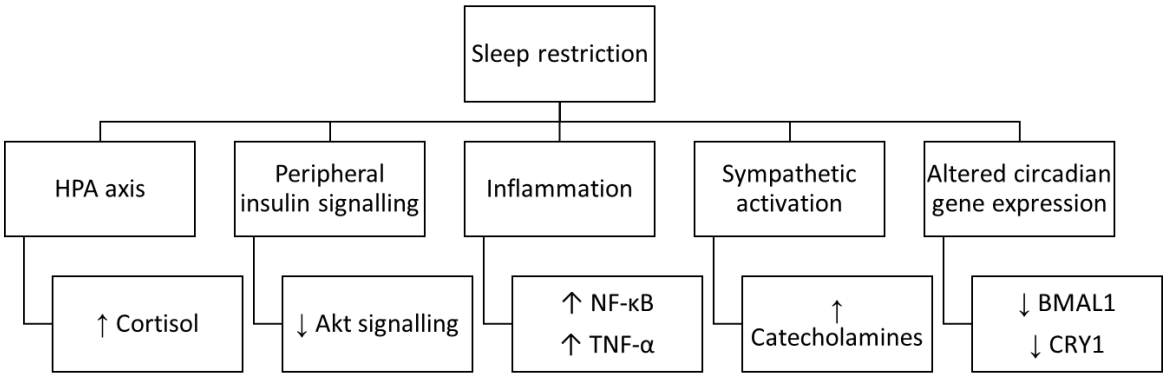


Figure 2.3. Potential mechanisms linking short sleep duration to impaired glycaemic control.

2.7 Glucose metabolism and exercise

2.7.1 Regular physical activity and glycaemic control

Regular exercise is well-known to be beneficial for overall health, decreasing the risk of early mortality and development of several diseases including cardiovascular disease, cancer, and diabetes (Warburton et al., 2006). Inactive individuals have an increased risk of developing type 2 diabetes independent of other confounding factors such as BMI, waist circumference, and energy intake (Ekelund et al., 2012).

The total volume of habitual physical activity is significantly associated with HbA1c and shows a significant inverse dose-response relationship in individuals who are at moderate or high risk for developing type 2 diabetes (Gay et al., 2016). Similarly, adults achieving more moderate-to-vigorous activity (MVPA) also have a lower HbA1c than those completing less moderate-to-vigorous activity (Gay et al., 2016) with each 30 minute per day increment of MVPA equating to a decrease in HbA1c of 0.07 mmol/mol (0.8%) (Bakrania et al., 2017). A reduction in HbA1c of around 0.5% is deemed to be clinically significant (Little and Rohlfing, 2013), suggesting that the additional 30 minutes of physical activity each day is beneficial for metabolic health. A recent meta-analysis of intervention studies which aimed to increase physical activity demonstrated that for each additional 100 minutes of physical activity completed each week, HbA1c was reduced by around 0.14% (1.5 mmol/mol) (Boniol et al., 2017). This reduction was present regardless of the type or intensity of exercise, suggesting that individuals who exercise more may have improved glycaemic control regardless of the type of exercise they choose to do, although it may be necessary to exceed an extra 100 minutes per week to bring about clinically meaningful differences

2.7.2 Acute exercise and glycaemic control

An acute bout of exercise can also exert positive effects on metabolic health. Improved insulin sensitivity is often observed after a just single session of exercise (Bird & Hawley, 2017). This increase in insulin sensitivity may last for up to 72 hours following the exercise bout, although research has displayed varying findings on the duration and extent of the improvement (Bird & Hawley, 2017).

Increased insulin sensitivity in healthy individuals has been demonstrated following various modalities of exercise, including resistance exercise (Breen et al., 2011), steady-state continuous exercise (Rynders et al., 2014), and high intensity exercise

(Ortega et al., 2015; Rynders et al., 2014). However, high intensity exercise may be superior to continuous steady state exercise for increasing insulin sensitivity. Rynders and colleagues (2014) observed larger decreases in insulin and glucose area under the curve after high intensity exercise, compared to work matched moderate intensity exercise. Similarly, sprint interval exercise has produced superior improvements in insulin sensitivity compared to continuous exercise when measured 30 minutes after cessation of exercise (Ortega et al., 2015).

2.7.3 Mechanisms mediating improved insulin sensitivity after exercise

Several potential mechanisms have been proposed to explain the metabolic benefit of regular exercise. These include altered storage of adipose tissue away from metabolically active tissues, reduced overall adiposity, and an accumulation of the effects of multiple bouts of acute exercise (Gay et al., 2016; Johnson et al., 2009). However, as the link between physical activity and HbA1c still persists when BMI and waist circumference are accounted for, it could be suggested that the improvement in glycaemic control in regularly active individuals is a cumulative result of repeated bouts of acute exercise which causes changes in metabolic pathways.

The mechanisms underlying the increase in glucose uptake and insulin sensitivity following acute exercise are likely multi-factorial. During exercise, muscle contraction promotes glucose uptake into the muscle, independent of insulin. This contraction-stimulated glucose uptake is thought to continue for a few hours after cessation of exercise, which may be partly responsible for the improvements in glycaemic control immediately after an exercise bout (Goodyear & Kahn, 1998). The elevated glucose uptake may be due to enhanced GLUT-4 translocation to the cell surface. GLUT-4 expression at the cell surface is significantly increased for 22 h

after a single bout of endurance exercise (Greiwe et al., 2000). Moreover, there is an increased sensitivity to insulin in skeletal muscle following exercise. Phosphorylation of AS160 and Akt, two key members of the insulin signalling pathway, are increased for up to 26 h after cessation of an exercise bout (Breen et al., 2011). Limited evidence also suggests AMPK may be involved in the improvements in insulin sensitivity response to exercise (Hawley & Lessard, 2008). As mentioned in the previous section, Akt is a part of the insulin signalling pathway which may be compromised after sleep restriction. Consequently, exercise may be able to attenuate the insulin sensitivity impairment after sleep duration through its ability to upregulate or change the expression of proteins in the insulin signalling pathway. Similarly, Saner and colleagues (2018) have suggested that mitochondrial adaptations to exercise may be able to attenuate the impairment in insulin sensitivity after sleep loss.

At present, only two studies have examined the impact of exercise on metabolic outcomes during periods of short sleep. Both of these previous studies were conducted in a setting of total sleep deprivation, with one using an acute bout of exercise (VanHelder et al., 1993) and the other using a two-week interval training protocol prior to sleep deprivation (De Souza et al., 2017). Two weeks of prior exercise was observed to alleviate the increases in glucose and insulin after total sleep deprivation, and acute exercise partially improved insulin responses to an OGTT after sleep deprivation (VanHelder et al., 1993). However, these studies arguably have limitations in that total sleep deprivation was used and exercise was conducted prior to sleep loss. It is likely that short sleep durations are more common than total sleep deprivation, and that on occasion sleep loss may be unplanned. Therefore, it would be of interest to investigate the effect of exercise following partial sleep restriction to address this gap in the literature. Given that acute exercise has a positive effect on glucose uptake in skeletal muscle and therefore enhances insulin

sensitivity, it may be a useful non-pharmacological intervention to prevent metabolic impairments occurring after periods of either acute or chronic sleep loss.

In addition to the insulin sensitivity benefit of exercise, as mentioned earlier exercise may have a beneficial effect on several markers of sleep quality including sleep efficiency and total sleep time. Hence, in situations where short sleep is unavoidable, a bout of exercise may be beneficial in producing a higher quality sleep, indirectly combating the impairment in glucose regulation by increasing insulin sensitivity through a better sleep.

2.8 Conclusion

Thus far, this literature review has highlighted the many ways in which sleep can impact health, including interactions with other health behaviours such as diet and exercise. The literature identifies a clear role of sleep in the maintenance of glucose homeostasis. However, previous studies used a wide variety of methodologies making comparison between studies challenging. Whether or not glycaemic control is altered in a linear fashion by repeated nights of sleep restriction remains elusive. Furthermore, the ability of exercise to positively impact glycaemic control has not been well studied in a sleep restricted population.

2.8.1 Aims and Objectives

This thesis shall explore the relationship between sleep and glycaemic control, and how exercise contributes to this relationship. Specifically, the aims of the thesis are:

- To understand the impact of the number of nights of sleep restriction has on the impairment in glycaemic control. This will determine how many nights of

sleep restriction are required to impair glycaemic control to inform further sleep restriction research and the cumulative impact of sleep restriction on the risk of type 2 diabetes,

- To assess whether an acute bout of exercise can attenuate the negative effects of sleep restriction on glycaemic control,

- To examine if the relationship between sleep, glycaemic control, and physical activity persists in the free-living environment when other behavioural factors which also influence glycaemic control are taken into consideration.

Chapter 3 - General Methods

This chapter will describe the methods which were used in the studies presented in Chapters 4, 5 and 6. An outline of the studies presented in this thesis is given in Figure 3.1 below.

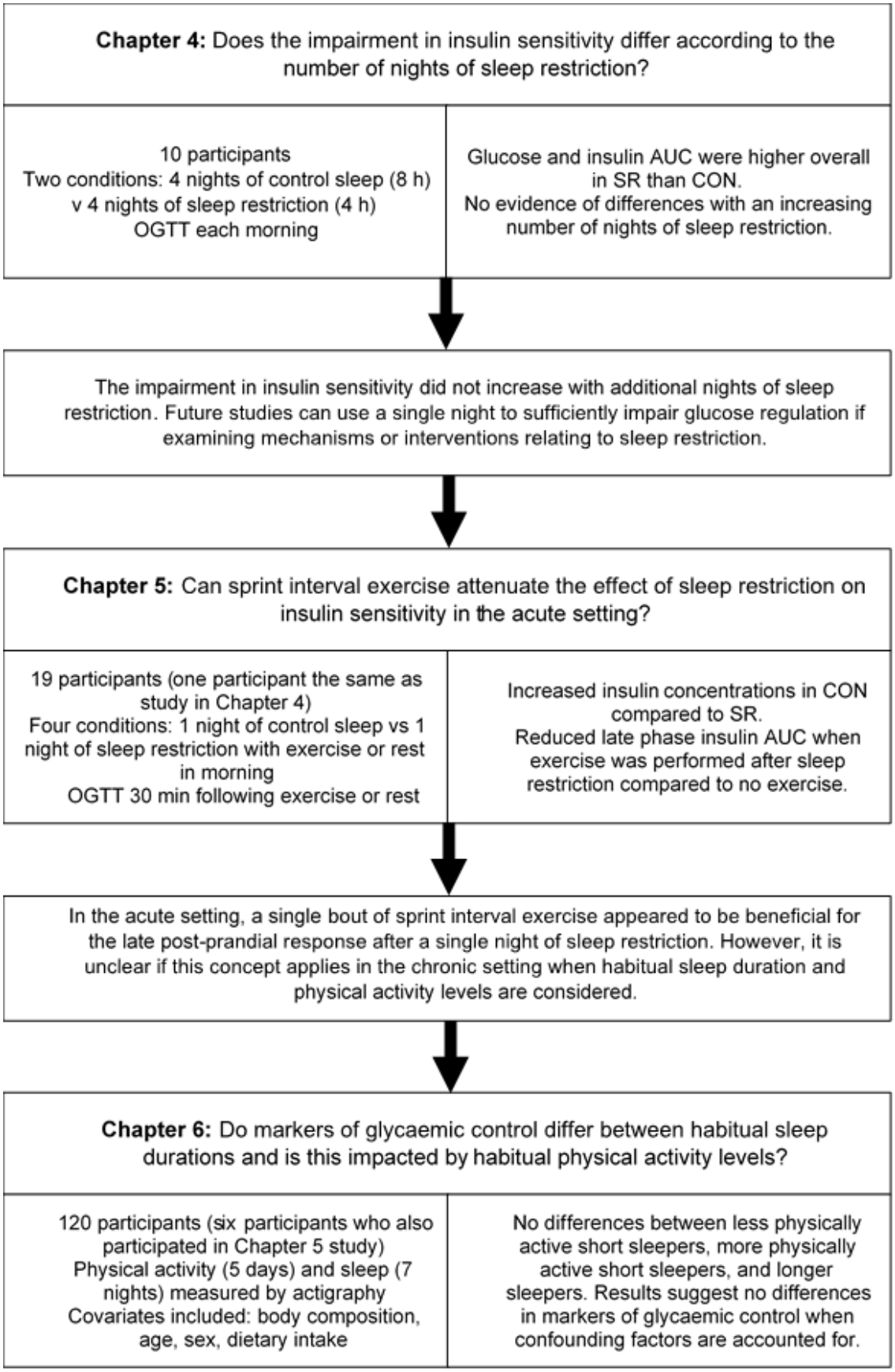


Figure 3.1. Outline of research studies undertaken. CON = control condition. SR = sleep restriction condition). OGTT = oral glucose tolerance test. AUC = area under the curve.

3.1 Recruitment

Recruitment for studies was conducted by placing poster advertisements around Northumbria University and the surrounding community, and through word of mouth. Individuals who expressed an interest in participating were provided with the Participant Information Sheet (Appendix A) and given a minimum of 24 hours to read it and decide whether or not they would like to take part in the study. If they decided to participate, a day and time was arranged for the initial visit, during which they would also have the opportunity to ask questions before completing written informed consent (Appendix B). All studies were conducted in accordance with the Declaration of Helsinki and study protocols were approved through the University of Northumbria Online Ethics System.

3.2 Anthropometric measures

For all studies presented in this thesis stature and body mass were measured. For these measurements, shoes were removed and light clothing was worn. Stature was measured to the nearest 0.1 cm using a free-standing stadiometer (Chapter 5 – SECA, Germany) or Harpenden wall-mounted stadiometer (Chapters 4 and 6 – Seritex, NJ, USA). Body mass was measured to the nearest 0.1 kg using digital scales (SECA). Body mass index (BMI) was calculated by dividing body mass in kilograms by stature in metres squared (kg/m^2).

3.3 Measurement of sleep duration

3.3.1 Polysomnography

Polysomnography was used in Chapter 4 to measure sleep in the laboratory. Polysomnographic recordings were obtained on the familiarisation night to screen for sleep disorders and nights 1 and 3 of each condition to determine sleep architecture. Surface electrodes were placed according to the international 10-20 system at sites Cz, C3, C4, Fpz1, Fpz2, F3, F4, P3, P4, O1, O2, A1 and A2 (Klem et al., 1999). Electrodes were also placed at the outer canthus of each eye and on the chin for EOG and EMG respectively. During the familiarisation stay, pulse oximetry, a snore microphone, and lower limb EMG were also used to enable any sleep disorders to be detected prior to the experimental trials. Any individuals with sleep disorders would be excluded from the study. To determine sleep stages recordings were scored automatically by DOMINO software (Somnomedics, Germany) using AASM criteria (Kushida et al., 2005).

3.3.2 Actigraphy

Actigraphy (GENEActiv, Activinsights, Kimbolton, UK) was used during studies to measure sleep during the experimental and entraining phases. Actigraphy monitors assess accelerations in three-dimensional axes over time to give an output of gravitational units. Algorithms can then be used to distinguish between sleep and wake.

The studies in Chapters 4, 5 and 6 used GENEActiv actigraphy monitors to monitor sleep duration. GENEActiv actigraphy monitors are tri-axial accelerometers, assessing acceleration in the x-, y-, and z-axis. The GENEActiv actigraphy monitors also contain light and temperature sensors to improve accuracy of wear time.

The absence of movement is used as a surrogate of sleep and therefore can give concurrent detail of sleep and activity periods. Actigraphy is commonly used as an alternative when total sleep time is required and PSG is not feasible (Marino et al., 2013). High levels of agreement (>90%) have been demonstrated between actigraphy and PSG when used to distinguish between sleep and wake periods (Ancoli-Israel et al., 2003).

In all studies throughout this thesis, actigraphy monitors were set up with a measurement frequency of 10 Hz. Participants were instructed to wear the actigraphy monitor continuously on the non-dominant wrist. For measurement of sleep, participants were instructed to press the button on the actigraphy watch upon going to bed with the intention to sleep and upon wakening in the morning. This allowed determination between intended sleep and time in bed. If participants forgot to press the button, bed and wake time were determined from the sleep diaries. Data were extracted in 60 s epochs and imported to the GENEActiv sleep macro available at open.geneactiv.org.

3.3.2.1 Validity of actigraphy for measurement of sleep time

Few studies have specifically assessed validity of the GENEActiv actigraphy monitor (Activinsights). Therefore, the GENEActiv actigraphy monitor (Activinsights) was assessed against polysomnographic derived total sleep time for criterion validity. Validity was measured using data obtained in the study presented in Chapter 4. Polysomnographic data and actigraphy data were collected simultaneously for two control nights (8 hour sleep opportunity) and two nights of sleep restriction (4 hour sleep opportunity) in the Northumbria Sleep Research Laboratory.

Upon completion of each trial period, actigraphy monitors were removed and data was extracted. For sleep data, files were converted to 60 s epochs before being

imported into the GENEActiv sleep macro (available at open.geneactiv.org). The macro uses the Sadeh algorithm (Sadeh et al., 1994) to determine sleep, and gives an output for variables including total sleep time, sleep efficiency, and activity periods during sleep time.

Statistical analysis was performed using Microsoft Excel (V.2016) and SPSS statistical software (IBM, New York, USA). Descriptive statistics for total sleep duration (min) and were calculated and are presented as mean ± SD. Inferential statistics were assessed by Bland-Altman plots to determine any systematic bias and paired t-tests to detect any differences between methods. Limits of agreements of ± 90 min were deemed to be satisfactory, as the subsequent studies which this validation study was designed to inform would have substantial differences in sleep restriction (> 2 h) between groups.

Due to technical issues with some actigraphy monitors and PSG equipment, concurrent actigraphy and PSG sleep data was available for 10 nights in control (CON) and 10 nights in sleep restriction (SR). Mean total sleep time during the control condition, determined by polysomnography, was 428 ± 18 min (7.1 ± 0.3 h) whereas mean total sleep time determined by actigraphy was 384 ± 53 (6.4 ± 0.9 h). The limits of agreement between the two assessment tools were -36 to 126 min (-0.6 to 2.1 h) with actigraphy showing underestimation by 45 min (0.75 h) (Figure 3.1). During the sleep restricted condition, mean total sleep time determined by polysomnography, was 218 ± 20 min (3.6 ± 0.3 h) whereas mean sleep time determined by actigraphy was 211 ± 15 min (3.5 ± 0.25 h). The limits of agreement were -23 to 35 min (-0.4 to 0.6 h) with actigraphy showing underestimation by 6 min (0.1 h) (Figure 3.2). Paired t-tests did not show any evidence of differences between methods (P = 0.221 in SR and P = 0.110 in CON).

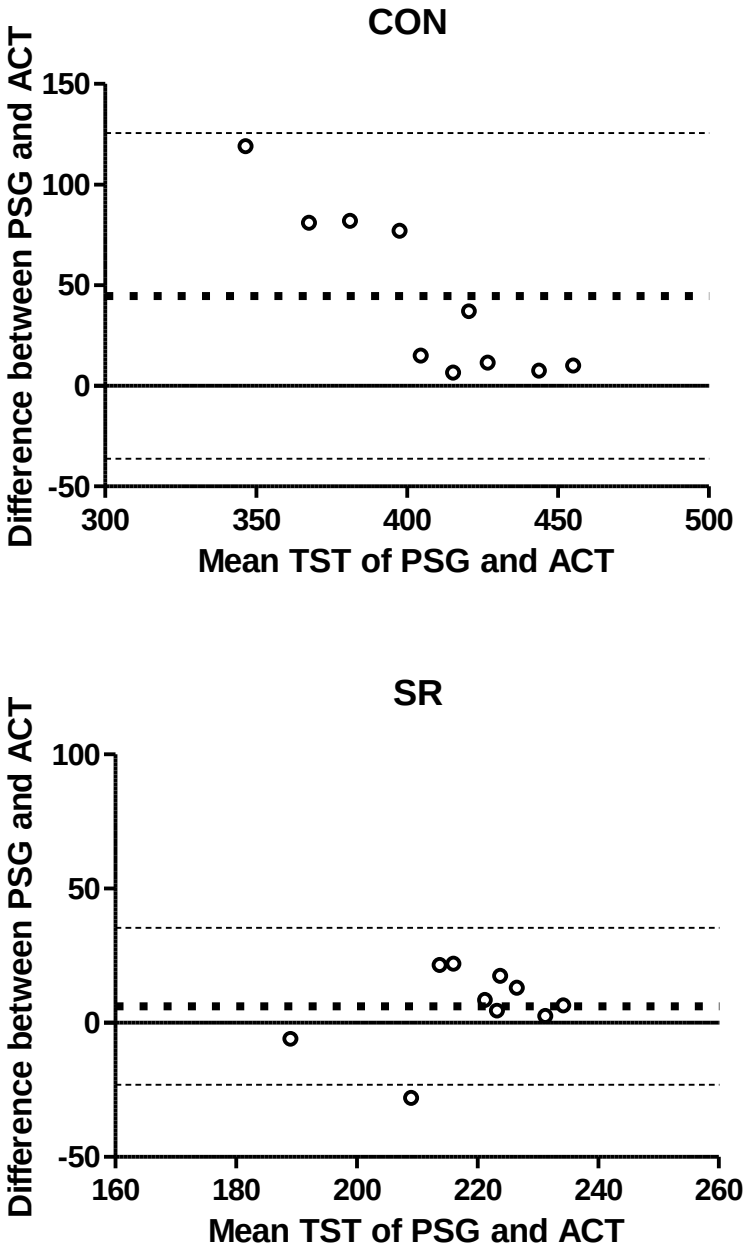


Figure 3.2. Bland-Altman plots for total sleep time (TST) measured by polysomnography (PSG) and actigraphy (ACT) in CON and SR. Bold dotted line represents mean bias. Dashed lines represent 95% limits of agreement (LOA). LOA are 45 ± 81 min (CON) and 6 ± 29 min (SR).

The difference in sleep duration between PSG and GENEActiv actigraphy during the sleep restricted condition shows very good agreement between the two methods. These results indicate that GENEActiv accelerometers can be used as a

valid tool to estimate total sleep duration when sleep is restricted to approximately 4 hours. The mean bias of 6 min during SR compared to 45 min during CON suggests that actigraphy is better at determining total sleep time in shorter sleep durations than longer durations. One possible explanation for this discrepancy may be that participants were less mobile because of a higher contribution of N3 due to sleep debt during the sleep restriction condition. This has also been suggested by Marino and colleagues (2013) who highlighted higher specificity of actigraphy in shorter sleep durations.

The degree of variation between PSG and accelerometers shown in the present study during the control condition is in contrast to those presented by Full and colleagues (2018) who have shown differences between 6 minutes underestimation to 4 minutes overestimation when comparing accelerometers to PSG for total sleep time. However, results from the present study are comparable to those from Van Hees et al. (2018) who showed a mean absolute error of 71 minutes between PSG and GENEActiv accelerometers for the sleep period time window duration. The limits of agreement observed in the current study are similar to previous studies comparing actigraphy to PSG (de Souza et al., 2003; Weiss et al., 2010) While our small sample size may be influenced by extreme scores, these results do highlight a degree of underestimation of total sleep time when using accelerometers. Future research should account for this mismatch when designing sleep studies when PSG is not feasible.

3.3.3 Self-reported measures

3.3.3.1 Sleep logs

Seven-day sleep logs were used in conjunction with actigraphy (sleep log shown in Appendix D). Participants were asked to complete these each morning approximately 30 minutes after waking. The sleep log was adapted from Carney

and colleagues (2012) to include the Karolinska Sleepiness Scale (Akerstedt & Gillberg, 1990). The Karolinska Sleepiness Scale has been previously used in sleep deprivation studies and is correlated with polysomnography measurements derived from EEG (Akerstedt & Gillberg, 1990).

3.3.3.2 Morningness-eveningness questionnaire

The morningness-eveningness questionnaire (MEQ) (Horne & Ostberg, 1976) was used to determine chronotype in the studies presented throughout this thesis. The MEQ consists of 19 questions. A number of points are given for each question depending on the answer selected, and these points are combined to give an overall total between 16 and 86. There are five categories in total: extreme evening type (16-30 points), moderate evening type (31-41 points), intermediate (42-58 points), moderate morning type (59-69 points), and extreme morning type (70-86 points). Any individuals who were classed as extreme morning or extreme evening type were excluded due to potential circadian misalignment.

