



An investigation into skeletal muscle insulin signalling and systemic inflammation as potential mechanisms responsible for the impairment in glucose regulation following acute sleep restriction in healthy males

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Abstract

Sleep restriction is associated with impaired glucose regulation, which is a risk factor for developing type 2 diabetes. However, the underlying mechanisms leading to this impairment are unknown. This thesis aims to examine the effects of partial sleep restriction on whole body metabolism, and investigate whether the impairment in insulin sensitivity observed after sleep restriction is coupled with changes in systemic inflammation and skeletal muscle insulin signalling.

A pilot study was carried out to assess whether or not two nights of partial sleep restriction would alter glucose regulation and substrate utilisation. 10 healthy males then participated in a separate randomised crossover study involving two nights of habitual sleep (control) and two nights of 50% of habitual sleep (sleep restriction). An oral glucose tolerance test was carried out after the second night of each condition to assess whole body glucose tolerance, insulin sensitivity and inflammation, and to examine skeletal muscle insulin signalling.

The pilot study findings confirmed that two nights of partial sleep restriction impaired glucose tolerance. Findings from the main study revealed no effect of trial on glucose tolerance ($P = .222$). Insulin sensitivity estimated by the Matsuda Index was 18.6% lower in the sleep restriction condition ($P = .010$). CRP and TNF α were similar between trials ($P > 0.05$). Fold change in PKB activity from baseline tended to be lower following sleep restriction at 30 min ($P = .098$) and 120 min ($P = .087$). AUC for insulin to PKB index was significantly higher in the sleep restriction condition ($P = .012$). Phosphorylated to total PKB was similar between conditions ($P = .217$).

Two nights of sleep restriction decreased insulin sensitivity in healthy males. This impairment was not coupled by increased systemic inflammation. Skeletal muscle insulin signalling showed conflicting findings, suggesting a possible disruption of skeletal muscle insulin signalling.

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Chapter 1

Introduction

In modern society many individuals often voluntarily curtail sleep due to increasing work or lifestyle demands (Luyster et al., 2012). Despite a recommended sleep duration of 7 to 9 hours each night, a recent report by The Sleep Council (2013) revealed that 70% of adults in the United Kingdom sleep for less than 7 hours per night, with an increasing number sleeping for only 5 to 6 hours each night. The reduction in sleep duration over the past few decades has been paralleled with an increased prevalence of type 2 diabetes and impaired glucose tolerance. This raises the possibility that there may be a connection between sleep duration and risk of developing diabetes. Indeed, research has shown that reduced sleep is associated with an increased risk of type 2 diabetes (Yaggi et al., 2006), which is a disorder characterised by insulin resistance and impaired glucose control. Both epidemiological and experimental studies (Knutson et al., 2007) provide evidence for impaired glucose control and insulin sensitivity after a period of sleep restriction. These data support the notion that there is a link between decreased sleep duration and an increased risk of impaired metabolism.

However, little is known about the underlying mechanisms. One plausible mechanism could be a dysfunction of glucose uptake in the peripheral tissues. This thesis is supported by evidence demonstrating decreased peripheral insulin sensitivity after sleep loss (Donga et al., 2010; Rao et al., 2015). Adipose tissue signalling has previously been shown to be affected by sleep restriction (Broussard et al., 2012). However, there are currently no published studies which examine glucose regulation in skeletal muscle after sleep restriction. Skeletal muscle accounts for approximately 80% of glucose uptake under insulin-stimulated conditions (Thiebaud et al., 1982), suggesting that it may be a key target for impaired glucose regulation following sleep loss. Therefore, research

investigating the responsiveness of insulin signalling pathways within skeletal muscle after sleep restriction is warranted.

The mechanisms responsible for impaired peripheral insulin sensitivity are unclear, however a number of factors have been proposed including alterations in autonomic function, hormonal profiles, and systemic inflammation (Mullington et al., 2009). Research shows increased levels of inflammation after sleep restriction (Vgontzas et al., 2004). Increased inflammation can induce insulin resistance (Shoelson et al., 2006), so it can be hypothesised that an increase in inflammation may also contribute to impaired insulin sensitivity following sleep restriction.

The general aim of this thesis is to explore the impairment of glucose regulation brought about by voluntary sleep curtailment. Specifically, the aims of the current project are to assess the effects of partial sleep restriction on:

- (a) whole-body glucose tolerance and insulin sensitivity,
- (b) skeletal muscle insulin signalling, and
- (c) systemic inflammation.

It is hypothesised that sleep restriction will lead to impaired whole body glucose tolerance and insulin sensitivity, and that this will be coupled with changes in skeletal muscle insulin signalling and an increase in systemic inflammation.

Chapter 2

Literature Review

2.1 – Overview of sleep

2.1.1 – The function of sleep

Originally believed to be of primary importance for brain function and memory, more recent research has demonstrated an important role of sleep in many other aspects of physiological health including energy conservation, immune function, appetite, and metabolism (Berger and Phillips, 1995; Irwin, 2002; Killick et al., 2012). During sleep, physiological changes occur in several different systems including cardiovascular, respiratory and endocrine changes. These changes alter many processes within the body, including cytokine expression and glucose metabolism.

Sleep deprivation studies have demonstrated that altering the normal sleep-wake pattern disrupts the diurnal pattern of immune function (Dickstein and Moldofsky, 1999), which may interfere with host defence mechanisms. Similarly, disruptions in glucose metabolism have been demonstrated when sleep is altered (Morselli et al., 2010). Taken together, it is apparent that sleep has a vital role in many biological processes and is not solely for cognitive function, as was thought previously.

2.1.2 – Sleep architecture

A period of sleep consists of cycles which tend to last approximately 70 to 90 minutes each (Feinberg and Floyd, 1979). Sleep cycles are comprised of rapid eye movement (REM) sleep and non-rapid eye movement (NREM) sleep. NREM sleep is subdivided into four stages – N1, N2, N3 and N4. N1 sleep occurs at the onset of sleep, followed by N2, N3, N4, and then REM sleep (Carskadon and Dement, 2011). These stages repeat for approximately 4 to 6 cycles throughout the night, depending on the total sleep time (Reutrakul and Van Cauter, 2014). During each cycle the percentage of each stage differs, with NREM sleep being predominant during the earlier cycles and decreasing as sleep progresses (Sharma and Kavuru,

2010). The deepest stage of sleep, N3, is often referred to as slow wave sleep and tends to decline as an individual ages (Van Cauter et al., 2000).

2.1.3 – Methods of measuring sleep duration

Several methods of measuring sleep variables exist including objective measures such as Polysomnography and actigraphy as well as subjective measures such as sleep diaries. Polysomnography (PSG) is considered the gold standard measurement method. PSG assesses a number of physiological parameters including brain activity and bodily movements to determine sleep architecture (Marino et al., 2013). Such measurements yield a great amount of detail throughout the sleep period including duration of individual sleep stages and total sleep time amongst others. Whilst PSG can provide an extremely detailed evaluation of sleep, it often requires the individual to sleep in a sleep laboratory so is less applicable to longitudinal or field based studies. Sleeping in an unfamiliar environment, such as a sleep laboratory, may disrupt sleep and may not necessarily reflect the sleep quality achieved in the home environment.

Other methods of evaluating sleep have been developed which are more practical for use in field and longitudinal studies. Actigraphy is a commonly used objective measurement of sleep which has been shown to have an accuracy and sensitivity in excess of 80% and 90%, respectively, when compared to polysomnography (Marino et al., 2013; Slater et al., 2015). Total sleep time estimated from actigraphy is also strongly correlated with PSG ($r = 0.79$ to $r = 0.94$) (Jean-Louis et al., 2001). Actigraphy involves estimation of sleep parameters from tri-axial movement. Although sleep architecture such as individual sleep stages cannot be determined using actigraphy; this method provides a reliable, objective measurement of sleep variables and may be an appropriate method for measuring sleep in settings where polysomnography is not feasible.

Sleep diaries are often used as a subjective measure of sleep either as a stand-alone measurement or in conjunction with an objective measurement. The completion of a sleep diary can allow estimation of quality of sleep as well as

estimating periods of wakefulness during the night, which may not always be detected by actigraphy. Self-reported measures of sleep contain an inherent risk of under- or over-reporting, whether purposely or not. Thus, using an objective measure, such as actigraphy, in combination with a sleep diary may address the limitations of each method. Actigraphy and sleep diaries can therefore provide a useful overview of sleep characteristics in studies where there is no access to PSG.

2.2 – Glucose regulation during sleep

Glucose regulation exhibits diurnal variations due to circadian rhythmicity (Van Cauter et al., 1997). Typically, glucose tolerance decreases as the day progresses, reaching a minimum during the first half of the night. However, differences in glucose tolerance also occur during sleep when compared to a state of wakefulness (Van Cauter et al., 1997). Glucose concentrations and insulin secretion rates have been observed to increase during sleep in the daytime as well as at night, indicating altered glucose regulation during sleep, independent of time of day (Van Cauter et al., 1991). During sleep, systemic glucose utilisation is reduced, primarily due to a decrease in brain glucose metabolism (Boyle et al., 1994). Therefore, when taken together, it is apparent that sleep is important for many aspects of glucose regulation, thus any alterations in sleep characteristics are likely to impact normal glucose regulation.

2.3 – Sleep duration and disease – Epidemiological studies

Epidemiological data has shown an association between both short and long sleep duration and an increase in mortality (Cappuccio et al., 2010). Compared to individuals achieving 7 to 9 hours of sleep, short sleepers have a 12% increased risk of early all-cause mortality (Cappuccio et al., 2010). This increased risk appears to be consistent across a range of populations including younger and

older adults, as well as individuals from a variety of geographical locations and ethnic backgrounds.

In addition to an increased risk of mortality, individuals with short sleep durations may have a higher risk of developing chronic diseases. The risk of developing cardiovascular disease is increased in individuals habitually sleeping less than 7 hours (Sabanayagam and Shankar, 2010). The odds of developing cardiovascular disease were increased when comparing six hours to seven hours of sleep, and increased even further for sleep durations of five hours or less.

Similarly, short sleep durations are also linked to an increased risk of metabolic diseases (Yaggi et al., 2006; Gottlieb et al., 2005; Gangwisch et al., 2007).

Epidemiological data have established a link between short sleep duration and increased risk of type 2 diabetes. The risk of developing type 2 diabetes has been shown to be more than doubled in those sleeping 5 hours or less compared to 7 hours, even when confounding variables were accounted for (Yaggi et al., 2006; Mallon et al., 2005). Interestingly, the increased risk appears to be sex specific. Several studies which have included only females, or which have categorised results by sex, have found that the risk of developing diabetes does not appear to be increased by short sleep durations in females (Ayas et al., 2003; Mallon et al., 2005). Why an increased risk occurs in males but not females remains unknown. Further research investigating the effects of short sleep specifically in females is warranted.

These data demonstrating an increased risk of developing type 2 diabetes in short sleep durations are consistent with laboratory studies which have repeatedly shown a relationship between sleep restriction and impaired metabolism. The increased risk of developing type 2 diabetes, an illness characterised by metabolic dysfunction leading to resistance to the actions of insulin, may cause a disruption to glucose homeostasis, and subsequently, lead to a myriad of health complications.

2.4 – Acute sleep restriction and impaired glucose clearance

Upon ingestion, glucose enters the bloodstream and must be taken up by cells in order to be utilised or stored for future use. However, the rate of glucose clearance can be impacted by sleep restriction. Six nights of 4 hours sleep was sufficient to decrease glucose clearance by almost 40% during an intravenous glucose tolerance test (IVGTT) in healthy males (Spiegel et al., 1999). Similarly, a decrease in glucose disposal during a hyperinsulinemic-euglycemic clamp has been demonstrated after just a single night of sleep restriction (Donga et al., 2010). A decreased rate of glucose disposal is suggestive of disrupted peripheral insulin sensitivity, and may also affect energy metabolism and substrate use.

2.5 – The relationship between sleep and resting metabolic rate and respiratory exchange ratio

Energy conservation is a primary function of sleep (Berger and Phillips, 1995). Resting energy expenditure (REE) is lower during sleep than wakefulness (Jung et al., 2011). During a period of sleep deprivation, 24-hour energy expenditure is increased as well as 8-hour energy expenditure during the night of sleep deprivation compared to a period of normal sleep (Jung et al., 2011). Despite studies showing changes in resting energy expenditure during the night of sleep restriction, conflicting findings have been demonstrated with regards to energy expenditure in the morning following sleep restriction. Several studies have measured resting energy expenditure in the morning following sleep loss ranging from three nights to two weeks, and observed no differences compared to control or baseline sleep (Nedeltcheva et al., 2009; Buxton et al., 2010; St-Onge et al., 2011; Shechter et al., 2014; Rao et al., 2015). One study observed a reduction in REE in the morning (Benedict et al., 2011), however they utilised total sleep deprivation, suggesting that REE may be affected only by total rather than partial sleep restriction.

