THE GLYCEMIC INDEX (GI) AND SLEEP: EFFICACY OF A HIGH GI MIXED MACRONUTRIENT MEAL TO IMPROVE SLEEP QUALITY

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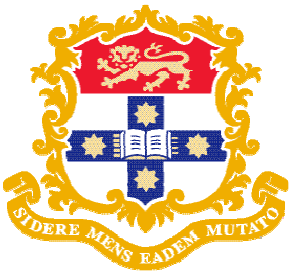

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This is to certify that the thesis entitled "**THE GLYCEMIC INDEX (GI) AND SLEEP: EFFICACY OF A HIGH GI MIXED MACRONUTRIENT MEAL TO IMRPOVE SLEEP QUALITY**" submitted by **Christopher P. Herrera** in fulfilment of the requirements for the degree of Doctor of Philosophy (PhD) in Health Sciences is in a form ready for examination.

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I, **Christopher P. Herrera**, hereby declare that the work contained within this thesis is my own and has not been submitted to any other university or institution as a part or a whole requirement for any higher degree.

I, **Christopher P. Herrera**, hereby declare that I was the principal researcher of all work included in this thesis, including work published with multiple authors.

In addition, ethical approval from The University of Sydney Human Ethics Committee was granted for each study presented in this thesis. Participants were required to read a participant information document and informed consent was gained prior to data collection.

Name Christopher P. Herrera

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Date _____

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ABBREVIATIONS

Area under the curve ..... AUC

Body mass index ..... BMI

Carbohydrate ..... CHO

Carbohydrate high GI ..... CHGI

Cholecystokinin ..... CCK

Cognitive behaviour treatment ..... CBT

Electrocardiogram ..... ECG

Electroencephalogram ..... EEG

Electromyogram ..... EMG

Electrooculargram ..... EOG

First night effect ..... FNE

Glycemic Index ..... GI

Glycemic Load ..... GL

Large neutral amino acids ..... LNAA

Mean difference ..... MD

Mixed macronutrient low GI ..... MLGI

Mixed macronutrient high GI ..... MHGI

Non-rapid eye movement sleep ..... NREMS

Platelet poor plasma ..... PPP

Polysomnography ..... PSG

Rapid eye movement sleep ..... REMS

Rapid eye movement sleep latency ..... REML

Serotonin 5HT

Selective serotonin reuptake inhibitor ..... SSRI

Sleep efficiency .....SE

Sleep onset latency .....SOL

Slow wave sleep .....SWS

Total sleep time .....TST

Tryptophan .....TRP

Wake after sleep onset..... WASO

Weighted mean difference.....WMD

ABSTRACT

Carbohydrate (CHO) ingestion, especially a high glycemic index (GI) CHO meal can improve sleep in healthy individuals, but evidence is lacking regarding its efficacy to improve the poor sleep quality inherent in insomnia. The efficacy of a high GI meal to improve sleep lies in its capacity to increase the availability of tryptophan (TRP), the amino acid precursor to serotonin (5HT), which is an intermediary product to melatonin, a primary neurohormone that influences sleep initiation. The addition of fat or protein to a CHO meal lowers the glycemic response. Thus, the effects of the GI of mixed macronutrient meals on sleep remain unknown. This thesis: (1) investigated the effects of a mixed macronutrient high GI (MHGI) and low GI (MLGI) meal compared to a carbohydrate only high GI (CHGI) meal on the postprandial availability of TRP to the brain, measured by it concentration relative to other large neutral amino acids (TRP/LNAA); and (2) evaluated the efficacy of a high GI mixed macronutrient meal to improve subjective sleep and objective sleep measures using polysomnography (PSG) in a group of men and women with insomnia.

A meta-analysis of the mean data on 9 PSG sleep variables commonly affected by the first night effect (FNE) was completed (Chapter 4); the potential moderating factors included the sleep setting (laboratory, home), recording montage (full PSG, EEG/EMG/EOG only, switched PSG; full PSG night 1 followed by EEG/EMG/EOG only on night 2), sleep period (*ad libitum*, fixed, unknown), and the age of the study population. A total of 24 studies were included in the meta-analysis reporting on 421 healthy sleepers. The data confirm the FNE in healthy sleepers and further describe the FNE by a prolonged SOL (4.46 min), delayed REML (20.55 min), an increase in

WASO (3.88%), and reductions in REMS (-2.17%), TST (-18.81 min), and SE (-4.66%; all $p < 0.01$). There was a two-fold increase in the delayed REML during laboratory compared to home based PSG (22.73 *v*s. 11.36 min, respectively, both $p < 0.01$).

The postprandial glucose and insulin responses to a high and low GI mixed macronutrient meal were compared to a CHO only meal on plasma TRP/LNAA concentration in healthy volunteers after an overnight fast (Chapter 5). The study was a repeated measures design where 10 healthy college-aged males underwent GI testing with additional blood collection to profile the 4 h postprandial amino acid responses to three test meals administered in a random order; carbohydrate only high GI (CHGI), an isoenergetic mixed macronutrient high and low GI meal (MHGI and MLGI, respectively). The energy from the mixed macronutrient meals (MHGI, MLGI) was nearly half that of the CHGI meal (~1915 vs. 3212 kJ, respectively) and the metabolic responses were improved (i.e., lower GI/GL, lower insulin release). There was a significant postprandial rise in plasma TRP/LNAA levels after all meals with a peak between 2-3 h ($p < 0.05$); the percentage rise from baseline was greatest after the CHGI meal (23%), followed by the MHGI (17%), and the MLGI meals (8%); the TRP/LNAA response was greater after CHGI compared to MLGI ($p = 0.04$) indicated by their respective total area under the curve (AUC).

The efficacy of the MHGI meal compared to the MLGI meal to improve subjective and PSG measures of sleep quality in men and women with insomnia was investigated in the final study (Chapter 6). The study was a repeated measure, cross-over design (n = 8, 4 men, 4 women) where participants underwent testing on 2 consecutive nights. Meals were given 3 h prior to individual habitual bedtimes. Subjective ratings of palatability, satiety, and sleepiness were assessed after each meal by a 10 cm visual analogue scale. Subjective measures of post-sleep quality were assessed using a 5-point Likert scale and the level of sleepiness post-study was assessed by a 10 cm visual analogue scale. Postprandial measures of TRP/LNAA and 5HT concentrations were assessed at baseline prior to meal consumption and for 3 h until bedtime. The average energetic load (kJ/kg) was greater for women (33.0 ± 4.1) than men (25.4 ± 3.8, $p = 0.03$). Participants were self-reportedly more rested after the MHGI (2.8 cm) compared to the MLGI meal (2.2 cm; $p = 0.01$) corresponding to "average" and "somewhat", respectively; this was clearly evident in women but not men. There were no significant differences between the MHGI or MLGI meals on averaged 2-night PSG sleep variables. Sex differences were present. The SOL tended to be shorter in women compared to men on all testing nights; PSG SOL values were lower than subjective SOL values. Men had more EEG arousals (AI) than women after the MHGI ($p < 0.001$) and MLGI meal ($p < 0.05$). Light sleep (NREM1) tended to be greater in men than women, especially after the MLGI meal. Both meals elicited a postprandial rise plasma TRP/LNAA levels; the percentage peak rise from baseline after the MHGI meal (17%) was 3.5 fold greater than after the MLGI (8%) meal despite marginal statistical significance; the AUC after the MHGI (1.73 ± 1.3) meal was also not statistically larger than after the MLGI (0.50 ± 0.46; $p = 0.12$) meal. There was no significant postprandial change in PPP-5HT concentrations.

The findings in the final study indicate the MHGI meal improves subjective sleep quality when provided as a regular evening meal 3 h prior to bedtime but only to women with insomnia. The PSG data demonstrated significant night-to-night variability in sleep quality in both men and women. This corroborates findings in the meta-analysis (Chapter 4). Remarkably, the postprandial increase in plasma TRP/LNAA concentration after the mixed macronutrient meal was identical in healthy participants (Chapter 5) and in the participants with insomnia (Chapter 6). However, despite an elevated TRP/LNAA response, especially after the MHGI meal, platelet poor plasma (PPP) 5HT concentration was not changed. The observed changes in blood glucose and insulin indicate the postprandial plasma TRP/LNAA response is likely mediated by the GL of a mixed macronutrient meal. Further studies are needed to evaluate a range of GL values from mixed macronutrient meals on the postprandial plasma TRP/LNAA concentration. Further studies are required to confirm the mechanisms underpinning related to improvements to sleep after high GI mixed macronutrient meals.

Parts of the work presented in this thesis have been published and/or presented in the following forums:

SUBMITTED PAPERS

C.M. Chow & C.P. Herrera (2010). Feeding and Sleep Behavior. *In press: International Handbook of Behavior, Diet, and Nutrition*. New York, Springer

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CONFERENCE PRESENTATIONS - ORAL

C.P. Herrera & C.M. Chow (2008). Shift work, Macronutrient Intake, and Sleeping Patterns: Staying Alert and Eating Healthy. *Symposium Chair & Speaker: American Association of Sleep Technologists Meeting*. Baltimore, MD, USA

CONFERENCE PRESENTATIONS – POSTER

C.P. Herrera, C.M. Chow (2008). A Meta-analysis of the First Night Effect (FNE) in Polysomnography – its implications for baseline measurements. *Abstract: Annual Meeting of the Associated Sleep Societies*. Baltimore, MD, USA

C.P Herrera, P. Ruell, H. O'Connor, C.M. Chow (2010). Influence of the glycemic load (GL) on subjective and objective measures of sleep quality in sleep initiation insomnia. *International Journal of Psychophysiology 77(3), p. 292.*

CHAPTER 1: Introduction

Insomnia represents one of the most commonly diagnosed sleep disorders in many western societies and is characterised by "the subjective dissatisfaction with the quantity, quality, and/or timing of sleep that occurs at least three times per week, for at least 1 month" while chronic insomnia refers to complaints occurring for at least three months (Morin and Espie 2003). Acute periods of sleeplessness often lead to daytime cognitive impairments and fatigue, whereas chronic insomnia impacts negatively on the quality of life including determinants of physical and social functioning, mental health, vitality, and pain (Hatoum, Kong et al. 1998; Katz and McHorney 2002). Higher absenteeism, impaired job performance, and increased health care utilisation contribute to the financial and economical burdens of insomnia in Australian and the USA (Hillman, Fanzca et al. 2006; Daley, Morin et al. 2009).

In Australia, the prevalence of insomnia is reportedly between 13-20% in a group of adults aged 21 years and over (Lack, Miller et al. 1988), however in a separate study 17% of Australian men and 25% of women reported difficulty sleeping in a community based sample (Olson 1996). The characteristic sleep pattern inherent in insomnia is fragile as there is considerable night-to-night variability (Vallieres, Ivers et al. 2005). On many nights of the week, pervasive, maladaptive cognitive thoughts and behaviours limit the total sleep duration. On other nights, sleep quality closely resembles a normal pattern or can be longer in duration due to the sleep homeostatic drive. Moreover, sleeping too little (<6 h) or too much (>9 h) is a significant risk factor for weight gain, insulin resistance, and type 2 diabetes (Taheri, Lin et al. 2004), and is associated with increased mortality (Knutson and Turek 2006).

Primary treatment for insomnia includes pharmacotherapy and behavioural treatment in the form of cognitive behavioural therapy (CBT). In the short term, pharmacotherapy (i.e., benzodiazepines, nonbenzodiazepines, and serotonin (5HT) agonists) can be highly effective to promote sleep onset or increase the total sleep time (Nowell, Mazumdar et al. 1997). However, these medications can be highly addictive and are associated with significant, negative, side-effects including persistent daytime drowsiness, cognitive impairment, and even fatal driving accidents (Berlin, Warot et al. 1993; Sateia and Nowell 2004; Gustavsen, Brarnness et al. 2008). CBT, as an adjunct to pharmacotherapy or delivered as a monotherapy, is highly efficacious in the management of insomnia symptoms since the aim is to prevent conditioned cognitive and behavioural patterns and thought from perpetuating insomnia (Morin and Espie 2003). However, CBT requires trained clinicians, is usually time-consuming (≥12 weeks) and is associated with high drop-out rates (Ong, Kuo et al. 2008). Despite the effectiveness of primary insomnia treatment, over 1.6 million adults in the USA use forms of complementary and alterative medicine (CAM) therapies as a self-directed treatment option for insomnia (Pearson, Johnson et al. 2006). CAM therapies include standardised psychological programs that can be worked on independently at home (van Straten, Cuijpers et al. 2009), biofeedback, prescribed exercise, relation techniques, and herbal or dietary products marketed to facilitate sleep (Morin, LeBlanc et al. 2006).

Recently, a link between the glycemic index (GI) and sleep initiation in healthy sleepers was established, but evidence is lacking regarding the effectiveness in those with sleep difficulties. Afaghi et al. (2007) showed that a high GI carbohydrate (CHO) compared to a low GI CHO meal given 4 h prior to bedtime shortened the time required to fall asleep by 50% (Afaghi, O'Connor et al. 2007). These findings are limited in application given the meals were large (~3200kJ) and primarily CHO (90% total energy) and therefore not representative of a typical evening meal. Also, the approximate GI (117) value corresponds to a glycemic load (GL 175) nearly 1.5 times higher than most persons consume for an entire day (GL 120) (Brand-Miller). As such these meals carry potentially significant unwanted metabolic responses, which make them unsuitable for everyday use, especially in those who are overweight or at risk for obesity or diabetes (Jenkins, Kendall et al. 2000; Pawlak, Ebbeling et al. 2002; Livesey 2005).

Whilst a lower energy meal may lower the glycemic response to the meal, it remains largely unknown to what extent the addition of fat and protein to this type of CHO meal will impact on changes to sleep behaviour and to what extent the GI may be used for the management of insomnia.

Thesis aims

The thesis aimed to evaluate the efficacy of a high GI mixed macronutrient meal to improve sleep quality in insomnia. In undertaking the study, the meal was analysed for its biochemical by-products that underpin the neurochemical regulation of sleep. The following specific aims described the project in more detail:

1. To develop a palatable, high and low GI version of a mixed macronutrient meal that was identical in appearance and suitable for daily use;
2. To quantify the postprandial glucose, insulin, and amino acid responses; and
3. To determine the efficacy of the high GI compared to the low GI mixed macronutrient meal to improve sleep quality.

Hypotheses

1. The high GI compared to the low GI mixed macronutrient meal would show a greater postprandial rise in plasma TRP/LNAA concentration.
2. The high GI compared to the low GI mixed macronutrient meal would result in better subjective sleep ratings for perceived sleep onset latency and sleep quality in participants with insomnia.
3. The high GI compared to the low GI mixed macronutrient meal would decrease the sleep onset latency and increase sleep efficiency measured objectively using polysomnography (PSG) in participants with insomnia.

Significance

Insomnia is a highly prevalent sleep disorder that often goes unrecognised and untreated despite significant adverse health outcomes (Benca, Obermeyer et al. 1992). This study represents a simple, cost-effective dietary manipulation of the GI of evening meals that could be employed as CAM therapy to manage acute or chronic insomnia. The results will provide empirical data on the effects of the GI on sleep in a mixed gender cohort with insomnia and will provide evidence for the biochemical mechanism underpinning the efficacy of high GI foods to promote sleep. Moreover, the application of the findings to primary healthcare are evident given sleep hygiene education, which includes awareness of dietary practices, is often included with other forms of psychological and behavioural treatment, but is currently not recommended as a single therapy due to insufficient evidence to assess the effectiveness (Morgenthaler, Kramer et al. 2006)

Thesis outline

Chapter 2 reviews the literature on the neurochemical regulation of sleep and the effects of dietary intake on sleep, highlighting the factors and biochemical by-products that influence postprandial sleepiness.

Chapter 3 provides specific descriptions of the protocols and related concepts used in the meal design (e.g. GI testing) and those related to PSG (e.g., sleep testing).

Chapter 4 reports a meta-analysis on the first night effect (FNE) describing the sleep quality on the first night during PSG compared to subsequent nights. This study was completed to aid in the design of the final sleep study.

Chapter 5 reflects the meal design and evaluation of the effects of isoenergetic high and low GI mixed macronutrient meals on postprandial blood glucose, insulin, and amino acid concentrations in healthy individuals.

Chapter 6 reports the findings on the effects of the high GI compared to the low GI mixed macronutrient meal on subjective and objective measures of sleep in a participant group of men and women with insomnia.

Chapter 7 provides a conclusion and the limitations of the thesis and recommendations for future dietary related sleep investigations.

CHAPTER 2: Literature Review

Parts of the work presented in this section have been submitted for publication as follows:

C.M. Chow & C.P. Herrera (2010). Feeding and Sleep Behavior. *In press: International Handbook of Behavior, Diet, and Nutrition*. New York, Springer

Introduction

Conceptually, sleep was once thought to be a period of constant inactivity. It is now known that sleep is a dynamic behaviour characterised by the cyclic pattern of non-rapid eye movement sleep (NREMS) and rapid eye movement sleep (REMS). This pattern of behaviour in humans is associated with specific central and peripheral nervous system activity that promotes protein synthesis to restore tissues and allows brain metabolic restoration (Oswald and Corner 1980; Adam and Oswald 1983). Sleep is tightly regulated by a homeostatic process and a circadian process (Borbely and Achermann 1999). The homeostatic drive for sleep is increased with prior wakefulness, whereas sleep-wake timing is under the influence of the master circadian clock located in the supra-chiasmatic nucleus (SCN) (Moore and Silver 1998).

The neurochemical regulation of sleep reflects the activities in many neuronal regions. In fact, sleep becomes continuous and waking does not exist without the ascending reticular activating system (Moruzzi 1964). The neural pathways extending both to and from this region, especially those in and around the hypothalamus are crucial for the regulation of sleep-wake states (Saper, Chou et al. 2001). Transitions from sleep-wake states therefore reflect the reciprocal interaction of neuropeptides, steroids, and neurotransmitters along the hypothalamaic-pituitary-somatotrophic (HPS) and the hypothalamaic-pituitary-adrenocortical (HPA) systems in the brain (Steiger 2007). Indeed, chronic insomnia is associated with hyperactivation of the HPA axis (Vgontzas, Bixler et al. 2001). Moreover, the lack of sleep exerts a modulating mechanism on endocrine function (Spiegel, Leproult et al. 1999), carbohydrate feeding behaviour (Wurtman and Wurtman 1995), and glucose metabolism (Knutson, Spiegel et al. 2007).

The relationship between sleeping and feeding is unique, as the behaviours are mutually exclusive, but demonstrate a reciprocal impact on human functioning. Sleeping and feeding are two crucial human behaviours necessary for survival since the absence of either will result in eventual death. The main dietary constituents that can readily influence sleep are the macronutrients of carbohydrates (CHO) and fat with opposing effects from protein. Whilst sleepiness is increased after CHO- or fat-based meals, macronutrient ingestion *per se* does not promote sleepiness. Postprandial sleepiness is primarily influenced by circadian timing (time of day) of the meals and the macronutrient content via neurochemical changes. Secondary factors include patterns of habitual feeding and gender. Because feeding can alter sleep patterns, it will become vital for dieticians, nutritionists, and other allied health practitioners to understand the acute effects of diet on sleep patterns, since poor sleep (seen in shift workers and in those with insomnia and depression) has implications for poor health outcomes. Figure 2.1 depicts the factors influencing postprandial sleepiness; the most significant factors are those at the base levels of the pyramid.

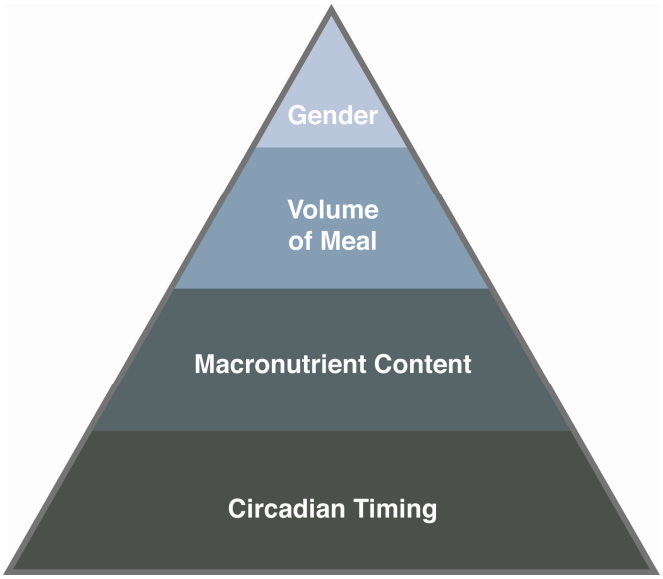


Figure 2.1. Factors influencing postprandial sleepiness.