3.3.3.3 Pittsburgh Sleep Quality Index

The Pittsburgh Sleep Quality Index (PSQI) (Buysse et al., 1989) is a questionnaire developed to measure sleep quality and pattern over the past 30 days. Questions relate to seven domains (subjective sleep quality, sleep latency, sleep efficiency, sleep duration, sleep disturbances, daytime dysfunction, and use of medication to assist sleep) and a global score is given, with a score of 5 or above indicating a "poor sleeper". In the studies in Chapters 4 and 5, the PSQI was used to exclude any individuals who were deemed to be poor quality sleepers.

3.3.3.4 Sleep Disorders Symptom Checklist-17

The Sleep Disorders Symptom Checklist-17 (SDS-CL-17) is an instrument developed to screen individuals for sleep disorders prior to participation in a research study (Klingman et al., 2017). Individuals are given a list of statements and

asked to rate how often this applies to them. Each question or group of questions relate to a specific sleep disorder; either obstructive sleep apnoea, restless leg syndrome, insomnia, narcolepsy, circadian rhythm disorder, or parasomnias. A score above the cut-point for a sleep disorder category indicates presence of that sleep disorder. In the study presented in Chapter 6, the SDS-CL-17 was used to exclude individuals with sleep disorders as this may influence study findings.

3.4 Physical activity

Actigraphy (GENEActiv, Activinsights) was used during studies to measure physical activity. Actigraphy is a useful tool to measure physical activity in the free-living environment. Algorithms can be used to give a description of intensity of activity in metabolic equivalents of a task (METs).

In all studies throughout this thesis, actigraphy monitors were set up with a measurement frequency of 10 Hz for measurement of physical activity. Participants were instructed to wear the actigraphy monitor continuously on the non-dominant wrist. Data were extracted in 60 s epochs, consistent with previous studies (Esliger et al., 2011), and imported to the GENEActiv Everyday Living macro available at open.geneactiv.org.

3.4.1 Validation of actigraphy for measurement of physical activity

Few studies have validated the GENEActiv monitor for physical activity (Esliger et al., 2011; Powell et al., 2017). When assessed on a mechanical shaker, the GENEActiv accelerometer has previously been shown to have good reliability (intra-CV of 1.4% and inter-CV of 2.1%) and excellent validity (r = 0.98) (Esliger et al., 2011). To assess the validity of the GENEActiv actigraphy monitor for physical activity, the monitors were assessed against indirect calorimetry assessments.

Indirect calorimetry is an accurate method for measurement of energy expenditure and is often used to assess validity of accelerometers (Ndahimana & Kim, 2017).

11 participants (4 males and 7 females) were recruited by advertisement and word of mouth at Northumbria University. Mean (±SD) age, stature and mass were 29 ± 10 yr, 170.7 ± 8.6 cm and 66.2 ± 12.0 kg respectively. Volunteers were eligible for the study if they were aged 18 to 40 yr, and in good health. Exclusion criteria included any symptoms of cardiac or respiratory conditions as assessed by a Physical Activity Readiness Questionnaire (PARQ). Participants were also excluded if they had any current or previous musculoskeletal injuries or any other contraindicators to exercise in the past 6 months. The study protocol was reviewed and approved by the Faculty of Health and Life Sciences Research Ethics Committee at Northumbria University (Ethics number: 17105) and written informed consent was obtained from all participants prior to beginning the study.

Participants arrived at the laboratory in a rested state and following a 12-hour fast having avoided vigorous activity for 24 hours prior to the laboratory visit. To determine resting metabolism, participants lay on a medical plinth for 15 minutes. Participants were then fitted with an actigraphy monitor on the non-dominant wrist and were fitted with a face mask to collect expired air. Participants then remained on the medical plinth and rested quietly for a further 15 minutes, during which, the last 5 minutes were collected for analysis. Expired air samples were collected into an online gas analyser (Oxycon Pro, Jaeger, Germany). Resting metabolic rate was determined using the abbreviated Weir formula (Weir, 1949). To validate physical activity intensity during a sedentary activity, participants sat at a desk to perform a computer-based task for 5 min. During this sedentary activity, participants were asked to type a written script onto a laptop and make formatting changes. During this period, laboratory conditions were kept constant at 23°C, 51% humidity and 1025 hPa.

Following the sedentary task, participants used a treadmill (h/p/cosmos, Germany) to validate light, moderate and vigorous activity. Participants were asked to complete a submaximal test. The test began with participants walking at a speed of 2 km/h. At 3-min intervals, the treadmill speed was increased by 2 km/h until the ventilatory threshold was reached. Ventilatory threshold was determined by a non-linear increase in minute ventilation (VE) relative to the volume of oxygen utilisation ($\dot{V}O_2$) (Reybrouck et al., 1986).

For physical activity data, files were converted into 15 s epochs before being imported into the GENEActiv everyday living macro (available at open.geneactiv.org). The cut-points used to define sedentary, light, moderate, and vigorous activity were <386, 386-439, 440-1811 and >1811 g/min respectively, based on values determined by a validation study (Esliger et al., 2011). Statistical analysis was performed using Microsoft Excel (V.2016) and SPSS statistical software (IBM).

Descriptive statistics for physical activity intensity (MET.min) were calculated and are presented as mean ± SD. Inferential statistics were assessed by Bland-Altman plots to determine any systematic bias and paired t-tests to detect any differences between methods. Limits of agreement of ± 4 MET.min were classed as satisfactory as it was thought this would be sufficient to detect differences between lower and moderate-to-vigorous intensity activities.

Physical activity intensity during the sedentary activity, determined by indirect calorimetry was 1.4 ± 0.2 MET.min, whereas actigraphy estimated the sedentary activity to be 1.2 ± 0.2 MET.min. The limits of agreement were -0.5 to 0.8 MET.min with actigraphy showing underestimation by 0.2 MET.min (Figure 3.3). Indirect calorimetry determined mean light activity intensity (2 km/h), moderate activity intensity (4 km/h) and vigorous activity intensity (8 km/h) to be 2.7 ± 0.6 MET.min,

4.7 ± 0.9 MET.min and 12.0 ± 3.0 MET.min respectively. The limits of agreement with actigraphy for light, moderate and vigorous activity were -1.3 to 2.0 MET.min, -2.5 to 3.0 MET.min, and -7.4 to 3.7 MET.min respectively, with actigraphy showing a bias of 0.4 MET.min, 0.2 MET.min, and -1.8 MET.min for light, moderate and vigorous activity (Figure 3.2). There were no significant differences between methods at any intensity (sedentary P = 0.097; light P = 0.193; moderate P = 0.574; vigorous P = 0.067).

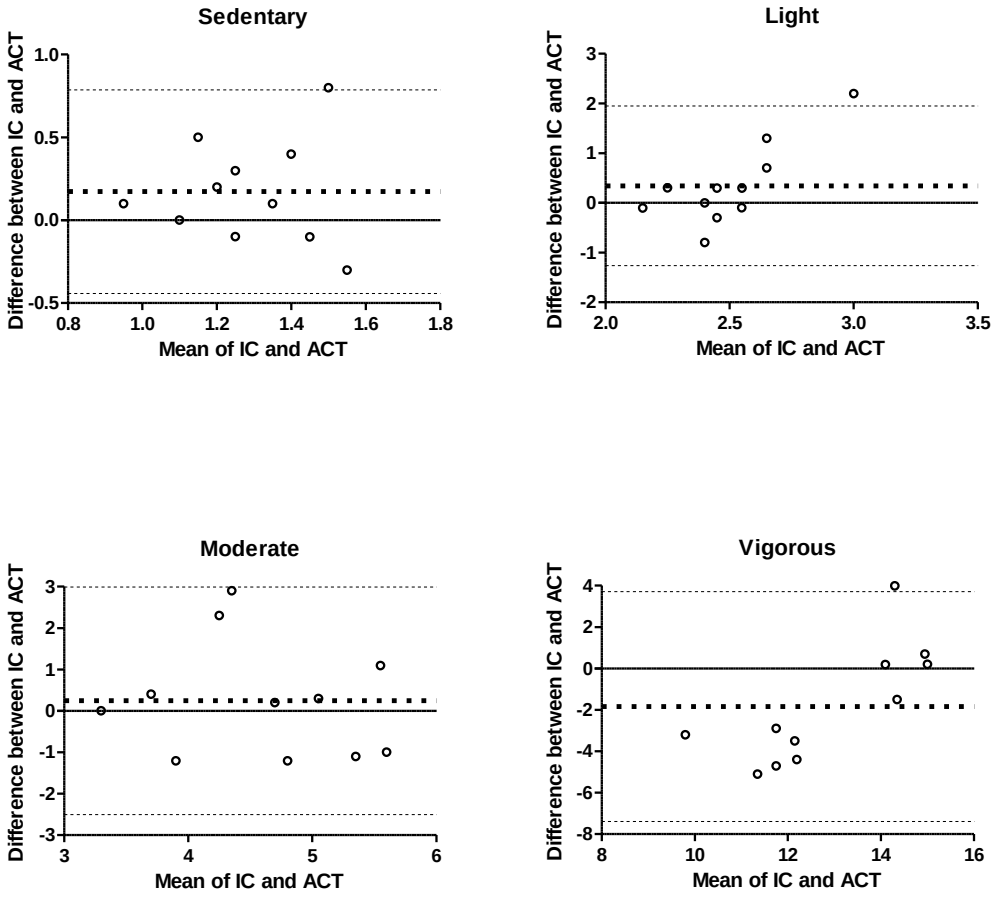


Figure 3.3. Bland-Altman plots for comparison of indirect calorimetry (IC) and actigraphy (ACT) for measurement of activity. Bold dotted line represents mean bias. Dashed lines represent 95% limits of agreement (LOA). LOA are 0.2 ± 0.6 MET.min (sedentary), 0.4 ± 1.6 MET.min (light), 0.3 ± 2.7 MET.min (moderate) and -1.8 ± 5.5 MET.min (vigorous).

Physical activity tended to show good agreement between measurement methods, with mean bias for all activity levels less than 2 MET.mins. While actigraphy showed very good agreement with indirect calorimetry at lower intensities, variation was greater during moderate and vigorous activity. Nonetheless, the average difference and limits of agreement are small for all levels of activity, indicating that GENEActiv accelerometers are a valid tool to assess MET.min.

3.5 Diet

In all studies presented in this thesis, 3-day food diaries (Appendix C) were used to determine habitual dietary intake. A recording period of three days was selected as this can provide an overview of habitual diet whilst ensuring compliance does not decrease due to a long recording period (Ortega et al, 2015). Participants were instructed to document every food or drink item consumed, the time at which it was consumed, the cooking method, and the estimated amount. Food diaries were completed on two weekdays and one weekend day Energy and macronutrient intake for the three days were calculated using Microdiet software (version 4.4; Downlee Systems Ltd., UK). An average daily intake from the three days was then calculated for energy (megajoules/d), carbohydrate (g/d), fat (g/d), and protein (g/d).

In the experimental studies (Chapter 4 and 5), participants were provided with individualised diets. Individualisation of diets to maintain habitual intake was possible due to the within-subjects design of the studies in these chapters, and enhances ecological validity compared to the use of standardised diets. In the study in Chapter 4 diet was provided throughout the four days of each experimental trial, and in the study in Chapter 5 diet was provided the day prior to each condition. Diets were matched for habitual energy intake, and macronutrients were kept within

10% of habitual intake. Diet was identical across conditions to prevent results being confounded by differences in dietary intake.

3.6 OGTT

Oral glucose tolerance tests (OGTT) were conducted in the studies presented in Chapters 4 and 5. For the OGTT, participants were provided with a beverage containing 82.5 g dextrose (MyProtein, UK) mixed with 300 ml water. They were instructed to consume the drink within 5 minutes. During the OGTT, blood samples were drawn prior to consumption (baseline (0)) and 15, 30, 45, 60, 90 and 120 min following consumption.

3.6.1 Repeatability of OGTT

To assess the repeatability of the OGTT, an intraindividual coefficient of variation was calculated (CV%) from the 4 control trials in the study presented in Chapter 4. Briefly, 10 participants underwent 4 consecutive day OGTTs conducted in a rested, fasted state. Blood samples were collected at regular intervals and analysed for glucose and insulin. CV% were calculated for peak glucose, total area under the curve of glucose and insulin as well as the early phase (0-60 min) and late phase (60-120 min) post prandial response. Repeatability measurements for the average CV(%) across the four control trials are displayed in Table 3.1.

Table 3.1. Repeatability of measures during consecutive day OGTTs.

Variable	CV(%)
Glucose AUC	
Total	10.2
Early	5.5
Late	16.5
Insulin AUC	
Total	20.5
Early	21.7
Late	29.3
Peak glucose	13.7

Glucose and insulin area under the curve (AUC) for total (0-120 min), early (0-60 min) and late (60-120 min) phase of the OGTT.

The daily variation observed in insulin AUC mimics previous research using consecutive day OGTTs (Gordon et al., 2011), which may suggest insulin has a higher natural biological variation than glucose. Additionally, the insulin assay had a higher variability than the glucose measurement method, which has been suggested to contribute to the higher variation observed in insulin compared to glucose responses (Utzschneider et al., 2007). The variability in glucose and insulin measures which we have highlighted must be considered when interpreting results from an intervention study. For a change to be meaningful it must lie outside of the natural variation. Work conducted by Gordon et al. (2011) shows that increases in glucose and insulin area under the curves of 63.5 mmol/L/120 min and 7061 pmol/L/120 min and decreases in glucose and insulin area under the curves of 80.9 mmol/L/120 min and 7237 pmol/L/120 min lie outside of the normal day-to-day variation and can be classed as clinically significant changes.

3.7 Blood collection and processing

In Chapters 4 and 5 the cannulation technique was used for blood collection to enable multiple blood draws. A cannula (20-gauge, Terumo, Japan) was inserted

into an antecubital vein and a connector (BD Connecta, Becton Dickinson, New Jersey, USA) was attached. For each sample a 10 ml syringe was used to transfer blood from the connector to a vacutainer. 8 ml of blood was collected for each sample into 10 ml serum vacutainers (Becton Dickinson,). Vacutainers were inverted 5 times to ensure thorough mixing. The samples were left to clot at room temperature for 30 min then centrifuged at 3500 rpm for 15 min at 4°C. Serum was aliquoted into microtubes. At this stage a small amount of serum from each sample was transferred into a capillary tube and placed into an Eppendorf tube containing 1 ml of hemolysing solution (EKF Diagnostics, Cardiff, UK) to allow determination of serum glucose. The remaining serum was frozen at -80°C until further analysis.

In Chapter 6 blood was collected by the venepuncture technique. A needle was inserted into the antecubital vein in the arm and approximately 10 ml of blood was drawn into a serum vacutainer (Becton Dickinson). The sample was allowed to clot for 30 min then centrifuged at 3500 rpm for 15 min at 4°C. Serum was aliquoted into a microtube. A small amount was taken up into a capillary tube and placed in hemolysing solution for measurement of glucose. The remaining serum was frozen at -80°C until further analysis.

3.7.1 Serum glucose

Serum glucose was measured immediately after centrifugation. The Biosen C-line (EKF Diagnostics) was calibrated using a standard of known concentration (12 mmol/L). The sample was then measured to determine glucose concentration in mmol/L. The Biosen C-line uses the enzymatic-amperometric method to determine glucose concentration. In this method an enzymatic reaction converts glucose to gluconic acid and hydrogen peroxide, and glucose is determined through measurement of hydrogen peroxide (Artigues et al., 2017).

3.7.2 Insulin

For measurement of serum insulin in Chapters 4 and 5, samples were defrosted on ice. Serum insulin was then measured using commercially available enzyme-linked immunoassays (Mercodia, Sweden) conducted according to manufacturer's instructions. Briefly, thawed samples were vortexed and added to a 96-well plate in duplicate. Standards were prepared according to manufacturer's guidance and added in duplicate. Samples and standards were incubated with enzyme conjugate before being manually washed 6 times. Substrate was then added and after incubation the reaction was stopped. A microplate reader (Anthos 2010, Biochrom, Cambridge, UK) and read at an optical density of 450 nm. A standard curve was created by plotting absorbance against concentration and a linear trendline was added. The concentration of each sample was determined, and an average of the duplicates was taken as the final concentration.

Chapter 4 - An Investigation into the Cumulative Effects of Sleep Restriction on Glycaemic Control

4.1 Introduction

It is well-known that insufficient sleep leads to changes in glycaemic control (Knutson et al., 2007). Adverse metabolic events are evident across a range of sleep restriction protocols. Early research demonstrated decreased glucose tolerance after 6 nights of 4 h sleep each night (Spiegel et al., 1999) and, more recently, a 19% reduction in whole-body insulin sensitivity, estimated by Matsuda Index, was observed when habitual sleep duration was reduced by 50% for 2 nights (Sweeney et al., 2017). Similarly, IVGTT-derived disposition index is decreased 30% after two weeks of 5.5 h compared to 8.5 h sleep, indicating increased insulin resistance (Nedeltcheva et al., 2009). Markedly, even a single night of sleep restriction appears to have metabolic consequences, manifest as a 20% reduction in glucose disposal rate during a hyperinsulinemic euglycaemic clamp (Donga et al., 2010).

Whilst previous research consistently demonstrates altered glucose metabolism and insulin sensitivity after sleep restriction, the varying sleep protocols and insulin sensitivity measurement methods employed do not enable accurate comparison between studies. However, it does appear that studies employing more nights of sleep restriction may show a greater impact on insulin sensitivity, with single night studies showing a 20% decrease in insulin sensitivity after sleep restriction (Donga et al., 2010), a two-night study showing a 40% increase in insulin after a mixed meal in the sleep restriction condition (Schmid et al., 2011), and a three-night study demonstrating increased insulin levels of around 60% after a meal (Klingenberg et al., 2013). The insulin sensitivity measurement method varies widely between these studies so whilst one can speculate, comparisons between these studies must be

interpreted with caution. Thus, it remains unclear if there is a cumulative effect of sleep loss on metabolism or if the alterations are similar regardless of severity, either in number of nights of sleep loss or hours per night.

The aim of the current study was to address the question: is the extent of the impairment in insulin sensitivity related to the number of nights of sleep restriction? This question has not yet been addressed in previous research and is important to inform the design of future studies aiming to examine mechanisms or interventions to attenuate metabolic impairments following sleep loss. It was hypothesised that each night of sleep restriction would lead to a greater decrease in insulin sensitivity.

4.2 Methods

4.2.1 Participants

12 volunteers were recruited by poster advertisement around Northumbria University to participate in this randomised crossover study. Individuals were eligible for the study if they were aged 18 to 40 yr, a non-smoker and had a regular sleep pattern (7 to 9 hr each night) as assessed using the PSQI (Buysse et al., 1989). Exclusion criteria were shift work or travel across time zones in the past 4 weeks, the presence of any sleep, metabolic, neurological, psychiatric, inflammatory disorder, or blood clotting disorders, a history of drug or alcohol abuse, a score greater than 5 on the PSQI, as this is indicative of poor sleep quality (Buysse et al., 1989), or a psychological or linguistic inability to give written informed consent. Individuals who were considered ‘extreme morning’ (score of 31 or less) or ‘extreme evening’ (score of 70 or more) types according to a morningness-eveningness questionnaire (Horne and Ostberg, 1976) were also excluded. Female participants

completed both experimental conditions during the follicular phase of the menstrual cycle, which was determined by a menstrual cycle questionnaire.

4.2.2 Study Design

The study employed a within-subject randomised crossover study design. Participants took part in a 1-night familiarisation stay and two 4-night experimental conditions – control (CON) and sleep restriction (SR). Participants were permitted to leave the laboratory during the days in the experimental conditions, and returned each evening at 1900. They were asked to avoid exercise, consumption of caffeine and alcohol, and napping whilst outside of the laboratory. Wrist actigraphy was used to ensure compliance during this time.

Randomisation was done using an online randomisation tool (www.randomization.com) to generate a list for the 12 participants to assign them to the first condition. As participants were recruited they were assigned to the list in chronological order. Four participants completed the control condition first. The study protocol was reviewed and approved by the Faculty of Health and Life Sciences Research Ethics Committee at Northumbria University (HLSES120117) and written informed consent (Appendix B1) was obtained from all participants prior to beginning the study.

4.2.3 Study conditions

The familiarisation night and both experimental conditions were carried out in the Northumbria Sleep Research Laboratory at Northumbria University. During CON, time in bed was 8 h/night (2300 – 0700), and 4 h/night (0300 – 0700) during SR. Time to bed was delayed during the SR condition to ensure that assessments of insulin sensitivity were conducted at the same time from wakening in both conditions. A washout period of at least 3 weeks, and a maximum of 5 weeks, was given between conditions to prevent carryover effects.

4.2.4 Experimental Protocol

4.2.4.1 Familiarisation

Prior to the first experimental condition, participants underwent a one-night sleep study in the laboratory for familiarisation. The purpose of this night was to allow participants to become accustomed to the sleep laboratory environment and wearing the polysomnographic equipment during sleep. This session also acted as a screening visit to identify and exclude any individuals with sleep disorders. Time in bed during familiarisation was kept consistent with self-reported habitual time in bed.

In the morning, stature and body mass were measured using a Harpenden wall-mounted stadiometer (Seritex) and digital scale (SECA), and BMI was calculated as detailed in Chapter 3.

4.2.4.2 Entraining

A 1-week entraining period was carried out prior to each experimental condition. During entraining, participants were instructed to keep a consistent bed time and wake time, which was individualised according to their habitual hours. Habitual bed time ranged from 2202 h to 0045 h and habitual wake time ranged from 0619 h to 0836 h. Compliance was measured by wrist actigraphy and a 7-d sleep log (Appendix D) which participants completed each morning 30 minutes after waking, as detailed in Chapter 3.

4.2.4.3 Experimental trials

Participants arrived at the laboratory at 1900 on the first evening of each experimental condition. After being briefed on their stay at the laboratory, they were told which condition they were assigned to upon arrival. Participants remained in the lounge until bed time and were permitted to carry out sedentary activities such as watching television, reading, or playing board games during this time. Researchers

were present at all times to ensure participants did not fall asleep before their specified bed time. On nights 1 and 3 of each condition, polysomnography was set-up 30 min prior to their permitted bedtime.

Participants were wakened at 0700 each morning. If necessary, electrodes were removed upon wakening. Participants were then taken to the physiology laboratory where they remained seated for 10 minutes before blood pressure and heart rate were measured using an automated blood pressure monitor (Dinamap V100, GE Healthcare, Illinois, USA). A cannula was then inserted into the antecubital vein to allow regular blood draws.

After insertion of the cannula, a baseline blood sample was obtained. Following this, an oral glucose tolerance test (OGTT) was conducted, as described in Chapter 3. Blood samples were drawn at regular intervals for 2 h.

Following completion of the OGTT, participants were provided with food and drink to consume for the remainder of the day. The food and drink provided were based on their habitual diet, as detailed in Chapter 3. Participants were only permitted to consume the food and drink provided, but were allowed water which they could consume ad libitum. Participants were then debriefed and thanked for their participation before leaving the laboratory at approximately 1030 each morning.

4.2.4.4 Blood collection and processing

For blood sampling the cannulation technique was used to enable multiple blood draws, as detailed in Chapter 3. During the OGTT, blood samples were drawn prior to consumption (baseline (0)) and 15, 30, 45, 60, 90 and 120 min following consumption of the drink. Glucose concentration was measured immediately using a Biosen glucose analyser (EKF Diagnostics). The remaining serum was frozen at -80°C until further analysis.

4.2.4.5 Blood sample analysis

As previously mentioned, serum glucose was measured immediately after centrifugation using the Biosen C-line automatic analyser (EKF Diagnostics). Insulin was measured using commercially available ELISA kits (Mercodia, Sweden), as detailed in Chapter 3. Intra- and inter- assay coefficients of variation were 6% and 14%, respectively.

4.2.5 Data Analysis

4.2.5.1 Sample Size Estimation

A sample size calculation was conducted using Minitab version 17 (Minitab, Pennsylvania, USA) based on previous data investigating insulin sensitivity following 2 nights of sleep restriction in healthy males (Sweeney et al., 2017). Based on a mean difference in insulin AUC of 998.5 and standard deviation of 1027, a sample size of 11 would give 83% power. We therefore aimed to recruit 12 individuals to allow for a dropout, however, due to one individual withdrawing from the study because of being uncomfortable having a cannula inserted and another being excluded due to issues obtaining blood samples, final power was 78%.