Sleep may also impact metabolism by altering substrate utilisation. At present, studies investigating substrate utilisation after sleep loss are limited, particularly

in the fasted state. In those studies that have, two have observed a decrease in respiratory exchange ratio (RER) following partial sleep restriction (Klingenberg et al., 2012; Shechter et al., 2014), and one has reported no difference (St-Onge et al., 2011). In normal glucose tolerant subjects, plasma glucose concentrations are usually related to glucose utilisation, with a higher plasma glucose concentration leading to a higher rate of glucose utilisation (Doberne et al., 1982). This concept would suggest that, in theory, if sleep restriction increases plasma glucose concentration, carbohydrate oxidation should also increase. The mixed findings regarding RER and substrate oxidation in these studies suggest that this may not always be the case, however additional research is warranted.

2.6 – Impact of sleep restriction on insulin and glucose metabolism

2.6.1 – Methods of measuring glucose tolerance and insulin sensitivity

Research investigating glucose tolerance and insulin sensitivity typically uses one or more of the following techniques: hyperinsulinemic-euglycemic clamp, intravenous glucose tolerance test (IVGTT), or oral glucose tolerance test (OGTT). The method chosen depends on factors including the type of study, setting, and variables of interest.

The hyperinsulinemic-euglycemic clamp is considered the gold standard technique for measuring insulin sensitivity (DeFronzo et al., 1979). This technique involves intravenous infusion of insulin and glucose to maintain a euglycemic state. Insulin sensitivity can be determined according to the rate of glucose infusion, and labelled glucose may be used to determine glucose regulation in specific tissues. Although the hyperinsulinemic-euglycemic clamp provides accurate measurements of insulin sensitivity in vivo, it can be expensive and impractical, therefore more feasible indirect methods of measuring insulin sensitivity and glucose tolerance have been developed.

An IVGTT uses the minimal model to estimate insulin sensitivity from blood samples obtained at regular intervals following an intravenous glucose bolus

(Muniyappa et al., 2008). Insulin sensitivity can be calculated based on the rate of glucose disappearance. The minimal model IVGTT approach has the advantage of using dynamic rather than steady-state data and can give measures of glucose effectiveness and beta-cell function in addition to insulin sensitivity (Muniyappa et al., 2008). Although the IVGTT technique is more feasible than the clamp technique, it still requires many blood samples and an intravenous infusion, making it impractical and time-consuming in some circumstances. Other methods, such as the OGTT, are less complex and have better ecological validity than the IVGTT.

The OGTT is a simpler test which can be used to estimate glucose tolerance and insulin sensitivity. A glucose drink is consumed and blood samples are drawn before and at regular intervals after the drink to determine measures of glucose tolerance and insulin sensitivity. The OGTT has been compared to the gold standard hyperinsulinemic-euglycemic clamp and has been shown to be a suitable estimate of glucose tolerance and insulin sensitivity (Matsuda and DeFronzo, 1999). As the OGTT is designed to assess glucose clearance, indexes have been developed to allow estimation of insulin sensitivity from this technique. The Matsuda Index (Matsuda and DeFronzo, 1999) and homeostatic model of assessment (HOMA) (Matthews et al., 1985) are widely used indices which can estimate insulin sensitivity. To calculate these indices the following equations are used:

$$\text{Matsuda index} = 10000 / \sqrt{(\text{fasting plasma glucose} \times \text{fasting plasma insulin}) \times (\text{mean glucose during OGTT} \times \text{mean insulin during OGTT})}$$

$$\text{HOMA} = (\text{fasting glucose} \times \text{fasting insulin}) / 22.5$$

The Matsuda index tends to reflect changes at the whole-body level including both peripheral and hepatic insulin sensitivity whereas the HOMA uses only fasting glucose and insulin values and therefore primarily reflects hepatic insulin sensitivity (Abdul-Ghani et al., 2007).

Therefore, in studies requiring measurement of whole body insulin sensitivity, the Matsuda index provides a good estimate when the hyperinsulinemic-euglycemic clamp is impractical.

2.6.2 – Sleep restriction and glucose tolerance

Spiegel and colleagues (1999) conducted the seminal study in the field, identifying an impairment in glucose metabolism following partial sleep restriction. Healthy young men were restricted to 4 hours sleep per night for 6 nights during a sleep restricted condition, which was followed by a recovery condition of 12 hours in bed for 6 nights. When sleep restricted, individuals displayed decreased rates of glucose clearance and decreased glucose effectiveness during an IVGTT and an increased glucose response to a breakfast meal despite similar insulin concentrations. Spiegel et al. (1999) noted that the glucose tolerance values seen in the sleep restriction condition in their study reflected values which were typical of older adults or individuals with impaired glucose tolerance. This study clearly demonstrates an impairment in glucose tolerance in the sleep restriction condition. However, the applicability of the results in more ecologically valid conditions could be questioned due to the comparison of the sleep restriction condition to a sleep recovery condition of 12 hours. Six nights of twelve hours sleep is likely unrealistic due to work and lifestyle demands. A comparison of sleep restriction to a sleep duration closer to the recommended duration of 7 to 8 hours would have been more ecologically valid.

More recent studies have also investigated glucose metabolism following sleep restriction. Impairments in glucose tolerance have been observed in several of these studies (Nedeltcheva et al., 2009; Buxton et al., 2010; Donga et al., 2010; Reynolds et al., 2012). However, there are also studies in which glucose tolerance was unchanged following sleep restriction (Bosy-Westphal et al., 2008; Klingenberg et al., 2013; Wang et al., 2016). One notable difference between these studies showing conflicting results is the number of nights of sleep restriction. Those studies which found changes in blood glucose concentrations restricted sleep for at least 5 nights, whereas the studies which did not observe

any differences used less than 5 nights of sleep restriction. It can therefore be speculated that there may be a minimum number of nights or hours of sleep restriction that is required to impair glucose tolerance. Despite this, Donga and colleagues (2010), observed changes in glucose clearance after a single night of sleep restriction. It is possible that the hyperinsulinemic-euglycemic clamp used for measurement of glucose tolerance is more sensitive and may detect subtle changes when compared to OGTT and IVGTT. It is therefore possible that the method of measurement may have impacted the results, however to confirm this further research is needed.

Another possibility as to why blood glucose concentrations remain unchanged in some studies may be due to an increased production of insulin. Glucose is tightly regulated, and often, additional insulin is produced in order to maintain homeostasis (DeFronzo and Tripathy, 2009). In many cases despite no obvious changes in glucose concentrations, an impairment in glucose metabolism may still be present and could be determined by measuring plasma insulin concentrations. This is demonstrated by Wang and colleagues (2016) who found no changes in plasma glucose concentrations during the OGTT following sleep restriction, however area under the curve for plasma insulin was higher, indicating an impairment in glucose metabolism.

2.6.3 – Sleep restriction and insulin sensitivity

Reduced insulin sensitivity is consistently found following sleep restriction (Nedeltcheva et al., 2009; Buxton et al., 2010; Donga et al., 2010; Broussard et al., 2012; Klingenberg et al., 2013; Rao et al., 2015; Wang et al., 2016). This finding is evident across a range of different methodologies, including IVGTT derived insulin sensitivity (Nedeltcheva et al., 2009; Buxton et al., 2010; Broussard et al., 2012), insulin sensitivity assessed by hyperinsulinemic euglycemic clamp (Donga et al., 2010; Rao et al., 2015) and insulin sensitivity determined by the Matsuda index following an OGTT (Klingenberg et al., 2013; Wang et al., 2016). However, the varying severities of sleep restriction and methods of measurement make direct comparisons difficult. Nevertheless, the mutual finding of reduced insulin sensitivity suggests that glucose homeostasis is

sensitive to even mild reductions in sleep duration, for example 1 to 3 hours of restriction each night (Wang et al., 2016).

Hepatic and peripheral insulin sensitivity are both affected by sleep restriction. A single night of 4.5 hours sleep restriction can reduce hepatic and peripheral insulin sensitivity by 22% and 20%, respectively (Donga et al., 2010). Recent research supports the reduction in peripheral insulin sensitivity (Broussard et al., 2012; Rao et al., 2015), however conflicting results have been observed regarding hepatic insulin sensitivity (Rao et al., 2015). It is unclear why these conflicting findings exist, although, as discussed previously, the severity of sleep restriction may be a contributing factor. The underlying mechanisms leading to a reduction in insulin sensitivity in the studies are unknown, so it can be speculated that these mechanisms differ depending on the severity of sleep restriction. Further study is therefore warranted to investigate the causes of insulin resistance following both acute and chronic sleep loss.

2.7 – Potential mechanisms underlying impaired glucose metabolism following sleep restriction

It is clear that acute sleep restriction leads to changes in glucose regulation which may have the potential to increase the risk of type 2 diabetes in individuals with chronic sleep loss. Before any interventions to prevent the increased risk of developing impaired glucose regulation can be developed, it is first necessary to identify the mechanisms which link sleep loss to these changes in metabolism. However, at present our understanding of these mechanisms remains elusive. Several theories as to why glucose metabolism is altered following sleep restriction have been proposed, including changes in autonomic function and hormone secretion (Mullington et al., 2009), increased inflammation (Mullington et al., 2010), and development of peripheral insulin resistance (Broussard et al., 2012; Rao et al., 2015). The underlying mechanisms are likely multifactorial and thus both of these proposed theories may be involved. It can be speculated that the mechanism of impairment could differ

depending on factors such as gender, age and even sleep restriction protocol. However, studies addressing the underlying mechanisms are limited, and in those that have findings are inconsistent, particularly regarding inflammation.

2.7.1 – Insulin signalling in peripheral tissues following sleep restriction

In the postprandial state the majority of glucose disposal occurs in the peripheral tissues (Woerle et al., 2003), therefore it seems sensible to hypothesise that an impairment in insulin sensitivity in peripheral tissues may be influential in the impaired whole body regulation following sleep restriction. Indeed, several laboratory studies have shown peripheral insulin resistance after sleep loss (Donga et al., 2010, Rao et al., 2015). Insulin signalling in adipocytes was investigated following four nights of partial sleep restriction in healthy individuals (Broussard et al., 2012). In addition to reduced whole body insulin sensitivity, they demonstrated an impairment in insulin signalling, manifested by a reduction in phosphorylation of protein kinase B (PKB).

In adipose and skeletal muscle tissue cells, PKB is key to the insulin signalling pathway. When insulin binds to the insulin receptors on the cell membrane, the insulin signalling pathway begins, ultimately leading to translocation of GLUT4 to the cell membrane (Zhang and Liu, 2014). This translocation of GLUT4 to the membrane facilitates glucose uptake into the cell. PKB is a key mediator in this pathway, therefore these data suggest that an impairment in insulin signalling in adipose tissue may contribute to the impairment in whole body glucose regulation.

Whilst adipose tissue is an important component of glucose control, it is likely that other peripheral tissues, such as skeletal muscle, may also be involved. Given that skeletal muscle is responsible for up to 80% of glucose uptake under insulin-stimulated conditions (Thiebaud et al., 1982), it can be theorised that a defect in skeletal muscle insulin signalling may promote impaired whole body glucose control following sleep loss. Peripheral insulin resistance, specifically in skeletal muscle, is thought to be the initial defect in the development of type 2 diabetes (DeFronzo and Tripathy, 2009). If sleep restriction leads to skeletal

muscle insulin resistance, this may explain the reduction in insulin sensitivity in acute sleep restriction studies; and may also explain the higher risk of type 2 diabetes which is shown in individuals who display chronic short sleep characteristics. At present there are no published studies investigating skeletal muscle insulin signalling after acute sleep restriction.

2.7.2 – Inflammation, insulin resistance, and sleep restriction

Chronic low-level inflammation is often present in individuals with insulin resistance (Shoelson et al., 2006). Sleep restriction has the potential to increase inflammatory processes (Irwin, 2002), which may contribute to the decrease in insulin sensitivity observed following sleep loss. A number of inflammatory markers that are associated with sleep restriction include interleukin-6 (IL-6), tumour necrosis factor alpha (TNF- α), and C-reactive protein (CRP) (Meier-Ewert et al., 2004; Vgontzas et al., 2004). Additionally, activation of the inflammatory regulator, NF- κ B is higher in peripheral blood mononuclear cells following a single night of sleep restriction (Irwin et al., 2008).