The factors influencing postprandial sleepiness are presented on the pyramid. The most significant factors are those at the base levels of the pyramid. Note it is possible that these factors may interact with each other at any level.

Neuropeptides, steroids, and sleep

Growth hormone-releasing hormone (GHRH) and corticotrophin-releasing hormone (CRH) play a major role in the HPS and HPA systems respectively. The neuropeptides and steroids known to exert specific effects on the sleep EEG include various peptides (GH, ghrelin, galanin, and neuropeptide Y) that promote sleep and others (CRH, somatostatin) that promote wakefulness (Steiger 2007).

Increased levels of neuropeptide Y act as a signal for sleep onset, especially in males (Antonijevic, Murck et al. 2000). Neuropeptide Y is though to represent a signal for sleep onset through antagonism of CRH (Steiger and Holsboer 1997). GHRH release predominates in the first half of the night stimulating the appearance of growth hormone (GH) and slow wave sleep (Steiger 2007). GH follows a circadian rhythm and can be stimulated by hypoglycaemia, stress, exercise, and protein depletion, but its release is inhibited by obesity, increased free fatty acids (FFA), and glucocorticoids (Merimee and Rabin 1973). There is a positive relationship between the presence of ghrelin and GH (Weikel, Wichniak et al. 2003) suggesting that ghrelin also promotes slow wave sleep. The neuropeptide galanin is involved in the regulation of NREM sleep via interactions with specific neurotransmitter (Saper, Chou et al. 2001), but it is less clear if this results in changes in the sleep EEG.

CRH is released during the second half of the night and is thought to impair NREMS and impacts on the release of cortisol (Steiger 2007). Cortisol also released from the HPA system affects sleep and mood (Gibson, Checkley et al. 1999). Cortisol shows a circadian rhythm with a peak during the early morning hours (0400 - 0800) (Knutsson, Dahlgren et al. 1997) corresponding to periods of REMS, but can be significantly

altered by meal composition (Gibson, Checkley et al. 1999) and changes in meal timing (Bogdan, Bouchareb et al. 2001). Levels of cortisol contribute to the maintenance of rapid eye movement sleep (REMS) through the feedback inhibition of CRH and certain neurotransmitters (Steiger 2007). A study involving patients with insomnia indicates nocturnal cortisol secretion is not impaired compared to healthy individuals, however, nocturnal melatonin section was significantly diminished (Riemann, Klein et al. 2002).

Melatonin, a neuropeptide, located in the pineal gland, is secreted at the onset of darkness, but suppressed by light. Levels of melatonin peak between midnight and 0200 h with the lowest levels in the afternoon (Kennaway and Voultsios 1998). Melatonin is considered a biological marker for the endogenous 24 h circadian rhythm (Ohashi, Okamoto et al. 1999). Melatonin increases sleep propensity and promotes sleep consolidation (Bermudez 1983). Periods of insomnia are associated with low nocturnal levels of melatonin, which results in elevated core body temperature (Dawson and Encel 1993). Indeed, exogenous administration of melatonin facilitates sleep onset (Brzezinski, Vangel et al. 2005) and even improves sleep in the blind who lack the environmental cue for its release (Jan, Espezel et al. 1994). It is suggested that in humans melatonin produces a hypnotic effect indirectly through thermoregulatory mechanisms since exogenous melatonin induces a lowering of the core body temperature (Dijk and Cajochen 1997). The core body temperature also acts as a marker for the endogenous circadian rhythm where periods of increasing temperature correlate with periods of alertness during the late afternoon, but temperature dips and nadir that occur around 0400 - 0600 h are associated with periods of sleepiness (Monk 2005).

Neurotransmitters and sleep

Figure 2.2 depicts the progression of synaptic activity and pre-empts the following description of the neurotransmitters activity involved in the regulation of sleep-wake states.

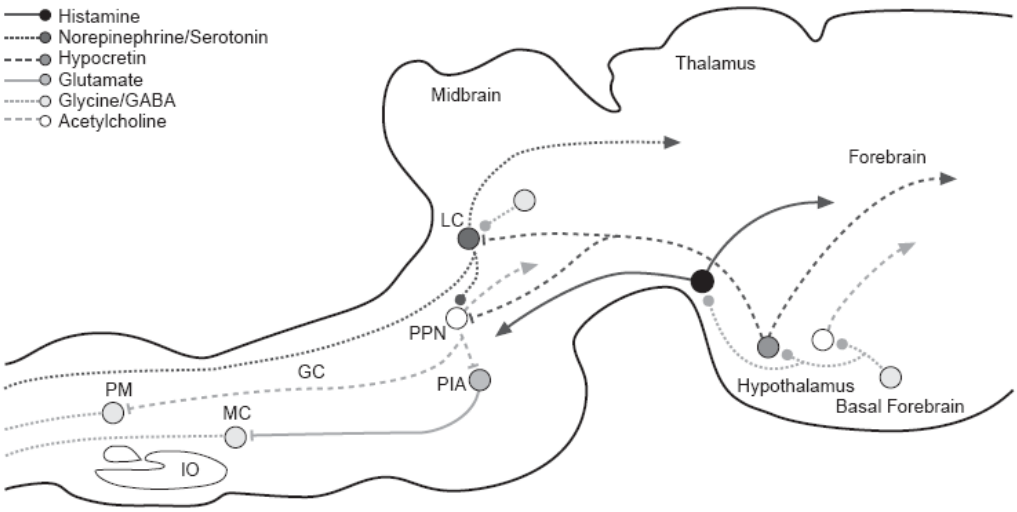


Figure 2.2. Synaptic relationships underlying the control of sleep and muscle tone; Adapted from Steigel 2004. This illustration represents a sagittal section of a cat's brain and illustrates the complexity of synaptic relationships involved in loss of consciousness and correlated loss of muscle tone that characterises normal sleep. Lines ending in solid dots indicate inhibitory output. Lines ending in arrows indicate excitatory output. Abbreviations: GABA, γ-aminobutyric acid; GC = nucleus gigantocellularis; IO, inferior olive; LC = locus coeruleus; MC = nucleus magnocellularis; PIA = pontine inhibitory area; MP, nucleus paramedianus; PPN, pedunculopontine nucleus.

Activity from neurons containing the neurotransmitters such as serotonin (5HT) and γ-aminobutyric acid (GABA) are especially important, however other neurotransmitters such as dopamine, norepinepherine, and acetylcholine specifically influence various stages of sleep (Siegel 2004).

Neurons that discharge with sleep are located primarily along the anterior hypothalamus and basal forebrain, which represents a prominent region involved in sleep (Siegel 2004). The neurons in the locus coeruleus of the pons contain norepinephrine, and the release of 5HT from the raphe nuclei, are similarly linked to sleep via mediation of muscle tone, resulting in a relaxed state. The release of 5HT triggers subsequent sleep promoting mechanisms by triggering activity in the neighbouring SNC, where melatonin is released (Dawson and Encel 1993). In fact, 5HT is the intermediary product in the production of melatonin and therefore its production may also promote sleep (Blum, Vered et al. 1992). Moreover, brain 5HT content is tightly controlled by the presence of the amino acid precursor, tryptophan, which is found in the normal dietary intake (Wurtman, Hefti et al. 1981). Evidence in the animal model suggests brain 5HT activity is linked to REMS by the regulation of muscle tone, especially the phasic activity during REMS (Wu and Boehmer 2004). Remarkably, insomnia follows after lesions are made in either the raphe system or the pre-optic area where 5HT is a major neurotransmitter (McGinty and Sternman 1968). Furthermore, pharmaceuticals like selective serotonin reuptake inhibitors (SSRIs) promote 5HT levels in the brain and are highly efficacious in the management of sleep difficulties such as those presented with insomnia (Perlis, Giles et al. 1997).

The activity of the serotonin system is widely mediated via projections from certain groups of neurons located in the basal forebrain and the anterior hypothalamus that contain the inhibitory neurotransmitter GABA (Siegel 2004). GABA is the most common inhibitory neurotransmitter in the brain and its release induces sleep by inhibiting the cortical activation involved in arousal (Saper, Chou et al. 2001). In fact, GABA also mediates the effects of various neuropeptides (e.g., galanin, somatostatin)

on the changes to sleep presented earlier. The discharge rate of GABA neurons in the basal forebrain and anterior hypothalamus increases at sleep onset and continues its activity while sleep progresses (Steiger 2007). In addition, about 25% of the neurons in the basal forebrain that project to the cortex to regulate sleep-wake states release the amino acid glutamate (Henny and Jones 2008).

The hypocretin/orexin (orexins) system represents another more recently discovered group of specialised neurons whose role may be to prevent unwanted transitions between sleep-wake states (Saper, Cano et al. 2005). Located anatomically between the prominent sleep (anterior hypothalamus and basal forebrain) and wake regions (posterior hypothalamus) in the lateral hypothalamus are neurons containing the neuropeptide orexins that can release both GABA and glutamate (Steiger 2007). These neurons have been linked to cataplexy, a symptom of narcolepsy in which unwanted sleep episodes are experienced with a complete disinhibition of muscle activity (Sakurai 2005). However, the mechanisms by which these neurons affect sleep-wake states are still controversial. Remarkable is the ability of orexins to be both inhibited, thereby promoting sleepiness, or disinhibited, thereby promoting wakefulness, locomotor activity and foraging (Burdakov, Luckman et al. 2005).

In contrast, those neurons located in the posterior hypothalamus represent a prominent region for the wake state (Siegel 2004). These neurons have ascending projections toward the cortex and descending projections toward the brainstem which are active during waking (Jones 2003). Other major connections involved in the control of wakefulness are located in the posterior hypothalamus, where neurons releasing histamine are abundant. Lesions to this region have been shown to produce a coma-

like state (Saper, Chou et al. 2001) and classic antihistamine medications are known to produce side-effects such as drowsiness (Meltzer 1990). Finally, the neurotransmitters acetylcholine in the basal forebrain also plays a major role in the maintenance of wakefulness in the brain. Cholinergic neurons stimulate muscle atonia during REMS (Jones 2003).

Circadian timing of meals

It is known that sleep and metabolism are tightly regulated by the circadian clock that is located in the SCN of the hypothalamus (Kovac, Husse et al. 2009). The body clock keeps time of all biological rhythms. Circadian rhythms are those with an oscillation of approximately 24 h. These rhythms not only display peaks and troughs of activity at different times of the day, but show phase relationships. For an example, the peak core temperature that occurs at around 1600-1800 h is linked to alertness, whereas the temperature nadir occurring at around 0400-0600 h is linked to sleepiness (Monk 2005). Not surprisingly, the time of day that meals are eaten exerts an influence on postprandial sleepiness. A biphasic response was observed after a high-fat, low-carbohydrate (HFLC) or a low-fat, high-carbohydrate meals (LFHC) meal provided at breakfast or lunch. The response showed an increase in alertness that lasted for up to 30 min followed by a shift towards sleepiness that was greatest between 1100 and 1400 h independent of the meal type (Wells, Read et al. 1998). The initial increase in alertness may be explained by the thermogenic effect of the meal, while the postprandial sleepiness coincides with the post-lunch dip, a circadian phenomenon (Monk 1987). This phenomenon has been examined in a study where a 3 h sleep opportunity was allowed after either a CHO liquid lunch meal or a no food condition. Sleep occurred in both conditions suggestive of a strong post-lunch circadian dip (Zammit, Kolevzon et al. 1995). Importantly, sleep duration was markedly increased following food intake, suggesting that in addition to the circadian effect, the meal itself impacted sleep.

Harnish and colleagues queried if postprandial sleepiness was due to the ingestion process related to gustatory, olfactory and/or visual cues associated with a meal (Harnish, Greenleaf et al. 1998). They explored this question using the Multiple Sleep Latency Test (MSLT) with four nap opportunities tested at 1700 h and terminating at 2100 h. They offered participants a mixed macronutrient meal (energy from fat 46% and from carbohydrates 42%) or a sham meal (chewed and expectorated) in a crossover study where the meal allocation was randomised and counter-balanced. There was only a small difference in the sleep period between the feeding and sham feeding conditions at 2 h postprandially. However, sleepiness was delayed with progression of the remaining nap studies, which likely reflects an increased state of alertness linked to the circadian temperature peak as well as the thermic effects of the meal.

Macronutrients and sleep

Meals high in CHO and fats are more effective in promoting sleep compared to protein meals. An increase in sleepiness has been reported at breakfast after high CHO meals compared to high protein meals (Leathwood and Pollet 1982/83). Postprandial sleepiness after HFLC and HCLF meals may be present for up to 4 h depending on the measurement tool used. Sleepiness measured by subjective scales was present at 60 min after meal consumption (Leathwood and Pollet 1982/83; Spring, Maller et al. 1982/83; Lloyd, Green et al. 1994; Lloyd, Rogers et al. 1996; Wells, Read et al. 1997; Wells, Read et al. 1998), whereas sleepiness measured by objective measures occurred between 1.5 to 4 h (Wells, Read et al. 1998). Increased sleepiness measured by the MSLT was present at 1.5 h after meal ingestion, whereas that measured by the Akerstedt EEG sleepiness test (spectral analysis of EEG) was present at 3 h 20 min.

Early evidence showed that SWS is immediately increased after intravenous administration at bedtime of either pure glucose or of an amino acid mixture, whereas increases in REMS occurred after glucose administration only (Lacey, Stanley et al. 1978). Dietary intake of CHO showed appropriate trends in REMS. A dose-response effect for increased REMS was present after isocaloric diets with low (100 g), medium (300 g), and high (600 g) CHO content (Phillips, Chen et al. 1975). Further, an increase in REMS was evident after a low calorie CHO snack given 45 min before the usual bedtime (Porter and Horne 1981). In accordance with these results, a reduction in CHO intake such as either 50g/day (Kwan, Thomas et al. 1986) or complete absence. (Afaghi, O'Connor et al. 2008) resulted in a reduction in REMS, total sleep time, and increased SWS. Recently, a link between the type of carbohydrate intake and sleep initiation has been presented. Afaghi et al. 2007 tested the efficacy of a high versus a

low GI CHO meal and the timing of the high GI meal (4 h versus 1 h before the usual bedtime) on the sleep patterns of healthy adults. The subjects took significantly less time to fall asleep after the high GI compared to the low GI isocaloric meal eaten 4 h before their usual bedtime. They also took less time to fall asleep after the high-GI meal ingested 4 h before bedtime than after that ingested 1 h before bedtime. Moreover, there were no differences to other stages of sleep (Afaghi, O'Connor et al. 2007). In a separate study, when a cereal-type bedtime snack was given just prior to bedtime there was a reduction in sleep latency and a more restful sleep reported in healthy subjects (Brezinova and Oswald 1972). Thus, it is apparent daily food choices, and especially CHO intake, may positively influence sleep quality by promoting sleep onset, REMS, and total sleep duration.

Macronutrients and neurochemicals

The link between CHO intake and increased sleepiness is dependent on the GI of a meal. The GI has been shown to dictate the availability of tryptophan (TRP) to the brain. Dietary TRP is a crucial amino acid that links diet and sleep, given it is the biological precursor to 5HT, an intermediary to the synthesis and release of melatonin. The availability of TRP to the brain depends on its concentration in relation to the other large neutral amino acids (LNAA), since each competes for a single transport protein across the blood brain barrier (Wurtman 1988). Postprandial sleepiness may, therefore, be linked to the post-absorptive mechanisms leading to changes to plasma TRP/LNAA concentration.

Figure 2.3 illustrates the postprandial change in TRP/LNAA levels after two CHO-based meals (sucrose, high GI; raw starch, medium GI) and one fat plus protein meal. There is a clear GI dose-dependent increase in TRP/LNAA levels after CHO-based meals, and a decline after a protein plus fat meal (Lyons and Truswell 1988). This response has been replicated elsewhere (Ashley, Liardon et al. 1985; Caballero, Finer et al. 1988; Wurtman, Wurtman et al. 2003). The time required for digestion and absorption of nutrients relates to the plasma appearance of TRP, since TRP/LNAA levels peaks between 2-4 h after ingestion. This peak rise in plasma TRP/LNAA concentration has been replicated for meals provided in the evening (Lyons and Truswell 1988) providing supportive mechanistic evidence for sleep improvement after high GI CHO ingestion.

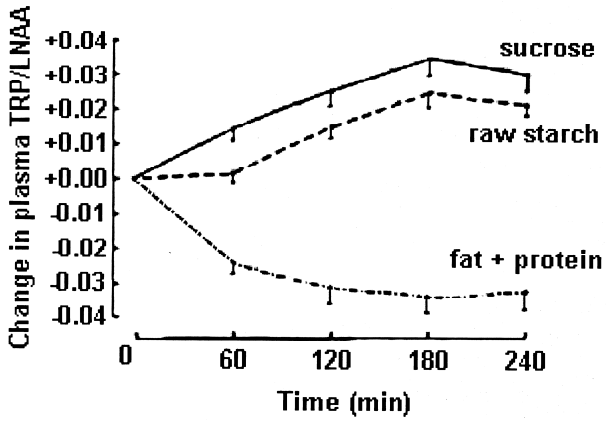


Figure 2.3. Postprandial change in TRP/LNAA levels after two carbohydrates and a fat plus protein meal. The mean change in plasma TRP/LNAA concentration in 13 subjects after sucrose (high GI), raw starch (medium GI), or fat+protein evening meals is shown. Vertical bars represent SEM. Values after sucrose were significantly different from corresponding raw-starch ($p < 0.05$, Neuman-Keul's test). Values after fat+protein were significantly different from both corresponding carbohydrate meals ($p < 0.001$). TRP: tryptophan. LNAA: large neutral amino acids. GI: glycemic index. Data are from Lyons and Truswell (1988), with permission from the Publishers.

High GI meals, through hastened entry of glucose into the blood, facilitate a greater insulin response that mediates the selective uptake of LNAA into skeletal muscle, leaving TRP that is largely bound to plasma albumin free to cross into the brain for conversion into 5HT (Fernstron and Wurtman 1972). Indeed, this cascade of events has been shown to lead to an increase in 5HT production. Figure 2.4 shows the postprandial levels of platelet-poor plasma (PPP)-5HT after a CHO, fat, or protein rich meal (Blum, Vered et al. 1992). As can be seen the level of PPP-5HT was significantly increased from baseline after the CHO-rich meal (4.47 fold) compared to after the fat-rich meal (1.66 fold), reflecting a reduction in the glycemic response when fat is added to a meal. Increased PPP-5HT is likely responsible for the increase in REMS after CHO intake, since increased levels of plasma tryptophan correlate positively with REMS in normal individuals (Chen, Kalucy et al. 1974).

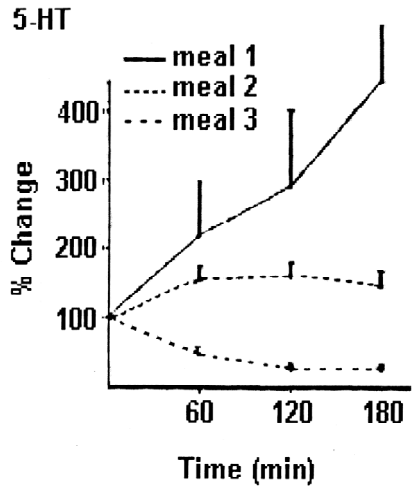


Figure 2.4. Effects of meal composition on platelet poor plasma serotonin (5HT) in healthy individuals. Percent change observed in platelet poor plasma 5HT levels after the administration of test meals: meal 1, carbohydrate; meal 2, protein-rich; meal 3, fat-rich. 5HT: 5-hydroxytryptamine or serotonin. Data are from Blum et al. (1992).