4.2.5.2 Statistical Analysis

All statistical analysis was performed using SPSS statistical software version 22 (IBM). $P < 0.05$ was used to indicate statistical significance. Descriptive statistics for participant characteristics and sleep duration were calculated and are presented as mean ± SD to show the variability of the values. Data for glucose and insulin are presented as mean ± SEM to give an indication of the sample mean in relation to the population mean. Total (0-120 min), early (0-60 min) and late (60-120 min) phase area under the curve for glucose and insulin were calculated for each OGTT using the trapezoidal rule. To estimate fasting level of insulin resistance, HOMA-IR (Matthews et al., 1985) was calculated using the following equation:

$$\text{HOMA-IR} = \frac{\text{fasting plasma glucose (mmol/l) x fasting plasma insulin (µIU/ml)}}{22.5}$$

An estimate of whole-body insulin sensitivity during the OGTT was determined using Matsuda index, which was calculated by:

Matsuda index =

$$\frac{10000}{\sqrt{(\text{fasting insulin (µIU/ml)} \times \text{fasting glucose (mmol/l)}) \times (\text{mean glucose (mmol/l)} \times \text{mean insulin (µIU/ml)})}}$$

To assess for differences in glycaemic control following sleep restriction, the data were checked for normality using Shapiro-Wilk tests prior to analysis. Any data which were found to be skewed were transformed appropriately. Linear mixed modelling was used to compare conditions and days. Post-hoc tests were conducted on significant main effects using the Sidak method.

4.3 Results

4.3.1 Participant characteristics

12 participants volunteered to take part in this study, however one participant was excluded and another withdrew, for reasons stated above. Participant characteristics for the remaining 10 participants are presented in Table 4.1.

Table 4.1. Participant characteristics

M:F	5:5
Age (y)	26 ± 5
Stature (cm)	174 ± 9
Body mass (kg)	73 ± 12
BMI (kg/m^2)	24 ± 2
PSQI score	3 ± 1
Morningness-eveningness score	52 ± 8

Data are mean ± SD. n = 10. PSQI score of 5 or below indicates good sleep quality. Morningness-eveningness score between 42-58 indicates intermediate, 41 or below indicates evening, and 59 or above indicates morning type.

4.3.2 Sleep characteristics

During the entraining periods mean bed and wake time measured by actigraphy was 2250 h and 0714 h, respectively. Mean time in bed (TIB) was 472 ± 43 min (7.9 ± 0.7 h), which did not differ from TIB in the control condition (480 ± 0 min [8.0 ± 0.0 h]; P = 0.88), but was significantly higher than TIB in SR (240 ± 0 min [4.0 ± 0.0 h]; P < 0.01). Likewise, total sleep time was similar between entraining and control (378 ± 53 min [6.3 ± 0.9 h] vs. 390 ± 60 min [6.5 ± 1.0 h]; P = 0.60), but was significantly less than entraining in SR (216 ± 13 min [3.6 ± 0.2 h]; P < 0.01).

A summary of sleep variables measured by polysomnography on the first and third night of each condition is presented in Table 4.2. Stage 3 sleep measured as a percentage of total sleep time on nights 1 and 3 of each condition was significantly higher in the sleep restriction condition compared to control (P < 0.001). However, there was no main effect of night or interaction effect (P = 0.145 and P = 0.940, respectively). Sleep efficiency showed a trend for a main effect of condition (P = 0.099), but no evidence of an effect of night (P = 0.278) or interaction effect (P = 0.278).

Table 4.2. Summary of sleep variables measured by polysomnography.

	C1	C3	SR1	SR3	p-value
TST (min)	392 ± 24	428 ± 11	219 ± 6	218 ± 6	< 0.001*
SE (%)	73 ± 9	88 ± 2	90 ± 2	90 ± 2	0.099
WASO (min)	62 ± 20	33 ± 4	17 ± 4	14 ± 4	0.010*
REM (%)	25 ± 3	20 ± 5	18 ± 4	15 ± 2	0.114
N1 (% TST)	22 ± 3	21 ± 5	20 ± 4	18 ± 2	0.358
N2 (% TST)	36 ± 2	37 ± 3	28 ± 3	31 ± 3	0.007*
N3 (% TST)	18 ± 2	19 ± 2	33 ± 4	36 ± 3	< 0.001*

C1 = control night 1; C3 = control night 3; SR1 = sleep restriction night 1; SR3 = sleep restriction night 3. Time in bed was 8 h/night in control and 4 h/night in sleep restriction condition. P-value for main effect of condition from conducting condition x day ANOVA. * denotes significant main effect of condition. Total sleep time (TST) is the total amount of sleep time in a sleep period. Sleep efficiency (SE) is the total time spent asleep compared to the total time in bed. Wake after sleep onset (WASO) refers to wake periods which occur following initial onset of sleep. REM, N1, N2 and N3 refer to sleep stages rapid eye movement, stage 1, stage 2 and stage 3 sleep, respectively.

4.3.3 Physical activity and blood pressure

Physical activity did not differ between conditions. Sedentary to light activity was significantly higher in SR compared to CON (mean difference +180 min; P = 0.006), but did not display evidence for an effect of day (P = 0.191) or interaction (P = 0.354). Moderate-to-vigorous physical activity was similar between conditions (P = 0.710) and days (P = 0.665), and did not show an interaction effect (P = 0.917).

Systolic and diastolic blood pressure were similar between conditions (P = 0.158 and P = 0.619) and showed no effect of day (P = 0.596 and P = 0.391) or interaction effect (P = 0.749 and P = 0.766).

4.3.4 Glucose and insulin

Findings for glucose and insulin are presented in Figure 4.1 and Table 4.3.

4.3.4.1 Glucose

When comparing glucose area under the curve between CON and SR, glucose showed a significant effect of condition ($P < 0.001$), but no evidence for an effect of day ($P = 0.620$), or day x condition interaction ($P = 0.152$) (Figure 3.1). The main effect of condition demonstrated higher glucose AUC in SR compared to CON ($P < 0.001$). Early phase glucose AUC tended to be higher in SR compared to CON ($P = 0.053$), and late phase AUC was higher in SR than CON ($P = 0.020$). Similar to total AUC, no differences were observed between days or interaction effects were observed for early or late AUC. Mean peak glucose was higher in the sleep restriction condition than the control condition (8.98 mmol/L in CON vs. 9.85 mmol/L in SR; $P = 0.003$). However, there was no evidence of an overall effect of day ($P = 0.367$), or interaction effect ($P = 0.131$).

4.3.4.2 Insulin

Results for total AUC for insulin are presented in Figure 4.1. Similar to glucose, insulin AUC showed a significant effect of condition ($P = 0.033$) when comparing CON with SR. However, no effect of day ($P = 0.863$) or interaction effect ($P = 0.285$). Early phase insulin AUC demonstrated a main effect of condition ($P = 0.001$) but no significant effect of day ($P = 0.265$) or interaction effect ($P = 0.445$). Late phase insulin AUC showed similar outcomes, with a significant difference between condition ($P = 0.005$) but not for day ($P = 0.986$) or condition x day ($P = 0.206$).

4.3.4.3 Matsuda and HOMA

No evidence of an effect of condition ($P = 0.276$), day ($P = 0.425$), or interaction effect ($P = 0.318$) were found for Matsuda index. Likewise, HOMA did not demonstrate any significant effect of condition ($P = 0.802$), day ($P = 0.285$), or interaction effect ($P = 0.388$).

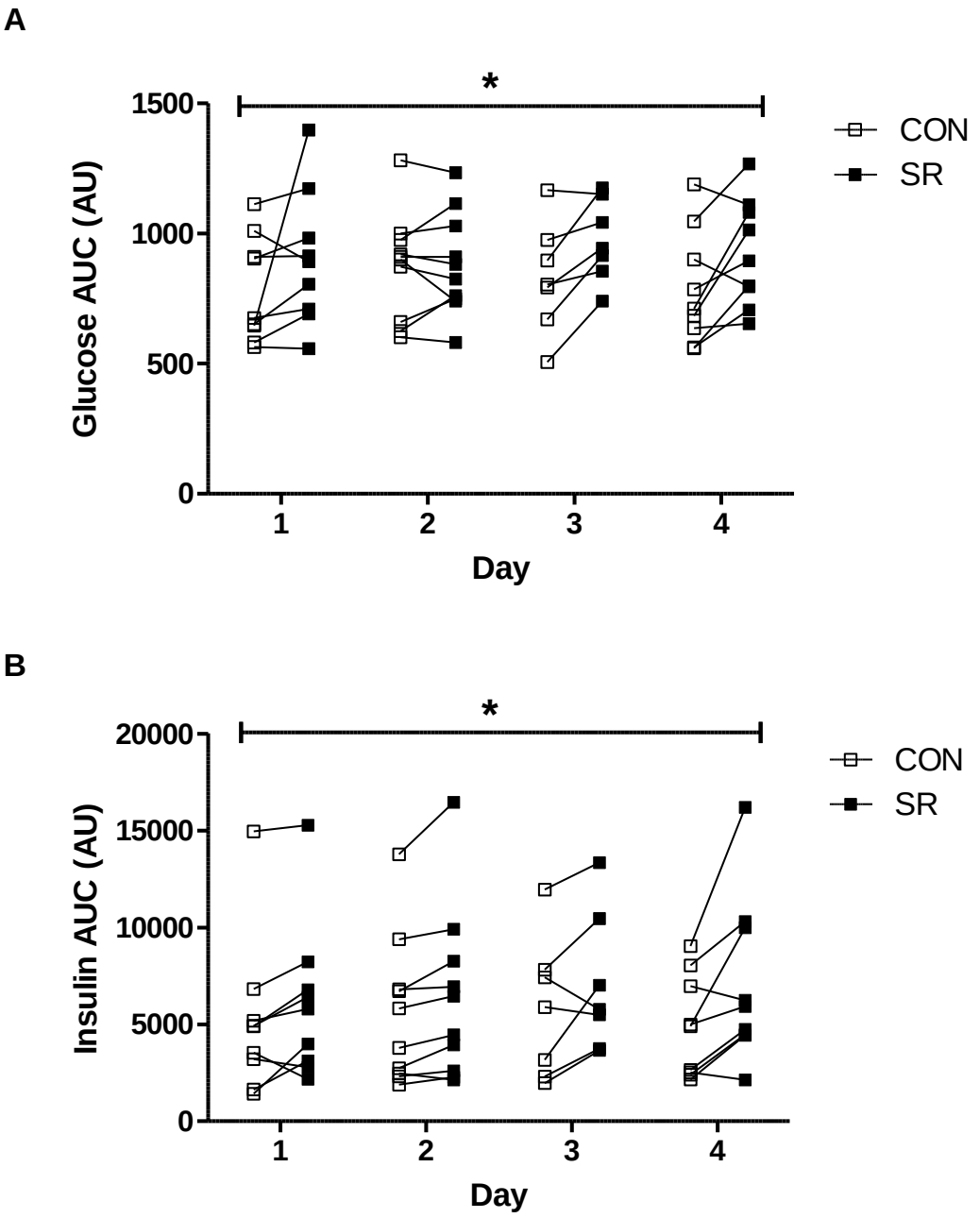


Figure 4.1. Glucose and insulin area under the curve (AUC) during a 120 min oral glucose tolerance test after 1-4 nights of 8 h time in bed (control – CON) or 4 h time in bed (sleep restriction – SR) each night. (A) Glucose AUC during CON and SR. (B) Insulin AUC during CON and SR. * indicates significant main effect of condition ($P < 0.05$). n = 10.

Table 4.3. AUC and insulin sensitivity indexes for glucose and insulin.

	CON1	CON2	CON3	CON4	SR1	SR2	SR3	SR4
Glucose (AU)								
Total	784 ± 64	875 ± 65	791 ± 72	776 ± 66	928 ± 81	883 ± 62	943 ± 49	925 ± 65
Early	433 ± 29	473 ± 35	420 ± 32	420 ± 29	489 ± 34	465 ± 26	483 ± 21	479 ± 26
Late	351 ± 39	403 ± 33	371 ± 42	356 ± 42	439 ± 51	418 ± 39	460 ± 31	446 ± 43
Insulin (AU)								
Total	5181 ± 1223	5573 ± 1144	5418 ± 1059	4717 ± 761	6141 ± 1145	6346 ± 1335	6832 ± 1077	7162 ± 1294
Early	2381 ± 499	2866 ± 612	2839 ± 636	2671 ± 530	2875 ± 539	3184 ± 867	3151 ± 496	3597 ± 753
Late	2800 ± 739	2707 ± 547	2580 ± 496	2046 ± 316	3266 ± 615	3162 ± 548	3680 ± 613	3565 ± 566
Matsuda	15.6 ± 5.4	8.8 ± 1.6	8.8 ± 2.0	10.2 ± 2.0	7.6 ± 1.6	8.9 ± 2.2	7.17 ± 4.8	8.8 ± 2.8
HOMA	1.1 ± 0.4	1.4 ± 0.4	1.4 ± 0.2	1.1 ± 0.2	1.1 ± 0.2	1.3 ± 0.3	1.0 ± 0.2	1.2 ± 0.2

Total (0-120 min), early (0-60 min), and late (60-120 min) AUC during oral glucose tolerance test for insulin and glucose, Matsuda index and HOMA during control (CON) and sleep restriction (SR) for each night. CON = 8 h/night and SR = 4 h/night time in bed. Data are presented as mean ± SEM. n = 10.

4.4 Discussion

The primary aim of this chapter was to assess if the impairment in glucose regulation following sleep restriction was impacted by the number of nights of restriction. The findings demonstrate that sleep restriction alters glucose regulation, increasing the area under the curve in both glucose and insulin concentrations when compared to the control condition. However, contrary to the hypothesis, the results did not show evidence that this impairment was affected by the number of nights of sleep restriction.

The increase in glucose AUC found following sleep restriction suggests altered glycaemic control. These findings are consistent with previous research (Donga et al., 2010; Nedeltcheva et al., 2009; Schmid et al., 2011; Spiegel et al., 1999). However, others have found no change in glucose profiles following sleep restriction (Bosy-Westphal et al., 2008; Wang et al., 2016). We have previously postulated that the varied findings in altered glucose profiles after sleep restriction was possibly due to differing methodologies relating to the severity of sleep restriction either in number of nights or hours of restriction each night (Sweeney et al., 2017). However, the evidence from this study does not support the hypothesis that cumulative sleep restriction would lead to larger impairments in glucose regulation. These results show that altered glucose profiles do not change in a linear manner.

Circulating glucose is tightly regulated by the actions of insulin. Therefore, in some cases, it is not surprising to observe no change in glucose profiles after a particular intervention due to alterations in insulin concentrations. Changes to insulin profiles and hyperinsulinemia have been shown to be an early marker of metabolic disease, independent of glucose concentrations (Dankner et al., 2009; Weir & Bonner-Weir, 2004). Indeed, an increased area under the curve for insulin in response to a standard glucose challenge would indicate reduced insulin sensitivity. The results of

the present study show increased insulin profiles during the OGTT following sleep restriction when compared to the control condition suggesting reduced insulin sensitivity. These results are consistent with previous work showing reduced insulin sensitivity following sleep restriction ranging from 1 to 14 nights (Broussard et al., 2012; Buxton et al., 2010; Donga et al., 2010; Klingenberg et al., 2013; Nedeltcheva et al., 2009; Schmid et al., 2011; Wang et al., 2016). Whilst an effect of condition was observed, there was no evidence for a cumulative effect of sleep restriction on insulin profiles, similar to the glucose profiles observed in the study.

The underlying mechanisms to explain the overall decrease in glucose regulation are unclear. Previous research has suggested peripheral insulin signalling may play a key role in the altered glucose metabolism following sleep loss (Broussard et al., 2012; Rao et al., 2015). Other mechanisms which have been proposed include increased inflammation and production of cortisol, however findings for these have not been consistent (Irwin et al., 2006; Nedeltcheva et al., 2009; Spiegel et al., 1999; Vgontzas et al., 2004). Taking into account the findings of the present study, it may be possible that any underlying mechanism could be active after the first night of sleep restriction with no further activation/impairments on subsequent nights.

The study was not designed to elucidate the underlying mechanisms, which may be viewed as a limitation. Further limitations include the small sample size and use of only healthy, young adults. Findings should therefore be interpreted with caution when extrapolating to other populations such as those with existing metabolic disorders and other age categories. However, the relatively homogenous sample may also be considered a strength, as findings are unlikely to have been confounded by factors such as age and health status. Furthermore, the replication of dietary intake across conditions also ensures changes in diet did not influence findings.

Taken together, the findings of the current study suggest that sleep restriction influences insulin sensitivity but may not do so in a cumulative manner. This may have implications for future studies involving sleep restriction, as it does not appear that the number of nights of sleep restriction is the main factor in the extent of the impairment in insulin sensitivity.

Chapter 5 – The Effect of Acute Sprint Interval Exercise on Glycaemic Control Following Sleep Restriction

5.1 Introduction

Sleep restriction can exert negative effects on glucose regulation and whole body insulin sensitivity (Knutson et al., 2007). A number of experimental studies have shown that sleep restriction results in impaired glucose control (Buxton et al., 2010; Donga et al., 2010; Rao et al., 2015; Reynolds et al., 2012; Spiegel et al., 1999). These findings are supported by findings in Chapter 4 of this thesis demonstrating that four nights of sleep restriction to 4 h each night impaired glycaemic control in healthy individuals. However, the findings did not demonstrate a cumulative effect of the number of nights of sleep restriction on the impairment in glucose regulation, suggesting that a single night of sleep restriction is sufficient to impair glucose regulation. This has also been previously shown by Donga and colleagues (2010), who demonstrated a 20% decrease in whole-body insulin sensitivity after a single night of 4 h sleep compared to a night of 8 h sleep.

One of the most commonly proposed mechanisms underlying the impairment in insulin sensitivity following sleep loss is altered peripheral insulin signalling (Broussard et al., 2012; Rao et al., 2015; Sweeney et al., 2017). Rao et al. (2015) demonstrated a 25% reduction in whole-body insulin sensitivity and 29% reduction in peripheral insulin sensitivity, but no significant change in hepatic insulin sensitivity after five nights of sleep restriction in healthy individuals. Insulin signalling in adipocytes and skeletal muscle may be impaired following four and two nights of sleep restriction, respectively (Broussard et al., 2012; Sweeney et al., 2017).

Additionally, increased levels of triglycerides are also indicative of metabolic impairment and may contribute to the impairment in insulin sensitivity after sleep

restriction. Lipid-induced insulin resistance is thought to occur in multiple ways. These include interfering with key components of the insulin signalling pathway in peripheral tissues such as PI3K and Akt phosphorylation (Yu et al., 2002), prohibiting glucose uptake via the Randle cycle, and inducing mitochondrial dysfunction through increased production of reactive oxygen species and therefore inhibiting phosphorylation of IRS-1 on the tyrosine residue (Martins et al., 2012). Research has demonstrated changes to the metabolic profile after sleep loss, favouring fatty acid transportation (Davies et al., 2014). Observational studies also show higher triglyceride concentrations in individuals with sleep durations less than 7 hours per night compared to those sleeping between 7 and 8 hours per night (Bjorvatn et al., 2007). Conversely, experimental findings are scarce and inconsistent. Lipid profiles measured over the course of a day were unaltered after five nights of 4 h sleep in one study (O'Keeffe et al., 2013) but improved in another (Reynolds et al., 2012). It is still unclear how lipid metabolism is impacted during an oral glucose tolerance test after sleep restriction, when the impairment in insulin sensitivity is most prominent.

Whilst research has investigated the mechanisms which contribute to the impairment in insulin sensitivity after sleep restriction, little is known about how to combat this impairment. Exercise is well known to improve insulin sensitivity (Borghouts & Keizer, 2000). An acute bout of exercise has the potential to upregulate insulin sensitivity for up to two days (Ortega et al., 2015). This effect is apparent with different exercise modalities, however high intensity exercise may be superior to continuous exercise for increasing insulin sensitivity (Ortega et al., 2015; Rynders et al., 2014). A single bout of sprint interval exercise has been shown to decrease glucose and insulin AUC and increase insulin sensitivity index by 142% during an IVGTT conducted 30 minutes post-exercise compared to rest (Ortega et al., 2015). Additionally, late phase insulin AUC has been shown to decrease by 26%

and minimal model derived insulin sensitivity increase by 85% following a single bout of high intensity exercise compared to no exercise in prediabetic adults (Rynders et al., 2014). However, while an improved postprandial response following exercise is well established, it is not clear if these improvements would be elicited in sleep restricted individuals.

Emerging evidence suggests exercise may be a promising intervention to alleviate the impairment in glycaemic control following sleep loss. It has been speculated that exercise may target similar molecular mechanisms to those which contribute to the impairment in insulin sensitivity following sleep restriction, such as signalling molecules in skeletal muscle, and therefore be beneficial following sleep loss (Saner et al., 2018). Indeed, recent research showed that two weeks of high intensity exercise training prior to a single night of total sleep deprivation attenuated the impaired insulin response to an OGTT (De Souza et al., 2017). However, total sleep deprivation is arguably less likely to occur than partial sleep restriction, which often occurs due to increased work and lifestyle demands of modern society (Basner et al., 2014). Moreover, two weeks of exercise training may not be feasible for situations where unplanned sleep restriction occurs.

Consequently, the primary aim of the current study was to examine if acute sprint interval exercise following a single night of sleep restriction could alleviate the effect of short sleep on insulin sensitivity. We hypothesised that in the sleep restriction and exercise condition, glucose regulation and insulin sensitivity would be improved compared to the sleep restriction and no exercise condition. The secondary aim of the study was to examine changes in triglyceride concentrations following sleep restriction to identify whether or not this contributed to the altered insulin sensitivity.

5.2 Methods

5.2.1 Participants

Recruitment for the study was conducted by placing poster advertisements around Northumbria University campus and through word of mouth. Individuals who expressed an interest in participating were provided with the Participant Information Sheet (Appendix A2) and given at least 24 h to read the information sheet before deciding if they wanted to take part.

Participants were healthy non-smoking males aged between 18 and 50 years old. Females were not included as the menstrual cycle can affect glucose regulation (Pulido & Salazar, 1999) and it would not have been feasible to control for menstrual cycle phase given the timescale of the study. Exclusion criteria were shift work, regular travel across time zones (classed as more than three times a year), travel across time zones in the past 4 weeks, any disorders which may influence glycaemic control (such as diabetes) or sleep (for example, obstructive sleep apnea), current or previous medication in the past year which may have impacted glucose metabolism or sleep, and a history of drug or alcohol abuse or eating disorders, following a specific diet such as intermittent fasting. Furthermore, individuals were excluded if they had alterated their sleep, diet, or physical activity pattern in the previous 3 months, had a habitual bedtime before 2200 h or after 0100 h, or a habitual wake time before 0600 h or after 0900 h.Individuals with poor sleep quality, classed as a Pittsburgh Sleep Quality Index (Buysse et al., 1989) score of above 5, or who were classed as extreme morning or evening types, assessed by the morningness-eveningness questionnaire (Horne & Ostberg, 1976), were also excluded from participating.

5.2.2 Study design

The study utilised a randomised crossover design, which consisted of a familiarisation session and four 1-night experimental conditions. Experimental conditions were control (CON), sleep restriction (SR), control plus exercise (CE), and sleep restriction plus exercise (SRE). Throughout the study participants slept in the home environment. Randomisation was done using an online randomisation tool (www.randomization.com) and participants were assigned to conditions in chronological order as they signed up to the study. The study protocol was reviewed and approved through the Northumbria University Ethical Approval System (13557) and written informed consent (Appendix B2) was obtained from all individuals prior to participation.

5.2.3 Study conditions

Experimental conditions differed in either sleep duration or exercise status. During CON and CE time in bed was 8 h (2300 - 0700). During SR and SRE time in bed was 4 h (0300 - 0700). In CON and SR participants rested in the morning upon wakening, and in SR and SRE they were required to carry out a bout of exercise 1 hour after wakening. All experimental visits were separated by at least 1 week to prevent carryover effects. Two nights of 8 h sleep has been shown to reverse the effects of five nights of sleep restriction on insulin concentrations (van Leeuwen et al., 2010). A maximum of 3 weeks was permitted between visits. Participants were not informed which condition they were assigned to until the day preceding the experimental night of control or sleep restriction.

5.2.4 Experimental Protocol

5.2.4.1 Familiarisation

At least one week prior to the first experimental visit participants attended the laboratory for screening and a familiarisation of the protocol. Upon arrival participants were briefed on the study protocol and given the opportunity to ask questions before completing an informed consent form. They were then asked to complete several screening questionnaires – a physical activity readiness questionnaire, the Pittsburgh Sleep Quality Index (Buysse et al., 1989), and a morningness-eveningness questionnaire (Horne & Ostberg, 1976). Following satisfactory completion of the screening questionnaires, they were issued with a 3-d food diary (Appendix C), actigraphy watch, and 7-d sleep diary (Appendix D). They were asked to start wearing the actigraphy watch and completing the sleep diary 1 week prior to the first experimental condition.

Body mass and stature were measured using balance scales (SECA) and a free-standing stadiometer (SECA). BMI was then calculated as described in Chapter 3.