Increased inflammation may lead to insulin resistance through a variety of mechanisms. TNF-alpha can increase serine phosphorylation of IRS-1, which reduces tyrosine phosphorylation and disrupts insulin signalling, thus promoting insulin resistance (Kanety et al., 1995). IL-6, another inflammatory marker which has been observed to increase after sleep restriction, can impact insulin signalling by also targeting IRS-1 (Chen et al., 2015). In addition, GLUT4 expression is reduced by IL-6, which will result in decreased glucose uptake into cells (Chen et al., 2015). Both TNF- α and IL-6 can regulate CRP (Castell et al., 1990), suggesting that the higher concentrations of CRP following sleep restriction may be a result of increases in other inflammatory markers. Levels of NF- κ B activation are also related to the presence of other inflammatory markers. Often, the activation of the NF- κ B pathway is regulated by TNF- α (Lawrence, 2009). Hence, the increase in TNF- α observed after sleep restriction may be responsible for the heightened activation of NF- κ B.

Since sleep restriction leads to increased inflammation, it is possible that inflammation promotes insulin resistance through a disturbance in insulin signalling. However, there is a lack of studies which have measured glucose metabolism and levels of inflammation in response to sleep restriction in the same study. So whilst we can hypothesise that there is a link, we cannot be certain until more research has been carried out.

2.7.3 – Stress hormones and sleep restriction

Another mechanism which has been proposed to contribute to impaired glucose metabolism following sleep restriction is an alteration in the secretion of the stress hormone cortisol. Evening cortisol concentrations are often increased following sleep restriction (Spiegel et al., 1999; Buxton et al., 2010; Reynolds et al., 2012). However, many studies have demonstrated no differences in morning and 24 hr cortisol between sleep restriction and control conditions (Vgontzas et al., 2004; Donga et al., 2010; Leproult et al., 2011; Schmid et al., 2011). It can therefore be suggested that increased cortisol may not be one of the primary underlying mechanisms leading to the impairment in glucose regulation following sleep restriction.

2.8 – Conclusions

This literature review covers many important aspects relating to sleep restriction and glucose metabolism. Whilst the wide variety of protocols make comparisons between studies difficult, there is a clear consensus that sleep is vital in glucose homeostasis, and that insufficient sleep can induce a wide variety of changes relating to glucose metabolism. However, whilst these ideas are consistent among the literature, the review raises several questions which need to be addressed to obtain a more complete understanding of the impact of sleep on glucose metabolism.

Arguably the most important question raised is what is causing the impairment in glucose metabolism following sleep restriction? Whilst several underlying mechanisms are beginning to be investigated, there is still much to uncover in

this area. Inflammation and peripheral insulin signalling appear likely to be involved, but evidence is lacking.

As an impairment in glucose metabolism increases the risk of developing type 2 diabetes, research to investigate the underlying mechanisms is warranted to gain a better understanding of why sleep restriction impacts glucose metabolism. This will then aid the development of interventions to alleviate the negative impacts of short sleep on glucose metabolism, and prevent an increase in the risk of type 2 diabetes.

The studies in this thesis are designed to further explore the relationship between sleep restriction and impaired glucose regulation and, more specifically, investigate whether the impairment in glucose regulation observed after sleep restriction is accompanied by changes in systemic inflammation and skeletal muscle insulin signalling. Two nights of sleep restriction are compared to two nights of control sleep in healthy individuals. Whole body glucose tolerance, insulin sensitivity, and systemic inflammation are measured from blood samples obtained during an OGTT following the second night of each condition. Collection of muscle tissue samples also allows skeletal muscle insulin signalling to be investigated. The findings of these studies will aid in understanding the underlying mechanisms and give scope for further work in the area.

Chapter 3

Acute sleep restriction reduces glucose tolerance and alters fasted substrate metabolism in healthy males: a pilot study

3.1 – Introduction

Short sleep is linked to a disruption in glucose metabolism (Knutson et al., 2007). Understanding the link between short sleep and impaired glucose metabolism is of importance to acute and chronic metabolic health (Knutson and Van Cauter, 2008). The studies investigating this topic vary widely in the methodology used. Sleep restriction ranges from a single night (Donga et al., 2010) to two weeks (Nedeltcheva et al., 2009). Whilst some studies have shown no change in glucose tolerance following sleep restriction (Bosy-Westphal et al., 2008; Schmid et al., 2009), the majority of studies have demonstrated disruptions in glucose or insulin metabolism after reduced sleep.

Varied results between studies could be due to differences in methodologies. Studies often differ in terms of sleep restriction severity, but also differ when employing a control condition. Previous studies have employed a control condition of a set duration that is likely to be longer than habitual sleep duration (for example 10 or 12 h). Since individual habitual sleep duration can differ, this raises the question if results can be altered by comparing sleep restriction to a control condition which is longer than habitual sleep duration. A recent study demonstrated that just two nights of extended sleep can reverse the effects of four nights of sleep restriction on glucose regulation (Broussard et al., 2016).

A large majority of studies have investigated glucose clearance and insulin sensitivity following sleep restriction, but less is known about substrate oxidation. It would seem plausible that sleep restriction would impair CHO oxidation to accompany impaired glucose clearance. However, studies that have investigated substrate oxidation following sleep restriction provide conflicting findings (St-Onge et al., 2011; Klingenberg et al., 2012; Shechter et al., 2014).

Consequently, the purpose of this pilot study was to design a sleep restriction protocol which would induce impaired glucose tolerance, accounting for individual differences in habitual sleep duration. This pilot study will also examine substrate oxidation as well as glucose tolerance following sleep restriction. The protocol will examine the impact of two nights of 50% sleep restriction under free-living conditions on glucose tolerance and substrate metabolism compared to a control condition of habitual sleep. We hypothesise that two nights of partial sleep restriction will impair glucose tolerance in healthy individuals.

3.2 – Methods

3.2.1 – Participants

10 participants were recruited by advertisement from the University to take part in this crossover study. Healthy males aged between 18 and 40 yr with a self-reported regular sleeping pattern were included. Exclusion criteria included the presence of any chronic illnesses such as diabetes or a history of any sleep disorders such as insomnia. Participants were fully informed of the study procedures and written consent was obtained prior to participation. The University of Stirling School of Sport Ethics Committee approved the study protocol.

3.2.2 – Experimental protocol

Prior to each trial participants were provided with a 5-day sleep diary. They were instructed to complete the sleep diary every morning for 3 days prior to and the 2 days during each trial period. The sleep diaries allowed analysis of habitual sleep characteristics, as well as being a measure of compliance. The sleep diary also asked participants to rate their 'sleepiness' on a scale of 1 to 10, with 1 being the least and 10 being the highest feeling of sleepiness. Participants were also asked to avoid caffeine and alcohol during the trial period.

The study consisted of a control (CON) trial and sleep restriction (SR) trial, which each lasted two consecutive nights. Trials were separated by a period of at least one week. During the control condition participants were instructed to go to bed and wake at their habitual time, as determined from the sleep diary. Sleep duration in the sleep restriction trial was 50% of habitual duration each night. Participants slept the second half of the night in SR, continuing to waken at their habitual time each morning. This ensured measurements were obtained at the same time after waking in both trials.

Participants visited the laboratory in a rested and fasted state on the final morning in each condition. On arrival at the laboratory stature and body mass were measured. Participants rested in the supine position for five minutes before a fingerprick blood sample was obtained for analysis of fasting blood glucose. RER and REE were then measured for 15 minutes using indirect calorimetry with a ventilated hood (Oxycon, CareFusion, Germany). The first 5 minutes of measurement were used to allow participants to become accustomed to wearing the hood and were therefore not included in the analysis.

After fasting measures of blood glucose, substrate oxidation and resting energy expenditure (REE) were obtained, an oral glucose tolerance test (OGTT) was carried out. Participants were given a drink containing 82.5g dextrose mixed with 300 ml water which they were instructed to consume all at once. Fingerprick blood samples were obtained at 15, 30, 45, 60, 90 and 120 min after the drink. Samples were immediately analysed for blood glucose using a glucose meter (LifeScan OneTouch, UK). Measurements of substrate oxidation and REE were also carried out by indirect calorimetry between 20-30, 50-60, 80-90 and 105-120 min following consumption the drink.

3.2.3 – Statistical Analysis

Data were analysed using Minitab V.17 statistical software (Minitab Ltd., United Kingdom). Data for participant and sleep characteristics are displayed as mean \pm SD. Data for blood glucose, RER, and REE are presented as mean \pm SEM. Blood glucose, RER and REE were analysed using repeated measures ANOVA and where

appropriate paired t-tests were carried out *post-hoc* to identify any differences within main effects. Any data which violated the assumption of normality were transformed prior to statistical testing. Area under the curve was calculated for plasma glucose and REE using the trapezoidal rule. A p-value of less than 0.05 was used for significance.

3.3 – Results

3.3.1 – Participant and sleep characteristics

10 participants completed the study. All participants were 21 years old. Mean stature was 184.7 ± 6.1 cm, and mean body mass was 84.5 ± 8.4 kg. The average BMI of the participants was 24.8 ± 2.9 kg/m².

Habitual sleep duration was 515 ± 53 min, which was similar to sleep duration in the control condition (496 ± 39 min, $P = .374$). Sleep duration was significantly lower in SR than CON (496 ± 39 min in CON and 252 ± 18 min in SR, $P < .001$).

3.3.2 – Plasma glucose

Plasma glucose concentrations throughout the OGTT are presented in Figure 1. Fasting plasma glucose was significantly lower in SR than CON (5.33 ± 0.12 mmol/L and 5.00 ± 0.13 mmol/L in CON and SR, respectively; $P = .018$). Main effects of time ($P < .001$) and trial x time ($P = .009$) were found and a trend was observed for a main effect of trial ($P = .069$). Plasma glucose was significantly higher in SR at 45 min ($P = .021$) and 90 min ($P = .010$), and tended to be higher at 60 min ($P = .093$). Plasma glucose peaked at 30 min in CON (8.54 ± 0.40 mmol/L) and 45 min in SR (9.23 ± 0.46 mmol/L). In both conditions glucose concentrations returned close to baseline by 120 min (5.09 ± 0.19 mmol/L in CON and 5.17 ± 0.21 in SR)(Figure 1A). Area under the curve for plasma glucose was 13% higher in SR than CON (784 ± 20 in CON and 883 ± 32 in SR, $P = .001$) (Figure 1B).

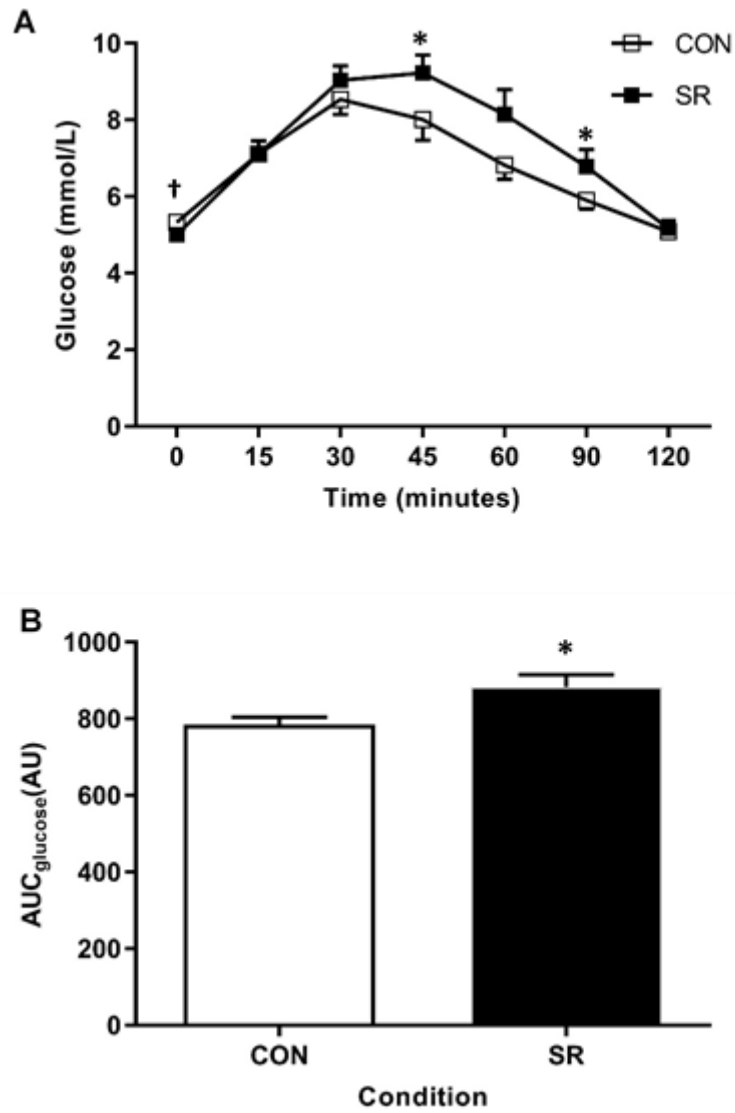


Figure 1. Blood glucose concentrations (Fig. A) and glucose AUC (Fig. B) during the OGTT following two nights of control sleep (CON) or 50% of habitual sleep (SR). (N=10). † indicates a significantly lower glucose concentration in SR than CON. * indicates significantly higher value in SR than CON. $P < .05$ used for significance.