The increase in postprandial sleepiness after fat-based meals may be explained by the release of the neuropeptide cholecystokinin (CCK) in response to the delivery of fatty acids and protein rich chyme to the duodenum. Increased CCK levels are present at approximately 3 h after digestion of HFLC meals or after intraduodenal infusion of lipids (Wells and Read 1995). In this study, increased sleepiness was present after both conditions compared to saline infusion. It is likely that postprandial sleepiness after ingestion of fat is mediated by vagal afferent signals to the brain (Schwartz, McHugh et al. 1993). Evidence in the animal model corroborates these findings (Murray, Clarke et al. 1993). Despite the soporific effects of high fat meals, increased fat intake may lead to positive energy balance and may contribute to weight gain, and thus should be avoided.

Habitual feeding behaviours, gender, and sleep

It is apparent that habitual food choices and gender both greatly influence subjective sleepiness. Meals that deviate from habitual intake with respect to the time of day, volume, or macronutrient content may cause a decline in subjective mood state and increased sleepiness (Lloyd, Green et al. 1994; Wells and Read 1994; Lloyd, Rogers et al. 1996). It is unclear if the carbohydrate feeding behaviour and choice of foods at breakfast have a physiological basis. However, it is possible that this behaviour is signalled by a low blood glucose level following an overnight fast (Robertson, Henderson et al. 2002) and may be synchronised with the sleep-wake cycle (Krauchi, Cajochen et al. 2002). After a LFHC breakfast meal, mood improves and fatigue decreases compared to a HFLC meal, a mixed macronutrient meal, and when no breakfast meal was provided (Lloyd, Rogers et al. 1996). In addition, subjective alertness declined after big lunches compared to small lunches irrespective of habitual lunch size (Craig and Richardson 1989). Further, solid meals, but not liquid meals increase sleepiness compared to an equal volume of water (Orr, Shadid et al. 1997).

Gender may also modify the dietary effects on sleepiness. In women, a decline in alertness is more prominent for those eating larger sized-meals compared with their usual sized lunch-time meals (Smith, Ralph et al. 1991). In another study, women tended to report greater postprandial sleepiness than males throughout the day (Wells and Read 1996). Other subjective mood states such as feelings of lethargy, boredom, and mental slowness have been reported across genders independent of meal type (Smith, Leekam et al. 1988). Thus, it appears that women may be more susceptible to postprandial sleepiness.

Application to health and disease

In summary, macronutrients may be suitable and used to an advantage in the management of sleep difficulties in shift workers and persons with insomnia. Sleep initiation and duration may improve by using moderately high GI foods when sleep is planned (i.e., after a night shift). Given that the efficacy of high GI foods on sleep is founded on the availability of tryptophan, natural foods rich in tryptophan (milk, yoghurt, spinach) are particularly suitable in combination with a CHO-based meal. Choosing a light meal low in GI with a portion of protein when alertness is desired (i.e., during a night shift) may circumvent postprandial sleepiness and lethargy as sleep propensity increases. Since high fat meals and the size of the meal promote sleepiness, avoiding such foods may reduce postprandial sleepiness.

CHAPTER 3: Study protocols and related concepts

The protocols for the dietary and sleep interventions and related concepts are described in this section. Detailed methodologies are described within the relevant study chapters.

Glycemic Index (GI) and glycemic load (GL)

The GI refers to a number scale from 0-100 which is used to express the extent of the postprandial rise in blood glucose in response to the ingestion of CHO from a meal (Thorburn, Brand et al. 1986). The relative CHO content of a meal contributes to the major rise in blood glucose. The addition of protein and fat to a meal reduces the peak rise and overall glycemic effect by increasing the time required for digestion and gastric emptying (Cooke 1975; Ercan, Gannon et al. 1994). High GI foods are rapidly digested and absorbed into the body resulting in a marked increase in blood glucose levels, whereas low GI foods produce only gradual increases in blood glucose levels (Brand-Miller 2009). Low GI foods improve overall metabolic control of diabetes through control of blood glucose, insulin, and lipid levels (Opperman, Venter et al. 2004). These foods have the potential to control weight gain by their ability to delay hunger (Radulian, Rusu et al. 2009). Because the GI is a relative ranking of foods containing the same amount of CHO it will always remain the same despite larger quantities being consumed (Brand-Miller 2009).

The glycemic load (GL) was introduced to describe the overall glycemic effect of a portion of food (Salmeron, Ascherio et al. 1997). This value takes into account the specific serving size and the GI of a food. Therefore, the GL can be used to describe the metabolic effect of different amount and types of CHO (Brand-Miller 2009). At least in healthy, young men, it appears the GL, and not the GI, is a more reliable measure of the overall glycemic response and insulin demand (Brand-Miller, Thomas et al. 2003). Thus, the higher the GL, the greater the expected glycemic and insulin response of the food (Foster-Powell, Holt et al. 2002).

The GI of a food can be determined after a series of test in which finger-prick blood samples are obtained from at least 10 healthy participants to measure levels of blood glucose for at least 2 h to construct blood glucose curves (Brouns, Bjorck et al. 2005). Participants are given a reference food and then a series of test foods in random order, each at least 48 h apart. The GI is calculated by comparing the incremental area under the curve (AUC) of the test food to the AUC of the reference food and multiplying by 100 (Figure 3.1 and Equation 1). The reference food, typically of 50 g pure glucose, must be equivalent to the available CHO (g) from the test food in order for accurate GI measurement. In the presented thesis, a 75 g glucose load was necessary because the mixed macronutrient meals each contained 75 g available CHO. These meals were designed to provide sufficient energy for adults when provided as a dinner meal.

Equation 1

$$\mathbf{GI} = \frac{\text{120 min AUC of test food}}{\text{Mean AUC from reference food}}$$

$$\mathbf{GL} = (\text{GI}/100) \times \text{available g CHO}$$

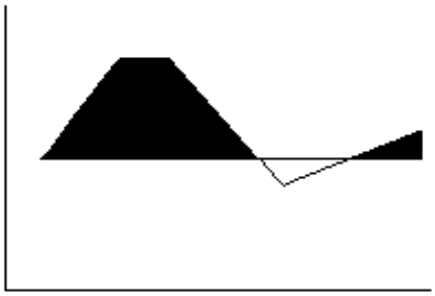


Figure 3.1. Determination of GI using the area under the curve method. This figure illustrates a typical 2 h blood glucose curve. The AUC of a meal represents the total area above baseline (shaded areas) only. Diagram adapted from Brouns et al. 2005.

Foods designated as high GI will have an index of at least 70, medium at least 56, and anything below is considered low GI (Foster-Powell, Holt et al. 2002). The cut-off for high GL is a value ≥ 20 and for low GL ≤ 10; medium GL is 0-19 (Brand-Miller, Holt et al. 2003). A high GL over an entire day is a value ≥ 120 (Brand-Miller 2009).

Research Diagnostic Criteria (RDC) for Insomnia

Standardised sleep questionnaires were used to establish research diagnostic criteria (RDC) for insomnia in accordance with published guidelines (Edinger, Bonnet et al. 2004). The Pittsburgh Sleep Quality Index (PSQI) and the Insomnia Severity Index (ISI) were administered to document the presence of insomnia as defined by the following research criteria: (1) a disturbed quality of sleep (PSQI ≥10); (2) sleep initiation of equal to or greater than 30 min, at least three times per week (PSQI, ISI); and an (3) ISI index of at least 11. The PSQI also established problems with sleep quality, duration, and efficiency, but were not required to meet the RDC for insomnia.

The PSQI (Buysse, Reynolds et al. 1989) contains 19 self-administered questions and five additional questions for the bed partner; the former only included in the overall score to assess sleep quality and disturbances over the last month (Appendix A). The PSQI score is grouped into seven subjective sleep components: sleep quality, sleep latency, sleep duration, sleep efficiency, sleep disturbances, the use of sleeping medications, and daytime dysfunction. The total score for the PSQI is from 0-21, "0" indicating no difficulty and "21" indicating severe difficulties in all areas. The PSQI is a validated instrument used to discriminate poor (PSQI ≥ 6) from good sleepers, but was not specifically designed to reflect the clinical assessment of insomnia (Bastien, Vallieres et al. 2001).

The ISI (Bastien, Vallieres et al. 2001) is a brief self-administered and validated questionnaire designed specifically to assess the perception of subjective symptoms of insomnia as well as the level of distress caused by these symptoms. The ISI contains 7 questions (Appendix A) to assess sleep onset and sleep maintenance difficulties, satisfaction of current sleep pattern, interference with daily functioning, noticeability of those problems by others, and the degree of distress or concern caused by these symptoms over the last two-week period. Each question is rated on a 0-4 scale with a total score of 28. Higher scores (above 22) suggest severe insomnia, scores between 15 and 21 suggest moderate insomnia, between 8 and 14 suggest sub-threshold insomnia, and between 0-7 are considered as no significant insomnia. Whilst a structured diagnostic interview remains the "gold-standard" for the evaluation and diagnosis of insomnia, the ISI is considered a reliable tool for screening and evaluation of insomnia in research and clinical settings (Morin and Espie 2003). A recent study has highlighted that the incidence and temporal pattern of insomnia is such that untreated patients experience a 'good sleep' as much as 50% of the time over a two-week period. Thus, a cut-off score of 11 was arbitrarily reflecting a value in the middle of the sub-threshold insomnia range.

Polysomnography (PSG)

PSG is considered the "gold-standard" procedure used to diagnose and evaluate treatment of various sleep disorders such as the forms of sleep disordered breathing (i.e., obstructive sleep apnoea) and periodic limb movements (Kushida, Littner et al. 2005). PSG is also indicated to support the clinical diagnosis of insomnia when there is a need to rule out other sleep disorders. During PSG, differential electrical potentials collected through scalp and skin surface electrodes are recorded. Comprehensive PSG can record up to 64 channels using sophisticated digital polygraph equipment and include measures such asl; brain wave activity, eye movement activity, skeletal muscle activity – typically of the masseter and anterior tibialis, respirations and respiratory effort, snoring sounds, plethysomnography (oxygen saturation) and others as indicated.

Objectively measured brain wave activity, electroencephalography (EEG), offers a dimension of sleep not attainable by subjective sleep measures. EEG measures reveal sleep is usually entered through Stage 1 drowsy sleep, followed by "true" sleep (Stage 2) characterised by the sleep EEG spindle structure, and followed by Stage 3 or slow wave sleep (SWS) and rapid eye movement sleep (REMS). Stages 1-3 do not exhibit rapid eye movements and are collectively known as non-rapid eye movement (NREM) sleep. In a normal adult sleep pattern, there are approximately 4-6 cycles of NREM and REM sleep; each cycle is between 90 to 120 minutes in duration (Roth 2004). During NREM sleep, cognitive activity is often reduced or fragmented and major body movements occur, however, skeletal muscle tone progressively decreases as this sleep state moves from light sleep of stages 1 and 2 to deep sleep of stage 3 or SWS. In contrast, during REMS, cognitive activity increases yet the body becomes nearly

paralysed with muscle atonia, and punctuated by phasic muscle twitches. Figure 3.2 is a hypnogram depicting the sleep pattern of an adult regular sleeper.

Both the SWS and REMS exhibit polarity during nocturnal sleep. SWS occurs in abundance during the first sleep cycle, whereas REMS predominates in the latter half as individual periods become longer, especially prior to waking. The distribution of the total sleep time for normal adult sleep stages is approximately 5% for NREM 1, 50% for NREM2, 20% for NREM 3, and 25% for REMS (Iber, Ancoli-Israel et al. 2007).

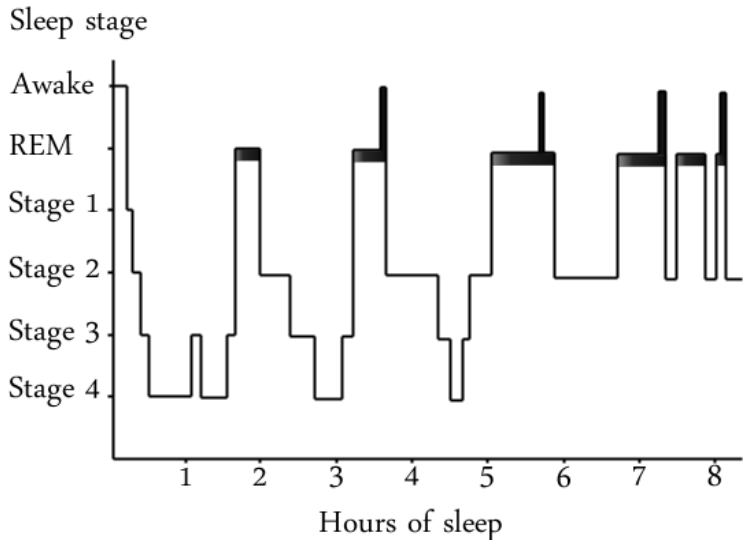


Figure 3.2. Hypnogram depicting the normal sleep pattern in adults. This figure illustrates the progression from awake through the various forms of sleep stages. The normal sleep pattern consists of 4-6 cycles of NREM and REM sleep. Note, stage 3 and stage 4 are now referred to as SWS collectively according the most recent guidelines set by the AASM (2007).

PSG Protocols

Recordings to collect EEG sleep data were conducted using the Compumedics S- and W-Series sleep system (Compumedics Pty Ltd, Australia) connected to a personal computer. A standardised procedure for the location and application of scalp and skin surface electrodes was completed according the American Academy of Sleep Medicine (Iber, Ancoli-Israel et al. 2007) and described in the subsequent section (Electrode Placement). Table 3.1 describes the PSG signal types, physiological responses monitored during all recordings and the type of electrode used.

Table 3.1. PSG signal types

Signal type	Physiological responses	Electrode Type
ECG	Heart rate	Adhesive, snap
EEG	Brain wave activity	Various scalp sites
EOG	Eye movement activity	Adhesive, snap
EMG	Skeletal muscle activity	Adhesive, snap
Snore	Audible snoring	Microphone
Airflow	Respirations	Thermocouple
Effort	Abdominal and thoracic respiratory Effort	Inductive-band plethysomnography
Sa02	Peripheral oxygen concentration	Finger monitor
Position	Body positions	Compumedics

Abbreviations: ECG, electrocardiogram; LOC, left ocular gram; ROC, right ocular gram; EMG, electromyogram; LLEG, left leg; RLEG, right leg; Airflow, airflow thermistor; Sa02, peripheral oxygen saturation.

Electrode Placement

The EEG scalp electrode placement was completed in all recordings in accordance with the guidelines of The International 10-20 System (Jasper 1958). This system documents the standard technique used worldwide to ensure the appropriate location, application, and terminology of EEG scalp electrodes to allow for accuracy of interpretation of EEG signals. Figure 3.3 illustrates the appropriate electrode sites and shows the appropriate final setup. The electrooculargram (EOG) channels designated LOC (left eye) and ROC (right eye) is not shown. These were placed 1cm laterally from the outer canthus of each eye and 1cm below and above, respectively. Modifications were made using Fz as a reference electrode (REF) and a grounding electrode (GND) in the middle forehead; the chin EMG electrodes were placed on the left (EMG1) and right (EMG2) side of the cheek to monitor muscle activity of the masseter. The electrocardiogram (ECG) was obtained using 2 adhesive type electrodes placed 1cm above the collar bone on the left (ECG1) and right (ECG2) side of the body. The detailed procedure is described as follows:

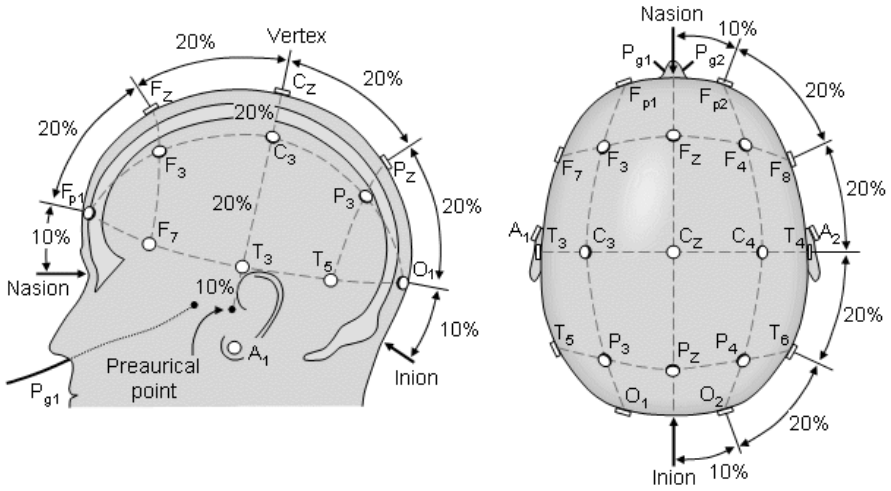


Figure 3.3. Appropriate electrode sites during PSG. This figure illustrates the nomenclature and site for standard PSG electrodes according to the International 10-20 System.

First, locate electrode sites by measuring the distance between the nasion and inion, and between the left and right preauricular points. Mark the appropriate electrode sites (C4, 01, REF, GND, A1, A2, Chin EMG 1 and 2) and prepare each by using abrasive gel (Nuprep, DO Weaver, USA) and cotton-tip applicator. Remove excess and clean skin of oils using alcohol wipe (Medi-Swab 70% Isopropyl alcohol). Collect EC2 electrode cream (Grass Technologies, Rhode Island, USA) neatly inside Ag/Cl cup electrode (Grass Technologies). Collect 2cm x 2cm gauze pad and cover with electrode cream, EC2, (Grass Technologies). Place electrode onto appropriate scalp site (C4, O1, and REF) securing with a 2cm x 2cm gauze pad. Collect adhesive electrode (Meditrace 100, Tyco Healthcare, USA), attach to snap wires (Grass Technologies), and place on appropriate head location (A1, A2, LOC, ROC, GND and Chin EMG). Collect all electrode wires and insert into appropriate signal location on the PSG system head box and connect to amplifier. Manoeuvre wires behind the closest side of the head securing behind the ear. Take wires at front of the head, underneath the chin and wrap 3 times with gauze strips at intervals of approximately 20 cm.

Tables 3.2 and 3.3 describe the recording montages used for the familiarisation (night 1) and test recording nights (nights 2-5), respectively. During the familiarisation night additional signals were placed according to published guidelines to screen for comorbid sleep disorders (i.e., obstructive sleep apnoea, PLMD).

Table 3.2. PSG recording montage for the familiarisation night

Channel	Polarity	Sensitivity (Zoom)	High Pass Filter (Hz)	Low Pass Filter (Hz)	Notch Filter (Hz)
ECG1-ECG2	Positive	4.0	0.3	30	50
C4 – A1	Negative	8.0	0.3	35	50
01 – A2	Negative	8.0	0.3	35	50
LOC – A2	Negative	8.0	0.3	35	50
ROC – A1	Negative	8.0	0.3	35	50
EMG1-EMG2	Positive	4.0	10	100	50
Snore microphone	Positive	4.0	10	100	50
LLEG1-LLEG2	Positive	10	10	100	50
RLEG1-RLEG2	Positive	10	10	100	50
Airflow	Positive	10-100	0.1	15	Off
Thoracic Effort	Positive	10-100	0.1	15	Off
Abdominal Effort	Positive	10-100	0.1	15	Off
Sa02	Positive	None	None	None	Off
Body Position	None	None	None	None	Off

Abbreviations: ECG, electrocardiogram; LOC, left oculargram; ROC, right oculargram; EMG, electromyogram; LLEG, left leg; RLEG, right leg; Airflow, airflow thermistor; Sa02, peripheral oxygen saturation. The ECG, Snore microphone, LLEG, RLEG, thoracic and abdominal effort, Sa02, and body position sensors were used to identify activity consistent with medical comorbity (i.e., sleep apnoea, PLMD).