Participants undertook an exercise familiarisation, which was a reduced version of the study exercise protocol. The familiarisation exercise bout consisted of a 5-minute warm-up at 70 W on the cycle ergometer, followed by two all-out 30-second sprints against 7.5% of body mass. Sprints were separated by 4.5 minutes of active recovery at a self-selected pace. A 5-minute cool down was then completed. Verbal encouragement was given during the sprints to ensure participants gave maximum effort. During the familiarisation session, seat height was self-selected by participants, with guidance from the researcher. Seat height was recorded to allow replication during experimental trials.

5.2.4.2 Experimental trials

An entraining period identical to that described in Chapter 4 was conducted prior to each experimental trial. Diet was provided for the day preceding each experimental trial and was replicated across conditions. Diet was individualised from food diaries as previously described in Chapter 3. Participants were instructed to eat only the foods provided and to avoid consumption of any caffeine or alcohol. Water was permitted ad libitum. Participants were also asked to refrain from exercise during this time. Wrist actigraphy was used throughout the study to ensure compliance. In addition, participants sent time-stamped text messages to the researcher between 2300 and 0300 in SR and SRE conditions.

After the night of control sleep or sleep restriction, participants arrived at the laboratory by public transport at 0800 each morning following a 10 h overnight fast. Upon arrival, participants either completed a bout of high intensity exercise (CE and SRE) or rested in a seated position for the equivalent duration (CON and SR). They were then given a 30 min recovery period in which they rested in a seated position. During the recovery period a cannula was inserted into the antecubital vein and a baseline sample was drawn at the end of the 30 min period. A 2 h oral glucose tolerance test was conducted, and blood samples were drawn at regular intervals. During the OGTT participants remained seated in the laboratory but were permitted to complete sedentary tasks such as watch films, read, or work. Participants were debriefed and thanked for their participation before leaving the laboratory at approximately 1130.

5.2.4.3 Exercise

The exercise protocol used in this study was based on previous research by Ortega et al (2015). Ortega and colleagues demonstrated a 142% increase in IVGGT-derived insulin sensitivity measured 30 minutes following sprint interval exercise in

healthy, physically active males recruited from a university population, which mirrors the population used in the present study.

All exercise was performed on a Monark Ergomedic 894E cycle ergometer (Monark, Sweden). Seat height was recorded and remained consistent across the two exercise experimental trials. All sprint interval exercise in the familiarisation and experimental trials was preceded with a 5-minute warm-up and cool down at 70 W.

The sprint interval exercise consisted of four all-out 30 s sprints interspersed with 4.5 minutes of active recovery. During the sprints resistance was set at 7.5% of body mass. During the active recovery period, participants cycled at a self-selected pace against a resistance of 1 kg, which was the lowest permitted by the ergometer. Cycle ergometers were connected to a computer equipped with Monark Anaerobic software (Monark) to conduct the sprints and record power output. Verbal encouragement was given throughout each sprint.

5.2.4.4 Blood collection and processing

Blood was collected at baseline (0), 15, 30, 45, 60, 90 and 120 minutes during the OGTT using the cannulation technique. Samples were collected and processed as described in Chapter 3.

Serum glucose was measured using the Biosen automatic analyser (EKF Diagnostics). Insulin was determined using commercially available ELISA kits (Mercodia), which were conducted according to manufacturers instructions. All conditions and timepoints for a single participant were analysed on the same plate. Intra- and inter-assay CV were 4.9% and 18%. Serum triglycerides were measured using the Randox Daytona automated analyser (Randox Laboratories, Crumlin, UK).

5.2.5 Data analysis

5.2.5.1 Sample size calculation

Sample size was calculated using G*Power software version 3.1 (Faul et al., 2007). Previously published data (Rynders et al., 2014) showing a mean difference of 1.5 mmol/L in 2-hour glucose values during an OGTT after high intensity exercise compared to control was used. 18 participants were required to achieve 90% power, therefore 20 participants were recruited to account for a potential dropout rate of 10%.

5.2.5.2 Statistical analysis

Descriptive data are presented as mean ± SD and metabolic data as mean ± SEM. Area under the curve (AUC) for glucose, insulin and triglycerides was calculated using the trapezoidal rule. Glucose and insulin AUC were calculated for total (2 hours), early phase (0-60 min) and late phase (60-120 min) during the OGTT. HOMA-IR (Matthews et al., 1985) and Matsuda Index (Matsuda & DeFronzo, 1999) were calculated as described in Chapter 3 to estimate insulin resistance and whole-body insulin sensitivity.

Data were analysed using SPSS Statistics version 22 (IBM). Shapiro-Wilk tests were used to check for normality and any data which violated the assumption of normality were transformed using a natural log transformation. Linear mixed modelling was used to compare glucose, insulin and triglyceride concentrations and AUC between conditions. Post-hoc tests were conducted on significant main effects using the Sidak method. $P < 0.05$ was used to indicate significance.

5.3 Results

5.3.1 Participant characteristics

Nineteen males completed the study, with one participant withdrawing due to feeling unwell whilst having a cannula inserted. Participant characteristics are displayed in Table 5.1.

Table 5.1. Participant characteristics.

Age (y)	25 ± 8
Stature (cm)	180 ± 7
Body mass (kg)	81 ± 12
BMI (kg/m^2)	25 ± 3
PSQI score	4 ± 1
Morningness-eveningness score	53 ± 9

Data are mean ± SD. n = 19. PSQI score of 5 or below indicates good sleep quality. Morningness-eveningness score between 42-58 indicates intermediate, 41 or below indicates evening, and 59 or above indicates morning type. n = 19.

5.3.2 Sleep

Actigraphy-derived sleep variables are presented in Table 5.2. Total sleep time (TST) was different between CON and SR (mean difference 142 min [2.4 h]; $P < 0.001$), CE and SR (mean difference 182 min [3.0 h]; $P < 0.001$), CE and SRE (mean difference 181 min [3.0 h]; $P < 0.001$) and CON and SRE (mean difference 141 min [2.4 h]; $P < 0.001$).

Table 5.2. Sleep variables for each experimental condition.

	CON	CE	SR	SRE
Bed time (hhmm)	2303 ± 0029	2306 ± 0023	0305 ± 0017	0255 ± 0017
Wake time (hhmm)	0658 ± 0017	0659 ± 0011	0700 ± 0010	0657 ± 0023
TIB (min)	472 ± 27	472 ± 22	236 ± 19	244 ± 15
TST (min)	337 ± 95	377 ± 61	195 ± 43*†	196 ± 37*†

Bed time, wake time, time in bed (TIB) and total sleep time (TST) in control (CON), control plus exercise (CE), sleep restriction (SR) and sleep restriction plus exercise (SRE) condition. Data are mean ± SD. * indicates difference from CON ($P < 0.05$). † indicates difference from CE ($P < 0.05$). n = 19.

5.3.3 Exercise

Peak power output (PPO) and work done during each of the 30-second sprints is outlined in Table 5.3. PPO did not differ between conditions ($P = 0.644$), but a difference was observed over time ($P < 0.001$), with PPO significantly higher in the first sprint compared to the third (mean difference 106 W; $P = 0.009$) and fourth (mean difference 118 W; $P = 0.007$), and higher in the second compared to the fourth (mean difference 60 W; $P = 0.041$). Total work done was similar between conditions (63666 ± 12029 J in CE and 65045 ± 11294 J in SRE; $P = 0.340$).

Table 5.3. Peak power output and total work done during sprints.

	PPO (W)		Total work (J)	
	CE	SRE	CE	SRE
1	857 ± 189	853 ± 187	18356 ± 2903	18355 ± 3471
2	818 ± 177	804 ± 160	16551 ± 2921	16580 ± 3139
3	762 ± 194	736 ± 172	15488 ± 2842	15424 ± 3237
4	733 ± 179	746 ± 144	14686 ± 2408	15502 ± 2490

Peak power output (PPO) and total work during 4 all-out 30 s sprints performed the morning after a night of 8 h (CE) or 4 h (SRE) time in bed. No significant differences were observed between conditions. Data are presented as mean ± SD. n = 19.

5.3.4 Glucose and insulin

Glucose and insulin concentrations during the OGTT are displayed in Figure 5.1. Total, early and late phase AUC for glucose and insulin are shown in Figure 5.2.

Glucose concentrations did not show evidence of an effect of condition ($P = 0.216$) or interaction effect ($P = 0.146$). However, there was a significant effect of time during the OGTT ($P < 0.001$). No significant difference between conditions were found for peak glucose ($P = 0.158$). There was no evidence of an effect of condition ($P = 0.207$) for total glucose AUC. Likewise, late phase and early phase glucose AUC did not show any significant differences between conditions ($P = 0.264$ and $P = 0.122$, respectively).

Insulin concentrations demonstrated a main effect of condition ($P = 0.019$), time ($P < 0.001$), and an interaction effect ($P = 0.014$). Post-hoc analyses revealed significantly higher insulin concentrations in SR compared to CON ($P = 0.022$), with SR showing higher concentrations than CON at 30 min (40.04 ± 23.17 and 25.79 ± 13.94 µIU/ml; $P = 0.004$). Insulin concentrations were higher in SR compared to SRE at 60 minutes (31.40 ± 4.42 and 21.03 ± 3.38 µIU/ml; $P = 0.042$), 90 minutes (19.47 ± 3.51 and 15.52 ± 4.94 µIU/ml; $P = 0.002$), and 120 minutes (14.12 ± 4.69 and 5.99 ± 1.67 µIU/ml; $P = 0.003$). Total insulin AUC displayed a trend for an effect of condition ($P = 0.075$), with SR tending to be higher than CON ($P = 0.064$). Early and late phase insulin AUC also showed significant main effects of condition ($P = 0.010$ and $P < 0.001$, respectively). The early phase insulin AUC was higher in SR than CON (1472 ± 186 and 2044 ± 1129; $P = 0.048$). Late phase displayed a lower AUC in SRE than SR (1267 ± 128 and 862 ± 135; $P = 0.004$).

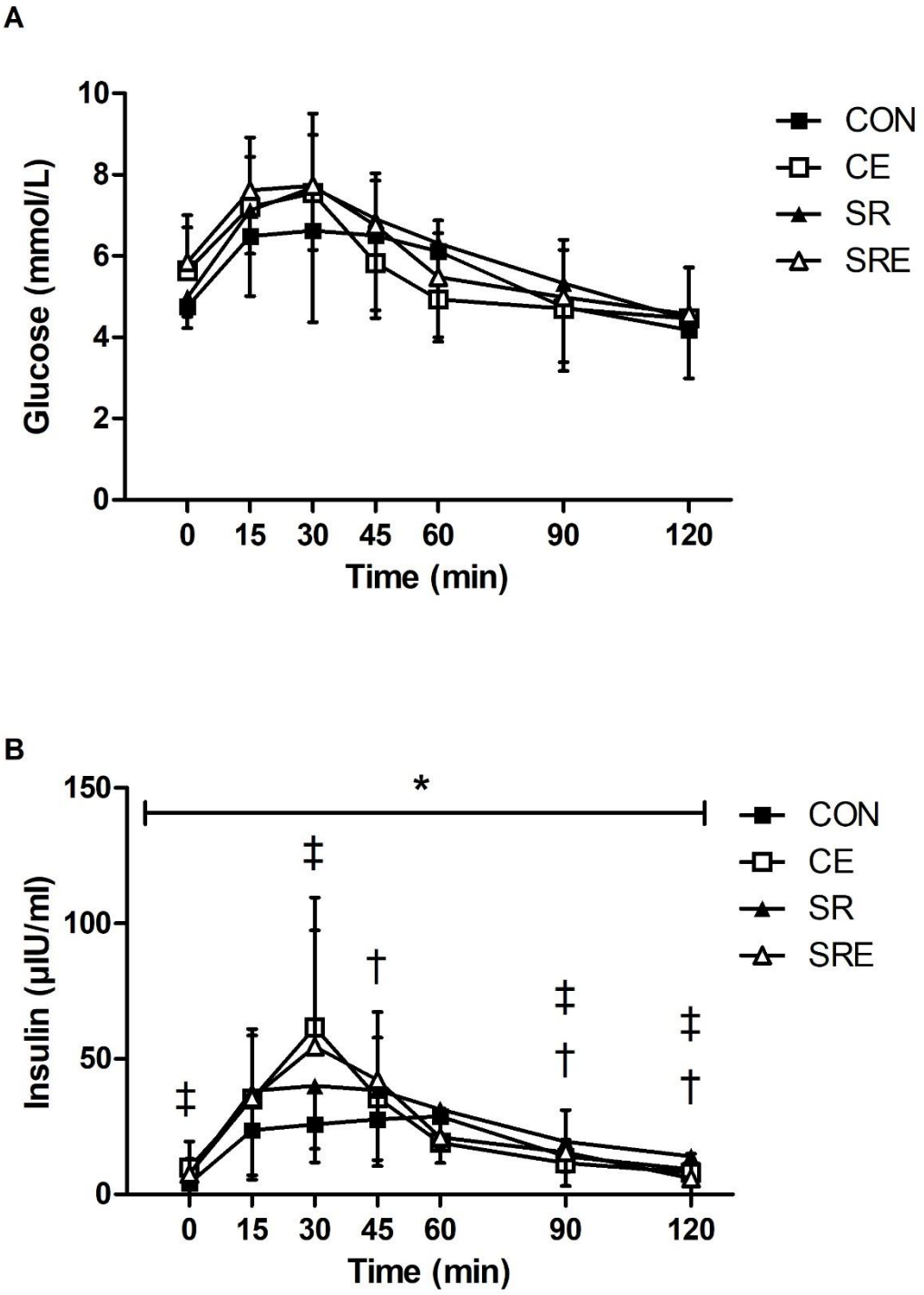


Figure 5.1. Glucose (A) and insulin (B) concentrations during the OGTT after one night of control sleep and rest (CON), sleep restriction and rest (SR), control sleep and exercise (CE) and sleep restriction and exercise (SRE). $P < 0.05$ indicates significance. * indicates main effect of condition. ‡ indicates difference between CON and SR. † indicates difference between SR and SRE. n = 19.

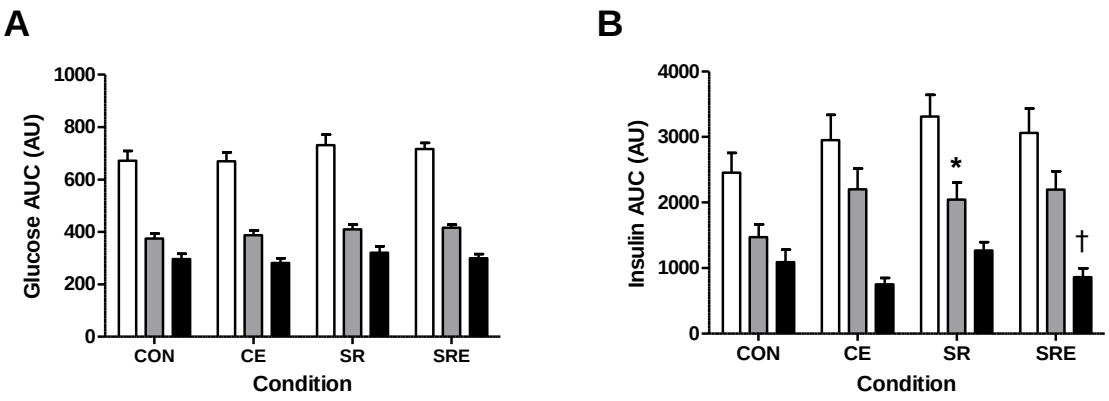


Figure 5.2. Total (white bars), early (grey shaded bars) and late (black bars) phase AUC for glucose (A) and insulin (B) during oral glucose tolerance test following one night of 8 h time in bed (control – CON), 4 h time in bed (SR) 8 h time in bed plus a session of sprint interval exercise in the morning (CE), and 4 h time in bed plus a session of sprint interval exercise in the morning (SRE). Data are presented as mean ± SEM. Main effect of condition for early (0-60 min) and late (60-120 min) AUC. * indicates significant difference (P <0.05) compared to CON in early phase. † indicates significant difference (P <0.05) compared to SR in late phase. n = 19.

HOMA-IR showed a main effect of condition (P = 0.019), with SR higher than CON (0.87 ± 0.23 and 1.64 ± 0.60; P = 0.029). Matsuda index was significantly different between conditions (P = 0.003), with SR displaying a lower index than CON (25.31 ± 4.77 and 12.11 ± 1.46; P = 0.020).

5.3.5 Triglycerides

Triglyceride concentrations during the OGTT (Figure 5.3) did not show evidence for a difference between conditions (P = 0.863) or interaction effect (P = 0.225). There was a significant effect of time (P < 0.001), with concentrations higher at 0, 30, and 60 min compared to 120 min (P < 0.05), and 30 and 60 min compared to 90 min (P < 0.05). Similarly, AUC was comparable between conditions (94 ± 8 mmol/L for CON, 97 ±8 mmol/L for CE, 104 ± 9 mmol/L for SR and 113 ± 17 mmol/L for SRE; P = 0.732).

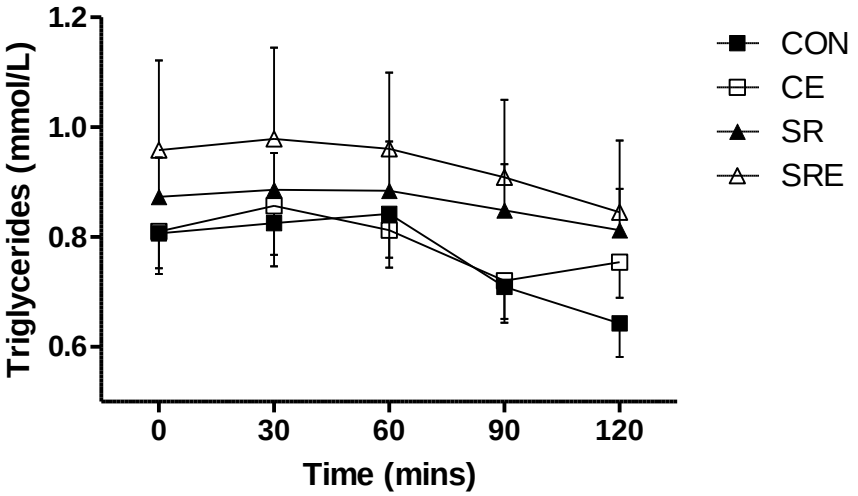


Figure 5.3. Triglyceride concentrations during the OGTT in control (CON – 8 h time in bed), control plus exercise (CE – 8 h time in bed), sleep restriction (SR – 4 h time in bed), and sleep restriction plus exercise (SRE – 4 h time in bed) conditions. No evidence of differences between conditions was found ($P > 0.05$). n=19.

5.4 Discussion

The current study aimed to determine if a single session of exercise could alleviate the impairment in insulin sensitivity that occurs following acute sleep restriction. The findings demonstrate that a single night of sleep restriction impaired insulin sensitivity, and that a single session of sprint interval exercise performed following sleep restriction may improve the late postprandial response.

Consistent with the findings in Chapter 4, participants displayed reduced insulin sensitivity after sleep restriction, as indicated by increased insulin concentrations during the OGTT and a decreased Matsuda index in SR compared to CON. This is in agreement with previous research demonstrating that one night of sleep restriction reduces whole-body insulin sensitivity in healthy individuals (Donga et al., 2010), as well as other studies which employed multiple nights of sleep restriction (Sweeney et al., 2017; Wang et al., 2016). Sleep restriction may impair whole body insulin sensitivity through alteration of peripheral insulin signalling, with five nights of sleep restriction to 4 hours per night reducing peripheral, but not hepatic, insulin sensitivity (Rao et al., 2015). Furthermore, sleep restriction has been shown to decrease Akt phosphorylation, which plays a key role in the insulin signalling pathway in peripheral tissues (Broussard et al., 2012).

It was hypothesised that a bout of sprint interval exercise would attenuate the impairment in insulin sensitivity in sleep-restricted individuals. Whilst total insulin AUC was not significantly altered after the exercise bout, findings suggest that there was an alteration to the late post-prandial response. Insulin AUC was lower during the second hour of the OGTT when exercise was performed after sleep restriction compared to sleep restriction alone. However, there did not appear to be evidence of an improvement in insulin or glucose profiles after exercise in the early phase of the OGTT following exercise. These findings are in contrast to Ortega and

colleagues (2015) who demonstrated improved insulin and glucose 60 minutes following high intensity exercise. Methodological differences may explain this discrepancy, as an IVGTT was employed in that study, whereas we used an OGTT. Gastric emptying plays a role during the OGTT whereas this is bypassed when glucose is injected rather than ingested orally. Gastric emptying rate may be slowed by intermittent high intensity exercise (Leiper et al., 2001), likely due to a reduction in splanchnic blood flow which plays an important role in gastric emptying. This may delay the absorption of the glucose drink and therefore findings may not be comparable between an OGTT and IVGTT. Alternatively, it may be possible that sleep restriction alters the metabolic response to exercise.

The findings of the present study reflect those by Rynders and colleagues (2014), who noted improvements in the late rather than total postprandial response after a bout of exercise. High intensity exercise is known to temporarily increase glucose due to gluconeogenesis, possibly for carbohydrate sparing and glycogen repletion (Marliss et al., 1992). Given that assessments were made only 30 minutes after cessation of exercise, the temporary alterations in glucose regulation which occur during exercise were still influencing our measurements. Insulin secretion in the early phase may impact the late-phase response by suppressing endogenous glucose production (Del Prato et al., 2002). Therefore, it is possible that increased early-phase insulin response after exercise due to gluconeogenesis may have played a role in the reduced late-phase response observed in SRE. Nonetheless, a reduced late-phase response during an OGTT may predict incident diabetes independent of early-phase response (Lorenzo et al., 2012), which suggests that although no change in total AUC was observed, the reduced late-phase response may be beneficial for metabolic health.

A secondary aim of the current study was to determine whether any changes occurred in serum triglycerides after sleep restriction compared to control sleep, and

if exercise altered this. The findings did not show any evidence for altered triglyceride concentrations between conditions. Epidemiological studies suggest that short sleep may impair lipid metabolism, however few studies have addressed changes in triglycerides following experimental sleep restriction. Reynolds and colleagues (2012) demonstrated decreased triglyceride concentrations after sleep restriction, but speculated this change may have been influenced by the study design which provided a diet which may have differed from the participants' habitual diet. As the diet provided in the current study was designed to match habitual diet it is unlikely the findings were influenced by a change in dietary intake. However, the findings of the current study mimic those of O'Keeffe et al. (O'Keeffe et al., 2013), who also did not find any evidence for a change in lipid profiles following sleep restriction in healthy individuals.

Whilst the present study suggests there is potential for exercise to positively influence the post-prandial response to an OGTT in sleep-restricted individuals, the underlying mechanisms remain elusive. There did not appear to be any changes in triglycerides between rest and exercise, suggesting any alterations in glycaemic control were mediated by other mechanisms. Previous research has suggested that insulin action is improved after exercise through altered phosphorylation of components of the insulin signalling pathway in peripheral tissues (Wojtaszewski & Richter, 2006). As sleep restriction is thought to negatively affect peripheral insulin sensitivity (Broussard et al., 2012; Rao et al., 2015), it can be speculated that exercise improves insulin sensitivity in sleep-restricted individuals through alteration of insulin action in peripheral tissues.

The present study has some limitations which should be noted. Firstly, the study population consisted only of healthy males, meaning it may not be feasible to extrapolate the findings to other populations such as females or those with existing metabolic abnormalities. Secondly, an OGTT does not enable measurement of

metabolic characteristics such glucose disposal and uptake, so gives limited information regarding the decrease in insulin sensitivity.

In summary, sprint interval exercise may offer some potential to attenuate the impairments in insulin sensitivity following a single night of reduced sleep. This may have implications for individuals facing sleep curtailment, whether voluntarily or otherwise. To our knowledge, this is the first study to explore the effect of acute exercise on insulin sensitivity following sleep restriction, therefore further exploration is warranted. Future research may investigate whether exercise modality, intensity, duration, or timing influences the change in glucose regulation in sleep-restricted individuals. Furthermore, the effect of exercise and sleep duration on glycaemic control should be investigated in the chronic setting, examining whether habitual sleep duration and physical activity levels have similar effects on metabolism in the free-living environment.

Chapter 6 – Markers of glycaemic control in habitual short sleepers with high and low physical activity

6.1 Introduction

Evidence demonstrates an association between sleep duration and metabolic health. Short sleepers have an increased risk of developing a metabolic disorder such as type 2 diabetes (Yaggi et al., 2006). Several experimental studies, including those in the previous two chapters, have examined the impact of acute sleep restriction on glucose metabolism using various methods, and repeatedly observed impaired metabolism after short sleep (Knutson et al., 2007). Controlled experimental studies are useful for determining initial findings or mechanisms, but due to their nature in design, they often lack ecological validity, and findings may not always transfer to the free-living environment where many external factors can influence measures.