3.3.3 – Resting energy expenditure

No effect of trial or trial x time were observed for REE ($P = .497$ and $P = .539$, respectively), however there was a main effect of time ($P = .006$). Fasting REE was similar between conditions (1.44 ± 0.04 and 1.48 ± 0.05 kcal/min for CON and SR, respectively; $P = .324$). There was also no difference between conditions for AUC (169 ± 5 and 171 ± 4 for CON and SR, respectively; $P = .598$).

3.3.4 – Respiratory exchange ratio and substrate oxidation

Respiratory exchange ratio and CHO and fat oxidation rates during the OGTT are displayed in Figure 2. Main effects of trial and time were found for RER ($P = .013$ and $P < .001$, respectively). There was no effect of trial x time ($P = .109$). Paired t-tests revealed a significantly lower RER at 30 min in the SR condition (0.85 ± 0.02 for CON and 0.79 ± 0.01 for SR; $P = .001$). Trends were also observed at fasting and 60 min ($P = .050$ and $P = .060$, respectively).

Main effects of trial ($P = .022$) and time ($P < .001$) were observed for CHO oxidation (Fig. 2B). There was a trend for an effect of trial x time ($P = .082$). Paired t-tests revealed a trend for lower fasting CHO oxidation in SR compared to CON (0.18 ± 0.03 g/min in CON and 0.10 ± 0.03 g/min in SR, $P = .055$), and significantly lower CHO oxidation in SR at 30 min ($P = .007$) and 60 min ($P = .039$). Main effects of trial ($P = .012$) and time ($P < .001$) were also observed for fat oxidation during the OGTT (Fig. 2C), however there was no effect of trial x time ($P = .836$). Fasting fat oxidation tended to be higher in SR than CON ($P = .055$). Paired t-tests also demonstrated significantly higher fat oxidation at 30 min ($P = .003$) and 60 min ($P = .037$).

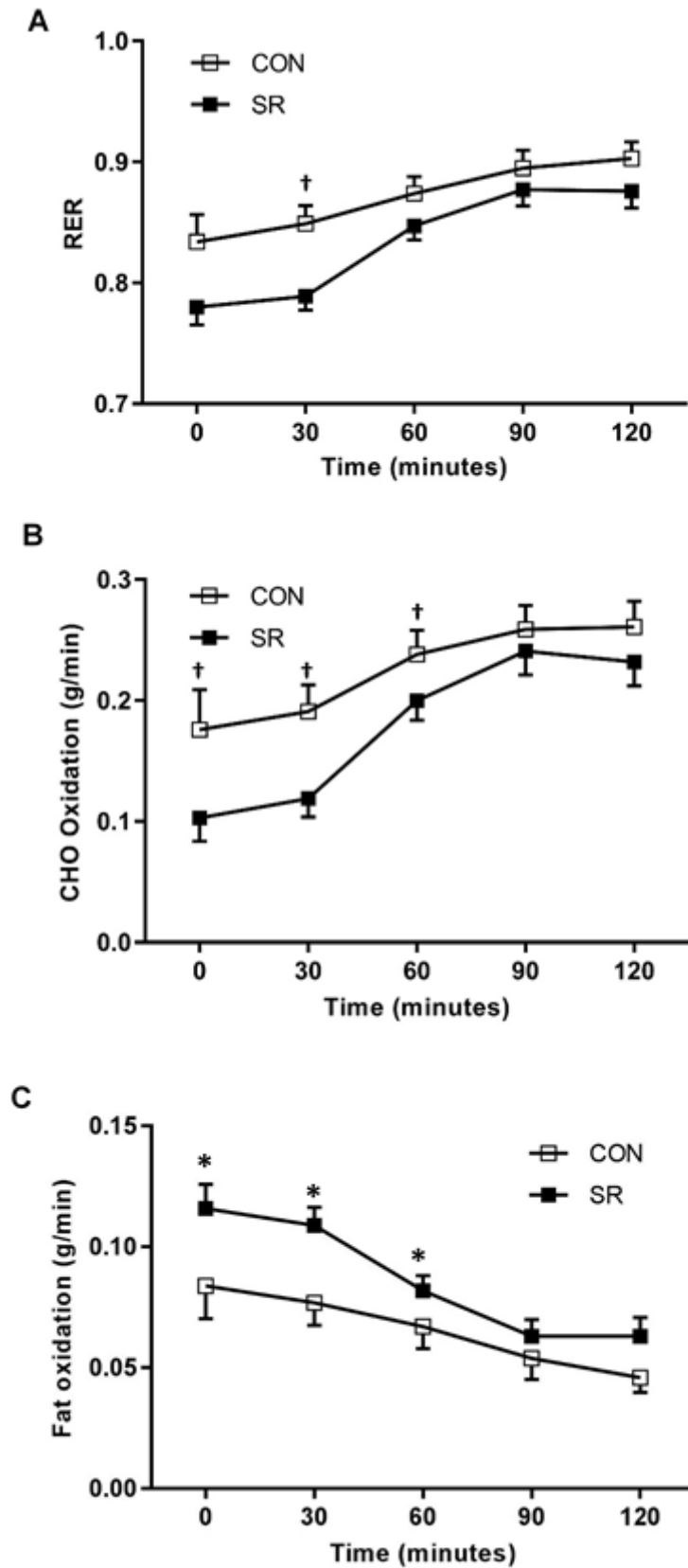


Figure 2. Respiratory exchange ratio (Fig. 2A), CHO oxidation (Fig. 2B), and fat oxidation (Fig. 2C) during OGTT following two nights of control sleep (CON) or sleep restriction (SR). N=10. * indicates a significantly higher value in SR. † indicates a significantly lower value in SR. P < .05 used for significance.

3.4 – Discussion

The aim of this pilot study was to determine whether two nights of 50% sleep restriction would impair glucose regulation in young healthy males. The findings demonstrate increased blood glucose concentrations in the sleep restriction condition compared to the control condition, indicating that two nights of partial sleep loss was sufficient to impair glucose tolerance in this population.

Glucose AUC and peak glucose concentration were both increased in the sleep restriction condition compared to the control condition. This increased glucose response may be due to reduced insulin sensitivity, which has been observed previously (Spiegel et al., 1999). The present study did not measure plasma insulin, therefore we can only speculate an altered insulin response.

Measurement of plasma insulin concentrations would have allowed better interpretation of results, therefore future studies should include measurement of insulin concentrations to determine whole-body insulin sensitivity.

Indirect calorimetry measurements showed no change in REE in the fasted state or during the OGTT. However, the findings did demonstrate a preference towards fat utilisation in the fasted state following sleep loss compared to normal sleep, as indicated by decreased respiratory exchange ratio and carbohydrate oxidation and increased fat oxidation.

Resting energy expenditure was not altered by sleep restriction in the present study which is in agreement with previous studies (Bosy-Westphal et al., 2008; Nedeltcheva et al., 2009; Buxton et al., 2010; St-Onge et al., 2011; Klingenberg et al., 2012; Shechter et al., 2014). The only study which observed a change in REE was that of Benedict and colleagues (2011), however sleep was deprived for an entire night in this study, suggesting that REE may be affected only by total sleep deprivation rather than partial restriction. The absence of slow wave sleep in total sleep deprivation may explain the discrepancies between studies using partial and total sleep deprivation. Slow wave sleep is an important contributor to energy conservation during sleep, and is often preserved even when sleep is restricted (Brunner et al., 1993; Belenky et al., 2003). It could therefore be

speculated that no change was observed in the studies using partial sleep restriction as slow wave sleep may not have been significantly less than in normal sleep, although without polysomnography to measure sleep architecture we cannot be certain this is the case.

The findings of this study indicate increased utilisation of fat in the fasted state after sleep restriction. Similarly, at measurements obtained between 20 and 30 min and 50 and 60 min during the OGTT, there was increased fat and decreased carbohydrate oxidation after sleep restriction compared to control sleep.

However, this difference was observed only at the first two time points after ingestion of the drink, suggesting that the switch in fuel preference may have been temporary. Indeed, previous studies have suggested that the decreased fasting RER observed after sleep restriction is likely due to the increased time spent awake in the fasted state, and does not reflect a true change in substrate use (Klingenberg et al., 2012; Shechter et al., 2014). The findings of the current study may also be representative of this theory as differences were observed only in the fasted state and early stages of the OGTT.

The length of measurement of REE and RER is a limitation of the present study. Although our findings are indicative of reduced carbohydrate oxidation in the fasted state and after ingestion of a glucose bolus, it is unknown if this would return to normal as the day progresses. A decrease in carbohydrate utilisation is typical of ageing individuals and is correlated with a higher body fat percentage and increased plasma glucose levels (Rizzo et al., 2005). However, whether the reduction in RER in the present study would have any clinical significance long term would require a longer period of measurement to determine how long it would take for RER values to return to values observed in the control condition.

This pilot study revealed interesting findings which would benefit from further investigation in a more controlled trial. Glucose regulation is impaired in healthy individuals following just two nights of sleep restriction. Given that impaired glucose regulation is a primary characteristic of type 2 diabetes (American Diabetes Association, 2006), arguably one of the most important questions raised from this is what is the mediator between sleep loss and impaired glucose

regulation? Future research should therefore explore this relationship further in an attempt to establish the underlying mechanisms. Developing knowledge of the underlying mechanisms may allow development of interventions to alleviate this impairment following sleep restriction, and therefore lowering the risk of type 2 diabetes.

The next study in this thesis was designed to extend the current knowledge by examining potential underlying mechanisms, specifically systemic inflammation and skeletal muscle insulin signalling, which may be responsible for impaired glucose tolerance after sleep restriction.

Chapter 4

A potential role of skeletal muscle insulin signalling but no effect of inflammation in impaired whole body insulin sensitivity following acute sleep restriction in healthy individuals

4.1 - Introduction

The link between sleep restriction and poor glucose metabolism is not well understood. Reduced insulin sensitivity is thought to be responsible for the decrease in glucose tolerance (Donga et al., 2010; Broussard et al., 2012; Rao et al., 2015; Wang et al., 2016). Reduced insulin sensitivity has been observed after restricting sleep for 1 to 3 hours for 3 nights (Wang et al., 2016). Likewise, a single night of sleep restriction is sufficient to reduce insulin sensitivity by up to 25% (Donga et al., 2010), suggesting that even mild sleep restriction may negatively impact glucose metabolism and insulin sensitivity.

Despite many studies showing an impairment in glucose metabolism and insulin sensitivity after sleep restriction, there is a lack of knowledge regarding the mechanisms responsible for this impairment. Proposed mechanisms include disruption of insulin signalling in peripheral tissues (Broussard et al., 2012; Rao et al., 2015) and increased inflammation (Padilha et al., 2011).

Recent work by Rao and colleagues (2015) demonstrated that 5 nights of 4 h sleep led to decreased peripheral, but not hepatic, insulin sensitivity compared to 5 nights of 8 h sleep. This work offers insight that the peripheral tissues may influence the impairment in glucose metabolism following sleep restriction. Consistent with this finding, previous research has revealed an impairment in insulin signalling in adipose tissue when whole body insulin sensitivity is reduced following sleep loss (Broussard et al., 2012). Given that skeletal muscle accounts for up to 80% of glucose uptake under insulin-stimulated conditions (Thiebaud et al., 1982), it is possible that skeletal muscle may also exhibit impaired insulin signalling and contribute to impaired whole body glucose control after sleep restriction.

Insulin resistance is often accompanied by increased low-grade inflammation (Shoelson et al., 2006). Sleep deprived individuals often display increased levels of inflammation (Meier-Ewert et al., 2004; Vgontzas et al., 2004; Irwin et al., 2006). However, some research has shown conflicting findings, observing increases in some but not all inflammatory markers measured (Shearer et al., 2001). The inconsistent findings suggest a dose-dependent relationship may exist between sleep loss and inflammation, therefore whether increased inflammation contributes to the impairment in glucose regulation after only two nights of sleep restriction is unknown.