Table 3.3. PSG recording montage for testing nights

Channel	Polarity	Sensivity/ Zoom	High Pass Filter (Hz)	Low Pass Filter (Hz)	Notch Filter (Hz)
C4 – A1	Negative	8.0	0.3	35	50
01 – A2	Negative	8.0	0.3	35	50
LOC – A2	Negative	8.0	0.3	35	50
ROC – A1	Negative	8.0	0.3	35	50
EMG1-EMG2	Positive	4.0	10	100	50

Abbreviations: ECG, electrocardiogram; LOC, left oculargram; ROC, right oculargram; EMG, electromyogram.

Physiological Calibrations

After completion of the electrode placement each subject was asked to lie on their back and instructed to perform various manoeuvres that are designed to simulate sleep-related behaviours. The resulting tracings were annotated on the personal computer and carefully examined to verify correct signal derivations and signal quality (i.e., electrical impedance $\leq$ 10 kOhm). Any indication of poor signal transmission, instrument failure, or the presence of interfering signals were addressed at this time. The physiological calibrations performed during PSG recordings are as follows: eyes closed for 30 s, eyes open for 30 s, look left and right for 5 s, look up and down for 5 s, blink eyes five times slowly, clinch jaw or grind teeth. In addition, on the first night (familiarisation), the following responses were measured; make a snoring sound three times slowly, flex left foot only, flex right foot only, hold breath for 10 s, breath through your nose only for 30 s, breath through your mouth only for 30 s.

Sleep and EEG arousal scoring

Standardised guidelines for the appropriate recognition and notation of sleep stages were followed according to the American Academy of Sleep Medicine (Iber, Ancoli-Israel et al. 2007) using standard 30 s epochs. An epoch is the term used to describe the digital polygraph on the personal computer and is comparable to what would be seen on paper using an analogue system. Table 3.4 describes the appropriate EEG, EOG, and EMG activity used to identify the individual sleep stages of NREMS (1-3) and REMS. Sleep scoring was completed by a researcher blinded to the experimental conditions (CMC).

Table 3.4. Sleep stage scoring criteria

Sleep Stage	EEG	EOG	EMG
Wakefulness, alert	Low voltage, mixed-frequency (beta rhythm)	Voluntary eye movements	Increased tonic activity
Wakefulness, drowsy	Low voltage, mixed frequency (alpha rhythm)	Voluntary eye movements	Increased tonic activity
NREM Stage 1	Theta waves / vertex waves, low voltage mixed frequency	Slow rolling eye-movements	Decreased tonic activity from wakefulness
NREM Stage 2	Sleep spindles, k-complexes, mixed frequency waves	Occasional slow rolling eye-movements then similar to EEG	Tonic activity, low threshold
NREM Stage 3 or SWS	Delta waves predominates, moderate voltage activity	No eye movements, follows EEG	Tonic activity, low threshold
REM	Low voltage, mixed frequency; saw tooth waves, theta bursts	Phasic rapid eye-movements; or periods of tonic activity	None; muscle atonia, phasic twitches

EEG rhythm frequency: beta > 14 Hz, alpha 8-13 Hz, theta 4-7 Hz, delta < 0.5-2Hz; sleep spindle, train of distinct waves of 11-16Hz with a duration of ≥ 0.5 s;

K-complex, distinctly sharp negative wave followed by a positive component with total duration ≥ 0.5 s

EEG arousals were scored using the criteria provided by the American Academy of Sleep Medicine (Iber, Ancoli-Israel et al. 2007). The general definition used to describe an EEG arousal is as follows:

> "An arousal is defined as an abrupt shift in EEG frequency that occurs during sleep and lasts between 0.5 and a maximum of 14.9 s."

The rules described below were also applied to the primary (C4) and secondary (O1) EEG channels and the EMG channel as appropriate to confirm EEG arousals:

- Subject must be asleep for at least 10 s and there must be at least 10 s of intervening sleep between arousals
- Arousals in NREMS may occur without concurrent increases in EMG activity
- Arousals in REMS must occur with an increase in EMG activity

PSG sleep variables

PSG sleep variables studies were computed as per the digital polygraph specifications (Compumedics Pty Ltd, Australia).

- Lights Out: start of recording (1 min after physiological calibrations)
- Sleep Onset Latency (SOL): time from lights out to the third consecutive 30 s epoch of NREM 1 or first epoch of any other stage. "Start of sleep."
- Rapid Eye Movement Latency (REML): time from SOL to the onset of the first REM sleep epoch
- Sleep Period: time from SOL to last 30 second epoch of sleep including intervening periods of wakefulness
- Non-Rapid Eye Movement Sleep (NREM): The sum of all NREM sleep stages (1, 2, and 3)
- NREM Stage 1: the sum of all stage 1 sleep time within the sleep period
- NREM Stage 2: the sum of all stage 2 sleep time within the sleep period
- NREM Stage 3: the sum of all stage 3 sleep time within the sleep period
- Rapid Eye Movement Sleep (REMS): the sum of all REM sleep time within the sleep period
- Total Sleep Time (TST): the sum of all sleep time including any sleep stage within the sleep period
- Wake After Sleep Onset (WASO): the sum of all time spent awake during the sleep period
- Arousal Index: the number of EEG arousals (see Arousal Scoring) per hour of sleep period

Respiratory events, limb movements, and snoring

During the familiarisation night additional rules were applied to the PSG channels of respiratory related events, limb movements, and peripheral oxygen saturation to identify the presence of sleep disordered breathing or periodic limb movement disorder (PLMD). Briefly, periods of reduced airflow and/or respiratory effort with a 3% drop in peripheral oxygen saturation (hypopnoea), periods without breathing (apnoea), snoring, and limb movements were noted and tabulated during the sleep period. Standardised guidelines were used to assess these data against the presence of obstructive sleep apnoea or PLMD as indicated by an appropriate index of 5 or 15 events/hour of sleep, respectively (Redline, Budhiraja et al. 2007).

CHAPTER 4: A meta-analysis of the first night effect (FNE)

The enclosed study at the time of writing this thesis was in review as follows:

C.P. Herrera & C.M. Chow (2010). A Meta-Analysis of the First Night Effect (FNE). *In revision: Journal of Clinical Sleep Medicine*. MS # JC-00030-09

Acknowledgement is made Dr. Helen O'Connor, The University of Sydney, Discipline of Exercise and Sport Science for her generous review of the previous manuscript versions. Further acknowledgement is made to the editor and reviewers of the Clinical Journal of Sleep Medicine during the 2009-2010 academic years.

ABSTRACT

Introduction: It is well established that sleep on the initial night during PSG is poor in relation to a subsequent night. To account for this first night effect (FNE) it is common practice to provide a familiarisation period and to discard data before subsequent analysis. To date, the characteristics and magnitude of the FNE have not been quantitatively reviewed.

Methods: A systematic literature search was performed to identify consecutively recorded data on PSG sleep variables. Meta-analysis was completed using MIX (Meta-analysis with Interactive eXplanations, Bax, Leon). The study population, sleep setting, recording montage, sleep period, and age were used as moderating factors.

Results: Meta-analysis was completed in healthy sleepers (a total of 24 studies reporting on 421 subjects). The FNE was characterised by a prolonged SOL (4.46 min), delayed REML (20.55 min), increased WASO (3.88 %), and reductions in REMS (-2.17 %), TST (-18.81 min), and SE (-4.66%; all $p < 0.01$). Studies conducted in the laboratory demonstrated a two-fold increase (Qb 6.83, $p < 0.01$) for the delayed REML compared to those in the home (22.73 vs. 11.36 min, respectively, both $p<0.01$).

Conclusions: This study provides the first quantitative analysis to confirm and describe the characteristics of the FNE in healthy sleepers. These findings show a clear estimate of the magnitude of change in 9 PSG sleep variables across the first two consecutively recorded nights. These data substantiate the common practice to treat the first recording night during PSG as a familiarisation period. Further research is needed before a concise statement about the FNE can be made in clinical groups of insomnia and depression.

Keywords: FNE, First night effect, PSG, Healthy, Methodology

INTRODUCTION

An early paper by Retschaffen and Kales (1964) reported an adaptation effect to the sleep laboratory. This study investigating the influence of monetary incentives for controlling dreaming in healthy men (20-30 y), showed a lower REM sleep duration on the first of four consecutive nights (Rechtschaffen and Verdone 1964). Agnew et al. (1966) described this phenomenon as the first night effect (FNE) reporting an increased wakefulness, less rapid eye movement (REM) sleep, and a delayed onset of slow wave and REM sleep (Agnew, Webb et al. 1966). Other studies have reported a delayed sleep onset, lower sleep efficiency and decreased total sleep time (Toussaint, Luthringer et al. 1997; Lorenzo and Barbanoj 2002; Curcio, Ferrara et al. 2003). Since these early reports it has become common practice to treat the first night of PSG as a familiarisation period and data is typically discarded before baseline measures are obtained and subsequent analysis is conducted. However, over the last 4 decades many attempts have been made to evaluate the FNE with respect to common methodological factors.

The sleep setting, type of participant, and age are among the most important factors influencing the FNE (Lorenzo and Barbanoj 2002). Compared to the laboratory, an attenuated FNE is often reported when studies are conducted in the home (Coates, George et al. 1981; Zucconi, Ferini-Strambi et al. 1996). Forms of sleep pathology such as insomnia and depression contribute to additional night-to-night variability in sleep quality (Edinger, Marsh et al. 1991) and sleep fragmentation (Mendels and Hawkins 1967), therefore it is as yet unclear if these clinical groups share the same FNE characteristics as healthy sleepers. Further, treating the first night as a

familiarisation period gives the opportunity to screen for sleep disorders in otherwise healthy subjects. However, the added respiratory effort bands, limb movement electrodes, and oximeter finger probe may impact negatively on sleep quality and contribute to a more pronounced FNE (Agnew, Webb et al. 1966; Johns and Dore 1978). Moreover, the removal of these electrodes on a subsequent night may differentially influence the sleep response. Another methodological factor that may influence the FNE may be the sleep period. During PSG studies the sleep period may be fixed or can be provided *ad libitum*. When the sleep period is provided *ad libitum* it incorporates usual circadian timing of sleep and waking and offers optimal sleep opportunity. On the other hand, a fixed sleep period in research studies may impose unfavorably on sleep onset latency or total sleep time, and may truncate REMS, potentiating a rebound on the subsequent night (Rotenberg, Kayumov et al. 1997).

Importantly, exclusion of first night PSG data corresponds to a substantial loss and significant additional cost with respect to personnel, equipment, and consumables (Woodward, Bliwise et al. 1996). To date, a quantitative review documenting the characteristics and magnitude of the observed FNE has not been conducted. Thus, the common familiarisation period during PSG has not been empirically validated. The present systematic review and meta-analysis examined the FNE observed during sleep investigations with consecutively recorded PSG data in healthy adults and patients with insomnia or depression. In addition, exploring key methodological factors (study population, sleep setting, recording montage, sleep period, and age) provides meaningful interpretations to aid in PSG study designs.

METHODS

Systematic Search

A systematic review of the literature was undertaken on the 1st December 2009 using an online search of the MEDLINE (from 1950) and PsychINFO (from 1967) databases. Keywords used to identify English language sleep investigations reporting data on the FNE were (sleep, EEG, polysomnography, or PSG) and (first night effect or FNE). Additional references were collected from a previously unpublished search.

Selection of published studies

Included studies reported nocturnal sleep data on at least two consecutive nights in adults aged greater than 17 y to minimise variance from childhood and adolescent sleep patterns (Ohayon, Carsadon et al. 2004). Sleep data were required on at least one of the following 9 sleep variables: sleep onset latency (SOL min), REM latency (REML min), total sleep time (TST min), wake after sleep onset (WASO, %TST), sleep efficiency (SE %), or the percentage of TST spent in the following stages: non-rapid eye movement stages 1 or 2 (NREM1, NREM2), slow wave sleep (SWS), and REM sleep (REMS). These variables were chosen since they are among those commonly impacted by the FNE. Also, these are among those recommended during PSG (Kushida, Littner et al. 2005). Exclusion was made as follows: First, all abstract and conference proceedings were excluded. Second, reports involving patients who had medical comorbidity (i.e., pain disorders, epilepsy, sleep disordered breathing, periodic limb movements) were excluded to minimise related effects on sleep. Third, a series of other exclusions were met if the reported data were: (1) after an initial adaptation period, (2) from non-consecutive recordings, (3) presented as an average of two nights,

(4) from experimental or comparative trials (e.g., placebo administration or comparing personality traits), or (5) involved participants with prior experience in sleep testing. Reports involving participants with prior experience of sleep testing were excluded as their familiarity with the environment or testing procedure may minimise the disruptions to their sleeping pattern (Clausen, Sersen et al. 1974).

All retrieved studies were assessed for possible inclusion and relevance by inspection of full-text papers by one reviewer. A second reviewer was consulted where inclusion or exclusion criteria could not be determined.

Assessment of study quality

A formal assessment of study quality was not performed as is recommended for a meta-analysis (Pai, McCulloch et al. 2004). The purpose of the present meta-analysis was to describe and estimate an effect from one night to the next among 9 PSG sleep variables within the same cohort. Therefore, the randomisation process, group allocation, and method of blinding of the participants were irrelevant. Moreover, the outcome measures were measured within the same individuals for all PSG variables. As such, the primary research question did not fit a traditional randomised control trial, cross-sectional, or case-control design. Thus, the most appropriate appraisal on the quality of the studies is to consider the clinical homogeneity within each study population.

Data abstraction and coding

Study and subject criteria were retrieved from each report independently by one author and tabulated. Ambiguous data was resolved by discussion with a second reviewer. Items retrieved were: authorship, date of publication, number of subjects, gender distribution, and the following moderating factors: study population, sleep setting, recording montage, sleep period, scoring method, and the average age of the sample. Moderating factors were grouped as follows; population as healthy sleepers, insomnia or depression; sleep setting as laboratory (hospital and sleep centre) or home; recording montage as either neuro (studies using only EEG, EOG, and EMG channels), full PSG (included the utility of respiratory and/or limb movement measures on both night), or switched (consisted of a full PSG on the first night, followed by only neuro channels on subsequent nights); sleep period as either ad libitum (allowed for habitual sleep periods), fixed (fixed timings), or unknown (did not report the available sleep period). The individual night 1 and night 2 data were coded in means and standard deviations (SD) for each sleep variable. A pooled SD was computed by taking the root mean square of the night 1 and night 2 SD, and the standard error (SE) was recorded.

Data synthesis and statistical analysis

The mean difference (MD) for each sleep variable across the first two nights (N1 minus N2) and the SE were meta-analysed using MIX (Meta-analysis with Interactive eXplanations, Bax, Leon)(Bax, Yu et al. 2006; Bax, Yu et al. 2008). Weighting was applied to each MD using the inverse variance method and the weighted mean difference (WMD) was calculated as a summary statistic to indicate the overall pooled MD among all included studies for each sleep variable. Statistical significance was assessed using the Z statistic, computed by dividing the WMD by its SE, resulting in

the overall 95% confidence interval (CI) for each sleep variable. Significance was met at an alpha level of $p < 0.05$. Both fixed and random effects model assumptions were followed and reported as appropriate following the Q-statistic (the sum of squares of each MD about the WMD) to test for homogeneity and dispersion of the overall WMD for each sleep variable (Egger, Smith et al. 1997). The Q-statistic follows an approximate Chi-squared distribution on k-1 degrees of freedom, where k is the number of MDs under the null hypothesis that the true effect is the same for all trials. When Q is significantly lower than the chi-squared critical value, the null hypothesis is accepted; therefore the variability across the WMD does not exceed what would be expected by sampling error alone (fixed effects model). A random effects model is reported when Q remains statistically large at $p < 0.05$, indicating poor homogeneity of the magnitude and direction of the WMD (Egger, Smith et al. 2001). Accordingly, the I^2 index was used as a second indicator of between-study (non-random) variance to quantify the extent of heterogeneity from the collection of WMD by comparing the Q statistic with its expected value assuming homogeneity (Higgins and Thompson 2002). Significance was assessed at I^2 values between 30-60%, 50-90%, and 75-90%, as moderate, substantial, and considerably high heterogeneity, respectively (Higgins, Thompson et al. 2003).

Step-wise exclusions were made for heterogeneous sleep variables to locate possible moderating factors (sleep setting, recording montage, and sleep period) influencing the FNE (Hedges and Olkin 1985). An individual WMD for each group within a moderator was calculated using the appropriate fixed or random effects model and tests for homogeneity across groups were conducted (Qw). The difference in the magnitude between each group WMD was determined using the Q between (Qb) statistic, where

the overall Q statistic is equal to the sum of the Qw and Qb. Significance for Qb was met at an alpha level of $p < 0.01$ to minimise Type 1 error rate (Hedges and Olkin 1985).

The influence of age on the overall WMD for each sleep variables was analysed by plotting each individual study MD as a function of the average age. The method of least squares was used to fit a linear equation. The coefficient of determination (r^2) was reported along with the proportion of variance (PVE% = r^2 x 100) shared by the two variables, indicating the extent that age influences the WMD.

Visual inspection of the individual sleep variable funnel plots (each MD versus the inverse standard error) was completed to identify significant outliers. Publication bias was formally assessed by testing the degree of asymmetry according to the Egger test (Egger, Smith et al. 1997).

RESULTS

Systematic Review

The systematic search identified 120 studies of further interest. An additional 38 studies were included from a previous search (unpublished data). After duplicates were removed there were a total of 83 studies identified for inspection of title and abstract for further consideration of inclusion. Because the search terms used were broad but inclusive with respect to the FNE many studies identified were non-PSG ambulatory sleep recordings, or involved animals, pediatric patients, or patients with serious medical comorbidity. Among the medical comorbidities were patients with sleep disordered breathing (Mosko, Dickel et al. 1988; Aber, Block et al. 1989; Redline, Tosteson et al. 1991), narcolepsy (Harsh, Peszka et al. 2000), epilepsy (Marzec, Selwa et al. 2005), nocturnal tumescence testing (Thase, Reynolds et al. 1987), post traumatic stress disorder (Ross, Ball et al. 1999), restless leg syndrome (Sforza and Haba-Rubio 2005), psychiatric disorders otherwise not classified (Kupfer, Weiss et al. 1974), chronic fatigue syndrome (Le Bon, Minner et al. 2003), cystic fibrosis (Milross, Piper et al. 2002), and pregnant women (Brunner, Magdalena et al. 1994). In addition, a number of studies cited using the FNE as a laboratory model for transient insomnia to evaluate various forms of treatment to improve sleep (Roehrs, Vogel et al. 1990; Erman, Loewy et al. 2004; Rosenberg, Caron et al. 2005; Suetsugi, Mizuki et al. 2007). Notably, two studies examined the phenomenon of readaptation to the sleep laboratory during long-term PSG studies (Schmidt and Kaelbling 1968; Schmidt and Kaelbling 1971; Stepanski, Roehrs et al. 1981), and one study (Wohlgemuth, Edinger et al. 1999) evaluated the short-term stability of sleep parameters but each failed to meet the inclusion criteria (e.g., data were averaged).

Thus, a total of 45 were found to be relevant and short listed for further consideration. After review of abstracts and full-text papers where necessary, a total of 33 studies met our inclusion criteria and were retained. Figure 4.1 details the systematic search strategy.