Cross-sectional studies have demonstrated associations between sleep duration and markers of glycaemic control (Lee et al., 2017; Nakajima et al., 2008; Ohkuma et al., 2013; Twedt et al., 2015). Other markers associated with glycaemic control such as adiponectin, cholesterol (total, HDL and LDL) and triglycerides have shown associations with sleep duration in some (Kaneita et al., 2008) but not all studies (Taheri et al., 2004; Williams et al., 2007). However, these studies have limitations which must be considered. Some included only diabetic participants (Ohkuma et al., 2013; Twedt et al., 2015) where findings may not necessarily be comparable at the sub-clinical level. Furthermore, cross-sectional studies often employ subjective measures of sleep (Hancox & Landhuis, 2012; Kaneita et al., 2008; Nakajima et al., 2008; Ohkuma et al., 2013), which can be useful in large-scale studies. However, individuals tend to overestimate their sleep duration by up to an hour compared to objective measures (Lauderdale et al., 2008). Assessment of physical activity, diet,

and/or body composition are also often measured by subjective means or methods known to be flawed such as BMI or 24-hour recall dietary questionnaires. The discrepancies in methodologies and assessment tools used may explain the inconsistent findings; hence, it remains unclear whether associations would exist when objective measures are used.

Physical activity is positively associated with glycaemic control (Warburton et al., 2006). Both cross-sectional and experimental trials have observed a positive impact of higher physical activity on markers of glycaemic control, including serum glucose, cholesterol, and triglycerides (Crichton & Alkerwi, 2015; Figueiró et al., 2019; Mann et al., 2014). Given that physical activity can improve markers of glycaemic control, it can be hypothesised that individuals who undertake regular physical activity may be less impacted by short sleep than those who are less physically active.

Accordingly, the aim of this study was to determine if markers of glycaemic control differed between individuals with short habitual sleep durations and individuals achieving longer sleep durations. Furthermore, this study examined whether or not habitual physical activity levels among shorter sleepers influenced glycaemic control. It was hypothesised that markers of glycaemic control would be negatively altered in less physically active short sleepers, but that differences would not be evident between the more active short sleepers and those achieving longer sleep durations.

6.2 Methods

6.2.1 Participants

120 volunteers were recruited by poster advertisement around Northumbria University and the surrounding areas. Individuals were eligible for the study if they were aged 18 to 50 years, and of white European ethnicity. Only individuals of white

European ethnicity were included as metabolic markers and circadian genes may be influenced by ethnicity (Egan et al., 2017) and this would potentially confound findings. Exclusion criteria were shift work, regular travel across time zones (>3 times each year) or travel across time zones in the past 4 weeks, any disorders or medication which may influence glycaemic control (for example PCOS, diabetes, metformin, etc.), a sleep disorder (assessed using the Sleep Disorders Symptom Checklist-17) (Klingman et al., 2017), extreme morning or evening chronotype (assessed by the MEQ) (Horne & Ostberg, 1976), alteration to physical activity, dietary or sleep behaviours in the previous 3 months, a history of drug or alcohol abuse or eating disorder, pregnant or up to 1 year post-partum, or a psychological or linguistic inability to give written informed consent.

6.2.2 Study design

This study involved two visits to the laboratory, separated by one week. During the first visit, information about the participant's health behaviours was collected using questionnaires. The second visit consisted of numerous physiological measurements. Participants were issued with an actigraphy watch (GeneActiv, Activinsights) to wear, and 3-day food diary (Appendix C) and 7-day sleep diary (Appendix D) to complete between visits. The study protocol was approved through the Northumbria University Online Ethics System (ethics number 4259) and written informed consent (Appendix B3) was obtained prior to participation.

6.2.3 Study Protocol

The first study visit took place at a time and day suitable for participants. Upon arrival at the laboratory, participants completed informed consent and were then given several questionnaires to complete. These questionnaires were the Sleep Disorders Symptom Checklist (SDS-CL-17; (Klingman et al., 2017), a screening questionnaire, morningness-eveningness questionnaire (Horne & Ostberg, 1976),

and the Hospital Depression and Anxiety Scale (HADS) (Zigmond and Snaith, 1983).

At the end of the first visit participants were issued with an actigraphy watch (GENEActiv, Activinsights), 3-day food diary, and 7-day sleep diary. They were instructed to wear the watch continuously for seven days to measure sleep and physical activity. Additionally, they were asked to press the button on the actigraphy watch when they intended to go to sleep and upon wakening in the morning. This button press added a marker on the data, which assisted with analysis of sleep if required. The sleep diary was completed in conjunction with the actigraphy monitor to assist with sleep analysis as well as yield subjective bed and wake time. The second visit was conducted one week after the first visit, and consisted of measurement of physiological variables which included basic anthropometric variables, body composition and serum metabolic markers.

6.2.3.1 Physical activity

Wrist actigraphy was used to capture activity data between visits. As outlined in Chapter 3 and by others (Esliger et al., 2011; Powell et al., 2017), the GENEActiv wrist-worn accelerometer is a valid tool to measure physical activity in healthy adults. Participants wore the GENEActiv accelerometer (Activinsights) on their non-dominant wrist from the laboratory visit on the first day until they arrived at the laboratory for the second visit. As the first and last day were incomplete days, five full days of activity data were collected for each participant. Data were collected using at 10 Hz and converted using 60 s epochs. Data were extracted and CSV files were imported into the GENEActiv everyday living macro for Microsoft Excel (https://open.geneactiv.org). Absolute and percent sedentary behaviour and light, moderate, and vigorous activity was determined for each full day of wear and averaged to give a daily value. Cut-off points for light, moderate, and vigorous activity were set at 483, 678 and 2264 g/min, as recommended by the manufacturer

and Esliger and colleagues (2011). Moderate and vigorous metabolic minutes per day (met.mins/day) were combined to obtain a measure of moderate-to-vigorous activity (MVPA).

6.2.3.2 Dietary intake

Dietary behaviours were obtained using 3-day food diaries. Participants were asked to complete the food diaries on two weekdays and one weekend day. They were asked to log the time of eating, what they ate, the cooking method, and the amount. Any liquids consumed, with the exception of water, were also recorded. Food diaries were analysed for energy, carbohydrate, fat, saturated fat, and protein intake using Microdiet dietary analysis software (version 4.4; Downlee Systems).

6.2.3.3 Body composition

Stature and body mass were measured using a Harpenden wall-mounted stadiometer (Seritex) and digital scales (SECA). Whole-body dual energy x-ray absorptiometry (DXA) scanning was used to determine body composition. All scans were conducted using the Hologic Horizon W DXA scanner (Hologic, Massachusetts, USA) by the same trained technician and analysed using Hologic APEX Software version 5.6.0.5. Recommendations set out by Nana and colleagues (2015) were followed to ensure consistency between scans. All participants wore loose, light clothing and any metal was removed. Participants lay in a supine position with hands in a mid-prone position and feet angled inwards. In cases where the arms did not fit inside the scanning area, reflexion scanning was utilised, with the left upper limb excluded from the original scan and automatically determined using the DXA analysis software by reflection of the right upper limb. Reflexion scanning is a useful alternative to whole-body scanning for broader individuals and provides comparable results (Moco et al., 2019). Scans were analysed

automatically by the software and verified manually by the same researcher for all participants.

6.2.3.4 Sleep variables

Wrist actigraphy (GeneActiv, Activinsights) was used for 1 week between visits to measure sleep variables in conjunction with physical activity. As previously described in Chapter 3, participants wore the actigraphs on their non-dominant wrist and were instructed to press the button to indicate intention to sleep and wake times. A 7-day sleep log was completed in conjunction with actigraphy. From the data, total sleep time (TST) was determined for each night of wear using the GeneActiv sleep macro for Microsoft Excel (https://open.geneactiv.org). The TST for all nights were combined and divided by the total number of nights to give an average nightly sleep duration. Any participants with less than 6 nights of wear were excluded from analysis.

6.2.3.5 Blood sampling

During the second visit a blood sample was obtained using the venepuncture technique to determine markers of glycaemic control including glucose, adiponectin, total cholesterol, HDL cholesterol, and triglycerides. Samples were processed then frozen as indicated in Chapter 3 until further analysis.

6.2.4 Data Analysis

A previous study (Krueger & Friedman, 2009) demonstrated approximately one third of adults have a sleep duration below 7 hours per night. Therefore, 120 participants were recruited for the current study to ensure the sample included a sufficient range of sleep durations.

The sample was divided into sextiles according to sleep duration. The participants in the bottom two sextiles (i.e. 40 individuals with shortest sleep duration) and top

sextile (20 individuals with longest sleep duration) were included in analysis. The 40 short sleepers were further divided into two groups according to MVPA met.mins/day. Therefore, three groups were included in analysis: less active short sleepers (LASS), more active short sleepers (MASS), and longer sleepers (LS).

Blood samples were tested to determine serum glucose, adiponectin, triglycerides, total cholesterol and HDL cholesterol. Glucose was measured immediately after centrifugation using the Biosen automatic analyser (EKF Diagnostics). Adiponectin was measured using a commercially available ELISA kit (Abbexa, Cambridge, UK). Triglycerides were determined using an automated analyser (Randox Daytona, Randox Laboratories). Total and HDL cholesterol were measured by the Reflotron Plus analyser (Roche, Switzerland). LDL cholesterol was calculated from total cholesterol, HDL cholesterol and triglycerides using Friedewald's formula (Friedewald et al., 1972):

$$LDL = total\ cholesterol\ (mmol/L) - HDL\ cholesterol\ (mmol/L) - \frac{triglycerides\ (mmol/L)}{2.19}$$

Triglyceride to HDL ratio was calculated by dividing triglyceride concentration by HDL cholesterol concentration.

6.2.4.1 Statistical analysis

Data were analysed using SPSS statistical software version 22 (IBM). Shapiro-Wilk tests of normality were conducted and any variables which violated the assumption of normality were natural log-transformed. Participant characteristics were compared between groups using one-way ANOVAs for continuous variables and Chi-squared tests for categorical variables. One-way ANCOVAs were conducted to determine differences between sleep durations for adiponectin, total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides (TG), TG-to-HDL ratio, and fasting

glucose. Any significant main effects were compared using the Sidak method . Analyses were adjusted for age, sex, lean mass, and energy intake.

6.3 Results

6.3.1 Participant Characteristics

Participant characteristics for each of the three groups are displayed in Table 6.1. MEQ score was 52 ± 5 in LS, 50 ± 9 in LASS, and 57 ± 12 in MASS. Anxiety and depression scores were 7 ± 3 and 3 ± 2 in LS, 6 ± 3 and 2 ± 2 in LASS, and 6 ± 3 and 2 ± 3 in MASS, respectively. Groups differed by sex ($P = 0.002$), BMI ($P = 0.045$), lean mass ($P = 0.048$), total sleep time ($P < 0.001$) and MVPA ($P < 0.001$) but did not show differences between age ($P = 0.825$), energy intake ($P = 0.449$), carbohydrate intake ($P = 0.736$), fat intake ($P = 0.964$), protein intake ($P = 0.863$), fat mass ($P = 0.062$), or visceral adipose tissue ($P = 0.144$). Post-hoc analyses did not show evidence for any differences between groups for BMI or lean mass. MVPA was higher in MASS than LASS and LS ($P < 0.001$ for both). Total sleep time was lower in both MASS and LASS than LS ($P < 0.001$ for both).

Table 6.1. Participant characteristics by group.

	LASS	MASS	LS
Age (y)	27 ± 7	28 ± 8	28 ± 8
Sex (M:F)	16:4	8:12	6:14
BMI (kg/m^2)	26 ± 5	23 ± 4	24 ± 3
Lean mass (%)	66 ± 6	66 ± 7	61 ± 7
FM (%)	30 ± 7	30 ± 7	34 ± 6
VAT (g)	416 ± 251	299 ± 163	335 ± 192
Energy (MJ/kg/day)	0.12 ± 0.04	0.12 ± 0.03	0.12 ± 0.04
CHO (g/kg/day)	3.04 ± 0.97	3.21 ± 0.96	2.95 ± 1.11
Fat (g/kg/day)	1.16 ± 0.54	1.20 ± 0.36	1.19 ± 0.43
Protein (g/kg/day)	1.51 ± 0.53	1.43 ± 0.60	1.43 ± 0.71
TST (mins)	340 ± 34*	341 ± 33	461 ± 24**
TST (h)	5.7 ± 0.6*	5.7 ± 0.6	7.7 ± 0.4**
MVPA (met.mins/day)	690 ± 212**	1337 ± 242*	663 ± 426

Participant characteristics for less active short sleepers (LASS), more active short sleepers (MASS) and longer sleepers (LS). BMI = body mass index; FM = fat mass; VAT = visceral adipose tissue; CHO = carbohydrate; TST = total sleep time; MVPA = moderate-to-vigorous physical activity. $P < 0.05$ used for significance. * indicates difference from LS. ** indicates difference from MASS. Data are mean ± SD. n = 20 for each group.

6.3.2 Metabolic markers

Metabolic markers for each group are presented in Table 6.2. There was a significant difference in HDL cholesterol between groups ($P = 0.024$). Post-hoc analyses revealed HDL cholesterol was significantly lower in LASS compared to LS ($P = 0.038$) and MASS ($P = 0.050$), but no differences were observed between MASS and LS ($P = 1.000$). No differences were observed between groups for adiponectin ($P = 0.315$), triglycerides ($P = 0.909$), total cholesterol ($P = 0.290$), LDL cholesterol ($P = 0.076$), fasting glucose ($P = 0.710$), and TG to HDL ratio ($P = 0.553$) (Table 6.2).

Table 6.2. Metabolic markers in each group.

	LASS	MASS	LS
HDL (mmol/L)†	1.12 ± 0.06	1.31 ± 0.06*	1.32 ± 0.05*
Triglycerides (mmol/L)	1.14 ± 0.10	1.13 ± 0.18	0.81 ± 0.06
Total cholesterol (mmol/L)†	4.14 ± 0.2	4.41 ± 0.2	4.5 ± 0.2
LDL (mmol/L)†	2.35 ± 0.21	2.73 ± 0.20	2.73 ± 0.18
TG:HDL	1.28 ± 0.16	1.03 ± 0.26	0.62 ± 0.06
Glucose (mmol/L)†	5.03 ± 0.14	5.15 ± 0.14	5.13 ± 0.13
Adiponectin (µg/mL)	10.13 ± 1.05	12.18 ± 1.18	13.71 ± 1.00

Data are adjusted means (†) or geometric means ± SEM. LASS = less active short sleepers, MASS = more active short sleepers, and LS = longer sleepers. Adjusted for sex, BMI, energy intake, fat mass, and visceral adipose tissue mass. P = 0.05 used to indicate significance.* indicates significantly different from LASS. n = 20 for each group.

6.4 Discussion

The current study aimed to examine if differences in habitual sleep duration and physical activity determined any differences in serum markers of glycaemic control. It was hypothesised that less physically active shorter sleepers would have altered markers of glycaemic control compared to longer sleepers, but that there would not be a difference between more active short sleepers and longer sleepers. The findings do not demonstrate any evidence of altered markers of glycaemic control between sleep durations and physical activity levels for most markers, with the exception of HDL cholesterol, which showed reduced levels of HDL cholesterol in the less physically active short sleepers compared to the more physically active short sleepers and longer sleepers.

There was no evidence of a difference between groups for glucose, adiponectin, total cholesterol, LDL cholesterol, triglycerides or TG-to-HDL ratio. This is in contrast to some previous studies showing differences in these markers between

sleep durations (Kaneita et al., 2008) but in agreement with others (Anujuo et al., 2015; Bjorvatn et al., 2007; Bos et al., 2019). Bjorvatn and colleagues (2007) initially showed significant relationships between short sleep duration and triglycerides and total cholesterol, however this association was mitigated when confounding factors were included. The findings of the present study did show HDL cholesterol was lower in short sleepers who were less active compared to short sleepers who were more active and longer sleepers. Research examining HDL cholesterol and sleep duration is limited, however a few studies have demonstrated positive associations between shorter sleep durations and lower HDL cholesterol (Kaneita et al., 2008; Lin et al., 2017; Potter et al., 2017). The findings of the present study suggest that physical activity may possibly improve HDL cholesterol in short sleepers.

Previous research has demonstrated mixed findings with regards to whether or not habitual short sleep duration affects markers of glycaemic control. Anujuo et al. (2015) did not observe associations between sleep duration and glycaemic control. However, significant differences in markers of glycaemic control according to sleep duration were found in multiple studies including those by Whitaker et al. (2018) and Kaneita et al. (2008). It is unclear why there are differences between study findings, however, it could be speculated that the discrepancies are due to the confounding factors included in the analysis, and the method by which variables were measured. Studies employing self-reported tools are at risk of overestimation of sleep duration and physical activity (Dyrstad et al., 2014; Lauderdale et al., 2008b). Additionally, a number of previous studies did not adjust for dietary intake. Short sleep is known to be associated with altered macronutrient intake, including increased total fat intake (Grandner et al., 2010). Fat intake shows positive associations with HbA1c (Harding et al., 2001), and therefore dietary intake should be considered in studies examining sleep duration and glycaemic control. Furthermore, body composition is known to be a factor associated with risk of developing type 2 diabetes (Yeung et al., 2019)

and should be adjusted for when analysing such data. The current study used objective measures to control for multiple confounding factors known to be associated with glycaemic control, such as DXA scanning for body composition and actigraphy for physical activity and sleep. The findings did not demonstrate differences between shorter and longer sleepers for any markers with the exception of HDL cholesterol, suggesting that confounding factors such as body composition, dietary intake, and physical activity may mediate the positive relationship between sleep duration and glycaemic control that was observed in previous studies.

An alternative explanation for the discrepancies in study findings could be related to sleep duration thresholds. Previous research that has observed differences in markers of glycaemic control examined individuals sleeping less than five hours compared to individuals sleeping 7 to 8 hours (Kaneita et al., 2008; Whitaker et al., 2018). However, in the current study, the short sleep duration groups slept between 5 and 6 hours, which was compared to 7 - 8 hours. It could be speculated that there is a threshold habitual sleep duration below which there are metabolic consequences, and that the short sleep durations in the present study were not short enough to observe these effects. In agreement with this, a previous study did not demonstrate significantly increased odds of developing metabolic syndrome when comparing a group with an average sleep duration of 5.88 hours to a group who had an average sleep duration of 7.26 hours (Potter et al., 2017), reflecting the durations in the current study.

The present study design has multiple strengths. Firstly, it was conducted in the free-living environment, and therefore has higher ecological validity than controlled laboratory-based studies. Whilst controlled laboratory studies are useful for determining preliminary findings and understanding mechanisms, the results may not always transfer to the free-living environment in which the environment influences lifestyle factors such as diet, physical activity and sleep. Secondly,

objective measures were used where possible, such as DXA scanning for body composition and actigraphy for sleep and physical activity. These measures remove the potential for underestimation and overestimation, which is often associated with self-reported measures (Dyrstad et al., 2014; Lauderdale et al., 2008b). In contrast, the study did not include more active longer sleepers. Therefore, it is unclear if the differences in markers of glycaemic control between sleep durations would be more pronounced if more active longer sleepers were included. Individuals who are generally more physically active tend to have improved markers of glycaemic control including cholesterol and triglycerides (Monda et al., 2009), and consequently, it would have been useful to examine any differences between more active longer sleepers and shorter sleepers. Furthermore, the sample size is small, and data were captured at only one timepoint. Thus, it is not possible to determine how markers would have changed over time if short sleep was continued for several months or years.

In summary, the findings of the present study suggest that overall markers of glycaemic control do not appear to be different between longer and shorter sleepers when confounding variables are accounted for, but that there may be a difference in HDL cholesterol between longer and shorter sleepers which is mitigated when habitual physical activity is higher.

Chapter 7 - General Discussion

7.1 Overview

Short sleep durations are common amongst adults in the United Kingdom, with almost three-quarters sleeping less than 7 hours each night (The Sleep Council, 2017). In parallel with the increase in short sleep durations is the rise in the prevalence of type 2 diabetes (Sharma et al., 2016). Observational studies have demonstrated associations between short sleep durations and increased risk of developing type 2 diabetes (Gangwisch et al., 2007; Yaggi et al., 2006); and experimental studies have established impaired insulin sensitivity after sleep restriction (Zhu et al., 2019). However, little research has focussed on solutions to combat these metabolic consequences of sleep restriction. Furthermore, research often overlooks other lifestyle factors that link poor sleep to impaired glucose control.

The research in this thesis has examined the interaction between short sleep, exercise, and glycaemic control in healthy individuals. Specifically, the primary aims were three-fold: i) to understand the impaired glycaemic response to glucose intake over consecutive nights of sleep restriction (Chapter 4); ii) to investigate if exercise has the potential to alleviate the sleep restriction induced impairment in insulin sensitivity (Chapter 5); iii) to determine if these factors act in a similar manner when transferred to the free-living environment (Chapter 6). A secondary aim was to investigate if changes in lipid profiles were involved in mediating the impairment in insulin sensitivity arising from sleep restriction in controlled (Chapter 5) and free-living settings (Chapter 6). Consistent with these aims, the main findings in this thesis were that: i) there did not seem to be a cumulative effect of the number of nights of sleep restriction on the impairment in glucose regulation; ii) acute exercise may be beneficial for the late postprandial response after acute short sleep; iii)

when confounding factors are accounted for, there does not appear to be an impact of habitual short sleep on markers of glycaemic control. There was also no evidence of changes in lipid metabolism after acute sleep restriction, suggesting alternate mechanisms may be responsible for these changes. Collectively, these findings indicate that exercise may have the potential for beneficial metabolic health in short sleepers in acute settings, but further study is required under chronic conditions.

7.2 Summary of chapters and main findings

Whilst there is an abundance of studies examining sleep restriction and glycaemic control (Zhu et al., 2019), it remained unclear if the impairment in glucose control was exacerbated with each consecutive night of sleep restriction. Due to the differing methodologies employed for inducing sleep loss and measuring glucose control, directly comparing existing studies is challenging. Determining the manner in which insulin sensitivity is impaired is of importance to inform future sleep studies that will examine potential mechanisms or studies that employ interventions to mitigate the metabolic consequences of sleep restriction. Chapter 4 aimed to address this gap in the literature and establish a foundation for the subsequent study which was presented in Chapter 5. A 4-night sleep restriction protocol was used with oral glucose tolerance tests conducted each morning to determine differences in serum glucose and insulin profiles. Consistent with previous research outlined in Chapter 2, four nights of sleep restriction to four hours per night significantly impaired insulin sensitivity. However, the findings indicated that the impairment in glycaemic control was similar across the four nights. This suggests that one night of sleep restriction to 4 hours could induce a metabolic impairment with no further decline following subsequent nights. This important novel finding informs future studies that employ sleep restriction protocols to induce metabolic

changes. Implementing a single night of sleep restriction may provide minimum burden to participants while still achieving intended outcomes.

Chapter 5 utilised the findings from Chapter 4 to explore if exercise could alleviate impaired glucose regulation after sleep restriction. In addition, this chapter also examined if changes in serum lipids contributed to this impairment. Recent evidence has shown that two weeks of exercise training can subsequently improve the insulin response to total sleep deprivation (De Souza et al., 2017). Accordingly, it was hypothesised that an acute bout of exercise would have similar outcomes following partial sleep restriction. After a single night of sleep restriction to four hours and either sprint interval exercise or no exercise, serum markers of glycaemic control were measured in response to an OGTT. As expected, sleep restriction increased the insulin response to glucose intake. Exercise after sleep restriction did not alter the total insulin AUC, but there was a reduction in late-phase insulin AUC when compared to sleep restriction alone. Late-phase AUC is an important aspect of postprandial metabolism. An improvement in late-phase AUC can be indicative of a reduced risk of developing type 2 diabetes (Hayashi et al., 2013; Lorenzo et al., 2012). A later peak or more prolonged increase in insulin concentrations suggest an increased beta-cell insulin secretory response due to diminished insulin sensitivity (Hayashi et al., 2013). Findings did not demonstrate any significant changes in triglycerides after sleep restriction or exercise, indicating that a change in triglycerides is unlikely to be a major contributor to the changes in glucose regulation after acute sleep restriction.