At present, no published studies have investigated the effects of sleep restriction on insulin signalling within skeletal muscle, or whether decreased insulin sensitivity is coupled with increased inflammation after two nights of partial sleep restriction. Therefore, this study was designed to address this gap in the literature. The aims of the study were to investigate (a) the effects of two nights of sleep restriction on whole body glucose tolerance and insulin sensitivity; (b) whether there were any changes in skeletal muscle insulin signalling after sleep restriction; and (c) whether any changes in insulin sensitivity were coupled with changes in levels of inflammation. We hypothesised that two nights of sleep restriction would decrease whole body glucose tolerance and insulin sensitivity, as well as increasing systemic inflammation and disrupting insulin signalling within skeletal muscle.

4.2 – Methods

4.2.1 – Participant and Study Design

Ten participants were recruited by advertisement to take part in this randomised crossover trial. The study involved three visits to the laboratory which comprised of a pre-screening visit and two main trial visits. The main trial visits were separated by a 3 wk washout period. Inclusion criteria were healthy males aged 18 to 40 yr with a regular sleeping pattern. Exclusion criteria included smokers, individuals that have travelled across times zones or carried out shift work in the

past four weeks, or individuals who reported any symptoms of poor sleeping behaviour. Individuals who reported the presence of any health conditions or who were taking any medications that may interfere with or be worsened by the study protocol, such as metabolic disorders, were also excluded. The study protocol was approved by the local University research ethics committee. All participants were fully informed of the study procedures and gave written consent prior to participation.

4.2.2 – Pre-screening

Participants arrived at the laboratory in the morning for the pre-screening visit. Stature and body mass were measured and body mass index was calculated. Participants then completed a standard health questionnaire, a morningness-eveningness questionnaire (Horne and Ostberg, 1976) and the Pittsburgh Sleep Quality Index (PSQI) (Buysse et al., 1989) to assess overall health and sleep characteristics prior to participation. Each participant was given a 7-day sleep diary and 3-day food diary which they were required to complete before the first main trial. A wrist watch actigraph (GENEActiv, Activinsights Ltd., Cambridgeshire, UK) was worn in conjunction with the 7-day sleep diary to obtain an objective measurement of habitual sleep duration.

4.2.3 – Entraining

Participants underwent one week of entraining prior to each main trial. During this period, participants were instructed to continue with their usual daily routines and to go to bed and wake at the same time every day, keeping times similar to their habitual sleeping behaviours. Wrist actigraphy was used throughout the entraining period to ensure compliance.

4.2.4 – Trial Conditions

Trials consisted of two consecutive nights of either control sleep (CON) or partially restricted sleep (SR). Control sleep duration was calculated as the mean habitual sleep duration determined by the 7-day sleep diary and actigraphy. Sleep duration in SR was 50% of the mean habitual sleep duration. During the

sleep restriction trial participants slept the second half of the night, continuing to wake at the same time each morning. A constant wake time ensured measurements were obtained the same length of time after wakening in each trial.

Permuted block randomisation was used to assign participants to the first study condition. Six participants completed the control condition first. During both trials wrist actigraphy was used to ensure compliance. In addition, in the sleep restriction trial participants sent time stamped SMS messages to the researcher every hour from their habitual bed time until their sleep restricted bed time.

During the trials participants were provided with a 48 h diet based on their habitual food intake determined from the 3-day food diary. This diet was replicated for each trial and participants were instructed to eat only the food provided during this period. Participants were asked to continue with their normal daily routines during each trial period but to avoid any vigorous physical activity or consumption of caffeine or alcohol.

4.2.5 – Laboratory Visits

Following the second night of control sleep or sleep restriction, participants arrived at the laboratory in a rested and fasted state. After measurement of stature and body mass, participants rested in the supine position before blood pressure and heart rate were measured using a digital blood pressure monitor (Hangzhou Sejoy Electronics & Instruments Co., LTD, China). A cannula was then inserted into the antecubital vein in the arm to allow blood samples to be drawn regularly. Participants then performed an oral glucose tolerance test (OGTT) to assess glucose clearance. Participants were given a drink containing 82.5 g dextrose mixed with 300 ml water, which they were instructed to consume all at once. Blood samples were collected before (0 min) and at 15, 30, 45, 60, 90 and 120 min following consumption of the drink. In addition, muscle tissue samples were obtained before (0 min) and 30 and 120 min after the ingestion of the drink.

4.2.6 – Blood collection and processing

All blood samples throughout the OGTT were obtained by the cannulation technique. For each sample 10 ml of blood was collected into EDTA treated and untreated vacutainer tubes. After collection, untreated serum samples were left at room temperature for 30 min and EDTA treated samples were immediately placed on ice. All samples were centrifuged at 3500 rpm for 10 min at 4°C. After centrifugation plasma was aliquoted into micro tubes and stored at -70°C until analysis.

4.2.7 – Blood plasma assays

Blood samples collected at 0, 15, 30, 45, 60, 90 and 120 min during the OGTT were analysed for plasma glucose. Plasma glucose was assayed in duplicate using an ILab automated analyser (Instrumentation Laboratory, Warrington, Cheshire, UK). Circulating insulin was measured at 0, 15, 30, 45, 60, 90 and 120 min using ELISA techniques from a commercially available ELISA kit (Demeditec, Kiel, Germany). Inflammatory markers were measured from blood samples collected at 0, 30 and 120 min during the OGTT also using commercially available ELISA kits for TNF- α (Invitrogen, Camarillo, CA) and CRP (Kalon Biological, Guildford, UK).

4.2.8 – Muscle collection and preparation

Muscle tissue samples during the OGTT were obtained from the *Vastus Lateralis* using the Bergstrom biopsy technique. The site was cleaned before 2% Lidocaine was injected to anaesthetise the local area. A tissue sample was then obtained. Approximately 60-100 mg of skeletal muscle tissue was obtained for each sample. Tissue samples were immediately cleaned with saline solution to remove blood and fat tissue. Cleaned samples were placed in micro tubes and snap frozen using liquid nitrogen before being transferred to a -70°C freezer, where they were stored until analysis.

4.2.9 – Muscle tissue processing

Approximately 30 mg of human skeletal muscle tissue was homogenized by a dounce homogeniser on ice in RIPA buffer [50 mmol/l Tris·HCl pH 7.5, 50 mmol/l

NaF, 500 mmol/l NaCl, 1 mmol/l sodium vanadate, 1 mmol/l EDTA, 1% (vol/vol) Triton X-100, 5 mmol/l sodium pyrophosphate, 0.27 mmol/l sucrose, and 0.1% (vol/vol) 2-mercaptoethanol and Complete protease inhibitor cocktail (Roche)]. Debris was removed by centrifugation at 4°C for 15 min at 13,000 *g*. The supernatant was then removed, and protein concentration was determined using the bicinchoninic acid protein assay according to the manufacturer's instructions (Sigma Aldrich, UK).

4.2.10 – Kinase assay

PanPKB kinase assays were carried out by immunoprecipitation for 2 h at 4°C in homogenization buffer [50 mM Tris·HCl pH 7.5, 0.1 mM EGTA, 1 mM EDTA, 1% (vol/vol) Triton X-100, 50 mM NaF, 5 mM NaPPI, 0.27 M sucrose, 0.1% β-mercaptoethanol, 1 mM Na₃(OV)₄, and 1 Complete (Roche) protease inhibitor tablet per 10 ml]. Protein G sepharose (2.5 μl per IP) was used to precipitate the immune complexes. Immune complexes were washed twice in high-salt washes (homogenisation buffer as above with 0.5 M NaCl added) followed by one wash in assay buffer (see below). Prior to carrying out the activity assay the immune-bead-complex was suspended in a total of 10 μl of assay buffer. All assays were carried out in a 50-μl reaction. Assays were started every 20 s by the addition of a hot assay mix, which consisted of assay buffer (50 mM Tris·HCl pH 7.4, 0.03% Brij35, and 0.1% β-mercaptoethanol), ATP-MgCl₂ (100 μM ATP + 10 mM MgCl₂), ³²γ-ATP (specific activity; 0.5 × 10⁶ cpm/nmol), and finally synthetic peptide substrates (Crosstide; GRPRTSSFAEG at 30 μM). Assays were stopped at 20-s intervals by spotting onto squares of p81 chromatography paper (Whatman; GE Healthcare, UK) and immersing in 75 mM phosphoric acid. Papers (p81) were washed three times for 5 min in 75 mM phosphoric acid and once in acetone. They were then dried and immersed in Gold Star LT Quanta scintillation fluid (Meridian Biotechnologies, Chesterfield, UK) and counted in a Packard 2200CA TriCarb scintillation counter (United Technologies). Assay results were quantified in nmol·min⁻¹·mg⁻¹ (U/mg). Blanks for background subtractions were carried out with immunoprecipitated kinases with no peptide included in the assay reaction. Activities assays for panPKB were carried out on cell lysates by IP from 300 μg of

cell lysate. The IP step was performed with 2 µg each of PKB α / β / γ antibodies (DSTT, Dundee University). Antibodies were used with 2.5 µl of protein G sepharose per IP to immunoprecipitate for 2 h at 4°C. The activity assay ran for 2 hr.

4.2.11 – Western blots

Protein was boiled in Laemmli sample buffer and separated on SDS-PAGE gels for 1.5 h at 150 V. Proteins were then transferred to PDVF membranes for 2 h at 30 V. Following transfer membranes were blocked for 1 h in BSA solution. Membranes were then incubated overnight at 4°C with the appropriate primary antibody diluted in TBST. Primary antibodies were phosphoPKB Thr308, total Akt and eEf2 (Cell Signalling). The membranes were rinsed three times for 5 min in TBST the next morning before being incubated with HRP-conjugated anti-rabbit secondary antibody (Cell Signalling) diluted in TBST. The membranes were washed three times for 5 min with TBST. Enhanced chemiluminescence was used to detect antibody binding. Band visualisation was carried out using a Chemidoc XRS system (Bio-Rad, Hertfordshire, UK). Bands were quantified by densitometry (Image Lab, Bio-Rad, Hertfordshire, UK). Membranes were stripped using stripping buffer and re-probed for total protein to express phosphoprotein relative to total protein.

4.2.12 – Statistical analysis

Data were analysed using Minitab v.17 statistical software (Minitab Ltd., United Kingdom). Participant characteristics and sleep time are presented as mean \pm SD. Blood variables and muscle PKB data are presented as mean \pm SEM. Repeated measures ANOVA (trial x time) were employed to analyse plasma glucose, insulin and inflammatory markers and muscle PKB phosphorylation and activity. Paired t-tests were carried out *post-hoc* to identify any differences in main effects and at 0 min for each variable to compare baseline values. Data which violated the assumptions of the statistical tests were transformed appropriately prior to analysis. A p-value of less than 0.05 was used to indicate significance. The Matsuda Index (Matsuda and DeFronzo, 1999) and homeostasis model

assessment (HOMA) (Matthews et al., 1985) were used to estimate insulin sensitivity. Area under the curve for glucose, insulin, inflammatory markers, PKB activity, and PKB phosphorylation were calculated using the trapezoidal rule and the area under the curve for plasma insulin to PKB activity ratio was determined. Cohen's d effect sizes and confidence intervals were calculated for PKB activity.

4.3 – Results

4.3.1 – Participant and Sleep Characteristics

Ten males completed the study. Participant characteristics are displayed in Table 1. Habitual time to bed ranged from 2120 h to 0245 h, with a mean time to bed of 2344 h. Participants' habitual wakening time was between 0544 h and 1020 h, with an average time of 0742 h. Mean habitual sleep duration was 484 ± 66 min. Mean sleep duration in the control condition was 442 ± 78 min, which was not significantly different from habitual sleep duration ($P = .124$). Mean sleep duration in SR was significantly less than in CON (235 ± 34 min for SR; $P < .001$). Mean time to bed and mean wakening time was 2349 h and 0711 h in CON and 0319 h and 0714 h in SR, respectively.

Participant Characteristics

| | |
|--------------------------------------|-------------|
| Age (yr) | 23 ± 4 |
| Stature (cm) | 181.6 ± 5.8 |
| Body mass (kg) | 82.2 ± 9.5 |
| Body mass index (kg/m ²) | 24.9 ± 2.1 |
| Systolic blood pressure (mmHg) | 125 ± 5 |
| Diastolic blood pressure (mmHg) | 70 ± 4 |
| Habitual energy intake (kcal/d) | 2369 ± 616 |
| PSQI score | 4 ± 1 |
| Morningness-eveningness score | 53 ± 8 |

Table 1. Participant characteristics at baseline. Data presented as mean ± SD. N=10. PSQI = Pittsburgh Sleep Quality Index. PSQI score equal to or greater than 5 is indicative of poor sleep quality. Morningness-eveningness score can range from 16 ('definite evening type') to 86 ('definite morning type'). A score of 42-58 is indicative of 'neither type'.