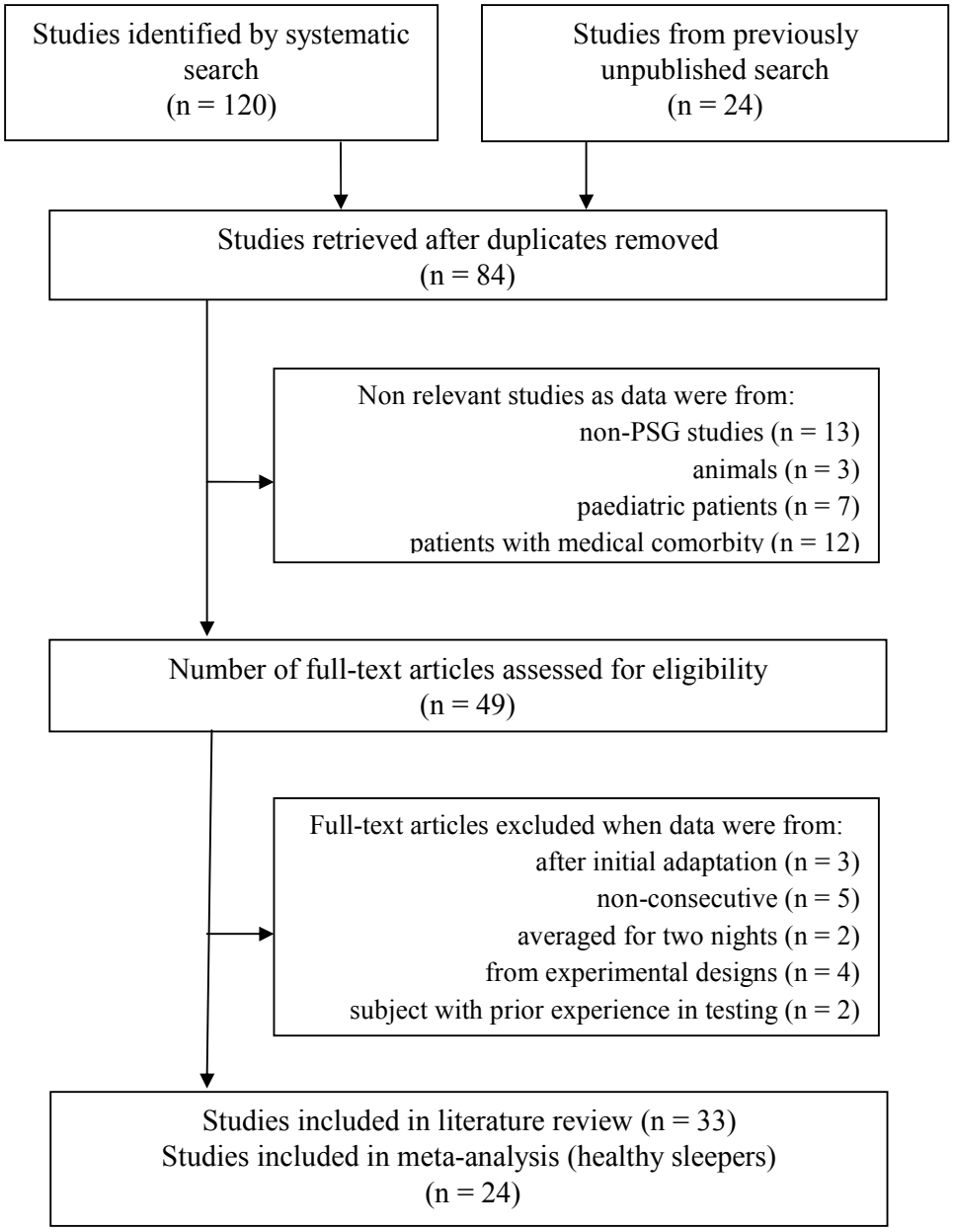


Figure 4.1. Systematic literature search strategy. This search resulted in a total of 33 studies involving healthy sleepers and patients with insomnia and depression. Meta-analysis was completed for healthy sleepers only due to poor diagnostic homogeneity among the insomnia and depression study populations (n).

There were a total of 24 studies involving healthy individuals (Agnew, Webb et al. 1966; Mendels and Hawkins 1967; Clausen, Sersen et al. 1974; Coble, McPartland et al. 1974; Johns and Dore 1978; Webb and Campbell 1979; Coates, George et al. 1981; Sharpley, Solomon et al. 1988; Kupfer, Frank et al. 1989; Wauquier, van Sweden et al. 1991; Kayumov 1994; Toussaint, Luthringer et al. 1995; Woodward, Bliwise et al. 1996; Zucconi, Ferini-Strambi et al. 1996; Rotenberg, Hadjez et al. 1997; Toussaint, Luthringer et al. 1997; Le Bon, Staner et al. 2001; Lorenzo and Barbanoj 2002; Riemann, Klein et al. 2002; Curcio, Ferrara et al. 2004; Heitkemper, Jarrett et al. 2005; Tamaki, Nittono et al. 2005; Feige, Scaal et al. 2007; Bolla, Lesage et al. 2008).

There were 9 studies involving patients with insomnia (Coates, George et al. 1981; Hauri and Olmstead 1989; Edinger, Marsh et al. 1991; Toussaint, Luthringer et al. 1995; Saletu, Klosch et al. 1996; Zucconi, Ferini-Strambi et al. 1996; Edinger, Fins et al. 1997; Toussaint, Luthringer et al. 2000; Riedel, Winfield et al. 2001).

There were 6 studies involving patients with depression (Reynolds, Newton et al. 1982; Ansseau, Kupfer et al. 1985; Ansseau, Kupfer et al. 1985; Kupfer, Frank et al. 1989; Toussaint, Luthringer et al. 1995; Rotenberg, Hadjez et al. 1997).

Study quality (clinical homogeneity)

The studies involving healthy individuals demonstrated clear clinically homogeneity. The diagnostic criteria for participant recruitment were measured according to common methodology. In most studies, these criteria were the absence of self-reported significant medical and psychiatric comorbidity. Other criteria included the use of a validated screening questionnaire (Coble, McPartland et al. 1974; Curcio, Ferrara et al. 2004), drug screen (Toussaint, Luthringer et al. 1995; Toussaint, Luthringer et al. 1997) or structured clinical interview (Toussaint, Luthringer et al. 1995; Zucconi, Ferini-Strambi et al. 1996; Toussaint, Luthringer et al. 1997; Toussaint, Luthringer et al. 2000). In one study healthy individuals also were asked to complete a survey to assess the anxiety related personality traits (Kajimura, Kato et al. 1998); these were either 'low' or 'high' anxiety traits; thus the 'high anxiety' group was excluded. Thus, there were only minimal differences and nearly all studies reportedly used a self-reported method to screen for diseased states.

The studies involving patients with insomnia and depression demonstrated substantial clinical heterogeneity. Although a structured or clinical interview was among the most common methodology used to diagnosis these conditions, insomnia has various sub-types and the depressive episode varies in severity. The clinical diagnosis of primary insomnia appropriately involved the assessment of the patients' self-reported disturbance to sleep initiation and/or to sleep maintenance, or, a non-restorative sleep (Morin and Espie 2003). However, most of the included studies did not report the insomnia sub-type. One study reported patients were with mixed difficulties initiating and maintaining sleep (DIMS) (Edinger, Marsh et al. 1991) and another reported DIMS with generalized anxiety disorder (Saletu, Klosch et al. 1996). Similarly, the

depressive episode can be variable and was appropriately assessed in most studies (Hamilton 1960). However, a threshold or cut-off score was not commonly reported. Also, one study reported on inpatients in a hospital (Toussaint, Luthringer et al. 2000). Thus, these differences introduce clear clinical heterogeneity among the recruited patients with depression. Therefore, a quantitative analysis was not conducted for both groups. Instead, a qualitative summary is provided below.

FNE in depression and insomnia

There was no consensus regarding the presence or characteristics of the FNE in the studies involving patients with insomnia or depression. It was noted in one study that a single night PSG may be inappropriate to fully convey the nature of a patient's sleep disturbance (Edinger, Marsh et al. 1991). Thus, the clinical populations do not manifest the FNE similarly to healthy sleepers. In depression, an attenuated FNE was initially observed (Mendels and Hawkins 1967). Moreover, most findings report a significant FNE for measures of REM sleep only (Reynolds, Newton et al. 1982; Ansseau, Kupfer et al. 1985; Rotenberg, Kayumov et al. 1997; Toussaint, Luthringer et al. 1998).

Noteworthy among the reports in insomnia were studies showing a clear reversed FNE (RFNE) in which sleep is actually of better quality on the first night compared to a subsequent night (Hauri and Olmstead 1989; Riedel, Winfield et al. 2001). The RFNE was characterised by a shorter SOL, reduced REML, and greater SE and TST. Hauri & Olmstead (1989) suggested the maladaptive associations present in the home sleep environment do not generalise to the sleep laboratory and therefore an RFNE was present. Given the sleep laboratory presents a stressful stimulus in healthy persons, it is

likely there is a relationship between anxiety levels, insomnia, and the FNE. Reidel & Winfield (2001) examined this relationship in older patients with insomnia. The data indicated that elevated anxiety levels on PSG night 1 contributed to a FNE, but a considerably proportion of the insomnia patients still show a clear RFNE. Hauri and Olmstead (1989) failed to identify any such difference in anxiety levels between the FNE and RFNE patient groups. Thus, it is unknown to what extent anxiety levels contribute to the differential reports in insomnia. Further research is needed to evaluate this differential response during the first two nights of PSG recording in these patient groups. Thus, it remains largely unknown whether a single familiarisation night or multiple nights is needed to account for the FNE in clinical patients.

Meta-Analysis

Meta-analysis was completed in healthy sleepers only but not in the insomnia and depression group due to poor homogeneity among the clinical diagnostic criteria. An abbreviated coding sheet showing non PSG study characteristics (author, year, sample size, moderating factors) of the included studies is reported in Table B1 (Appendix B). There were a total of 421 subjects (163 males, 201 females, 57 in mixed gender reports) included in the meta-analysis. There were 19 studies (79%) conducted in the laboratory compared to 5 studies in the home. A total of 13 studies used a neuro montage, 8 used a full PSG on both night, and 3 followed a switched montage. The sleep period was provided *ad libitum* in 13 studies, fixed in seven studies, and unknown in the remaining four.

A total of 16 studies reported using the scoring guidelines of Retschaffen & Kales (Rechtschaffen and Kales 1968), 2 studies used the system of Dement & Kleitman (Dement and Kleitman 1957), 2 studies used the system of Agnew (Agnew, Webb et al. 1966), one study used a validated auto scoring system (Sharpley, Solomon et al. 1988), and the other four did not report the scoring guidelines (Coble, McPartland et al. 1974; Johns and Dore 1978; Kayumov 1994; Zucconi, Ferini-Strambi et al. 1996).

A summary of the WMD for each sleep variable, 95% CI, Q statistic, and significance level is reported in Table 4.1. Two studies (Agnew, Webb et al. 1966; Mendels and Hawkins 1967) did not report a measure of variance therefore the median SD was used (Furukawa, Barbui et al. 2006).. The cumulative forest plots (Figure 4.2) sorted by publication date for the 6 significant FNE sleep variables is presented on the following pages as a summary of the individual and cumulative data present above. The individual study MD is represented by a point with respective 95% CI (horizontal line); the overall WMD is represented the diamond apex with 95% CI (diamond end points).

The data confirm the FNE in healthy sleepers indicating on the first night compared to a subsequent night there is a prolonged SOL (4.46 min), a delayed REML (20.55 min), an increase in WASO (3.88%), and reductions in TST (-18.81 min), SE (-4.66%), REMS (-2.17%; all $p < 0.01$). A trend was seen for a small but significant increase in NREM1 (0.84%, $p = 0.07$). A random effects model was used for SOL, REML, TST, and SE (Q $p < 0.05$) indicating that factors other than the recording night may be responsible for the observed variability. Between study variance (I^2) remained considerably high for REML (89%), TST (88%), and SE (67%), and substantial for SOL (46%).

Table 4.1. Summary of each WMD for PSG sleep variables of the FNE in healthy sleepers

Sleep Variable	k	n	WMD	95% CI	Z	Q	I^2
SOL (min)	21	383	4.46 b	2.49 to 6.43	4.44*	37.29*	46%
REML (min)	21	370	20.55 b	12.6 to 28.5	5.07*	177.1*	89%
WASO (%TST)	17	337	3.88 a	2.74 to 5.03	6.64*	23.65	32%
TST (min)	14	225	-18.81 b	-28.55 to -9.06	3.78*	109.91*	88%
SE (%)	17	264	-4.66 b	-6.59 to -2.73	4.73*	42.2*	67%
NREM1 (%TST)	17	274	0.84 a	0.06 to 1.73	1.83	8.52	0%
NREM2 (%TST)	17	294	0.44 a	-0.86 to 1.75	0.66	7.1	0%
SWS (%TST)	19	318	-0.81 a	-1.85 to -0.23	1.52	10.47	0%
REMS (%TST)	20	326	-2.17 a	-3.11 to -1.22	4.49*	9.07	0%

K, number of studies; n, number of subjects; WMD, weighted mean difference; a, fixed effect model, b random effect model, * denotes $p < 0.05$.

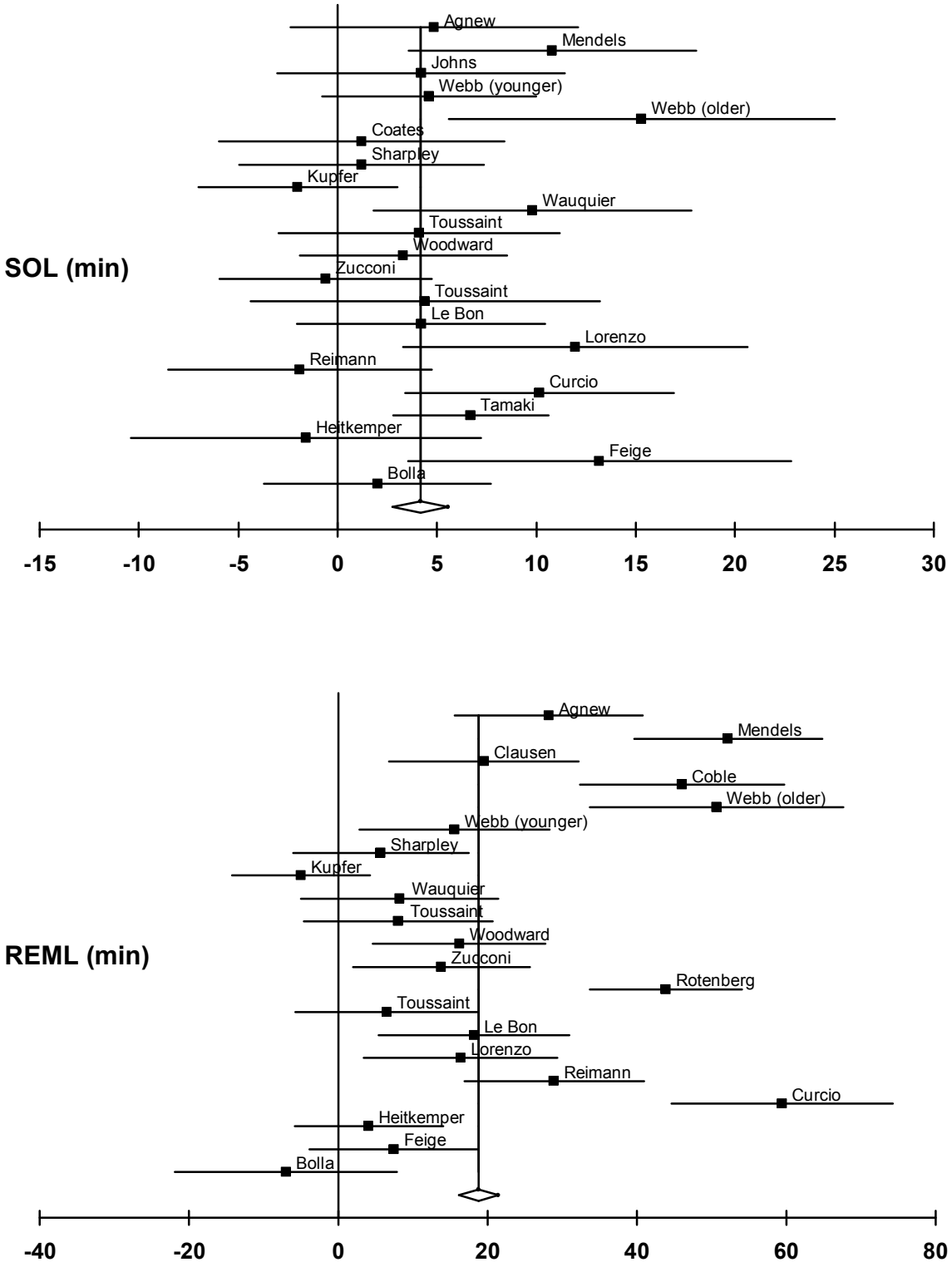


Figure 4.2. Forest plots of PSG sleep variables of the FNE in healthy sleepers. These forest plots (sorted by publication date) representing individual study mean differences (points), their respective 95% CI (horizontal lines), and the overall weighted mean difference (diamond apex, line) and 95% CI (diamond end-points).

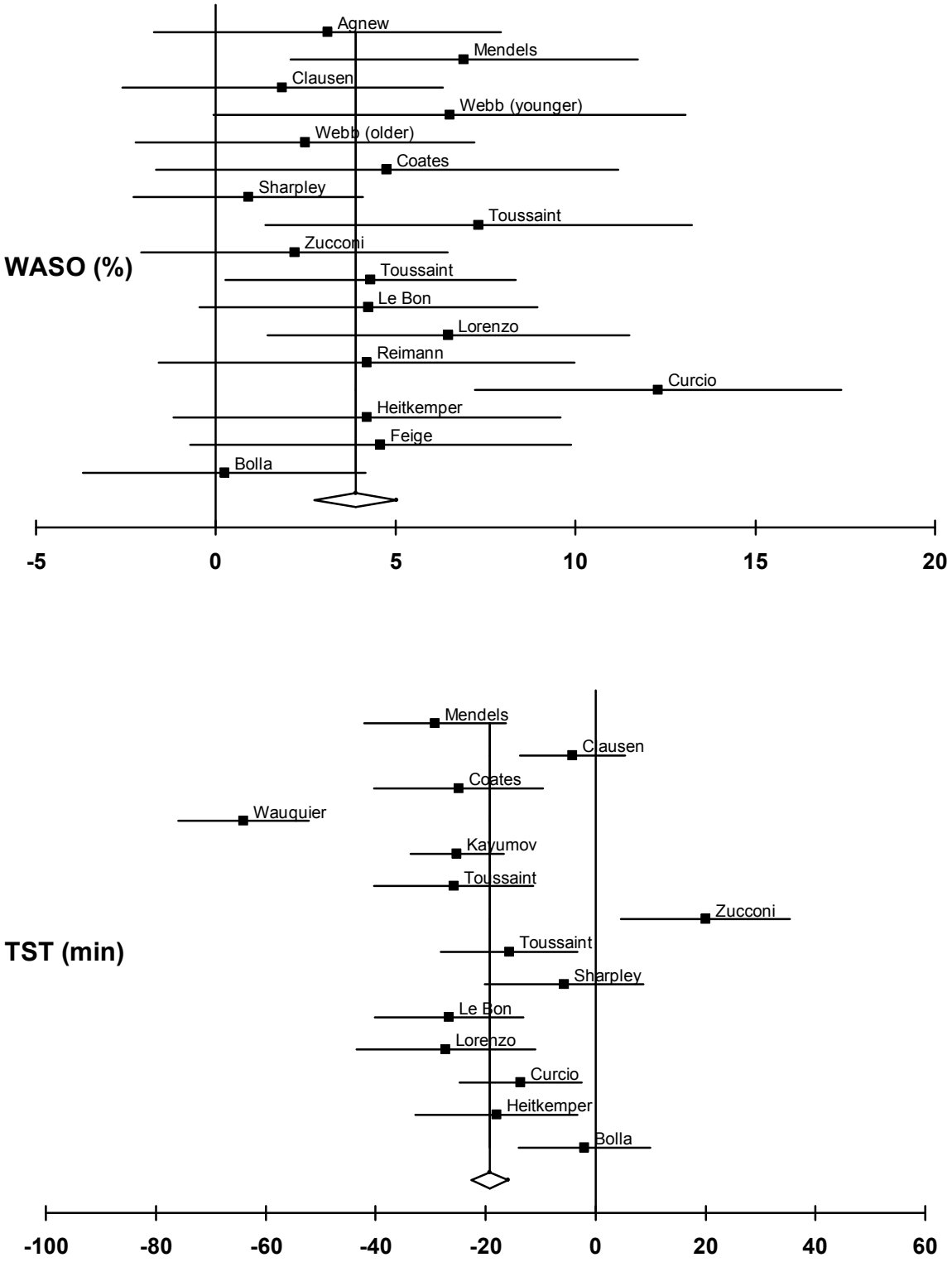
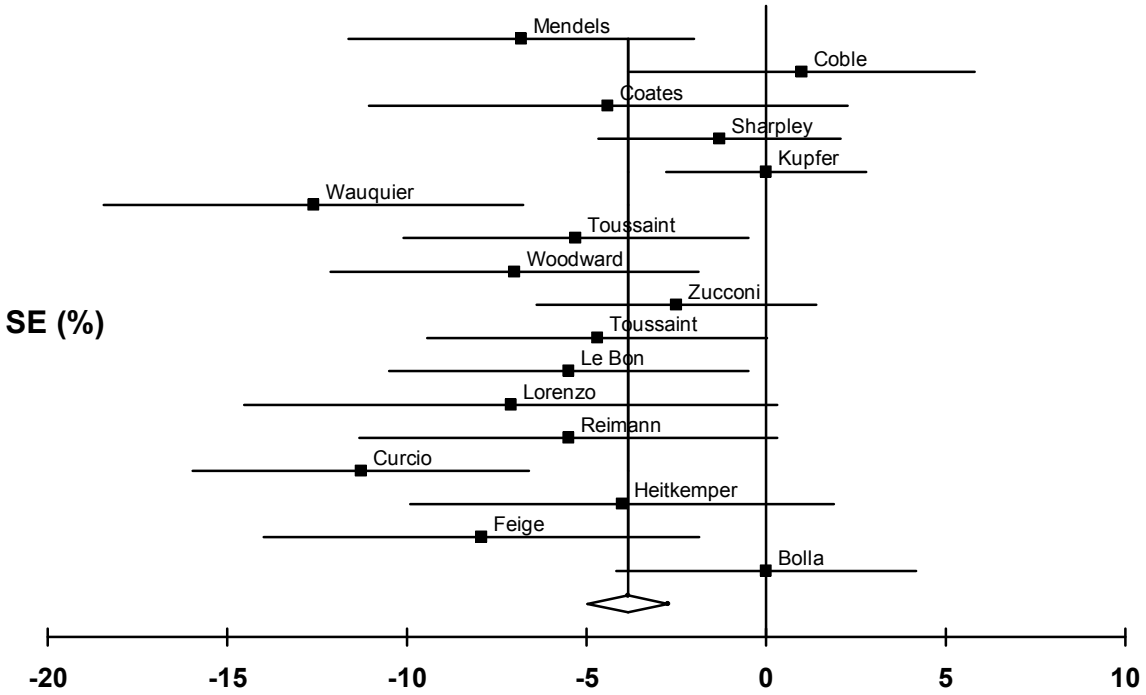


Figure 4.2 continued. Forest plots of PSG sleep variables of the FNE in healthy sleepers. These forest plots (sorted by publication date) representing individual study mean differences (points), their respective 95% CI (horizontal lines), and the overall weighted mean difference (diamond apex, line) and 95% CI (diamond end-points).