Short-term intervention studies, using controlled measures, are very useful to determine physiological responses. However, it is important to explore how well these findings transfer to the free-living environment when behaviours are uncontrolled and influenced by many external factors. Chapter 6 aimed to investigate differences in glycaemic control between individuals of different habitual

sleep durations as well as those with differing physical activity levels in a free-living setting. Multiple measures were used to obtain an overview of the major factors that can influence glycaemic control, including diet and body composition. Groups were formed according to habitual sleep duration and short sleepers were further split according to habitual physical activity levels. The results revealed a lack of significant differences between groups for markers of glycaemic control. These findings agree with some previous work indicating that habitual short sleep duration may not be primarily responsible for the increased risk of metabolic disorders. However, HDL cholesterol concentrations were lower in less active short sleepers compared to longer and more active short sleepers, suggesting that physical activity potentially rescues the decrease in HDL cholesterol during habitual short sleep.

7.3 Reduced sleep quantity and impaired glycaemic control

Chapters 4 and 5 add to the current body of literature by highlighting changes in glycaemic control after a single night of sleep restriction to four hours. Whilst this has been previously shown (Donga et al., 2010), this was not systematically addressed over multiple nights of restriction. The exact mechanisms which lead to this impairment are yet to be elucidated. Arguably, the most strongly suggested mechanism is alterations in peripheral tissue insulin sensitivity. Rao and colleagues (2015) demonstrated changes in peripheral, but not hepatic, insulin sensitivity after five nights of four hours sleep in healthy individuals. This change was accompanied by increased non-esterified fatty acids (NEFAs), suggesting that a change in lipid metabolism may have played a role in altering peripheral insulin sensitivity. Furthermore, we and others have previously shown potential changes in the insulin signalling pathway in peripheral tissues after sleep restriction (Broussard et al., 2012; Sweeney et al., 2017). Akt activity and phosphorylation in skeletal muscle and

adipocytes were reduced after partial sleep restriction to two nights of 50% of habitual duration and four nights of 4.5 hours sleep per night, respectively, in healthy males.

Altered lipid metabolism, including increased NEFA and triglyceride concentrations, can negatively impact peripheral insulin signalling (Saltiel & Kahn, 2001), and it could therefore be suggested from previous literature that an alteration in lipid metabolism induces changes in peripheral insulin sensitivity after sleep loss. In Chapter 5, triglycerides were measured to assess altered lipid metabolism as a potential mechanism and no significant changes in triglycerides between conditions were observed. However, the possibility that changes in lipid metabolism were contributing to the reduced insulin sensitivity cannot be ruled out. Rao and colleagues (2015) demonstrated decreased triglycerides in combination with increased NEFAs, and proposed that this combination may have been due to preference for gluconeogenesis rather than de novo lipogenesis during sleep loss. Therefore, it is possible that detecting changes may require simultaneous measurement of triglycerides and NEFAs.

Habitual short sleep is often also associated with impaired metabolism (Iftikhar et al., 2015). Proposed mechanisms include altered appetite hormones and timing of food intake, changes in body composition, and underlying sleep disorders (Balkau et al., 2010; Moller-Levet et al., 2013; Schmid et al., 2015; Taheri et al., 2004). The study presented in Chapter 6, however, demonstrated contrasting findings, displaying no differences in glycaemic control between individuals sleeping 5-6 hours each night and those sleeping 7-8 hours per night. There are several potential explanations for the differences between study findings, one being that confounding factors may mediate such differences. For example, in some studies, significant relationships were observed initially but were reduced or completely mitigated when confounding factors, such as sex and BMI, were included in analysis (Ayas et al.,

2003; Bjorvatn et al., 2007). An alternative explanation may be due to the nature of the study design (i.e. an observational study). With such studies, it is difficult to establish cause and effect, and may indeed be a reverse causality issue. Insulin resistance may lead to disrupted sleep or even short sleep duration. Evidence shows that insulin resistance is associated with an increased risk of developing sleep disorders such as obstructive sleep apnea (Balkau et al., 2010), which is often characterised by disrupted and short sleep.

7.4 Exercise, physical activity and metabolic function in sleep restricted individuals

The role of exercise and physical activity within sleep and metabolism is an underexplored area that is only just beginning to be developed. The findings in Chapter 5 have added to the body of literature, illustrating for the first time that sprint interval exercise can improve late-phase postprandial insulin response after sleep restriction to four hours. However, Chapter 6 revealed no differences in biomarkers of glycaemic control between more and less physically active individuals sleeping 5-6 hours.

Acute exercise is known to improve glycaemic control through insulin dependent and independent mechanisms (Wojtaszewski & Richter, 2006), particularly in the peripheral tissues. Esbjörnsson-Liljedahl et al. (2002) have shown that sprint interval exercise on a cycle ergometer reduces muscle glycogen content by more than 50%. As a result, glucose uptake into skeletal muscle is increased, with an estimated 40% of an oral glucose bolus used for this purpose (Hickner et al., 1997; Kelley et al., 1988). Using a similar protocol to Esbjörnsson-Liljedahl and colleagues, Chapter 5 has demonstrated changes in the late-phase postprandial

insulin response after exercise, which has also been observed by others (Rynders et al., 2014). However, total AUC for insulin was not altered which may be due to the timepoint at which the OGTT was conducted. Exercise inflicts a temporary increase in glucose concentrations, which may be caused by increased gluconeogenesis and increased catecholamines. These temporary changes appear to last around thirty minutes after a session of high intensity exercise (Rynders et al., 2014). Thus, it is likely that any possible improvements in insulin sensitivity did not become apparent until longer after cessation of exercise and the collection of glycaemic markers.

Chronic physical activity is also well established to have positive effects on cardiometabolic markers (Grgic et al., 2018). More specifically, moderate to vigorous physical activity is associated with decreased risk of type 2 diabetes incidence (Sternfeld et al., 2019). Given the association between sleep duration and risk of developing type 2 diabetes, Chapter 6 aimed to investigate the role of MVPA in individuals with short sleep duration. Findings from the chapter did not demonstrate an effect of sleep duration or physical activity on biomarkers of glycaemic control. While it is difficult to determine why this is the case, it could be speculated that since no differences were observed between groups of different sleep duration, the role of physical activity to attenuate any impairment in glycaemic control would be minimal. Furthermore, the physical activity levels of each group were much greater than the minimum recommended level (less active group ~690 MET.min/day vs. recommended activity ~86 MET.min/day) (Kyu et al., 2016). Given such differences, it is unlikely that increases in physical activity will yield large benefits in glycaemic control. Kyu et al. (2016) have detailed a dose response curve for physical activity and diabetes risk in a recent meta-analysis. Results from the study indicate that major benefits occurred at around 571 MET.min/day with diminishing benefits from greater levels of physical activity. Increases in those with

high physical activity levels (from 1285 MET.min/day to 1714 MET.min/day) reduced the risk of diabetes by only 0.6%.

Findings from Chapter 6 have revealed reduced HDL cholesterol in the less active short sleepers compared to longer sleepers. In addition, physical activity in the short sleepers improves HDL cholesterol. Few studies have investigated physical activity, sleep and cholesterol. However, studies reallocating sleep time with moderate-to-vigorous physical activity show possible benefits on metabolic biomarkers (Grgic et al., 2018). Replacing thirty minutes of sleep each day with MVPA showed more favourable HDL cholesterol, triglycerides, glucose, insulin, and insulin sensitivity (Buman et al., 2014). Similarly, Chastin et al., (2015) showed that whilst sleep is beneficial for obesity and blood pressure, it may have negative consequences for metabolic biomarkers if sleep is favoured over MVPA.

Taken together, these findings suggest that exercise may exhibit beneficial effects on glycaemic control in sleep restricted individuals in the acute setting but that the effect of chronic physical activity and short sleep duration on glycaemic control is less clear, and therefore warrants further study.

7.5 Limitations

Whilst specific limitations have been highlighted in each chapter, there are some limitations which are common between studies. Firstly, the studies in this thesis were designed to focus on healthy individuals. The findings therefore cannot be extrapolated to individuals with existing metabolic disorders such as type 2 diabetes. Additionally, the samples used were relatively homogenous in that the majority of participants were aged 18 to 50 years old. Although similar findings between sleep duration and glycaemic control have been observed in younger and older age groups, results have been mixed and other factors may begin to contribute to a decline in glycaemic control in ageing individuals (i.e. those over 60

years old (Imbeault et al., 2003; Shimokata et al., 1991). All participants in the studies presented in this thesis were nocturnal sleepers and did not have any sleep disorders. In populations where sleep is misaligned with the natural circadian rhythms, such as shift workers, insulin sensitivity may be influenced by factors other than sleep duration. For example, circadian misalignment in shift workers has been demonstrated to disrupt insulin sensitivity independent of sleep duration (Leproult et al., 2014). The findings in this thesis should therefore be interpreted with caution when referring to populations other than those used in the three studies presented.

In the studies presented in Chapters 4 and 5, glycaemic control was measured using an OGTT, and insulin sensitivity was estimated using the Matsuda index and HOMA. Whilst it has been demonstrated that these surrogate measures of insulin sensitivity relate well to insulin sensitivity measures obtained from the hyperinsulinemic-euglycaemic clamp method, it is not possible to obtain detailed information about glucose uptake and effectiveness from an OGTT. Therefore, the exact nature of the impairment in glucose regulation which occurred in these studies is unclear. However, it may be argued that the OGTT is ecologically superior to a method such as the hyperinsulinemic-euglycaemic clamp due to oral ingestion of the glucose rather than an intravenous infusion. Furthermore, in these chapters many aspects of lifestyle which are known to influence glycaemic control were controlled and replicated across conditions. Whilst a controlled design is useful for examination of novel concepts, it should be acknowledged that this does not fully reflect a free-living setting in which food intake and physical activity are likely to differ.

7.6 Conclusion and future research directions

The main aim of this thesis was to explore the link between short sleep, glycaemic control, and physical activity. The studies presented in this thesis have confirmed previous research demonstrating an impairment in glycaemic control after acute sleep restriction, highlighting the importance of sleep in addition to diet and physical activity for modulating insulin sensitivity. However, when transferred to the chronic setting, findings were contradictory, showing no evidence of differences in markers of glycaemic control, except for HDL cholesterol. This adds to the mixed findings in the current body of literature, suggesting further work in this area is required to elucidate the role sleep plays in long term metabolic health. This thesis provides preliminary data which suggests a potential benefit of exercise in attenuating the negative metabolic consequences of short sleep in an acute, controlled setting.

From a practical perspective, it can be suggested that sufficient sleep should be included as part of a healthy lifestyle to prevent declines in metabolic health. In those individuals who experience acute sleep restriction, it may be advantageous to conduct a bout of sprint interval exercise following the night of short sleep to counteract the impairment in insulin sensitivity which occurs after sleep loss.

However, as the research examining this topic is just beginning to emerge, there are many unanswered questions which provide scope for future research. These include investigating the effect of different exercise modalities, timings, and durations after sleep restriction, and examining the time-course of improved insulin sensitivity following an exercise bout in sleep-restricted individuals. Whilst improvements in glycaemic control have been observed with sprint interval exercise, resistance exercise may be superior to sprint interval exercise for reducing insulin AUC and improving insulin sensitivity (Tong et al., 2017). Furthermore, the impairment in glucose regulation after insulin sensitivity may be due to

compromised insulin signalling within skeletal muscle (Sweeney et al., 2017). Acute resistance exercise can enhance glucose uptake in skeletal muscle and research has shown increased phosphorylation of components of the insulin signalling pathway in skeletal muscle for up to 26 hours following cessation of exercise (Breen et al., 2011). Hence, resistance exercise may provide superior effects on insulin sensitivity after sleep restriction compared to sprint interval exercise, and thus is an area which warrants further study.

It would also be of interest to examine the time course of impaired glycaemic control following sleep restriction, and to determine if underlying mechanisms differ according to the severity of sleep duration, either in number of nights or duration of restriction each night. Future research could therefore use different sleep restriction protocols and simultaneously measure variables which are proposed to contribute to the impairment in glucose regulation after sleep restriction, such as insulin signalling in skeletal muscle and adipose tissue, systemic inflammation, and circadian gene expression. This would enable interventions to be targeted towards a specific mechanism or mechanisms.

Future research may also focus on the link between sleep, physical activity, and glycaemic control in the chronic setting. Longer term studies using objective measures such as actigraphy to measure sleep and physical activity are warranted, as well as tracking how changes in sleep duration and physical activity over a prolonged period impacts glycaemic control. Studies such as these could employ more robust measures of glycaemic control such as an OGTT or continuous glucose monitoring, rather than obtaining a single sample as was done in the study presented in Chapter 6. This would enable more detailed information to be collected about glycaemic control status.

To summarise, this thesis has provided novel findings which show that i) there does not appear to be a linear increase in the impairment of glycaemic control with an increasing number of nights of sleep restriction, ii) acute sprint interval exercise may be beneficial for improving the late postprandial response to an OGTT after sleep restriction, and iii) the link between habitual sleep duration and glycaemic control may be influenced by confounding factors, suggesting that a multi-factorial approach should be taken to achieve optimal metabolic health. Future research can build upon these findings to address the gaps in the literature which will advance understanding in the field of sleep, exercise and glycaemic control.

Appendices

Appendix A1- Participant Information Sheet (Chapter 4)

Study Title: Sleep restriction and impaired glucose regulation – a dose-response study
Investigator: Emma Sweeney

Participant Information Sheet

You are being invited to take part in this research study. Before you decide it is important for you to read this leaflet so you understand why the study is being carried out and what it will involve.

Reading this leaflet, discussing it with others or asking any questions you might have will help you decide whether or not you would like to take part.

What is the Purpose of the Study

This study aims to determine how partial sleep restriction impacts blood glucose control, insulin sensitivity and inflammation within the body.

Studies have shown that lack of sleep can have negative effects on metabolic function, particularly the control of blood glucose levels. However, it is unknown whether the effects worsen if sleep loss increases or if the effect is the same regardless of how much sleep is lost. Why blood glucose control is affected by sleep restriction is also unknown, but increased inflammation may be one possibility. This study will therefore aim to investigate blood glucose control and levels of inflammation in the body after each night of sleep restriction. We will also investigate whether sleep structure (amount of deep sleep, how long it takes to fall asleep, etc.) changes in response to sleep loss.

Why have I been invited?

You have been invited to participate in this study because you:

- ✓ Are a healthy individual aged between 18 and 40 years old
- ✓ Have a normal sleep pattern (7-9 hr sleep per night)
- ✓ Do not smoke

Due to the nature of the study you cannot take part if you suffer from a neurological disorder, psychiatric disorder, metabolic disorder (e.g. impaired glucose tolerance or diabetes), inflammatory disorder (e.g. inflammatory bowel disease), sleep disorder or blood clotting disorder (e.g. hemophilia). If you are taking medication which impacts metabolism (e.g. metformin or other blood glucose control medication) or inflammation (e.g. immunosuppressants) you will also be unable to participate. You will also not be able to take part if you are a shift worker, have travelled over time zones in the past 4 weeks or have recently been involved in drug or alcohol abuse, as these may affect your circadian rhythm which will influence study results. If you have an allergy to dextrose (a type of sugar) you cannot take part as this will be used in the oral glucose tolerance test.

Do I have to take part?

No. Taking part in this study is completely voluntary and it is your decision whether or not you wish to take part. If you decide to take part you may withdraw from the study at any time without questioning. Participation is voluntary so no judgement will be made should you decide you do not wish to take part either before or during the study.

What will happen if I take part?

This study will involve a 2-week preliminary phase and 2 experimental phases, during which sleep will be restricted to 4 or 8 hours per night. The preliminary phase will involve a one night stay in the sleep laboratory to measure your sleep structure, and each experimental phase will involve a four night stay in the sleep laboratory. Experimental visits will be separated by a period of at least 3 weeks. During your stay in the sleep laboratory you will be allowed to leave once testing has finished (around 10am) each day, but must return to the sleep laboratory at 7pm each night for your overnight stay. During the times you are not in the sleep laboratory you will not be allowed to do any physical activity, nap or consume any foods except those provided by the researchers. If you are sleep restricted please also avoid driving as your reaction times may be slower than usual.

Preliminary Phase

During the 2 week preliminary phase you will be issued with an actigraphy watch which you must wear on your wrist at all times and a sleep diary to complete every morning for the two weeks. You will also be given a 3 day food diary to complete in week one which will allow us to individualise your diet to your likes and dislikes during your stays in the laboratory. Females will also be required to complete a menstrual cycle questionnaire so that both experimental trials can be conducted during the same phase of the menstrual cycle. During the preliminary phase you will be asked to have around 7 to 9 hours of sleep each night, and to keep a consistent wake time in the mornings. In the middle of the 2-week preliminary phase you will stay over in the sleep laboratory for one night, where your sleep will be measured (explained below). This will give us an indication of your sleep structure and identify

any possible sleep problems. This preliminary phase will be repeated prior to the second experimental phase.

Experimental Phase

Each experimental phase will involve 4 nights in the sleep laboratory. In the 24 hr prior to attending you will be asked to avoid any vigorous exercise or caffeine consumption. You will arrive at the sleep laboratory in the evening before the first night of experimental sleep.

Each morning an oral glucose tolerance test (OGTT – procedure explained below) will be carried out. During the OGTT blood samples will be obtained at regular intervals. The procedures for these are also outlined below.

You will then be allowed to leave the sleep laboratory and return at 7pm. Each night you will sleep for either 4 or 8 hours depending on the condition you have been assigned to. As your bed time will be delayed in the sleep restriction condition you may watch television, do work, read, etc. during this time. On the first and third night in each condition sleep structure will be measured. You will also be asked to complete appetite scales before and after each meal every day. After the OGTT on the final morning you will be instructed to have a recovery sleep if you have been sleep restricted.

Oral Glucose Tolerance Test

For the OGTT you will be given a drink containing 82.5 g dextrose (a type of sugar) mixed with 300 ml of water, which you will be asked to consume within 5 minutes. Following this, blood samples will be collected at regular intervals, as outlined below.

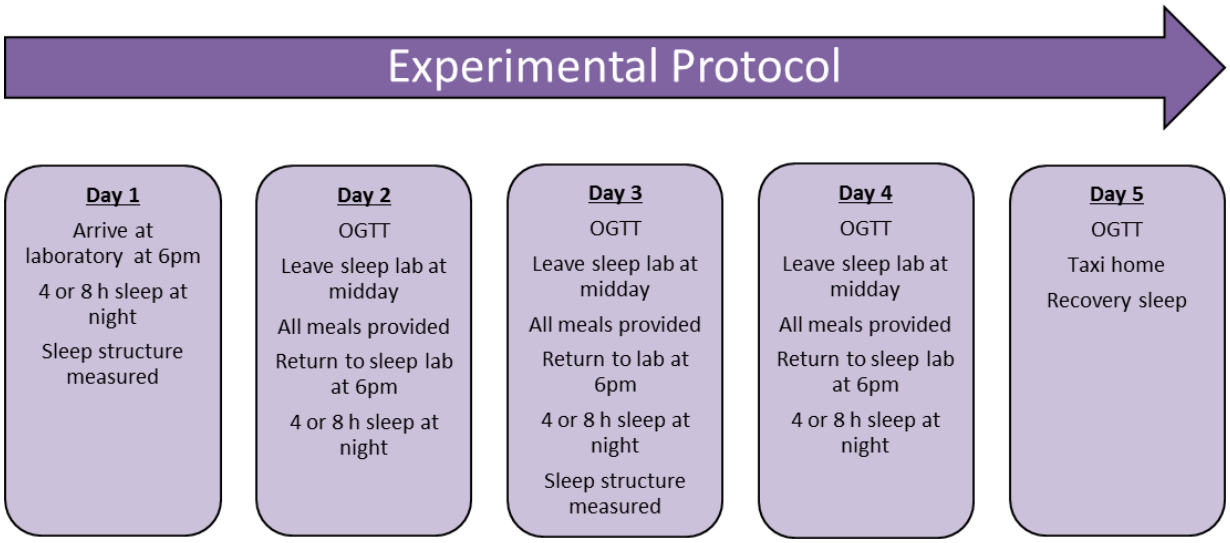
Blood sampling

Blood samples will be obtained via the cannulation technique. A cannula will be inserted into the antecubital vein in the arm to allow regular blood samples to be drawn. Blood samples will be drawn at baseline (0), 15, 30, 45, 60, 90 and 120 min following consumption of the dextrose drink.

Measurement of sleep structure

Measurement of sleep structure involves the attachment of electrodes to the head in order to gain measures such as total sleep time, time spent in each stage of sleep and sleep efficiency. This is a painless process. All electrodes will be attached by a trained researcher before you go to sleep and you will then be left to sleep uninterrupted until the morning. Electrodes will then be removed upon wakening.

The Sleep Laboratory



The sleep laboratory, pictured above, is fully equipped with a bedroom, en-suite bathroom, kitchen, and lounge. During your stay you will not be permitted to leave but may use the facilities within the sleep laboratory and may also bring your laptop/tablet/etc. There will be a staff member around at all times should you have any questions or problems during your stay. You should have also been given an information sheet for overnight stays in the sleep laboratory – please read that in conjunction with this information sheet before deciding if you would like to participate as it contains more detail on what your stay would entail.

Does the study involve any discomfort or embarrassment?

This study involves collection of blood samples using the cannulation technique. There may be slight discomfort whilst the cannula is inserted however any discomfort experienced should be mild and only last a short period of time. Whilst

the cannula is left in for the duration of the OGTT discomfort should be minimal, often not being felt at all. A risk assessment has been carried out for the collection of blood samples. Please note that should any abnormalities be found during sleep or from blood samples you will be advised to inform your general practitioner of these findings. No psychological embarrassment will be involved in this study.

What are the possible benefits of taking part?

By taking part in this study you will be contributing to research to develop insight into the way sleep duration can impact metabolic health. This research may have implications for individuals at risk of developing metabolic disorders and those who regularly curtail their sleep.

Will I be reimbursed for participating in the study?

Due to the large time commitment involved, you will be issued with £160 of Love2Shop gift cards for participating in the study. These can be spent at a large number of leading retailers. A full list of retailers can be found online (https://www.highstreetvouchers.com/gift/where-to-spend-love2shop-cards)

Will my taking part in this study be kept confidential and anonymous?

Yes. You will be assigned a unique reference number for the study which means you will remain anonymous. All data will be anonymized. When the data is used it will be collated with that of the other participants and will in no way be linked back to you. Only the researchers will have access to the individual data sheets. Data sheets will be stored separately from consent forms.

How will my data be stored?

All data which is in paper form will be stored in a locked filing cabinet within the Faculty of Health & Life Sciences at Northumbria University. Electronic data will be stored on a password protected computer. Data will be stored in accordance with University guidelines and the Data Protection Act (1998) will be followed.

How will the results of this study be used?

On completion of the study results will be collated with those of the other participants and will in no way be linked back to you. Findings may be published in scientific papers or presented at conferences. If you would like a copy of the summary of findings you may contact the researcher at the email address given below and you will be issued a copy once the study has ended. Please note that individual data will not be issued and collated findings will not be available immediately after completion of the study.

Who is Organizing and Funding the Study?

This study has been organised by researchers within the Department of Sport, Exercise and Rehabilitation at Northumbria University. The principal investigator is Emma Sweeney (doctoral researcher). No funding has been sought from external sources.

Who has reviewed this study?

This study has been reviewed and approved by the Faculty of Health and Life Sciences Research Ethics Committee at Northumbria University.
Should you require any information about the ethical aspects of this study please contact:
Dr Mick Wilkinson
Northumberland Building
Northumbria University
Newcastle upon Tyne
NE1 8ST
Email: mic.wilkinson@northumbria.ac.uk

Contact for further information:

Emma Sweeney (principal researcher) – emma.sweeney@northumbria.ac.uk

Name of another person who can provide independent information or advice about the project:
Mick Wilkinson – mic.wilkinson@northumbria.ac.uk

Appendix A2 – Participant Information Sheet (Chapter 5)

Faculty of Health & Life Sciences

PARTICIPANT INFORMATION

TITLE OF PROJECT: The effect of exercise on glucose regulation following acute sleep restriction

Participant ID:

Principal Investigator: Emma Sweeney (PhD researcher)

Investigator contact details: Email: emma.sweeney@northumbria.ac.uk

1. What is the purpose of the project?

Sleep duration has been shown to impact health, with insufficient sleep being associated with a higher risk of developing metabolic disorders such as diabetes. Sleep restriction can impair blood glucose control even in healthy individuals. However, it remains unknown whether exercise, which has positive effect on glucose metabolism, can help the impairment in blood glucose regulation after sleep restriction.

2. Why have I been selected to take part?

You have indicated that you are:

- interested in taking part in the study
- male
- healthy
- aged 18-50 years (inclusive)
- non-smoker

Additionally, please read the next section which outlines the exclusion criteria.