4.3.2 – Plasma glucose and insulin

Mean plasma glucose and insulin concentrations during the OGTT and area under the curve for plasma glucose and insulin are displayed in Figure 3. A repeated measures ANOVA revealed a main effect of time for plasma glucose ($P < .001$). There was no effect of trial ($P = .222$) or trial x time ($P = .985$). Mean baseline plasma glucose concentrations during CON were similar to SR (4.92 ± 0.10 mmol/L and 5.02 ± 0.06 mmol/L, respectively; $P = .221$). In response to the dextrose drink, plasma glucose peaked at 45 minutes in both conditions (7.69 ± 0.75 mmol/L and 8.57 ± 0.69 mmol/L for CON and SR, respectively). Plasma glucose returned to values similar to baseline at 120 min in both conditions (5.55 ± 0.37 mmol/L and 5.85 ± 0.30 mmol/L for CON and SR, respectively). Similarly, AUC was not different between conditions ($P = .391$).

Mean plasma insulin concentrations showed main effects of trial and time during the OGTT ($P = .006$ and $P < .001$, respectively). Plasma insulin values were similar at baseline in CON and SR (18.7 ± 4.9 μ IU/mL and 20.0 ± 5.0 μ IU/mL, respectively; $P = .172$). In response to the dextrose drink plasma insulin peaked at 60 min in CON and at 45 min in SR (69.0 ± 12.5 μ IU/mL at 60 min in CON and

83.9 ± 10.2 μIU/mL at 45 min in SR). SR displayed significantly higher insulin concentrations than CON at 15 min (59.1 ± 9.0 μIU/mL and 48.5 ± 9.5 μIU/mL for SR and CON, respectively; P = .031) and at 45 min (83.9 ± 10.2 μIU/mL and 67.3 ± 10.8 μIU/mL for SR and CON, respectively; P = .028). During the OGTT there was a 14% higher insulin AUC in the sleep restriction condition compared to the control (7069 ± 1018 vs. 8068 ± 901 for CON vs. SR, respectively; P = .013). Matsuda Index was 18.6% lower in SR than CON (4.19 ± 0.71 vs. 3.41 ± 0.51 for CON and SR, respectively; P = .010). There was no difference in HOMA between conditions (4.05 ± 1.02 and 4.42 ± 1.08 for CON and SR, respectively; P = .139).

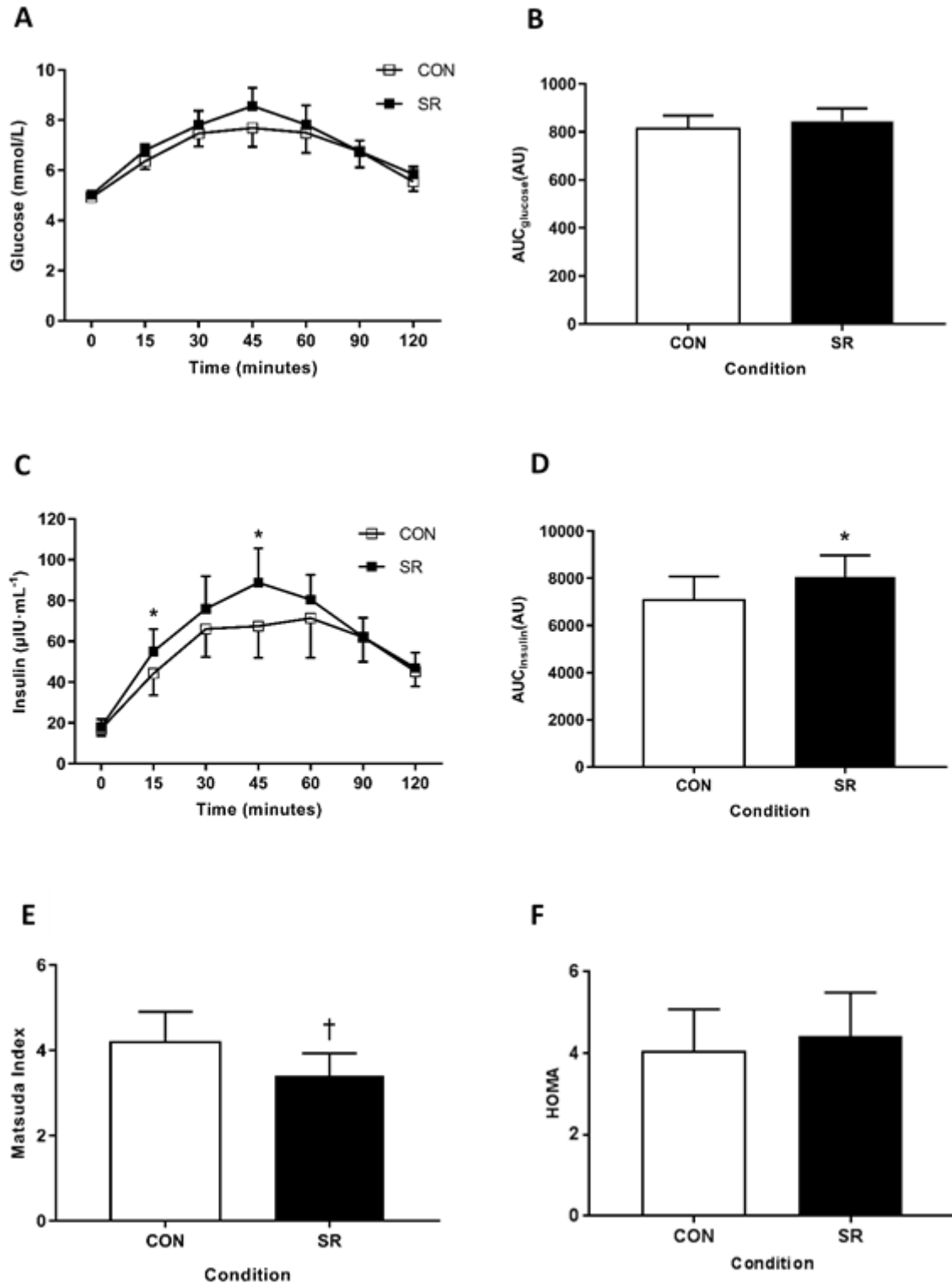


Figure 3. Mean plasma glucose and insulin concentrations during the OGTT following two nights of normal sleep (CON) or sleep restriction (SR) (n=10). (A) Mean plasma glucose concentrations for CON and SR. (B) Area under the curve for plasma glucose. (C) Mean plasma insulin concentrations for CON and SR. (D) Area under the curve for plasma insulin. (E) Matsuda Index calculated from 0, 30, 60, 90 and 120 min during the OGTT. (F) Homeostasis model assessment (HOMA). * indicates significantly higher values in SR condition. † indicates significantly lower values in SR condition. P<0.05 was used to indicate significance.

4.3.3 – Plasma inflammatory markers

Concentrations of circulating CRP and TNF- α are displayed in Table 2. CRP data was unavailable for one participant, therefore n=9 for CRP results. A trend for a main effect of trial was observed for CRP ($P = .094$), however there was no effect of time ($P = .685$) or trial x time ($P = .537$). CRP at baseline tended to be lower in SR than CON ($P = .092$), however this trend was no longer visible at 120 min ($P = .202$). An analysis of variance revealed no effect of trial ($P = .421$), time ($P = .912$), or trial x time ($P = .697$) for TNF- α . Similarly, there was no difference in baseline concentrations of TNF- α between conditions ($P = .858$).

| Inflammatory marker | CON | SR |
|--|-----------------|-----------------|
| CRP (mg/L) | | |
| 0 min | 1.6 \pm 0.3 | 0.8 \pm 0.2 |
| 120 min | 1.6 \pm 0.2 | 1.0 \pm 0.2 |
| TNF-α (pg/ml) | | |
| 0 min | 1.82 \pm 0.06 | 1.84 \pm 0.05 |
| 120 min | 1.81 \pm 0.04 | 1.83 \pm 0.06 |

Table 2. Circulating inflammatory markers before (0 min) and 120 min during an OGTT following two nights of control sleep (CON) or sleep restriction (SR). Data presented as mean \pm SEM. N = 9 for CRP and N = 10 for TNF- α .

4.3.4 – Skeletal muscle insulin signalling

PanPKB Activity

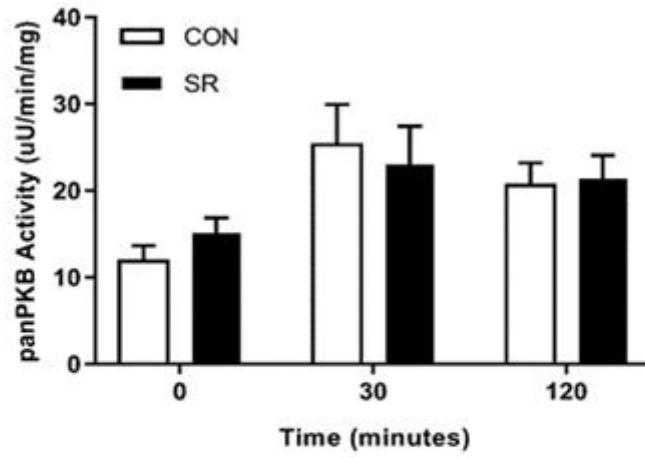
PanPKB activity is shown in Figure 4. One participant was excluded from the results for PKB activity due to analysis problems, therefore, n=9 for all panPKB activity results. A main effect of time was observed for panPKB activity ($P = .001$) and there was a trend for trial x time ($P = .087$). There was no effect of trial ($P = .589$). PanPKB activity (Fig. 4A) during CON was 12.37 \pm 1.93 μ U/min/mg at baseline and was significantly increased in response to the dextrose drink at 30

min ($26.67 \pm 4.96 \mu\text{U}/\text{min}/\text{mg}$; $P = .007$) and 120 min ($21.47 \pm 2.75 \mu\text{U}/\text{min}/\text{mg}$; $P < .001$). At baseline panPKB was 27% higher in SR than CON, demonstrating a medium effect size ($15.72 \pm 1.81 \mu\text{U}/\text{min}/\text{mg}$ in SR; 95% CI -1.91, 8.61; Cohen's $d = 0.63$). PanPKB in SR displayed a 16% lower response than CON at 30 min, showing a small effect size ($22.29 \pm 4.88 \mu\text{U}/\text{min}/\text{mg}$ at 30 min in SR; 95% CI -9.60, 0.85; Cohen's $d = 0.32$). PanPKB activity was similar between conditions at 120 min ($21.47 \pm 2.75 \mu\text{U}/\text{min}/\text{mg}$ and $21.40 \pm 2.69 \mu\text{U}/\text{min}/\text{mg}$ for CON and SR, respectively; Cohen's $d=0.01$).

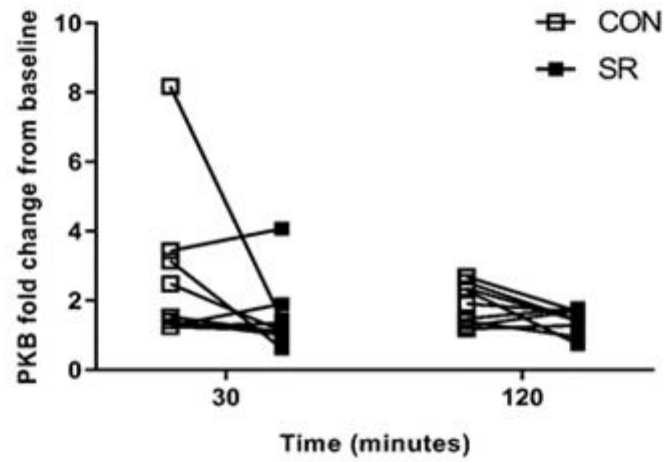
PanPKB fold change (Fig. 4B) from baseline to 30 min tended to be lower in SR than CON (2.7 ± 0.7 fold in CON and 1.5 ± 0.3 fold in SR; 95% CI -2.91, 0.65; $P = .098$, Cohen's $d = 0.69$). Likewise, there was a tendency for a lower fold change from baseline to 120 min in SR than CON, displaying a large effect size (1.9 ± 0.2 fold in CON and 1.4 ± 0.1 fold in SR; 95% CI -1.03, 0.04; $P = .087$; Cohen's $d = 1.08$).