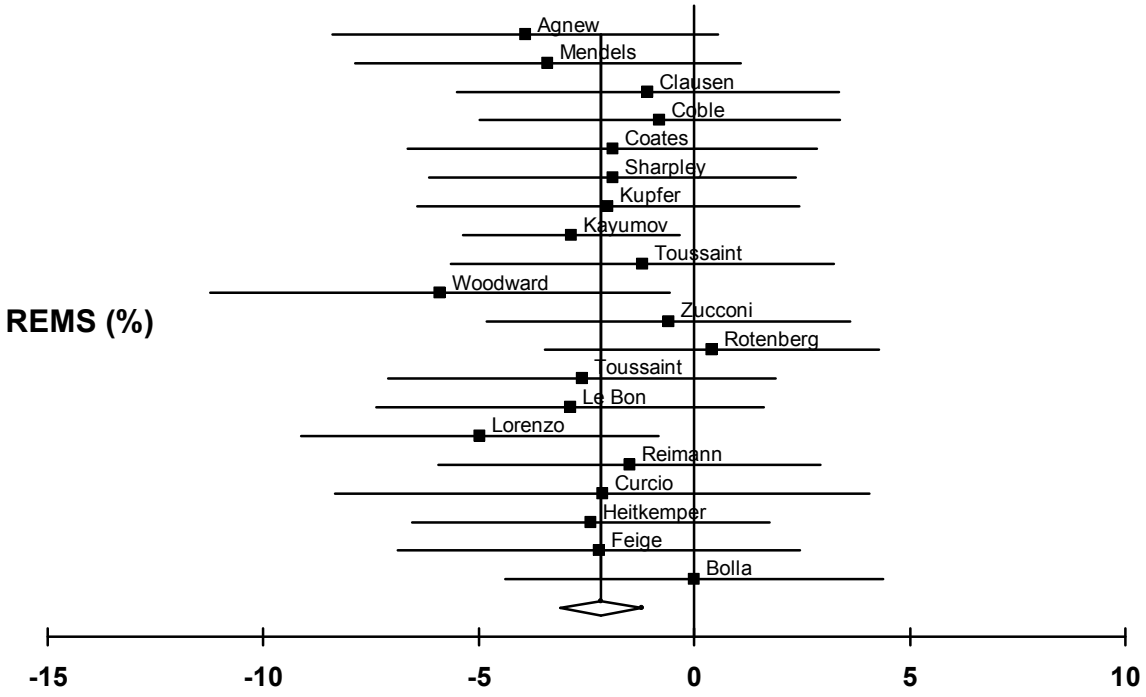


Figure 4.2 continued. Forest plots of PSG sleep variables of the FNE in healthy sleepers. These forest plots (sorted by publication date) representing individual study mean differences (points), their respective 95% CI (horizontal lines), and the overall weighted mean difference (diamond apex, line) and 95% CI (diamond end-points).

Moderating Factors

Sleep Setting

The FNE was more pronounced during laboratory (prolonged SOL, delayed REML, increased WASO, and decreased TST, SE, and REMS) compared to the home (delayed REML, increased WASO, and reduced SE) as indicated by the greater number of WMD (Table 4.2). The delayed REML in the laboratory (22.73 min) was greater (Qb 6.83, $p < 0.01$) than in the home (11.36 min; both $p < 0.05$). Significant heterogeneity was present in both locations, especially for TST (Q laboratory 23.33, home 81.78, both $p < 0.05$) and SE (Q laboratory 30.47, home 11.72, both $p < 0.05$). Additional heterogeneity was indicated in the laboratory for SOL (Q = 30.30) and for REML (Q = 157.89, both $p < 0.05$). Other sources besides the sleep setting may be responsible for the substantial between study variance (I^2) in the laboratory among REML (90%), TST (66%), and SE (70%), and in the home for TST (95%) and SE (66%).

Table 4.2. Influence of the sleep setting on heterogeneous PSG sleep variables of the FNE in healthy sleepers

Sleep Variable	k	WMD	Q	Qw	Qb	I^2
SOL (min)					nil	
laboratory	16	5.06b*	30.3*			51%
home	5	2.42a	5.09	$p < 0.05$		21%
REML (min)					$p < 0.01$	
laboratory	17	22.73b*	167.89*			90%
home	4	11.36a*	2.38	$p < 0.01$		0%
WASO (%TST)					nil	
laboratory	13	4.54a*	18.69			36%
home	4	2.31a*	1.96	$p = 0.15$		0%
TST (min)					nil	
laboratory	9	-17.38b*	23.33*			66%
home	5	-20.45b	81.78*	$p < 0.01$		95%
SE (%)					nil	
laboratory	12	-4.62b*	30.47*			70%
home	5	-4.83b*	11.72*	$p < 0.05$		66%
REMS (%TST)					nil	
laboratory	16	-2.25a*	8.39			0%
home	4	-1.78a	0.54	$p = 0.96$		0%

K, number of studies; WMD, weighted mean difference; a fixed effect model, b random effect model; Q, Q statistic; Qw, Q within group; Qb, Q between groups; I^2, I^2 statistic;

* denotes $p < 0.05$.

Recording Montage

The FNE was more pronounced when the neuro (prolonged SOL, delayed REML, increased WASO, and reduced TST, SE, and REMS) compared to the full PSG (prolonged SOL, delayed REML, increased WASO, and reduced SE and REMS) and switched montage was used (increased WASO) as indicated by the greater number of significant WMD (Table 4.3). The delayed REML was greater (Qb $p < 0.05$) when the neuro (25.56 min) montage was used. Significant heterogeneity was present in all montages for REML, TST, and SE (Q statistics, Table 4.3). Additional heterogeneity was present for the neuro montage for SOL (Q = SOL 20.72) and WASO (Q = 18.39; both $p < 0.05$). Other sources besides the recording montage may explain the substantial between study variance (I^2), especially for measures of REML, TST, and SE (all >73%) and to a moderate degree for WASO and SOL (both >30%, Table 4.3).

Table 4.3. Influence of the recording montage on heterogeneous PSG sleep variables of the FNE in healthy sleepers

Sleep Variable	k	WMD	Q	Qw	Qb	I^2
SOL (min)					nil	
neuro	12	4.76b*	20.72*			47%
full PSG	7	3.76a*	8.62			30%
switched	3	2.79b	7.10	$p < 0.01$		72%
REML (min)					$p < 0.05$	
neuro	11	25.56b*	128.14*			92%
full PSG	7	16.07b*	29.25*			79%
switched	3	13.22	10.6*	$p < 0.01$		81%
WASO (%)					nil	
neuro	10	4.71b*	18.39*			51%
full PSG	4	2.85a*	4.10			0%
switched	3	4.34a*	0.01	$p < 0.05$		0%
TST (min)					nil	
neuro	9	-17.77b*	19.14*			58%
full PSG	5	-20.10b	88.57*			95%
switched	0			$p < 0.01$		73%
SE (%)					nil	
neuro	8	-4.49b*	21.09*			72%
full PSG	7	-4.47b*	18.88*			74%
switched	2			$p < 0.01$		
REMS (%TST)					nil	
neuro	11	-2.12a*	3.33			0%
full PSG	6	-2.23a*	5.60			29%
switched	3	-1.38a	0.26	$p = 0.98$		0%

K, number of studies; WMD, weighted mean difference; a fixed effect model, b random effect model; Q, Q statistic; Qw, Q within group; Qb, Q between groups; I^2, I^2 statistic; * denotes $p < 0.05$. neuro: studies using only EEG, EOG, and EMG channels; full PSG: included the utility of respiratory and/or limb movement measures on both nights; switched: consisted of a full PSG on the first night, followed by neuro channels on subsequent nights

Sleep Period

The FNE was similar whether the sleep period was provided *ad libitum* or fixed (prolonged SOL, delayed REML, increased WASO, and decreased TST, SE, and REMS), but both were more pronounced than when the sleep period was unknown (increased WASO and decreased REMS) as indicated by the number of significant WMD (Table 4.4). There were no significant differences across groups (all, Qb nil). Significant heterogeneity was present in all sleep periods for REML (Q *ad libitum* 87.01, fixed 31.54, unknown 52.66, all $p<0.05$). Additional heterogeneity was indicated when the sleep period was provided *ad libitum* for WASO (Q = 18.42), TST (Q = 78.46), and SE (Q = 28.81, all $p < 0.05$) or when the sleep period was unknown for TST (Q = 29.31), SE (Q = 9.30, both $p < 0.05$). Between-study variance (I^2) remained high for REML across all sleep periods, but was minimal when sleep period was fixed for the other six sleep parameters (Table 4.4).

Table 4.4. Influence of the sleep period on heterogeneous PSG sleep variables of the FNE in healthy sleepers

Sleep Variable	k	WMD	Q	Qw	Qb	I^2
SOL (min)						
ad libitum	12	5.02a*	14.56			24%
fixed	4	5.05a*	2.95			0%
unknown	4	4.56b	14.15*	p<0.01	nil	79%
REML (min)						
ad libitum	11	22.24b*	87.01*			89%
fixed	6	20.22b*	31.54*			84%
unknown	4	16.90b	52.66*	p<0.01	nil	94%
WASO (%)						
ad libitum	10	3.69b*	18.42*			51%
fixed	4	5.35a*	1.02			0%
unknown	3	4.34a*	2.07	ns	nil	3%
TST (min)						
ad libitum	8	-19.83b*	78.46*			91%
fixed	3	-21.8a*	1.65			0%
unknown	3	-12.01b	29.31*	p<0.01	nil	93%
SE (%)						
ad libitum	9	-4.79b*	28.81*			75%
fixed	4	-5.58a*	0.44			0%
unknown	4	-3.79b	9.30*	p<0.01	nil	78%
REMS (%TST)						
ad libitum	9	-1.86a*	2.15			0%
fixed	6	-2.37a*	5.6			11%
unknown	5	-2.37a*	1.05	ns	nil	0%

K, number of studies; WMD, weighted mean difference; a fixed effect model, b random effect model; Q, Q statistic; Qw, Q within group; Qb, Q between groups; I^2, I^2 statistic; * denotes $p < 0.05$. Ad libitum refers to a habitual sleep period, fixed refers to fixed research timing, and unknown refers to when the sleep period was not reported.

Age

The average age of the study sample moderately contributed to the variance in FNE among the following sleep variables: TST (52%), SE (40%), and to a lesser but meaningful degree for SWS (21%), and SOL (15%). The reduced TST ($r^2 = 0.52$, $p < 0.005$) and reduced SE ($r^2 = 0.40$, $p < 0.01$) were negatively associated with increasing age (solid lines, Figure 4.3). However, when the study by Waquier et al. (1992) was excluded as an outlier (mean age 85 y), the age-related trends were absolved; TST ($r^2 = 0.09$, $p = 0.32$) and SE ($r^2 = 0.17$, $p = 0.11$). Post-hoc comparison of age indicated that when an average age cutoff of 35 y was applied (younger, < 34.99 y vs. older, > 34.99 y) there was a trend showing a decreased SWS among the older group only (WMD fixed effects, older -1.89%, $p = 0.08$).

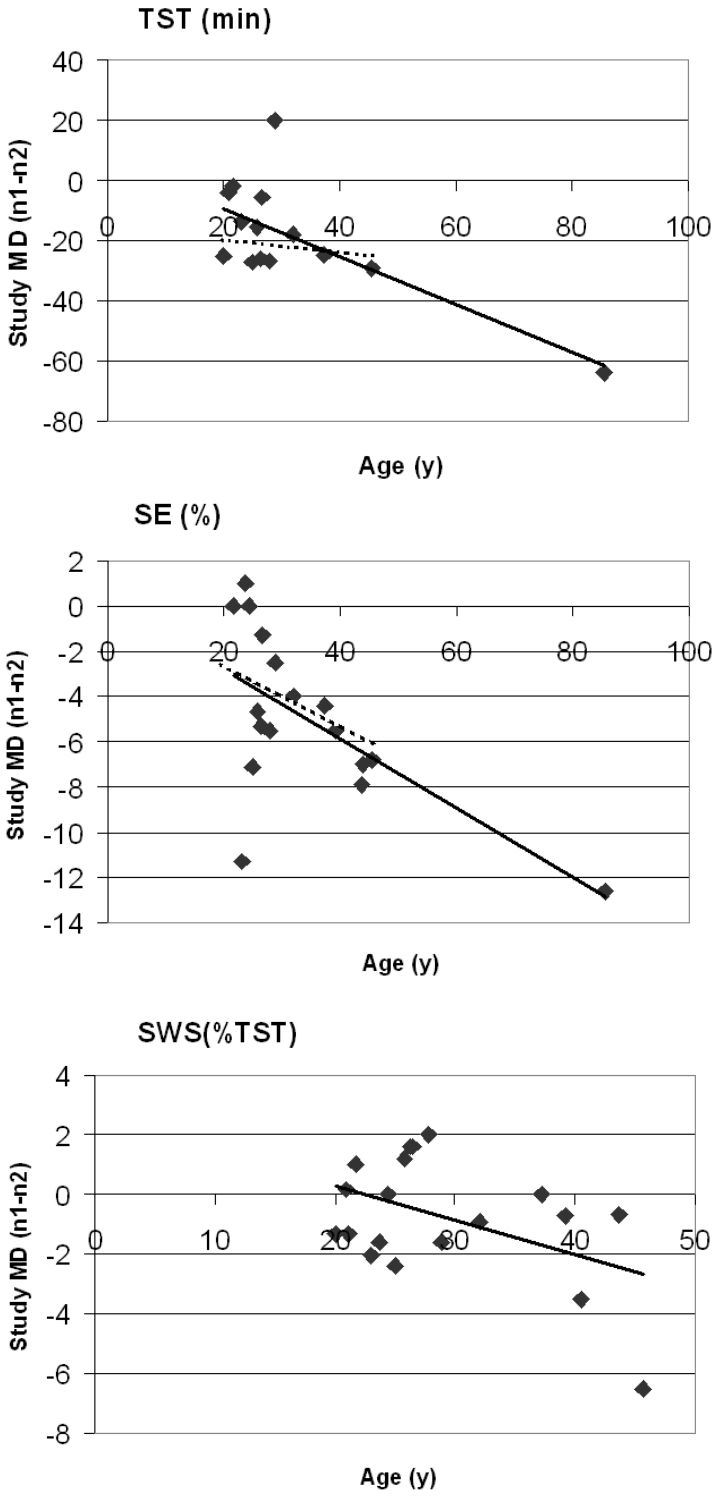


Figure 4.3. Age-related trends for PSG sleep variables of the FNE in healthy sleepers. Age-related trends (solid line) for the weighted mean difference of TST, SE, and SWS. When an age-related outlier was removed for the study by Wauquier et al. 1992 (mean age 85 y) the age-related trends were absolved (dotted line).

Publication Bias

Visual inspection of individual funnel plots (1/SE) showed outliers were present for REML, WASO, TST, and SE (Figure B1, Appendix B). Formalised tests for asymmetry indicated significant publication bias for SE (intercept -3.65, $p = 0.03$) and WASO (intercept 3.92, $p < 0.01$), with a trend for REML (intercept 9.01, $p = 0.06$). Post-hoc analyses (for these 4 variables) were conducted to examine if a small sample size accurately explained the publication bias. Sample size (n) was designated as small ($n < 11$; $k = 11$), medium ($n = 12$-20; $k = 8$), or large ($n > 20$; $k = 6$) where at least 3 MDs were present. The delayed SOL was limited to studies with medium and large samples only (WMD small, random effects, 2.69 min, $p = 0.20$; medium 5.05 min, large 5.71 min, both fixed effects, $p < 0.05$). The reduced TST followed the same pattern (WMD small, random effects, -17.63 min, $p = 0.14$; medium, random effects, -17.06 min; large, fixed effect, -19.93 min, both $p < 0.01$). The delayed REML and decreased SE were present in all conditions; however the random effects model was used for studies with small sample sizes.

DISCUSSION

To our knowledge this is the first meta-analysis to quantitatively confirm the FNE in healthy sleepers. The utility of a meta-analysis to examine data from accumulated sleep reports has been shown elsewhere (Ohayon, Carsadon et al. 2004; Zhang and Wing 2006; Kobayashi, Boarts et al. 2007). The FNE in healthy sleepers was characterised by a prolonged SOL, delayed REML, increased WASO, and reductions in REMS, TST, SE. Among the moderating factors, differences in the sleep setting, but not the recording montage or sleep period, accounted for part of the variability. Further, publication bias contributed to part of the heterogeneity among measures of REML, WASO, and SE but age was not a major factor influencing the characteristics of the FNE.

Noteworthy are the calculated weighted mean difference (WMD, Table 4.1) values for each sleep variable. Each WMD provides a clear estimate for the magnitude and direction of the FNE in healthy sleeper. The delayed SOL was small (4.46 min) and likely reflects a ceiling effect in healthy sleepers (Coble, McPartland et al. 1974). However, the importance of this finding during intervention studies where small changes to SOL are expected is noteworthy. Although not empirically examined, differences in the application of scoring criteria for SOL remain a possible source of heterogeneity among sleep variables reflecting time domains (SOL, REML, TST, and SE). These findings point to the importance of implementing standardised scoring rules as presented in the American Academy of Sleep Medicine Manual (Iber, Ancoli-Israel et al. 2007). However, the use of change scores (WMD) across nights minimised this confounding factor. Further, one study which exclusively investigated the FNE on the

sleep onset period showed similar results using either the first occurrence of stage 1 or stage 2 as definitions of SOL (Tamaki, Nittono et al. 2005). Along with the delayed SOL, the data indicate an increased WASO (3.88%) and considerable reduction to SE (-4.66%). These factors may have contributed to the large reduction in TST (-18.81 min).