3. What are the exclusion criteria (i.e. are there any reasons why I should not take part)?

Participants complying with at least one of the following criteria will not be eligible:

- Participants diagnosed with at least one of the following will not be eligible:
  - diabetes (type I or type II), or any other disorder likely to affect metabolism
  - blood clotting disorder

- sleep disorder (such as obstructive sleep apnea)

- Taking any medication known to affect metabolism or sleep
- Working in shift schedules (ex: nurse, baker, etc.)
- Regular travel across time zones (>3 times a year) or travel across time zones in the past 4 weeks
- A bedtime before 22:00 or after 01:00, or a wake time before 06:00 or after 09:00
- A history of alcohol or drug abuse
- Eating disorders: anorexia and bulimia or unstable dietary pattern;
- A psychological or linguistic inability to sign the informed consent;

If you are unsure about your eligibility then please contact the research team and we can discuss any issues or ambiguity.

We cannot list every individual medical condition and medication in the exclusion criteria; if you have any health issues or are taking any medications (prescribed or over the counter) you are encouraged to check with the research team that these do not exclude you from the study before you attend the lab. These criteria are study specific, so whilst one study may allow certain medications, another may not.

4. What will I have to do?

The study will involve a prescreening/exercise testing session and 4 experimental trials which each require one visit to the laboratory, at least 1 week apart. There will be a maximum of 2 weeks between visits. A summary of the experimental visits is presented in the diagram below.

<u>Prescreening/Exercise familiarisation – 1 hour</u>

During the first visit you will have the opportunity to ask any questions you may have about the study before completing a written consent form if you are happy to take part. You will then be asked to complete several questionnaires related to your sleep habits and given a 3 day food diary (2 weekdays and 1 weekend day) to complete before the first experimental trial. Following this, you will complete an exercise session to become familiarised with the type of exercise which will be used in the experimental trials. For the familiarisation exercise, you will complete a 5 minute warm up, followed by two maximum effort 30 second sprints on the cycle ergometer, followed by a 5 min cool down.

<u>Experimental trials</u>

There are 4 experimental trials – a control trial, a control plus exercise trial, a sleep restriction trial, and a sleep restriction plus exercise trial. You will complete all 4 trials in a random order and will not be told which trial you are on until the day prior to the night of sleep restriction or control sleep.

Prior to each trial you will have a 1 week entraining period, which involves keeping a consistent sleep wake schedule for 7 days. During this period you will be asked to wear an actigraphy watch which will track your sleep and complete a sleep diary.

Following the entraining period you will have either a night of 8 hour sleep (control and control plus exercise conditions) or 4 hour sleep (sleep restriction and sleep restriction plus exercise conditions). On the day prior to the 4 or 8 h sleep you will be required to keep a consistent diet across the 4 conditions. We will provide you with a diet for this day based on your habitual intake as determined from your food diary. You must also avoid alcohol, caffeine, and exercise on this day.

In the 4 h sleep conditions, in addition to wearing the actigraphy watch, you will be required to send text messages to the researcher every hour between 11pm and 3am to ensure you are awake. You may also receive a phonecall from the researcher for this purpose.

Upon wakening after your night of 4 or 8 hour sleep, you will come to the laboratory in a rested, fasted state. This means you will be asked to avoid all food and drink except water for 10 hours prior to arrival at the laboratory. When making your way to the laboratory please use transport where possible, unless you live within the immediate vicinity of the university. This is because any physical activity may influence the measures in the study. If you are sleep restricted please do not drive as you may have increased tiredness.

You will then either rest or complete a high intensity cycling exercise protocol. The exercise protocol in the experimental conditions will be similar to the familiarisation, except that four maximum effort 30 second sprints will be completed rather than two. Sprints will be separated by 4.5 minutes of unloaded recovery. Warm up and cool down will remain the same.

Following the rest or exercise, you will be given a 30 min recovery period. An oral glucose tolerance test (OGTT) will then be conducted. The oral glucose tolerance test involves insertion of a cannula into the antecubital vein in your arm. A fasted blood sample will be drawn from the cannula, then you will be given a glucose drink to consume. Following consumption of the glucose drink, blood samples will be drawn at regular intervals for 120 min. During the 2 hours you must remain in the laboratory however you may do work/read/watch a film between blood samples.

You will also be asked to collect urine samples at several points during each experimental visit. The timepoints for urine samples are show on the diagram below and outlined in section 7. These will be the second urination of the day on the day prior to each testing day and each testing day. You will also be asked to provide spot samples when you arrive at the laboratory, before the OGTT, and at the end of the OGTT.

All experimental visits will follow this similar protocol, but differ in the length of sleep or whether you are exercising or resting in the morning.

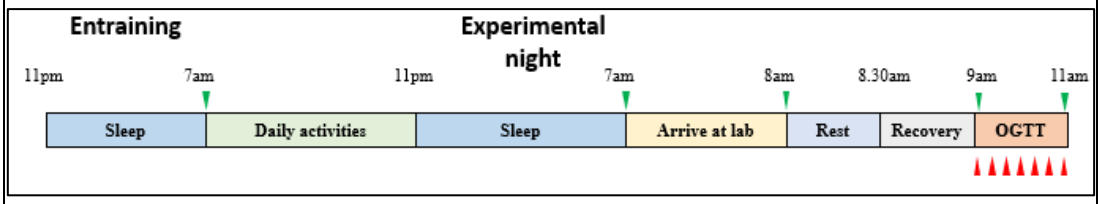


Figure 1. Timeline of activities for experimental trials (visits 2,3,4 and 5). Red arrows indicate blood samples. Green arrows indicate urine samples. Sleep on experimental night will be from 11pm-7am in control conditions and 3am-7am in sleep restriction conditions. 8-8.30am will be rest in the two rest conditions and exercise in the two exercise conditions (rest shown as an example of timings).

5. Will my participation involve any discomfort or embarrassment?

This study involves collection of blood samples using the cannulation technique. There may be slight discomfort whilst the cannula is inserted, however any discomfort experienced should be mild and only last a short period of time. Minor bruising may also occur following cannulation. A risk assessment has been carried out for the collection of blood samples and the samples will be collected by trained researcher following standard operating procedures. Please note that should any abnormalities be found from the blood sample you will be advised to inform your general practitioner of these findings.

The nature of the exercise sessions in this study are designed to be difficult. You may therefore find the exercise challenging.

As you will be sleep restricted in 2 of the visits, we advise that you do not drive or carry out any tasks which require a high level of alertness on these days. You should have an extended sleep the night following sleep restriction to ensure you are recovered.

No psychological embarrassment will be involved in this study.

6. Will my participation result in any benefits to me?

By taking part in this study you will be contributing to research to develop insight into the way sleep and exercise can impact metabolic health. Whilst there will be no direct benefit to you, the study results are intended to extend knowledge on the relationship between sleep, exercise, and metabolic health which may benefit others in the future.

7. Will I have to provide any bodily samples (i.e. blood, saliva)?

Blood samples will be obtained by the cannulation technique during the OGTTs in visits 2, 3, 4 and 5. All samples will be obtained by a trained researcher. 10ml will be obtained for each sample and an additional 5 ml at the beginning and end of the OGTT, so 80 ml will be collected in total for each visit.
You will also provide urine samples. You will collect these yourself from the second urination of the day on the day prior to each testing day and each testing day. You will also be asked to provide spot samples when you arrive at the laboratory, before the OGTT, and at the end of the OGTT.

8. How will confidentiality be assured?

You will be assigned a unique reference number for the study which means you will remain anonymous. All data will be anonymized. When the data is used it will be collated with that of the other participants and will in no way be linked back to you. Only the researchers will have access to the individual data sheets. Data sheets will be stored separately from consent forms.

All data which is in paper form will be stored in a locked filing cabinet within the Faculty of Health & Life Sciences at Northumbria University. Electronic data will be stored on a password protected computer. Data will be stored in accordance with University guidelines and the Data Protection Act (1998) will be followed. Data will be stored for 5 years after completion of the study.

9. Who will have access to the information that I provide?

Only the principal investigator (Miss Emma Sweeney) and the research team will have access to the information that you provide. Any data that leaves the site will only be identifiable by an identification number and it will not be possible for anybody outside of the investigational site to identify you.

10. What categories of personal data will be collected and processed in this study?

We will collect contact details, date of birth, ethnicity and blood/urine markers of glycaemic control.

11. What is the legal basis for processing personal data?

The legal basis for processing the personal data required for the purposes of this study is that the research is being conducted in the public interest, necessary for scientific research purposes.

12. How will my information be used in the future?

On completion of the study results will be collated with those of the other participants and will in no way be linked back to you. Findings may be published in scientific papers, presented at conferences, and used to form part of a thesis.

13. Has this investigation received appropriate ethical clearance?

Yes, this study has received ethical approval from the Faculty of Health and Life Sciences Ethics committee. If you require confirmation of this please contact the Chair of this Committee, stating the title of the research project and the name of the principal investigator:

Mick Wilkinson, Chair of Faculty of Health and Life Sciences Ethics Committee, Northumberland Building, Northumbria University, Newcastle upon Tyne, NE1 8ST

14. Will I receive any financial rewards / travel expenses for taking part?

No.

15. How can I withdraw from the project?

Participation in this study is completely voluntary. If you wish to withdraw at any point you can do so by contacting the principal researcher (Emma Sweeney) at the contact details given in section 17. Should you decide to withdraw no judgement will be made and you do not have to state your reason for withdrawal if you do not wish to do so.

16. What are my rights as a participant in this study?

You have the right of access to a copy of the information comprised in your personal data (to do so, submit a Subject Access Request). You have a right, in certain circumstances, to have inaccurate personal data rectified, and a right to object to decisions being taken by automated means. If you are dissatisfied with the University's processing of personal data, you have the right to complain to the Information Commissioner's Office.

17. If I require further information who should I contact and how?

If you need more information you should contact the principal investigator - Emma Sweeney:

Email: emma.sweeney@northumbria.ac.uk

Tel: 0191 243 7018

Name and contact of Data Protection Officer at Northumbria University: Duncan James (dp.officer@northumbria.ac.uk)

Appendix A3 – Participant Information Sheet (Chapter 6)

Faculty of Health & Life Sciences

PARTICIPANT INFORMATION

TITLE OF PROJECT: Sleep duration and glycaemic control

Participant ID:

Principal Investigator: Emma Sweeney (PhD researcher)

Investigator contact details: Email: emma.sweeney@northumbria.ac.uk

1. What is the purpose of the project?

Sleep duration has been shown to impact health, with insufficient sleep being associated with a higher risk of developing metabolic disorders such as diabetes. However many studies which have investigated this have not taken into account the physical activity status, dietary intake, or body composition of the participants. All of these lifestyle factors may influence glucose control. Therefore, the aim of this study is to examine the relationship between sleep duration and glycaemic control whilst taking into account lifestyle factors which may modify this relationship.

Additionally, part B of this study (optional – see section 4 for more information) will investigate if there is a difference in metabolite profile between shorter and longer sleepers. Identifying differences in metabolite profile will allow us to see which metabolic pathways are altered by sleep duration, which may help to explain why short sleepers have been shown to be at greater risk of developing chronic diseases such as type 2 diabetes.

2. Why have I been selected to take part?

You have indicated that you are:

- interested in taking part in the study
- healthy
- aged 18-50 years (inclusive)

Additionally, please read the next section which outlines the exclusion criteria.

3. What are the exclusion criteria (i.e. are there any reasons why I should not take part)?

Participants complying with at least one of the following criteria will not be eligible:

- Participants diagnosed with at least one of the following will not be eligible:
  - diabetes (type I or type II), or any other disorder likely to affect metabolism (eg. PCOS)
  - blood clotting disorder
  - sleep disorder (such as obstructive sleep apnea)
  - any other pathology which, according to investigator's judgment, is likely to affect the study parameters
- Taking any medication known to affect metabolism or sleep
- Ethnicity which is not white, European descent (due to differences in circadian genes).
- Working in shift schedules (ex: nurse, baker, etc.)
- Regular travel across time zones (>3 times a year)
- A bedtime before 22:00 or after 01:00, or a wake time before 06:00 or after 09:00
- Change in smoking, physical activity, sleep or dietary habits in the past 3 months
- A history of alcohol or drug abuse
- Eating disorders: anorexia and bulimia or unstable dietary pattern;
- Pregnancy in progress (or suspected)
- A psychological or linguistic inability to sign the informed consent;

If you are unsure about your eligibility then please contact the research team and we can discuss any issues or ambiguity.

We cannot list every individual medical condition and medication in the exclusion criteria; if you have any health issues or are taking any medications (prescribed or over the counter) you are encouraged to check with the research team that these do not exclude you from the study before you attend the lab. These criteria are study specific, so whilst one study may allow certain medications, another may not.

4. What will I have to do?

You will be required to attend the laboratory on 2 occasions:

Visit 1 (approx. half an hour)

On arrival you will be met by the researcher who will go over the study protocol and give you the opportunity to ask any questions you may have. Providing you are still happy to participate you will be given an informed consent form to complete and the researcher will confirm your eligibility. Once eligibility has been confirmed you will be given several questionnaires which will ask about your sleep and lifestyle habits. At the end of this visit you will be provided with a food and physical activity diary, sleep diary, and actigraphy watch. You will be given instructions on how to complete/wear these until your next visit.

Part B: There is an optional second part to this project (part B). If you agree to participate in the second part, you will also be given a tube to collect a urine sample prior to the second visit. Please note that if you wish to participate in part B, it does not necessarily mean that your blood and urine sample will be used for this part of the study. The reason for this is that we are only using 60 participants for part B, and these participants will be the 30 people who sleep for the shortest amount of time and the 30 people who sleep for the longest amount of time. Therefore if you have provided samples for this part of the study and do not fall into these categories your data will not be analysed for metabolite profile.

Visit 2 (approx. half an hour)

This visit will take place 7 days after visit 1. Please arrive in a fasted state (no food or drink except water for 10 h prior) and bring your completed food and sleep diaries to this visit along with your actigraphy watch. You will be required to rest in a chair for 5 minutes then blood pressure will be measured. You will then have a DXA scan, which is an accurate method of measuring body composition. Following the DXA scan you will be required to provide a single blood sample which will be obtained from the antecubital vein in your arm by a trained researcher.

Throughout participation (between visits 1 and 2)

You will be given a 7-day sleep diary and 3-day food, physical activity and light exposure diary to complete and an actigraphy watch to wear between visits. You will be requested

not to change your habits (dietary, sleeping and smoking habits, usual physical activity, etc.) during participation; However, if any changes occur, it will have to be reported to the investigator. On the morning of your second visit you will be required to collect a urine sample from your second void of the day and bring this with you to the laboratory.

DXA scan

A DXA scan is considered a gold-standard method of measuring body composition. You will be asked to lie still on the scanner table for approximately 7 minutes whilst the scan is carried out. For the DXA scan you will be required to wear comfortable clothing which doesn't contain any metal, and remove any jewellery. To allow for an accurate reading, you need to be hydrated so please ensure you drink plenty of water before arriving at the laboratory.

DXA scans emit a small dose of radiation – each whole-body scan is equivalent to less than 2 days UK background radiation. You will therefore be required to complete a DXA screening form prior to the scan.

5. Will my participation involve any discomfort or embarrassment?

This study involves collection of blood samples using the venepuncture technique. There may be slight discomfort whilst the needle is inserted however any discomfort experienced should be mild and only last a short period of time. A risk assessment has been carried out for the collection of blood samples and the sample will be collected in a sterile environment. Please note that should any abnormalities be found from the blood sample you will be advised to inform your general practitioner of these findings. No psychological embarrassment will be involved in this study.

6. Will my participation result in any benefits to me?

By taking part in this study you will be contributing to research to develop insight into the way sleep can impact metabolic health. Whilst there will be no direct benefit to you, the study results are intended to extend knowledge on the relationship between sleep and metabolic health which may benefit others in the future.

7. Will I have to provide any bodily samples (i.e. blood, saliva)?

You will be required to provide a single blood sample on visit 2. This will be obtained using the venepuncture technique, which involves a needle being inserted into the

antecubital vein in your arm. Should any abnormality be found from the blood sample you will be advised to visit your GP.

Should you agree to participate in Part B, a urine sample (second void of the day) is also required on the morning of your second visit. You will collect this using the equipment provided by the researchers and bring it with you to the laboratory. As outlined above, only those 30 participants who sleep for the shortest amount of time and those 30 participants who sleep for the longest amount of time will have their data analysed for part B. Therefore even if you opt in to this part of the study your data will not be used for metabolite profile analysis if you do not fall into either of these categories.

8. How will confidentiality be assured?

You will be assigned a unique reference number for the study which means you will remain anonymous. All data will be anonymized. When the data is used it will be collated with that of the other participants and will in no way be linked back to you. Only the researchers will have access to the individual data sheets. Data sheets will be stored separately from consent forms.

All data which is in paper form will be stored in a locked filing cabinet within the Faculty of Health & Life Sciences at Northumbria University. Electronic data will be stored on a password protected computer. Data will be stored in accordance with University guidelines and the Data Protection Act (1998) will be followed. Data will be stored for 5 years after completion of the study.

9. Who will have access to the information that I provide?

Only the principal investigator (Miss Emma Sweeney) and the research team will have access to the information that you provide. However, any data that leaves the site will only be identifiable by an identification number and it will not be possible for anybody outside of the investigational site to identify you.

10. What categories of personal data will be collected and processed in this study?

We will collect contact details, date of birth, ethnicity, blood markers of glycaemic control and metabolite profile (Part B only).

11. What is the legal basis for processing personal data?

The legal basis for processing the personal data required for the purposes of this study is that the research is being conducted in the public interest, necessary for scientific research purposes.

12. How will my information be used in the future?

On completion of the study results will be collated with those of the other participants and will in no way be linked back to you. Findings may be published in scientific papers or presented at conferences.

13. Has this investigation received appropriate ethical clearance?

Yes, this study has received ethical approval from the Faculty of Health and Life Sciences Ethics committee. If you require confirmation of this please contact the Chair of this Committee, stating the title of the research project and the name of the principal investigator:

Chair of Faculty of Health and Life Sciences Ethics Committee, Northumberland Building, Northumbria University, Newcastle upon Tyne, NE1 8ST

14. Will I receive any financial rewards / travel expenses for taking part?

No.

15. How can I withdraw from the project?

Participation in this study is completely voluntary. If you wish to withdraw at any point you can do so by contacting the principal researcher (Emma Sweeney) at the contact details given at the top of this information sheet. Should you decide to withdraw no judgement will be made and you do not have to state your reason for withdrawal if you do not wish to do so.

16. What are my rights as a participant in this study?

You have the right of access to a copy of the information comprised in your personal data (to do so, submit a Subject Access Request). You have a right, in certain circumstances, to have inaccurate personal data rectified, and a right to object to decisions being taken by automated means. If you are dissatisfied with the University's processing of personal data, you have the right to complain to the Information Commissioner's Office.

17. If I require further information who should I contact and how?

If you need more information you should contact the principal investigator - Emma Sweeney:

Email: emma.sweeney@northumbria.ac.uk

Tel: 0191 243 7018

Name and contact of Data Protection Officer at Northumbria University: Duncan James (dp.officer@northumbria.ac.uk)

Appendix B1 – Informed consent form (Chapter 4)

INFORMED CONSENT FORM

Project Title: Sleep restriction and impaired glucose metabolism – a dose-response study

Principal Investigator: Emma Sweeney (doctoral resesarcher)

	please tick or initial where applicable
I have carefully read and understood the Participant Information Sheet.	☐
I have had an opportunity to ask questions and discuss this study and I have received satisfactory answers.	☐
I understand I am free to withdraw from the study at any time, without having to give a reason for withdrawing, and without prejudice.	☐
I agree to take part in this study.	☐

REMOVAL AND STORAGE OF TISSUE
I agree that the following tissue or other bodily material may be taken and used for the study:

Tissue/Bodily material	Purpose	Removal Method
Blood	*Glucose, insulin, inflammatory markers*	*Cannulation*

I understand that if the material is required for use in any other way than that explained to me, then my consent to this will be specifically sought. I understand that I will not receive specific feedback from any assessment conducted on my samples, but should any kind of abnormality be discovered then the investigator will contact me.

I understand that the University may store this tissue in a Licensed Tissue Bank only for the duration of the study, it will then be destroyed.

Method of disposal:

Clinical Waste ✓
Other
If other please specify.....

Signature of participant..... Date.....……..

(NAME IN BLOCK LETTERS).....…….

Signature of researcher..... Date.....……..

(NAME IN BLOCK LETTERS).....…….

Appendix B2 – Informed consent form (Chapter 5)

INFORMED CONSENT FORM

Project Title: The effect of exercise on glucose regulation following acute sleep restriction

Principal Investigator: Emma Sweeney (doctoral researcher)

	please tick or initial where applicable
I have carefully read and understood the Participant Information Sheet.	☐
I have had an opportunity to ask questions and discuss this study and I have received satisfactory answers.	☐
I understand I am free to withdraw from the study at any time, without having to give a reason for withdrawing, and without prejudice.	☐
I agree to take part in this study.	☐

REMOVAL AND STORAGE OF TISSUE
I agree that the following tissue or other bodily material may be taken and used for the study:

Tissue/Bodily material	Purpose	Removal Method
Blood	*Markers of glycaemic control and metabolite profile*	*Cannulation*
Urine	*Metabolite profile*	*Participant collection*

I understand that if the material is required for use in any other way than that explained to me, then my consent to this will be specifically sought. I understand that I will not receive specific feedback from any assessment conducted on my samples, but should any kind of abnormality be discovered then the investigator will contact me.

I understand that the University may store this tissue in a Licensed Tissue Bank only for the duration of the study, it will then be destroyed.

Method of disposal:

Clinical Waste ✓
Other
If other please specify.....

Signature of participant..... Date.....……..

(NAME IN BLOCK LETTERS).....…….

Signature of researcher..... Date.....……..
(NAME IN BLOCK LETTERS).....…….

Appendix B3 – Informed consent form (Chapter 6)

INFORMED CONSENT FORM

Project Title: Sleep duration and glycaemic control

Principal Investigator: Emma Sweeney (doctoral researcher)

	please tick or initial where applicable
I have carefully read and understood the Participant Information Sheet.	☐
I have had an opportunity to ask questions and discuss this study and I have received satisfactory answers.	☐
I understand I am free to withdraw from the study at any time, without having to give a reason for withdrawing, and without prejudice.	☐
I agree to take part in this study.	☐

REMOVAL AND STORAGE OF TISSUE
I agree that the following tissue or other bodily material may be taken and used for the study:

Tissue/Bodily material	Purpose	Removal Method
Blood	*Markers of glycaemic control*	*Venepuncture*

I understand that if the material is required for use in any other way than that explained to me, then my consent to this will be specifically sought. I understand that I will not receive specific feedback from any assessment conducted on my samples, but should any kind of abnormality be discovered then the investigator will contact me.

I understand that the University may store this tissue in a Licensed Tissue Bank only for the duration of the study, it will then be destroyed.

Method of disposal:

Clinical Waste ✓
Other
If other please specify.....

Signature of participant..... Date.....……..

(NAME IN BLOCK LETTERS).....…….

Signature of researcher..... Date.....……..

(NAME IN BLOCK LETTERS).....…….

Northumbria University
NEWCASTLE

Appendix C – Food log

Diet Log

Name:

Date:

Nutritional Questionnaire

Do you have any food allergies or intolerances?

_____
_____
_____
_____
_____

What type of milk do you normally use?

_____
_____

What type of bread do you normally eat?

_____
_____

What type of spread do you normally use?

_____

Do you regularly drink tea or coffee and if so, how often and what with?

_____
_____
_____
_____

Do you take any supplements including vitamins and minerals?

_____
_____
_____
_____

Guidance notes

The aim of this booklet is to allow the investigator to attain an understanding of your daily dietary intake. For 3 days, you will need to record ***EVERYTHING*** you eat and drink.

Diet Logs

One page is allocated for each day's nutritional intake. Please provide detailed information when describing food and drink intake.

- Provide precise weights of foods / volumes of fluids.
- Record the cooking method (eg. fried, boiled)
- Record the 'brand' of food and fluid consumed (e.g. Tesco, Uncle Ben's etc).

It is extremely important to be completely honest when recording your food and drink intake.

Examples are provided in the grey boxes in the table provided overleaf.