The area under the curve for plasma insulin to PKB activity index ratio is displayed in Figure 4C. AUC for plasma insulin:PKB activity index was 2.67 ± 0.51 in CON and was significantly greater in SR at 3.33 ± 0.48 (95% CI 0.19, 1.14; $P = .012$, Cohen's $d = 0.47$).

A



B



C

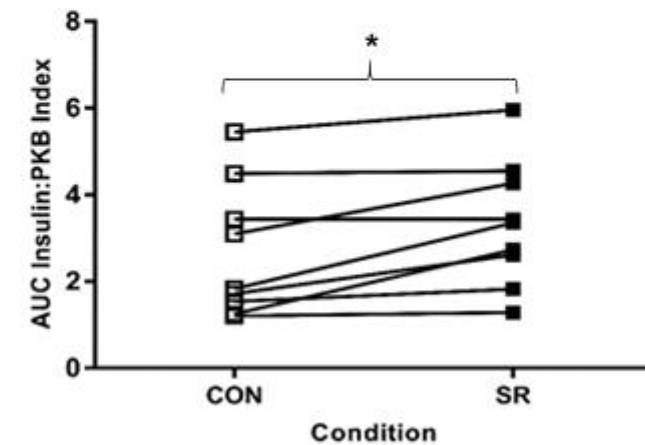


Figure 4. panPKB activity during the OGTT following two nights of normal sleep (CON) or sleep restriction (SR) (n=9). (A) panPKB activity at each timepoint. (B) Fold change from baseline in panPKB activity at 30 and 120 minutes. (C) AUC Insulin:PKB index. * indicates a significant difference between conditions. P<0.05 used for significance.

PKB phosphorylation

PKB phosphorylation results are displayed in Figure 5. No main effects of trial or trial x time were observed for phosphorylated to total PKB ($P = .217$ and $P = .845$, respectively), however there was a main effect of time ($P = .007$). The AUC for phosphorylated to total PKB ratio (Fig. 5B) was similar between conditions (274 ± 69 in CON and 296 ± 50 in SR; $P = .433$).

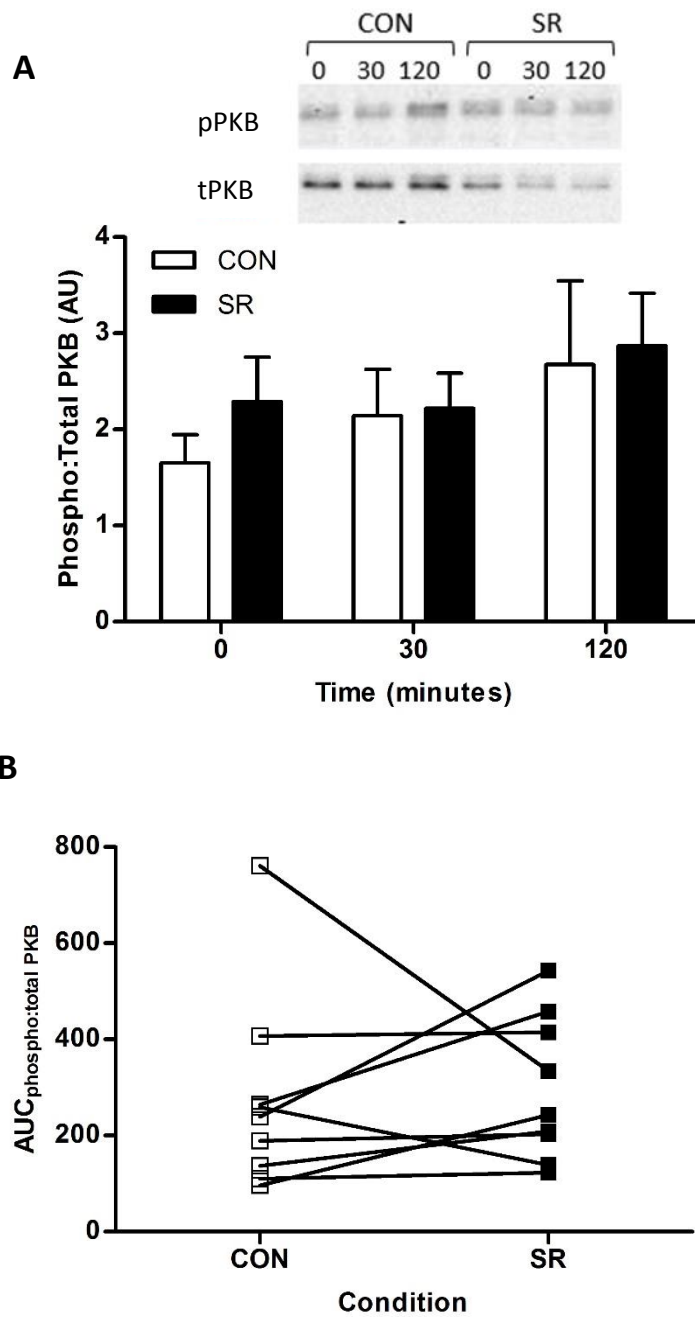


Figure 5. PKB phosphorylation during OGTT following two nights of control sleep (CON) or sleep restriction (SR) (n=9). (A) Blot showing phosphorylated (pPKB) and total (tPKB) PKB. Bar graph demonstrates phosphorylated to total PKB ratio at 0, 30, 120 min following OGTT. (B) AUC for phosphorylated to total PKB during OGTT.

4.4 – Discussion

The current study aimed to investigate if the reduction in insulin sensitivity observed after sleep restriction is coupled with any changes in skeletal muscle insulin signalling and whole body inflammation. The findings confirm that two nights of sleep restriction reduced whole body insulin sensitivity. However, contrary to the hypothesis, systemic inflammation did not increase, as shown by measurements of circulating CRP and TNF- α . Findings regarding skeletal muscle insulin signalling are conflicting. The PKB kinase assay results point towards a reduction in PKB activity following sleep restriction. However, Western blot analysis demonstrated no differences in phosphorylation of PKB between conditions. Collectively, these findings demonstrate that two nights of 50% sleep restriction impairs whole body insulin sensitivity in healthy males, and that this impairment does not seem to be caused by increased inflammation, contradicting what has been suggested previously. It is still unclear whether a defect in skeletal muscle is a contributing factor or not, although some of our findings suggest the presence of mild changes in skeletal muscle insulin signalling after sleep restriction which may be responsible for the observed impairment.

Plasma glucose concentrations were unchanged following sleep restriction in the current study. Fasting plasma glucose was similar between the sleep restriction and control condition, which is consistent with previous studies (Donga et al., 2010; Schmid et al., 2009; Schmid et al., 2011; Reynolds et al., 2012; Rao et al., 2015; Wang et al., 2016). Likewise, during the OGTT, plasma glucose concentrations were comparable between conditions. Several other studies have also found no changes in plasma glucose following sleep restriction (Zielinski et al., 2008; Schmid et al., 2009; Rao et al., 2015; Wang et al., 2016). However, others have observed altered glucose metabolism after sleep restriction (Spiegel et al., 1999; Nedeltcheva et al., 2009; Buxton et al., 2010; Reynolds et al., 2012). While it is unknown why there are differences between studies, the severity of sleep loss may explain the differences between findings. Those studies that have demonstrated changes in glucose metabolism after sleep loss utilised a more

severe sleep restriction protocol than those studies that observed no changes, including the current study. It is not known if a dose-response relationship between sleep restriction and impaired glucose metabolism exists; whilst it seems likely, it has not been confirmed, therefore additional research in this area is required. Often studies have compared sleep restriction to an extended duration of sleep beyond the recommended 7 to 9 hours and used a fixed duration of sleep restriction for all participants. Using such a sleep restriction protocol limits the applicability of the findings as individual variations in habitual sleep durations may influence how the individuals react to the conditions.

The plasma glucose findings in the current study also differ from the pilot study presented in the previous chapter of this thesis. Plasma glucose was significantly higher in the pilot study, whereas no significant changes were demonstrated in this study. An important difference between studies was the blood sampling method used. The sample size in the current study was small and may be underpowered to detect any changes in plasma glucose when venous blood sampling is used, which could explain the discrepancies between studies. Despite no significant increase in plasma glucose in this study, the peak plasma glucose concentration was almost 1 mmol/L higher in the sleep restricted condition compared to the control condition, which may still be of physiological importance.

The present study demonstrates increased plasma insulin concentrations and area under the curve during the OGTT in the sleep restriction condition compared to the control condition. This finding is consistent with previous studies (Buxton et al., 2010; Donga et al., 2010; Broussard et al., 2012; Rao et al., 2015; Wang et al., 2016). The increased plasma insulin response to the drink in the sleep restriction condition was not accompanied by any changes in plasma glucose, indicating a reduction in whole body insulin sensitivity. Supporting this, we observed a lower Matsuda index value in the sleep restriction condition compared to the control condition. HOMA, which primarily reflects hepatic insulin sensitivity in the fasted state (Abdul-Ghani et al., 2007), was unchanged

following sleep restriction, suggesting that the impairment stems from the peripheral, rather than hepatic, tissues.

Sleep restriction has been shown to alter insulin sensitivity in peripheral tissues (Broussard et al., 2012; Rao et al., 2015). Given this evidence, the present study investigated whether a disruption in skeletal muscle insulin signalling was responsible for the impairment in glucose regulation after sleep restriction. Assessment of PKB activity did not reveal any statistically significant differences between the sleep restriction and control condition; however, a lower fold change from baseline at 30 min and 120 min during the OGTT was observed, pointing towards a defective response to an oral glucose load. A lower insulin signalling response to glucose intake combined with increased circulating insulin revealed an increase in the AUC insulin:PKB index after sleep restriction compared to control sleep. An increased AUC insulin:PKB index may be indicative of reduced insulin sensitivity. Reduced PKB activity in skeletal muscle is often noted in individuals with type 2 diabetes (Krook et al., 1998). Although the changes in PKB activity in the present study are limited, we speculate that this initial defect may lead to comparable changes if continued over a longer period.

Despite observing small differences in PKB activity, no changes were observed in phosphorylation of PKB relative to total PKB content. This finding contradicts the results from Broussard and colleagues (2012) who demonstrated a reduction in phosphorylated PKB in adipose tissue following sleep restriction. It is unclear why there are differences between the studies. One possible reason could be a difference in insulin sensitivity between peripheral tissues. However, it is also useful to note that Broussard and colleagues used a five night sleep restriction protocol, while we employed only two nights of sleep restriction. As mentioned previously, it may be that sleep restriction exerts cumulative effects on insulin signalling in peripheral tissues. If a dose-response relationship between reduced PKB phosphorylation and sleep restriction exists, changes may not have been as severe in this study.

Our findings show a discrepancy between Western blot analysis and panPKB activity. The findings from activity assays point towards small changes in skeletal

muscle insulin signalling, whereas no changes were detected using western blot analysis. Discrepancies in results between the activity assay and western blot methods assessing protein signalling have been shown previously (McGlory et al., 2014). The protein kinase assay method may be able to detect changes of a smaller magnitude or in smaller sample sizes compared to the Western blot method. Therefore, it is possible that changes in PKB phosphorylation may be detected if sleep restriction is more severe either in the number of nights or length of restriction each night.

The current study assessed concentrations of circulating CRP and TNF- α , which are representative of whole body inflammation. Taking into consideration the findings of previous studies, one hypothesis of the current study was that an increase in whole body inflammation would be present following sleep loss. However, in contrast to previous research (Meier-Ewert et al., 2004; Vgontzas et al., 2004; Irwin et al., 2006), the findings do not support this hypothesis. Similar to the plasma glucose findings, it seems likely that a dose-response relationship may exist between sleep restriction and whole body inflammation. Two of the three studies that observed increased inflammation employed a more severe sleep restriction protocol, with at least six nights of restriction (Meier-Ewert et al., 2004; Vgontzas et al., 2004). In the study which employed only a single night of sleep restriction (Irwin et al., 2006), increased monocyte expression of inflammatory markers was observed immediately after wakening. However, this change was not present later in the day, suggesting that the difference may have only been temporary rather than a true reflection of changes in whole body inflammation. Thus, it seems highly plausible that a dose-response relationship exists, and that in the present study whole body inflammation was not responsible for the impairment in insulin sensitivity.

To summarise, the findings of the present study indicate that two nights of 50% sleep restriction impairs whole body insulin sensitivity in healthy, young males. It is unlikely that this impairment is caused by whole body inflammation, however skeletal muscle insulin signalling may be a potential contributor. The findings of this study have implications for those individuals who voluntarily curtail sleep as

the impairment in insulin sensitivity from sleep restriction may increase the risk of developing type 2 diabetes. Additional research is therefore warranted to clarify the underlying mechanisms leading to the disruption in glucose regulation after sleep restriction.