A prominent feature of the FNE is the disruptions to REM sleep. The data indicated a marked delay in REML (20.55 min), reflecting all but two of the included studies (Kupfer, Frank et al. 1989; Bolla, Lesage et al. 2008). Additionally, there was a significant, but small reduction in REMS overall (-2.17%, $p < 0.01$). These characteristics of the FNE are likely explained by the tendency to miss the first and shortest REMS period during the first sleep cycle (Agnew, Webb et al. 1966; Mendels and Hawkins 1967; Webb and Campbell 1979; Toussaint, Luthringer et al. 1995; Toussaint, Luthringer et al. 1997). The NREM sleep measures showed relative stability across nights evident by each WMD that was close to zero: NREM1 (0.84%), NREM2 (0.44%), and SWS (-0.81%). In the present study the use of a WMD could not be avoided but it may be misleading to estimate the change in NREM sleep across the first two nights. The forest plots for NREM2 and SWS (Figure B2, Appendix B) show that each individual study MD is on either side of the zero axes and the corresponding 95% CI is large. Thus, a WMD that is close to zero reflects very large differences in opposite directions. Thus, it is equally likely that NREMS measures will show a relative increase or relative decrease across the first two consecutively recorded PSG studies.

The step-wise analysis using various methodological factors was warranted among measures of SOL, REML, TST, and SE given substantial between-study variance (Hedges and Olkin 1985). The FNE was more pronounced in the sleep laboratory than in the home as indicated by the number of significant WMD (Table 4.2). Indeed, there was a two-fold greater delay in REML in the sleep laboratory. It remains unclear to what extent the recording montage affects the FNE. The data indicate a stronger response in the neuro setting where only EEG and EOG signals were monitored, compared to a full PSG setup that was expected to contribute to more disruptions. Publication bias was evident, especially for REML, WASO, and SE as demonstrated by the tests for asymmetry (including post-hoc analysis). Thus, it is likely that those studies not reporting a significant FNE (Clausen, Sersen et al. 1974; Coble, McPartland et al. 1974; Coates, George et al. 1981; Wauquier, van Sweden et al. 1991; Zucconi, Ferini-Strambi et al. 1996) were underpowered.

Limitations

The present meta-analysis suffers from some limitations. First, this study was unable to examine the interaction of two moderating factors at one time. The resultant groupings would have been too small to provide meaningful interpretations. Second, the analysis only reviewed measures of sleep macro architecture that were common to the FNE literature. One study (Curcio, Ferrara et al. 2004) reviewed the impact of the FNE on quantitative EEG data (i.e., spectral analysis) which may be important during investigations of sleep micro structure. Third, there are many potential moderating factors that could be examined such as the various other clinical groups (e.g. sleep apnea, PLMD). With respect to gender influences, De Gennaro et al. (2002) reported a greater delay in REML and greater reduction in REMS in males compared to females

(De Gennaro, Ferrara et al. 2002) but this study was not included in the present analysis as it incorporated groupings of otherwise healthy subjects across personality factors.

Future research

There were various other topics addressed within the examined studies but these were outside the framework of the present study. A prominent feature of the FNE in healthy sleepers is the disruption to REM sleep which may remain for periods up to three (Agnew, Webb et al. 1966; Mendels and Hawkins 1967; Schmidt and Kaelbling 1971; Webb and Campbell 1979; Toussaint, Luthringer et al. 1995; Toussaint, Luthringer et al. 1997) or four nights (Le Bon, Staner et al. 2001) suggesting a longer familiarisation period may be necessary. This is of particular importance in research settings where changes to REMS are considered as part of the research question and especially when applied to clinical populations where altered REM sleep is often reported (Le Bon, Staner et al. 2001). Unfortunately, as it was not a primary objective to examine the FNE extending past the second night there were insufficient study numbers to warrant additional analysis.

Equally important, the impact of re-entry into the sleep laboratory during long-term experimental studies was examined. Lorenzo et al. (2002) investigated the FNE across multiple sleep laboratory sessions, each lasting 4 consecutive nights. The FNE was only present for the initial period described as the "very first night effect". These authors concluded that the first night PSG data after re-entry within a specific protocol need not be excluded before subsequent analysis in healthy sleepers (Lorenzo and Barbanoj 2002).

Finally, the conceptual theories for the presence of the FNE were reported. The FNE is thought to represent a normal but heightened arousal response to a new stimulus, either that of the sleep laboratory (Agnew, Webb et al. 1966) or the sleep equipment itself (Mendels and Hawkins 1967). Given the sleep laboratory and PSG recording can be considered a stressful response in healthy individuals, it was suggested the FNE reflects a miniature model of psychophysiological insomnia, in which sleep is characterised by increased vigilance (Curcio, Ferrara et al. 2004). In fact, clinical studies investigating treatment efficacy on transient-type insomnia have incorporated the FNE into the study design as noted in the systematic search results. Other studies have proposed that the FNE represents the age-related adaptability of the central nervous system given there were greater disturbances with increasing age (Wauquier, van Sweden et al. 1991; Toussaint, Luthringer et al. 1995). These theoretical viewpoints, although likely substantiated, cannot be evaluated within the framework of the present analysis.

Summary and application to PSG study design

In summary, we believe this study was warranted and will serve as a valuable tool to aid in PSG study design. This study provides quantitative data to substantiate the need to allow for at least 1 night for familiarisation during PSG studies. Moreover, this study provides an estimate on the magnitude of observed changes to 9 PSG sleep variables across the first two consecutively recorded nights. This has important implications not only in research involving healthy individuals, but also in clinical studies where a control group is mandatory.

CHAPTER 5. Postprandial tryptophan availability after a high and low GI mixed macronutrient compared to a carbohydrate only meal

The enclosed study at the time of writing this thesis was in review as follows:

C.P. Herrera, K. Smith, F. Atkinson, P. Ruell, C.M. Chow, H. O'Connor, & J. Brand-Miller (2010). High glycemic index meals increase the availability of tryptophan in healthy volunteers. *In revision: British Journal of Nutrition: MS #: BJN-2010015521*

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ABSTRACT

Introduction: High GI carbohydrate ingestion results in a significantly elevated postprandial plasma TRP/LNAA concentration. The TRP/LNAA concentration describes the availability of tryptophan to the brain where it is converted to serotonin, a neurotransmitter involved in sleep regulation. It is largely unknown to what extent a mixed macronutrient meal will impact on TRP/LNAA concentrations compared to a carbohydrate only meal.

Methods: 10 healthy males (22.9 ± 3.4 y, BMI 23.5 ± 1.6 $kg.m^{-2}$) underwent GI testing. Postprandial glucose, insulin (both 2 h), and amino acid (4 h) responses to three test meals administered in random order: a carbohydrate only high GI (CHGI) and each of an isoenergetic mixed macronutrient high and low GI (MHGI, LHGI respectively) meal. ANOVA with a priori contrasts (two-factor, meal x time) were used to examine the postprandial TRP, LNAA, and TRP/LNAA responses. The incremental area under the curve (AUC) was computed for the TRP/LNAA response. Paired samples t-tests were used to compare the GI, GL, II, and AUC between meals.

Results: GI, GL, and II values were all significantly different between meals; the CHGI meal had the largest values followed by the MHGI and MLGI meals respectively. Postprandial TRP concentration initially increased after the MHGI and MLGI meals but steadily declined after the CHGI meal. Postprandial LNAA concentration declined after all meals but fell lower and more sharply after the CHGI meal (both comparisons, $p < 0.05$). Postprandial plasma TRP/LNAA concentration peaked at 2 h after each meal ($p < 0.05$); corresponding percentage rise from baseline was approximately 23% (CHGI), 17% (MHGI), and 8% (MLGI). The AUC after the CHGI meal was 3-fold greater than the MLGI meal and showed marginal statistical difference ($p = 0.05$); there were no differences between the CHGI and MHGI meals ($p = 0.38$) or between the MHGI and MLGI meals ($p = 0.43$).

Discussion: This study demonstrated that the postprandial TRP/LNAA response was dose-dependent of the GL values. It is likely the GL may be useful to predict the postprandial TRP/LNAA response to mixed macronutrient meals. These data confirm the underlying mechanism for improvements to sleep initiation after the CHGI meal in healthy individuals. Importantly, the MHGI meal represents a more balanced meal than the carbohydrate only meal. But the efficacy to improve sleep onset remains unknown.

Keywords: glycemic index, glycemic load, tryptophan, amino acids, carbohydrate

INTRODUCTION

The postprandial concentration of tryptophan (TRP) in the brain importantly dictates the synthesis of central nervous system serotonin (Spring 1984), a key neurotransmitter involved in the regulation of satiety (Lawton, Wales et al. 1995), mood (Orosco, Rouch et al. 2002), and sleep (Siegel 2004). The availability of dietary TRP to the brain depends on its concentration relative to other large neutral amino acids (LNAA) which compete for a common transport molecule across the blood brain barrier (Wurtman, Hefti et al. 1981). Dietary carbohydrate (CHO) promotes insulin secretion which enhances peripheral, skeletal muscle uptake of LNAA, except for TRP which is largely albumin bound. This cascade of peripheral events results is a relatively higher plasma TRP/LNAA concentration (McMenamy and Oncley 1958). Postprandial TRP/LNAA levels after predominately CHO ingestion range from an approximate 20-50% increase from baseline levels (Lieberman, Caballero et al. 1986; Lyons and Truswell 1988; Wurtman, Wurtman et al. 2003). These findings indicate the postprandial TRP/LNAA response to CHO based meals is largely dependent of the glycemic index (GI). Ingestion of a high compared to a low glycemic index (GI) CHO food promotes greater insulin release and a larger postprandial rise in the plasma concentration of TRP/LNAA (Lyons and Truswell 1988).

Recently, the GI of ingested CHO was shown to significantly influence sleep onset in healthy sleepers. Afaghi et al. (2007) reported a 50% reduction in the time required to fall asleep after a CHO rich high GI compared to an isoenergetic low GI meal when consumed 4 h before bedtime in healthy men with normal sleep patterns (Afaghi, O'Connor et al. 2007). Although not measured in this study, this was most likely due to

greater plasma insulin secretion and an increased plasma TRP/LNAA concentration after consumption of the high GI CHO rich meal.

The effect of the GI of regular mixed macronutrient meals with a mixed macronutrient profile on the postprandial TRP/LNAA levels is largely unknown. Addition of fat to a meal retards gastric emptying (Wells and Read 1995) and lowers the peak glycemic response (Ercan, Gannon et al. 1994) therefore insulin secretion would be decreased. Thus, addition of fat to a meal lowers the postprandial TRP/LNAA response (Lyons and Truswell 1988). Moreover, protein ingestion contributes to increased dietary amino acids. It has been suggested that the proportion of high GI CHO to protein of approximately 5:1 would neither raise nor lower the plasma LNAA concentration in humans because the direct contribution of LNAA from the added protein would be matched by the LNAA-lowering effect of insulin as a result of the CHO source (Berry, Growdon et al. 1991).

Therefore, the aim of this study was to measure the postprandial glycemic, insulin, and amino acid responses of two isoenergetic high and low GI mixed macronutrient (MHGI and MLGI) meals compared to a CHO only (CHGI) meal. The study hypothesis was that the MHGI meal will result in a greater postprandial rise in the plasma TRP/LNAA concentration compared to the MLGI meal and that both responses would be less than the CHGI meal.

METHODS

Participants

Ten healthy, young (22.9 ± 3.4 y), mixed ethnicity men of normal weight (BMI 23.5 ± 1.6 $kg.m^{-2}$) were recruited from a university student population. Exclusion criteria included self-reported current or past history of medical, psychiatric or sleep disorders, current use of prescribed medication, recreational drug use, allergy related to the study protocol, or habitual use of a limiting/restrictive diet. The study was approved by the Human Research Ethics Committee of The University of Sydney (Appendix C) and participants provided written informed consent (Appendix E) prior to participation.

Meals

Energy and macronutrient composition of the three test meals, carbohydrate only high GI (CHGI), mixed macronutrient high GI (MHGI) and mixed macronutrient low GI (MLGI), is summarised in Table 5.1. The CHGI meal (3212 kJ) was replicated from a previous study (Afaghi, O'Connor et al. 2007) and consisted of a large portion of rice (Jasmine GI ~ 109, Riviana Foods, Sydney, Australia) served with a tomato based vegetable puree. The mixed macronutrient meals (MHGI, MLGI) were isoenergetic (~1915 kJ), and consisted of rice (MHGI: Jasmine GI ~ 109; MLGI: Doongara GI ~ 46; Riviana Foods) served with a sachet of Sundried Tomato Chicken (Lean Cuisine™, Nestle Australia Ltd; 965 kJ, 7.9 g fat, 14.1 g protein, 24.7 g CHO). Meals were prepared in the University of Sydney Human Nutrition Unit kitchens. Uncooked rice (raw weight of CHGI: 200 g; MHGI: 64.7 g; MLGI: 64.5 g) was prepared using an electric rice cooker prior to the testing day with a ratio of rice to water of 1:1.5. Cooked rice was frozen (-20°C) in individual portions and reheated in a microwave

prior to serving. Frozen Lean Cuisine™ sachets were heated in the microwave according to manufacturer recommendations and poured over the rice immediately prior to serving. All meals were presented to participants with 250 mL of cool tap water and consumed within 15 min.

Table 5.1. Energy and macronutrient composition of the CHGI, MHGI, and MLGI meals

Meal	kJ	Fat g (% energy)	Protein g (% energy)	CHO g (% energy)	GI (GL)
CHGI[$]	3212	0.4 (1.6%)	16.8 (8%)	171.4 (90.4%)	117 (200)
MHGI	1916	7.9 (16.2%)	18.2 (17.2%)	75 (66.6%)	79 (59)
MLGI	1913	7.9 (16.1%)	18.6 (17.5%)	75 (66.4%)	51 (38)

All values expressed as mean. GI calculated from glucose area-under-curve (AUC) data; GL calculated by multiplying the GI by the amount of available carbohydrate (g) in each food.
[$] Indicates the GI for the CHGI meal was approximate due to larger CHO content of this test meal compare to the reference glucose drink providing 75 g CHO (Brouns 2005). It was determined unethical to use a glucose reference drink of 171.4 g CHO. Abbreviations: CHGI, carbohydrate only high GI; MHGI, mixed macronutrient high GI; MLGI, mixed macronutrient low GI meals.

Procedure

Participants presented in the morning by at least 10 am, having fasted overnight for a period of at least 8 h. Participants were required to avoid vigorous exercise for at least 24 h prior to testing, abstain from alcohol (within 48 h), and avoid over or under eating. Smokers were advised to abstain on the morning before testing. Participants were instructed to consume a high CHO, low fat evening meal devoid of legumes the night before each test session to avoid extreme hunger and variation in basal blood glucose concentration. Self-reported compliance with these instructions was evaluated by a researcher (CH & KS) each morning prior to testing.

Participants initially completed three, separate, reference tests at least 48 h apart using a glucose standard drink (77.1 g Glucodin^{TM} powder, dissolved in 250 g of cold water; 75 g available CHO) to calculate average glucose and insulin response. A 75 g glucose load was used instead of a typical 50 g test load to appropriately calculate the GI of the MHGI and MLGI meals, which both provided 75 g of CHO (Chapter 3). Test meals were administered in a randomised and counterbalanced order at least 48 h apart. Participants were blinded to the GI of the MHGI and MLGI meals which were identical in appearance. A questionnaire comprising multiple 10cm visual analogue scales (VAS) was administered immediately after meal consumption to assess palatability (0 = poor taste, 10 = good taste), satiety (0 = still hungry, 10 = completely full), and participant sleepiness (0 = alert, 10 = sleepy (Appendix F).

Calculation of Glycemic Index (GI), Insulin Index (II), and Glycemic Load (GL)

GI and II were calculated according to published guidelines (Brouns, Bjorck et al. 2005) using the mean data from the participants and the following ratio; the 120 min incremental area under the curve (AUC) of test meal compared to the mean AUC from the reference food multiplied by 100. The GL was calculated using the equation, GL = (GI/100) x g available CHO.

Biochemical Analysis

Finger-prick blood samples were collected into separate eppendorf tubes at baseline (5 min and 1 min prior to meal consumption) and after 15, 30, 45, 60, 90 and 120 min for analysis of glucose (Hitachi 912 automatic analyser, Boehringer Mannheim) and insulin (solid-phase antibody-coated tube radioimmunoassay, 'Coat-A-Count' Insulin RIA kit, Diagnostic Products Corporation, Los Angeles, CA, USA). All samples were immediately centrifuged at 7900 rpm for 45 s, with plasma stored at -20^{o}C for subsequent analysis.

After test meals, but not reference test, additional (2 mL) blood samples were collected by venepuncture from a forearm vein into a lithium-heparinised tube at 0 (baseline), 120, 180, and 240 min and immediately centrifuged (2500 rpm for 15 min at 4^{0}C) to separate plasma for subsequent analysis of amino acids: TRP and five LNAA (valine, leucine, isoleucine, phenylalanine, tyrosine) to calculate the TRP/LNAA ratio (McMenamy and Oncley 1958). Plasma samples were collected into separate eppendorf tubes and stored at -80^{0}C until analysis by reverse phase high performance liquid chromatography (HPLC, Shimadzu). All samples were analysed in duplicate following established methodology (Huq, Thompson et al. 1993).

Statistics

Data are reported as means ± one standard deviation (SD). All data were tested for normality of distribution and statistical procedures were completed using SPSS for Windows (SPSS Inc, version 15.0, Cary, NC, USA). Student's paired samples t-tests were used to identify differences between meals in palatability, satiety and sleepiness (VAS), the GI, GL, II, and the AUC of the TRP/LNAA response. A two-factor (meal x time), repeated measures analysis of variance (ANOVA) was used to test for the postprandial effect of time post meal, meal type, and interaction on TRP, LNAA, and TRP/LNAA concentrations. In ANOVA, normality was satisfied using Mauchly's test of sphericity unless reported with Greenhouse-Geisser value. Significance was set at $p < 0.05$.

RESULTS

Palatability, satiety, and sleepiness (VAS)

Ratings for palatability after the MHGI and MLGI meals were almost identical with mean positive scores (favouring good taste) of 8.3 ± 1.4 cm and 8.3 ± 1.4 cm, respectively; $p = 0.96$). Both mixed macronutrient meals were significantly more palatable than the CHGI meal (3.2 ± 2.2 cm; $p < 0.001$ for both comparisons). Satiety was slightly higher after the MHGI meal (6.2 ± 1.6 cm) compared to the MLGI meal (4.9 ± 2.4 cm) but this difference was not statistically different ($p = 0.05$). These ratings were significantly lower than after the CHGI meal (8.7 ± 1.1 cm, both comparisons, $p < 0.001$). There were no statistical differences between sleepiness ratings assessed immediately after each meal (CHGI 6.2 ± 1.7; MHGI 5.6 ± 1.9; MLGI 4.8 ± 2.4, all $p > 0.05$).

Glycemic Index (GI), Glycemic Load (G), and Insulin Index (II)

Data were omitted from one participant due to an abnormal fasting glucose concentration (Brouns, Bjorck et al. 2005). Postprandial levels for blood glucose and insulin are shown in Figure 5.1. A summary of the GI, GL, and II values is shown in Figure 5.2.