Contact

If you unsure how to complete this booklet and require further information at any time, please contact:

Emma Sweeney

Email: emma.sweeney@northumbria.ac.uk

DAY 1: Date: _____

TIME	FOOD / BRAND	COOKING METHOD	QUANTITY
Example 7.00 am	Weetabix Semi-skimmed milk Tea with 2 sugars		2 200 ml 1 cup

DAY 2: Date: _____

TIME	FOOD / BRAND	COOKING METHOD	QUANTITY
Example 7:15 am	Brown toast with flora margerine Tea with 2 sugars		2 slices 1 cup

DAY 3: Date: _____

TIME	FOOD / BRAND	COOKING METHOD	QUANTITY
Example 8:30 am	Walls sausages Eggs (medium) Tea with 2 sugars	Grilled Scrambled	2 3 1 cup

Sleep Diary

Please fill out this sleep diary every morning about 30 minutes after getting up. Guess the approximate times. Do not worry if your figures are not exactly correct. We are interested in your opinion of how you slept.

	Day 1 Date ___/___/____	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Did you take any naps yesterday? If so, how long were they?							
What time did you go to bed last night?							
How long did it take you to fall asleep?							
How many times did you wake up during the night?							
How many minutes were you awake during the night?							
What time did you wake up this morning?							
What time did you get out of bed this morning?							
How many hours of sleep did you get last night?							
How well did you sleep? (1-10=best)							
Rate your sleepiness (below):							

1 = Extremely alert
2 = Very alert
3 = Alert
4 = Rather alert
5 = Neither alert nor sleepy
6 = Some signs of sleepiness
7 = Sleepy, but no effort to keep awake
8 = Sleepy, some effort to keep awake
9 = Very sleepy, great effort to keep awake
10 = Extremely sleepy, falling asleep

Appendix E – Publication arising from study presented in Chapter 5

Accepted author manuscript version from International Journal of Sport Nutrition and Exercise Metabolism, 2019, https://doi.org/10.1123/ijsnem.2019-0235

Impaired Insulin Profiles Following a Single Night of Sleep Restriction: The Impact of Acute Sprint Interval Exercise

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Running head: Exercise and insulin sensitivity following sleep restriction

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Abstract

Experimental sleep restriction has demonstrated reduced insulin sensitivity in healthy individuals. Exercise is well-known to be beneficial for metabolic health. A single bout of exercise has the capacity to increase insulin sensitivity for up to two days. Therefore, the current study aimed to determine if sprint interval exercise could attenuate the impairment in insulin sensitivity after one night of sleep restriction in healthy males. Nineteen males were recruited for this randomised crossover study which consisted of four conditions – control (CON), sleep restriction (SR), control plus exercise (CE), and sleep restriction plus exercise (SRE). Time in bed was 8 h (2300 – 0700) in the control conditions and 4 h (0300 – 0700) in the sleep restriction conditions. Conditions were separated by a 1 wk entraining period. Participants slept at home and compliance was assessed using wrist actigraphy. Following the night of experimental sleep, participants either conducted sprint interval exercise or rested for the equivalent duration. An oral glucose tolerance test was then conducted. Blood samples were obtained at regular intervals for measurement of glucose and insulin. Insulin concentrations were higher in SR than CON ($P = 0.022$). Late-phase insulin AUC was significantly lower in SRE than SR (862 ± 589 and 1267 ± 558; $P = 0.004$). Glucose AUC was not different between conditions ($P = 0.207$). These findings suggest that exercise improves the late post-prandial response following a single night of sleep restriction.

Key Words: Glucose metabolism, sleep loss, high intensity exercise

Introduction

Short sleep durations are becoming increasingly common, with almost 75% of adults in Great Britain sleeping outside the recommended 7-9 hours each night (Hirshkowitz et al., 2015). Chronic short sleep is associated with increased risk of developing many diseases, including type 2 diabetes (Yaggi et al., 2006). Experimental studies demonstrate impaired glucose control following sleep restriction (Knutson et al., 2007), with a single night of restriction reducing whole-body insulin sensitivity by 20% (Donga et al., 2010).

The proposed mechanisms underlying impaired glucose regulation following sleep restriction include altered peripheral insulin sensitivity (Broussard et al., 2012; Rao et al., 2015) and changes to the metabolic profile, favouring fatty acid transportation (Davies et al., 2014) which may interfere with insulin signalling. However, while the detrimental effects of sleep restriction are clear, few studies have focussed on strategies to counter the impairment.

Exercise may have the potential to alter these proposed underlying mechanisms (Saner et al., 2018). A single bout of exercise positively impacts glucose regulation for up to 24 hours (Koopman et al., 2005). This improvement in glucose regulation is apparent with various types of exercise (Breen et al., 2011; Gillen et al., 2012); although high intensity exercise may to be superior to moderate intensity exercise for improving insulin sensitivity (Rynders et al., 2014; Ortega et al., 2015). Sprint interval exercise has been shown to produce improvements in insulin area under the curve (AUC) and the insulin sensitivity index when measured 30 min post cessation of the exercise bout in healthy males (Ortega et al., 2015). Whether or not

improvements such as these would occur in sleep-restricted individuals remains unknown.

Exercise appears to be a promising intervention to alleviate the impairment in insulin sensitivity following total sleep deprivation. Two weeks of high intensity exercise training has been shown to attenuate the insulin response to an oral glucose tolerance test after one night of total sleep deprivation (de Souza et al., 2017). However, it remains unclear if a single bout of exercise performed after partial sleep restriction can produce similar outcomes.

Consequently, this study aimed to investigate the effect of a single bout of sprint interval exercise on whole-body insulin sensitivity, following a single night of sleep restriction. We hypothesised that insulin sensitivity would be reduced following sleep restriction, and that this effect would be attenuated when exercise was performed.

Methods

Participants

Nineteen healthy males (mean ± SD; age 25 ± 8 y; body mass 81.4 ± 12.0 kg; stature 180 ± 7 cm) participated in this study. Exclusion criteria included shift workers, regular travel across time zones (>3 times a year) or in the past 4 weeks, presence of any disorders which may influence glycaemic control (such as diabetes) or sleep (such as obstructive sleep apnea), current or previous medication in the past year which may have impacted on glucose metabolism or sleep, alteration of sleep, dietary, or physical activity patterns in the previous 3 months, a history of drug or

alcohol abuse or eating disorders, following a specific diet which may influence the results, such as intermittent fasting, a habitual bedtime before 2200 or after 0100, or a habitual wake time before 0600 or after 0900. Individuals were also excluded if they had a contraindication to exercise, poor sleep quality (defined as a Pittsburgh Sleep Quality Index [PSQI] (Buysse et al., 1989) score of above 5), or were classed as extreme morning or evening types, assessed by the morningness-eveningness questionnaire [MEQ] (Horne & Ostberg, 1976). The study protocol was approved through the Northumbria University Ethical Approval system. All participants provided written informed consent prior to participation.

Study Design

This randomised crossover trial consisted of a familiarisation visit and four one-night experimental trials. The experimental conditions were control (CON), control plus exercise (CE), sleep restriction (SR) and sleep restriction plus exercise (SRE). Each condition involved a single night of either 8 h time in bed from 2300 to 0700 (CON and CE) or 4 h time in bed from 0300 to 0700 (SR and SRE). Four hours sleep has been shown to reduce insulin sensitivity in previous research (Donga et al., 2010). The morning following the control or restricted sleep, participants either rested (CON and SR) or undertook a bout of sprint interval exercise (CE and SRE). All experimental conditions were separated by at least 1 week to prevent carryover effects (van Leeuwen et al., 2010), but no more than 3 weeks.

Throughout the study participants slept at home. Experimental trials were preceded by a one-week entraining period, in which participants were asked to keep a consistent bed and wake time. Wrist actigraphy (GeneActiv, Activinsights Ltd, UK) was used in conjunction with time-stamped text messages to ensure compliance.

Participants sent hourly messages to the researcher between 2300 and 0300 in SR and SRE conditions. Participants were informed which condition they were on the day before each laboratory visit.

Diet was provided for the day preceding each experimental trial and was replicated across conditions. Diet was individualised from food diaries issued during the familiarisation session and total energy, carbohydrates, fat, and protein were kept within 10% of habitual intake. Participants were instructed to eat only the foods provided and to avoid consumption of any caffeine or alcohol, however were permitted to drink water ad libitum. Participants were also asked to refrain from exercise and napping during this time.

Study protocol

Familiarisation

One week prior to the first experimental visit participants attended the laboratory for a screening and familiarisation visit. Upon arrival participants were briefed on the study protocol and given the opportunity to ask questions before completing written informed consent. They completed several screening questionnaires – a physical activity readiness questionnaire, the PSQI (Buysse et al., 1989), and the MEQ (Horne & Ostberg, 1976). Following satisfactory completion of the screening questionnaires, they were issued with a 3-day food diary, actigraphy watch, and 7-day sleep diary.

Body mass and stature were measured using balance scales (SECA, UK) and a free-standing stadiometer (SECA, UK). Stature was measured to the nearest 0.1 cm and body mass to the nearest 0.1 kg.

Participants then underwent an exercise familiarisation, which was a reduced version of the study exercise protocol. The familiarisation exercise bout consisted of a 5-minute warm-up at 70 W on a cycle ergometer (Monark, Sweden), followed by two all-out 30-second sprints against 7.5% of their body mass. Sprints were separated by 4.5 minutes of active recovery at a self-selected pace. A 5-minute cool down was then completed.

Experimental trials

Participants went to bed at 2300 or 0300, depending on the condition. After getting up at 0700, participants arrived at the laboratory by public transport in a rested state at 0800, following a 10-hour overnight fast. Upon arrival, participants either completed a bout of sprint interval exercise (CE and SRE) or rested in a seated position for the equivalent duration (CON and SR). In all conditions participants were given a 30-minute recovery period, resting in a seated position. After the recovery period a 2-hour oral glucose tolerance test (OGTT) was conducted, and blood samples were drawn at regular intervals. The OGTT consisted of 82.5 g dextrose (MyProtein, UK) mixed with 300 ml water, consumed within 5 minutes. During the OGTT participants remained seated in the laboratory but were permitted to complete sedentary tasks. Participants left the laboratory at approximately 1130.

Exercise

The exercise protocol used in this study was based on previous research by Ortega et al (2015) who demonstrated a 142% increase in IVGTT-derived insulin sensitivity measured 30 minutes following sprint interval exercise in healthy males. All exercise was performed on a cycle ergometer (Monark Ergomedic 894E, Sweden), preceded and proceeded with a 5-minute warm-up and cool-down at 70 W.

The sprint interval exercise consisted of four all-out 30-second sprints against 7.5% of body mass, interspersed with 4.5 min of recovery. During the active recovery period, participants cycled at a self-selected pace against a resistance of 1 kg, which was the lowest permitted by the ergometer. Verbal encouragement was given throughout each sprint by the same researcher.

Blood collection and processing

Blood was collected at baseline (0), 15, 30, 45, 60, 90 and 120 minutes during the OGTT using the cannulation technique. For each sample, 8 ml was drawn into a syringe and transferred to a 10 ml serum vacutainer (Becton Dickinson, Sweden). Vacutainers were inverted to ensure thorough mixing. The samples were left to clot at room temperature for 30 minutes before being centrifuged at 3500 rpm at 4°C for 15 minutes. Serum was aliquoted into microtubes. A small amount of serum from each sample was taken up into a capillary tube and placed in microtubes containing 1 ml hemolysing solution (EKF Diagnostics, UK) for determination of glucose concentration. The remaining serum was frozen at -80°C until further analysis.

Serum glucose was measured using the Biosen C-line automatic glucose analyser (EKF Diagnostics, Germany). Serum insulin was measured using commercially available ELISA kits (Mercodia, Sweden), conducted according to manufacturers instructions. Intra- and inter-assay coefficients of variation were 6% and 14%, respectively.

Data analysis

Sample size calculation

Sample size was calculated using G*Power version 3.1.9.2 (Faul et al., 2007). Based on previous work showing a difference of 26.9 mg/dL in 2-hour glucose values during an OGTT between control and exercise conditions (Rynders et al., 2014), 18 participants were required to achieve 90% power.

Statistical analysis

Data are presented as mean ± SD. Area under the curve (AUC) for glucose and insulin were calculated using the trapezoidal rule. AUC was calculated for total (2 hours), early-phase (0-60 minutes) and late-phase (60-120 minutes) during the OGTT. HOMA-IR (Matthews et al., 1985) and Matsuda Index (Matsuda & DeFronzo, 1999) were calculated to estimate insulin resistance and whole-body insulin sensitivity.

Data were analysed using SPSS Statistics version 22 (IBM, UK). Shapiro-Wilk tests were used to check for normality and any data which violated the assumption of normality were transformed using a natural logarithm transformation. Linear mixed modelling was used to compare glucose and insulin between conditions. $P < 0.05$ was used to indicate significance.

Results

Sleep

Average time to bed, wake time, time in bed (TIB), and total sleep time (TST) in each condition are presented in Table 1. Differences in TST were observed between CON and SR (mean difference 142 min; $P < 0.001$), CE and SRE (mean difference 182 min; $P < 0.001$), CE and SRE (mean difference 181 min; $P < 0.001$) and CON

and SRE (mean difference 141 min; $P < 0.001$). No differences were observed between CON and CE ($P = 0.581$) or SR and SRE ($P = 1.000$).

Exercise

Peak power output (PPO) during each of the 30-second sprints is outlined in Table 2. PPO did not differ between conditions ($P = 0.644$), but a difference was observed over time ($P < 0.001$), with PPO significantly higher in the first sprint compared to the third (mean difference 106 W; $P = 0.009$) and fourth (mean difference 118 W; $P = 0.007$), and higher in the second compared to the fourth (mean difference 60 W; $P = 0.041$). Total work done was similar between conditions (63666 ± 12029 J in CE and 65045 ± 11294 J in SRE; $P = 0.340$).

Glucose and insulin

Glucose and insulin concentrations during the OGTT are displayed in Figure 1. Total, early- and late-phase AUC for glucose and insulin are shown in Figure 2.

Glucose concentrations did not show evidence of an effect of condition ($P = 0.216$) or interaction effect ($P = 0.146$). However, there was a significant effect of time during the OGTT ($P < 0.001$). No significant difference between conditions was found for peak glucose ($P = 0.158$). There was no evidence of an effect of condition ($P = 0.207$) for total glucose AUC. Likewise, late- and early-phase glucose AUC did not show any significant differences between conditions ($P = 0.264$ and $P = 0.122$, respectively).

Insulin concentrations demonstrated an overall difference between conditions ($P = 0.019$), time points ($P < 0.001$), and an interaction between conditions and time points ($P = 0.014$). Post-hoc analyses on these overall differences revealed significantly higher insulin concentrations in SR compared to CON ($P = 0.022$), with SR showing higher concentrations than CON at 30 min (40.04 ± 23.17 and 25.79 ± 13.94 μIU/ml; $P = 0.004$). Insulin concentrations were higher in SR compared to SRE at 60 minutes (31.40 ± 13.99 and 21.03 ± 10.67 μIU/ml; $P = 0.042$), 90 minutes (19.47 ± 11.11 and 15.52 ± 15.63 μIU/ml; $P = 0.002$), and 120 minutes (14.12 ± 14.84 and 5.99 ± 5.27 μIU/ml; $P = 0.003$). Total insulin AUC displayed a trend for an effect of condition ($P = 0.075$), with SR tending to be higher than CON ($P = 0.064$). Early- and late-phase insulin AUC also showed significant main effects of condition ($P = 0.010$ and $P < 0.001$, respectively). The early-phase insulin AUC was higher in SR than CON (1472 ± 811 and 2044 ± 1129; $P = 0.048$). Late-phase displayed a lower AUC in SRE than SR (1267 ± 558 and 862 ± 589; $P = 0.004$).

HOMA-IR showed a main effect of condition ($P = 0.019$), with SR higher than CON (0.87 ± 0.99 and 1.64 ± 2.60; $P = 0.029$). Matsuda index was significantly different between conditions ($P = 0.003$), with SR displaying a lower index than CON (25.31 ± 20.80 and 12.11 ± 6.38; $P = 0.020$).

Discussion

The present study demonstrated that one night of sleep restriction impaired insulin sensitivity, and that performing sprint interval exercise after sleep restriction may improve the late post-prandial response.

Participants displayed reduced insulin sensitivity after sleep restriction, indicated by increased insulin concentrations during the OGTT and decreased Matsuda index in SR compared to CON. This is consistent with previous research that has demonstrated reduced whole-body insulin sensitivity after a single night (Donga et al., 2010), and multiple nights of sleep restriction (Sweeney et al., 2017; Wang et al., 2016). Sleep restriction may impair whole-body insulin sensitivity through alteration of peripheral insulin signalling, with five nights of sleep restriction reducing peripheral, but not hepatic, insulin sensitivity (Rao et al., 2015). Furthermore, sleep restriction reduces Akt phosphorylation, which plays a key role in the insulin signalling pathway in peripheral tissues (Broussard et al., 2012).

We hypothesised that a bout of sprint interval exercise would attenuate the impairment in insulin sensitivity in sleep-restricted individuals. Whilst total insulin AUC was not significantly altered after the exercise bout, our findings suggest that there was an alteration to the late post-prandial response when comparing the sleep restricted conditions. However, we did not observe an improvement in insulin or glucose profiles after exercise in the early-phase of the OGTT. Our findings are in contrast to Ortega and colleagues (2015) who demonstrated improved insulin and glucose 60 minutes following high intensity exercise. Methodological differences may explain this discrepancy, as an IVGTT was employed by Ortega and colleagues, whereas we used an OGTT. Gastric emptying plays a role during the OGTT whereas this is bypassed when glucose is injected rather than ingested orally. Gastric emptying rate may be slowed by intermittent high intensity exercise (Leiper et al., 2001), delaying the absorption of the glucose drink and therefore findings may not be comparable between an OGTT and IVGTT. Alternatively, it may be possible that sleep restriction alters the metabolic response to exercise.

Our findings reflect those by Rynders and colleagues (2014), who noted improvements in the late rather than total postprandial response after a bout of exercise. High intensity exercise is known to temporarily increase glucose due to gluconeogenesis and possible carbohydrate sparing for glycogen repletion (Marliss et al., 1992). It may be possible that as glucose regulation was measured 30 minutes after cessation of exercise, the temporary alterations in glucose regulation which occur during exercise were still influencing our measurements. Additionally, although not statistically significant, it appears that insulin concentrations in the early-phase of the OGTT were increased after exercise compared to rest in the current study. An increase in early-phase insulin secretion may influence late-phase responses, potentially through suppression of endogenous glucose production (Del Prato et al., 2002). This early-phase response may therefore contribute to the lower late-phase insulin AUC which was observed in SRE compared to SR. The late-phase of the OGTT has been shown to predict incident diabetes independent of the early-phase (Lorenzo et al., 2012), suggesting that despite no overall change, a decrease in late-phase insulin AUC may be beneficial.

Whilst the present study suggests there is potential for exercise to positively influence the post-prandial response to an OGTT in sleep-restricted individuals, it was not designed to identify the possible mechanisms. Previous research has suggested that insulin action is improved after exercise through altered phosphorylation of components of the insulin signalling pathway in peripheral tissues (Wojtaszewski & Richter, 2006). As sleep restriction is thought to negatively impact peripheral insulin sensitivity (Broussard et al., 2012; Rao et al., 2015), it can be speculated that exercise improves insulin sensitivity in sleep-restricted individuals through alteration of insulin action in peripheral tissues.

The present study has some limitations which should be noted. Firstly, the study population consisted only of healthy males, meaning it may not be feasible to extrapolate the findings to other populations including females and individuals with metabolic abnormalities. Secondly, an OGTT does not enable measurement of metabolic characteristics such glucose disposal and uptake, so gives limited information regarding the decrease in insulin sensitivity.

In summary, sprint interval exercise may offer some potential to attenuate the impairments in insulin sensitivity following a single night of reduced sleep. This may have implications for individuals facing sleep curtailment. To our knowledge, this is the first study to explore the effect of acute exercise on insulin sensitivity following sleep restriction. Therefore, future research may investigate whether exercise modality, intensity, duration, or timing influences the change in glucose regulation in sleep-restricted individuals. It would also be beneficial to investigate the time course of improved glucose regulation to determine whether the benefit of exercise persists for multiple hours as is the case in non-sleep-restricted individuals.

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Author contributions

The study was designed by ELS, IHW, DP and JGE; data were collected and analysed by ELS, IK, TH, and IHW; data interpretation and preparation of manuscript were conducted by ELS, IHW, and DP; all authors provided comments on the manuscript and approved the final version.

Conflicts of interest

The authors have no conflicts of interest to declare.

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Table 1. Sleep variables for each experimental condition.

	CON	**CE**	**SR**	**SRE**
Bed time (hhmm)	2303	2306	0305	0255
Wake time (hhmm)	0658	0659	0700	0657
TIB (min)	472 ± 27	472 ± 22	236 ± 19	244 ± 15
TST (min)	337 ± 95	377 ± 61	195 ± 43*†	196 ± 37*†

Bed time, wake time, time in bed (TIB) and total sleep time (TST) in control (CON), control plus exercise (CE), sleep restriction (SR) and sleep restriction plus exercise (SRE) condition. Data are mean ± SD. * indicates difference from CON ($P < 0.05$). † indicates difference from CE ($P < 0.05$).

Table 2. Peak power output and total work done during sprints.

	PPO (W)		Total work (J)	
	CE	**SRE**	**CE**	**SRE**
1	857 ± 189	853 ± 187	18356 ± 2903	18355 ± 3471
2	818 ± 177	804 ± 160	16551 ± 2921	16580 ± 3139
3	762 ± 194	736 ± 172	15488 ± 2842	15424 ± 3237
4	733 ± 179	746 ± 144	14686 ± 2408	15502 ± 2490

Peak power output (PPO) and total work during 4 all-out 30 s sprints performed the morning after a night of 8 h (CE) or 4 h (SRE) time in bed. No significant differences were observed between conditions. Data are presented as mean ± SD.

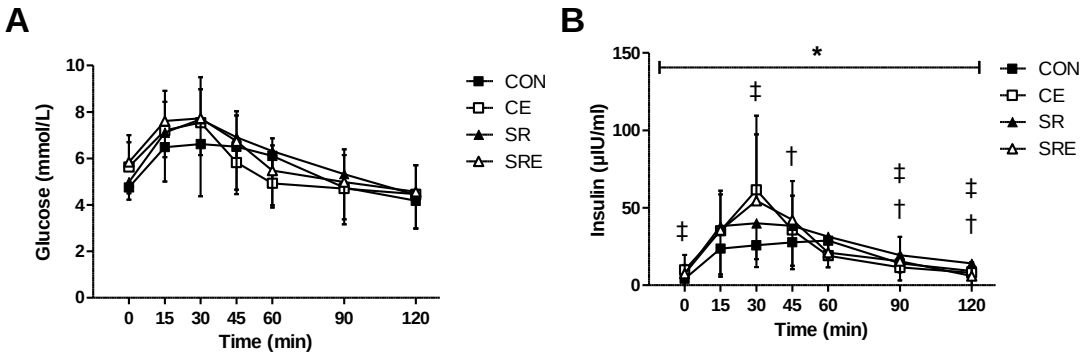

Figure 2. Glucose (A) and insulin (B) concentrations during the OGTT. $P < 0.05$ indicates significance. * indicates main effect of condition, with SR higher than CON. ‡ indicates difference between CON and SR. † indicates difference between SR and SRE.

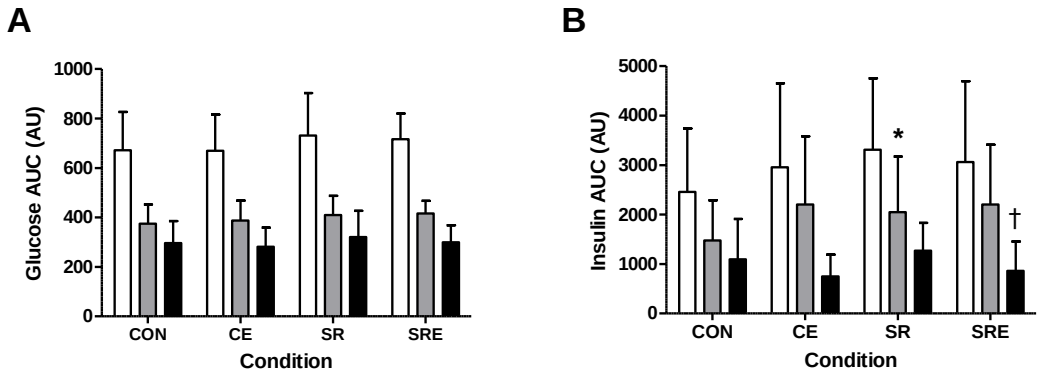

Figure 3. Total (white bars), early (grey shaded bars) and late (black bars) phase AUC for glucose (A) and insulin (B). Data are presented as mean ± SEM. Main effect of condition for early and late AUC. * indicates significant difference (P <0.05) compared to CON in early phase. † indicates significant difference (P <0.05) compared to SR in late phase.

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