Chapter 5

Summary

5.1 – General discussion

The findings presented throughout this thesis clearly demonstrate a disruption in whole body metabolism after two nights of sleep restriction, confirming the relationship between sleep and glucose metabolism (Morselli et al., 2010).

However, consistent with earlier studies (Bosy-Westphal et al., 2008; Nedeltcheva et al., 2009; Buxton et al., 2010; St-Onge et al., 2011; Klingenberg et al., 2012; Shechter et al., 2014), sleep restriction does not appear to alter resting energy expenditure. An increased preference towards fat utilisation was observed in the fasted state and early stages of the OGTT in the sleep restricted condition but, as suggested previously, this may be due to the increased time spent awake in the fasted state, rather than reflecting a true change in substrate metabolism (Klingenberg et al., 2012; Shechter et al., 2014).

The research presented in this thesis has expanded on the existing knowledge by exploring the underlying mechanisms leading to impaired glucose regulation after sleep restriction. Markers of inflammation and skeletal muscle insulin signalling were investigated as potential mechanisms. The findings suggest that whole body inflammation is unlikely to play a role in this situation as insulin sensitivity was impaired despite no increase in the concentration of circulating TNF- α or CRP. The notion that impaired skeletal muscle insulin signalling is a potential mechanism is uncertain as PKB signalling within skeletal muscle demonstrated conflicting findings, depending on the method of analysis. There also appears to be a discrepancy between studies when examining potential underlying mechanisms. Hence, it can be proposed that the mechanisms leading to impaired glucose regulation after sleep restriction are likely multi-factorial and may differ due to factors such as specific tissue insulin sensitivity, method of analysis and the length of sleep restriction employed.

A key strength of the studies presented here is the sleep restriction protocol utilised. Many previous studies have employed an absolute duration for the

control and sleep restriction conditions. Furthermore, previous studies often used extended durations as a control condition, such as 10 or 12 h (Buxton et al., 2010; Reynolds et al., 2012). Individual habitual sleep duration will differ, which may impact results. We overcame this limitation by using a sleep duration close to habitual duration in the control condition, and restricted sleep relative to habitual duration in the sleep restriction condition. Each individual therefore experienced a similar degree of sleep restriction compared to their habitual sleep patterns.

The findings presented in this thesis provide novel insights into the mechanisms underlying the impairment in insulin sensitivity following acute sleep restriction. However, there are several limitations to the studies presented here which should be considered. Firstly, the study samples consisted of only white males. Glucose regulation is known to vary between individuals of different ethnicities (Osei and Schuster, 1994) and sex (Varlamov et al., 2015), thus findings cannot be generalised to other populations due to possible metabolic variations. Secondly, sleep patterns were measured by actigraphy which, although validated against PSG for overall sleep duration (Jean-Louis et al., 2001), cannot give as much detail about sleep architecture as would be given by PSG. Therefore, the effect of sleep restriction on sleep quality and individual sleep stages cannot be determined. Lastly, the OGTT is an indirect measurement of insulin sensitivity. Although the OGTT correlates well with the gold standard hyperinsulinemic-euglycemic clamp for overall insulin sensitivity and glucose tolerance, it cannot give precise detail about the nature of insulin resistance.

The findings of these studies have implications for individuals who voluntarily curtail sleep, perhaps because of work or social pressures. Impaired insulin sensitivity and hyperglycemia are risk factors for the development of type 2 diabetes. Insulin resistance within skeletal muscle is thought to be the initial defect in the development of type 2 diabetes (DeFronzo and Tripathy, 2009). Therefore, frequent or prolonged periods of sleep restriction may have deleterious effects on glucose regulation, possibly leading to the development of type 2 diabetes.

It is questionable that impaired glucose regulation following short, infrequent periods of sleep restriction has any clinical relevance. Evidence demonstrates that following sleep restriction by periods of catch-up sleep of extended duration may attenuate the effects of sleep restriction on glucose metabolism (Killick et al., 2015). Similarly, napping in the day following the night of sleep restriction may also be beneficial in alleviating the increase in inflammation which has been shown after sleep loss (Vgontzas et al., 2007). Thus, the increased risk of developing impaired glucose tolerance or type 2 diabetes after sleep restriction may be more applicable to chronically sleep-deprived individuals who do not repay their sleep debt by extended sleep or naps.

5.2 – Future study

The findings of the study presented in this thesis taken together with the existing literature identifies several areas which would benefit from further investigation. A dose-response study is needed to identify whether there is a cumulative effect of sleep restriction on impaired glucose regulation. Further exploration into the underlying mechanisms is also warranted, particularly surrounding the possibility of disrupted insulin signalling within skeletal muscle. Skeletal muscle insulin signalling findings in the present study were inconsistent, and the sample size was small, therefore replication of a similar protocol with a larger sample size is warranted.

Interventions to prevent impaired insulin sensitivity following sleep restriction are also needed. Exercise is one possible intervention which should be explored. Glucose regulation is improved by exercise and this effect can last up to 24 hr (Koopman et al., 2005), suggesting that a bout of exercise prior to sleep restriction may counteract the effect of sleep restriction on glucose regulation. If exercise proves successful in attenuating the impairment in insulin sensitivity after sleep loss, it may be used as a possible therapy for shift workers and others who experience sleep loss.

5.3 – Conclusion

To conclude, the work presented in this thesis provides novel information which aids our understanding of the mechanisms responsible for impaired insulin sensitivity following acute sleep restriction. Many questions have been raised throughout which give scope for future research. Arguably the most important of these are: (a) what are the mechanisms responsible for the impairment? and (b) how can we prevent the impairment in insulin sensitivity in situations where sleep restriction is unavoidable? So, whilst our work has built on current knowledge in the field, there is still much to learn regarding sleep loss and its effect on glucose regulation.

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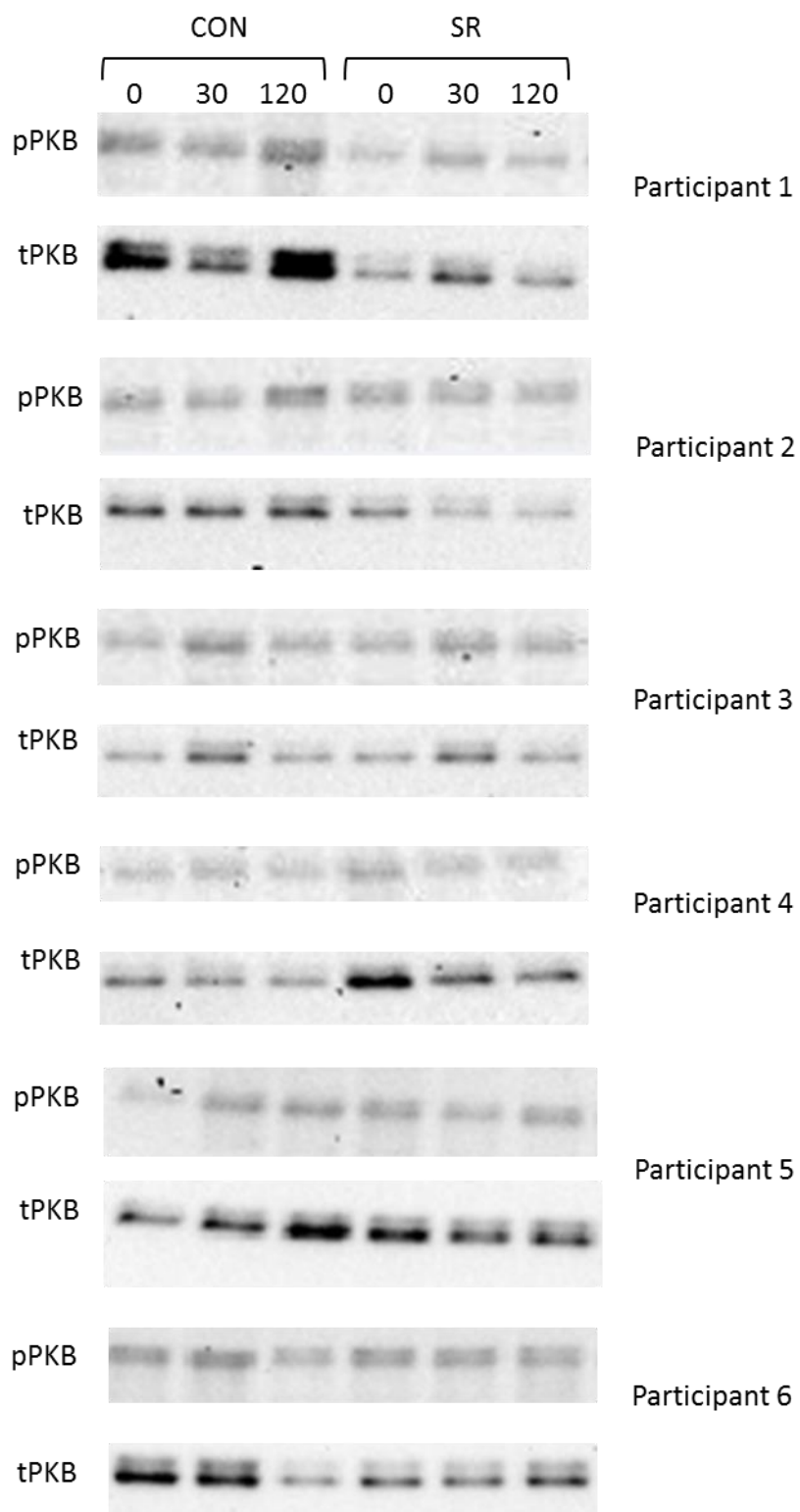
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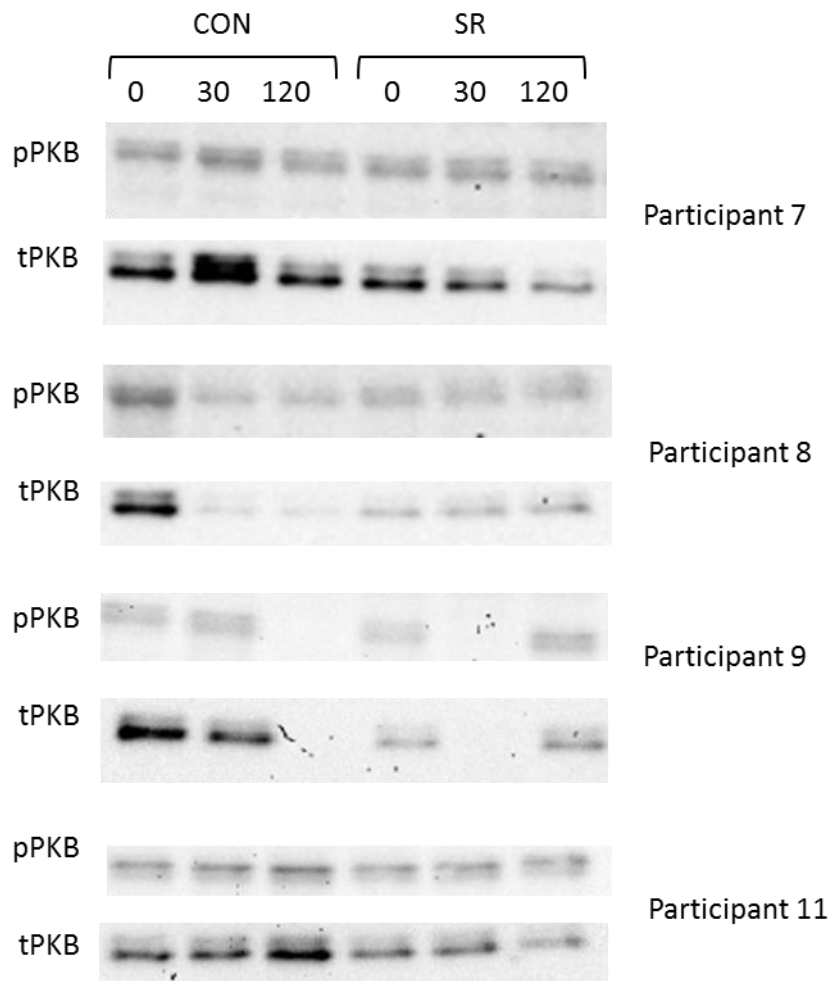
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Appendices

Appendix 1 – Individual Western Blot results showing phosphorylated PKB (pPKB) and total PKB (tPKB) in skeletal muscle at 0, 30 and 120 min during an





OGTT following two nights of control sleep (CON) or sleep restriction (SR).