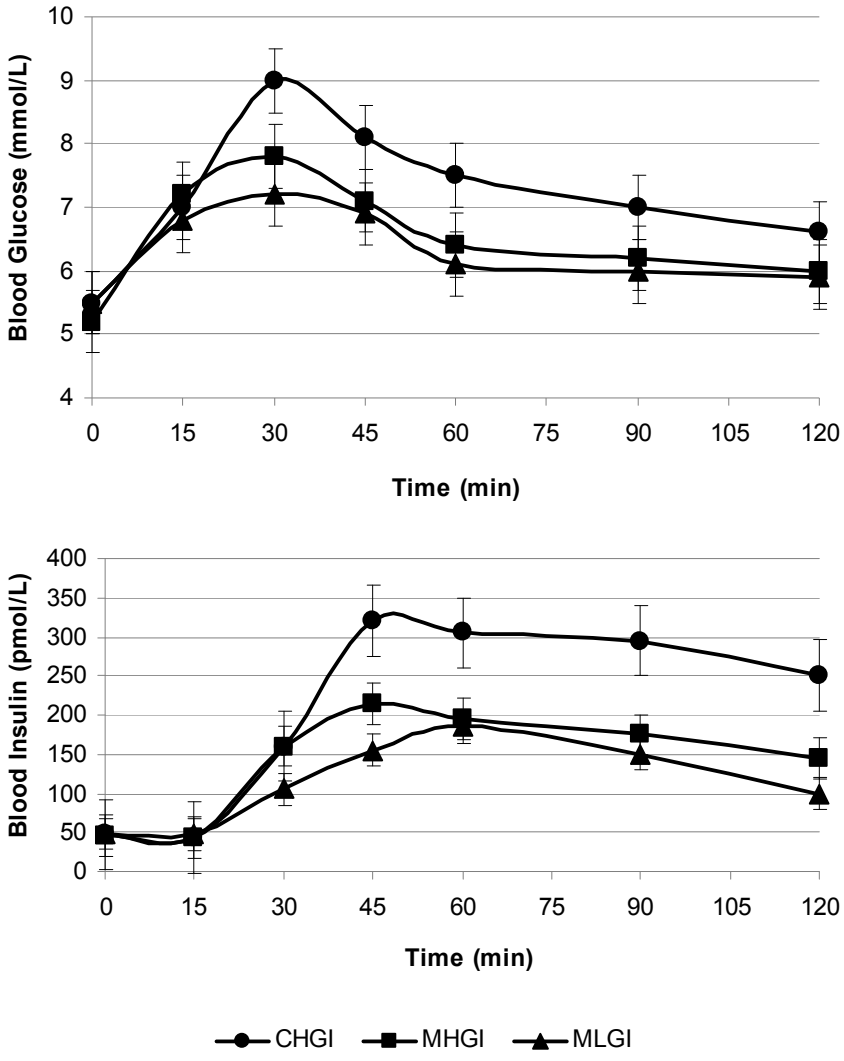

Figure 5.1. Postprandial glucose and insulin responses after the CHGI, MHGI, and MLGI meals in healthy participants. Postprandial glucose was maximal at 30 min in all meal conditions before steadily declining to levels slightly above baseline at 120 min. Postprandial insulin peaked at approximately 45 min after CHGI and MHGI but at approximately 60 min after MLGI followed by a gradual reduction. CHGI, carbohydrate only high GI; MHGI, mixed macronutrient high GI; MLGI, mixed macronutrient low GI.

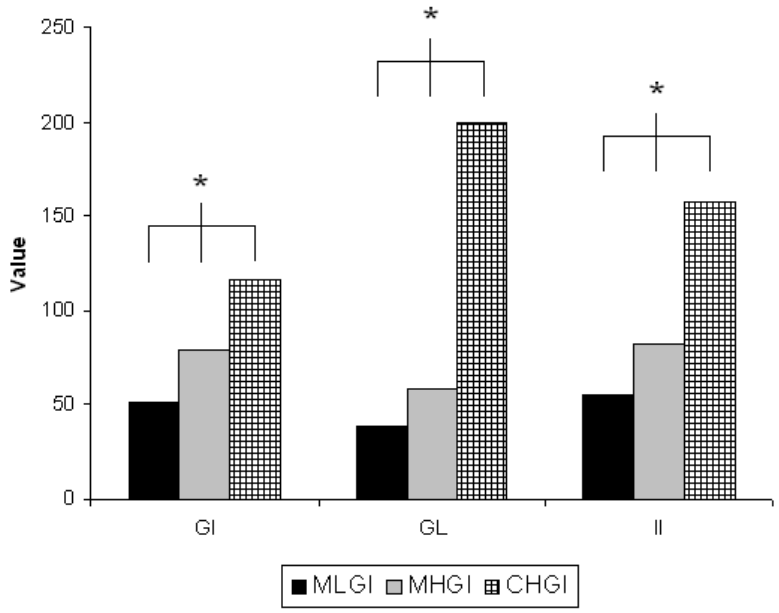

Figure 5.2. Summary of GI, GL, and II values for the CHGI, MHGI, and MLGI meals. The corresponding measures in each meal condition were statistically different from one another (all $p < 0.05$). CHGI, carbohydrate only high GI; MHGI, mixed macronutrient high GI; MLGI, mixed macronutrient low GI.

GI, GL, and II values were all significantly different between meals; the CHGI meal had the largest values followed by the MHGI and MLGI meals respectively (all comparisons $p < 0.05$; Figure 5.2). The GI value was greatest after the CHGI meal (116.6 ± 30.1), followed by the MHGI meal (78.6 ± 17.8), and the MLGI meal (50.8 ± 20.4) respectively. The GI for the CHGI meal was approximate as it was determined unethical to use a glucose reference drink of 171.4 g CHO. Calculated GL values were clinically different (Brand-Miller 2009) comparing the CHGI meal (~200), MHGI (58.9), and MLGI meals (38.2). The II value was greatest after the CHGI meal (158 ± 59.9), followed by the MHGI meal (81.9 ± 37.2), and the MLGI meal (55.7 ± 25.6) respectively.

Amino acid concentrations

Analysis of amino acid was completed on seven participants; two participants failed to complete all venepuncture procedures. Postprandial TRP and LNAA responses are shown in Figure 5.3. Basal TRP and LNAA concentrations were not statistically different between meals. TRP concentration after MHGI and MLGI were initially elevated compared to basal levels at 120 min ($p = 0.02$) and 180 min ($p < 0.001$) before declining, whereas TRP concentration after the CHGI meal showed a steady decline (time; $F(1.2,7.4) = 7.2$, $p = 0.026$ Greenhouse-Geisser). There was a significant postprandial decline in LNAA concentration throughout the study period (time; $F(3,18) = 43.2$, $p < 0.001$). The decline after the CHGI meal was significantly greater than after the MLGI ($p = 0.04$). The rate of change in LNAA concentration between meals was significantly different (meal x time; $F(6,36) = 8.7$, $p < 0.01$). The decline in LNAA concentration after the CHGI meal was more rapid than after both the MLGI ($p = 0.001$) and MHGI meals ($p = 0.004$).

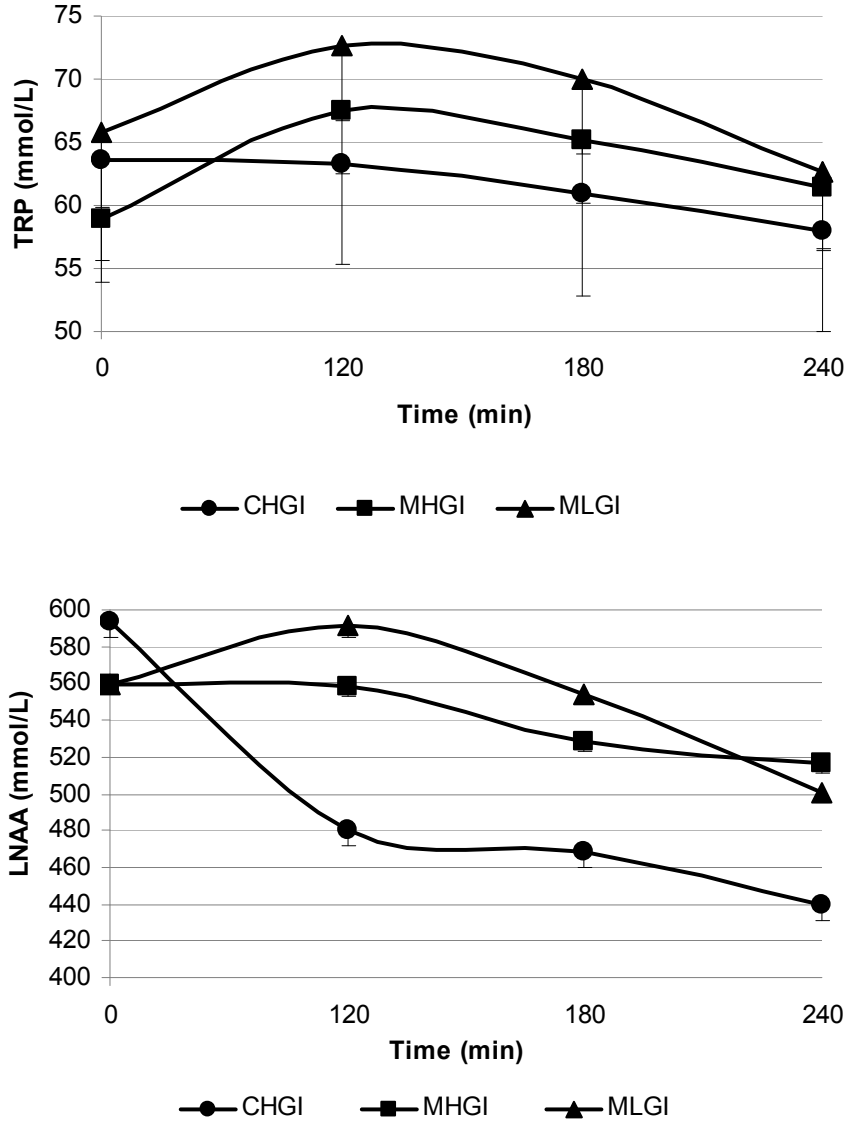

Figure 5.3. Effects of the CHGI, MHGI, and MLGI on postprandial TRP and LNAA levels in healthy participants. Postprandial TRP steadily decreased after CHGI but showed a relative increase at 120 min after both HGI and LGI before declining to near baseline. Postprandial LNAA concentrations dropped after CHGI and HGI but showed a relative increased at 120 min after MLGI before declining. CHGI, carbohydrate only high GI; MHGI, mixed macronutrient high GI; MLGI, mixed macronutrient low GI.

The postprandial TRP/LNAA response is shown in Figure 5.4 and the concentration values are indicated in Table 5.2. Basal TRP/LNAA concentrations were not statistically different between meals. Postprandial concentrations were significantly elevated after all meals (time; F (1.7,10.0) = 17.3, p = 0.001 Greenhouse-Geisser); corresponding percentage rise from baseline was approximately 23% (CHGI), 17% (MHGI), and 8% (MLGI). The AUC was greatest after the CHGI meal (4.4 ± 2.6), followed by the MHGI (2.8 ± 3.7) and MLGI meal (1.4 ± 1.4) but these differences were not statistically significant. There was a strong tendency for the AUC after the CHGI meal to be greater than the MLGI meal (p = 0.05). The AUC data were not statistically different between the CHGI and the MHGI meals (p = 0.38) or between the MHGI and MLGI meals (p = 0.43).

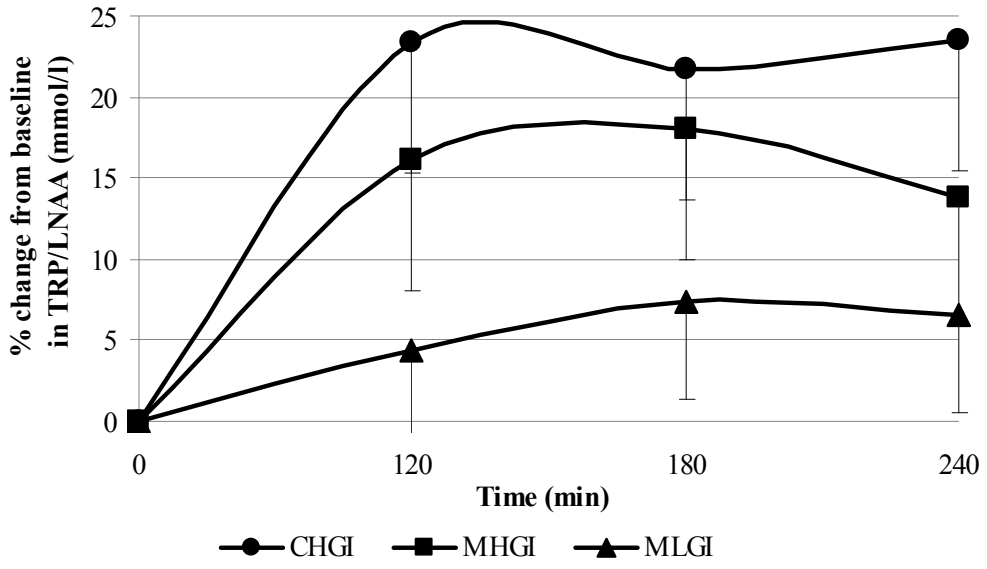

Figure 5.4. Effects of the CHGI, MHGI, and MLGI on postprandial TRP/LNAA levels in healthy participants. There was a significant postprandial rise after each test meal with a peak percentage increase between 180 and 240 min after meal consumption. The corresponding peak percentage rise was approximately 23% after CHGI, 17% after MHGI, and 7% after MLGI. CHGI, carbohydrate only high GI; MHGI, mixed macronutrient high GI; MLGI, mixed macronutrient low GI.

Table 5.2. Mean postprandial LNAA and TRP/LNAA concentrations after the CHGI, MHGI, and MLGI meals in healthy participants

Meal	val *mmol/L*	ile *mmol/L*	leu *mmol/L*	trp *mmol/L*	phe *mmol/L*	tyr *mmol/L*	TRP/LNAA *mmol/L*
CHGI							
0 min	244 ± 34	76 ± 9	140 ± 14	64 ± 9	57 ± 4	76 ± 13	0.1077 ± 0.014
120 min	217 ± 22	50 ± 6	93 ± 11	63 ± 8	51 ± 3	68 ± 10	0.1328 ± 0.022
180 min	216 ± 29	46 ± 2	88 ± 7	61 ± 9	51 ± 5	67 ± 13	0.1308 ± 0.020
240 min	204 ± 23	42 ± 5	83 ± 10	58 ± 7	47 ± 3	63 ± 9	0.1326 ± 0.018
MHGI							
0 min	234 ± 35	66 ± 13	133 ± 22	59 ± 7	54 ± 6	72 ± 16	0.1075 ± 0.022
120 min	237 ± 28	69 ± 7	125 ± 10	67 ± 11	55 ± 5	73 ± 17	0.1215 ± 0.020
180 min	230 ± 25	63 ± 8	116 ± 9	65 ± 11	51 ± 7	69 ± 18	0.1235 ± 0.020
240 min	225 ± 28	61 ± 6	116 ± 14	61 ± 9	49 ± 6	65 ± 16	0.1198 ± 0.019
MLGI							
0 min	230 ± 25	66 ± 11	132 ± 11	66 ± 5	57 ± 4	74 ± 15	0.1179 ± 0.009
120 min	243 ± 24	76 ± 10	136 ± 10	73 ± 9	58 ± 4	78 ± 16	0.1233 ± 0.016
180 min	235 ± 17	68 ± 8	123 ± 9	70 ± 7	55 ± 5	73 ± 14	0.1269 ± 0.015
240 min	216 ± 23	59 ± 10	111 ± 9	63 ± 6	49 ± 5	67 ± 15	0.1255 ± 0.012

Values are mean ± sd. LNAA are as follows: valine (val), isoleucine (ile), leucine (leu), trptophan (trp), phenylalanine (phe), tyrosine (tyr). CHGI, carbohydrate only high GI; MHGI, mixed macronutrient high GI; MLGI, mixed macronutrient low GI.

DISCUSSION

This study was conducted to examine the biochemical responses leading to an increased postprandial availability of tryptophan after an isoenergetic high and low GI mixed macronutrient meal compared to a CHO only high GI meal. The macronutrient profile and energetic load (kJ), after the MHGI and MLGI meals were improved compared to the CHGI meal. The postprandial glycemic and insulin responses after the MHGI and MLGI meals were substantially reduced compared to the CHGI meal. In addition, plasma TRP levels after the MHGI and MLGI meals were initially increased contrasting a steady decline after the CHGI meal. The plasma LNAA levels were significant reduced after all meals, however plasma LNAA concentration dropped lower and more quickly after the CHGI meal compared to the MHGI and MLGI meals. The magnitude of the corresponding postprandial increase in the plasma TRP/LNAA responses was greatest after the CHGI meal, followed by the MHGI, and MLGI meals respectively.

The MHGI and MLGI meals were identical in macronutrient composition and demonstrate improvement from the CHO only CHGI meal (Table 5.1). Palatability ratings for the MHGI and MLGI meals were similar and favoured a good taste whereas ratings after the CHGI meal were below average. The validity of VAS scales to assess subjective ratings of palatability after meal consumption is well established (Flint, Raben et al. 2000; Rahemtulla, Baldwin et al. 2005). The mixed macronutrient composition of these meals with a relative energy contribution (~ 1915 kJ) is more closely representative of a typical adult meal and consistent with westernised dietary guidelines (Australia 2003; America 2005).

It has been reported that regular consumption the CHGI meal (with GL 175) would elicit potentially damaging postprandial glucose and insulin responses for those at risk for diabetes or obesity (Jenkins, Kendall et al. 2000; Pawlak, Ebbeling et al. 2002; Livesey 2005). The lowered glycemic and insulin responses after the MHGI and MLGI meals compared to the CHGI meal carry important metabolic significance regarding the potential for regular meal consumption. Although, the GL of the MHGI (59) and the MLGI (38) are still greater than the recommended high GL value for an individual meal (20), each is considerably below the recommended total daily high GL value (120) (Brand-Miller). Thus, compared to the CHGI meal, the MHGI and MLGI are more suitable for regular consumption. Further, given the meals were identical in appearance, future blinding during experimental studies would be easily achieved.

Noteworthy are the individual TRP and LNAA responses between the mixed macronutrient meals and the CHGI meal. Postprandial TRP concentration after the MHGI and MLGI meals was initially increased contrasting a steady postprandial decline after the CHGI meal (Figure 5.3). The responses of the MHGI and MLGI meals mimic data reported in after a 500 kCal CHO and TRP (400 mg) evening meal (Ashley, Barclay et al. 1982). Compared to the CHGI meal, the mixed macronutrient meals contributed to a higher level of TRP derived from a dietary protein source (e.g. chicken breast)(Harada, Hirotani et al. 2007). Dietary TRP is largely albumin bound and therefore the presence of insulin does not promote significant skeletal muscle uptake (McMenamy and Oncley 1958). Thus, the MHGI and MLGI meals provided a greater amount of albumin bound TRP compared to the CHGI meal, which resulted in a differential TRP response.

The rate of LNAA uptake after the CHGI meal was significantly greater than both the MHGI and MLGI meals. Given the CHO:protein ratio was identical for the MHGI and MLGI meals (~ 4:1), the significant postprandial decline in LNAA concentration after the MHGI and MLGI meals is in direct contrast to the predicted nil postprandial LNAA response presented earlier (Berry, Growdon et al. 1991). In addition, after the MHGI meal plasma LNAA levels steadily declined compared an initial rise after the MLGI meal (Figure 5.3). The available amount of CHO in the CHGI meal (171.4 g) was markedly greater than both the MHGI and MLGI meals (each 75 g). By using a high GI rice (Jasmine GI ~ 109) for the CHGI and MHGI meals, and a low GI rice (Doongara GI ~ 46) for the MLGI meal, the resulting meal GI and GL values were significantly different (Table 5.1). This resulted in marked differences in the postprandial insulin response. The release of insulin (II value) was greatest after the CHGI meal, followed by the MHGI and MLGI meal respectively (Figure 5.2). These findings indicate the type and amount of CHO used in each meal substantially influenced the postprandial LNAA response. Thus, the GL value is critical in consideration of postprandial LNAA concentration given it reflects both the type and amount of CHO in a meal (Chapter 3). Indeed, at least in healthy sleepers, the GL is a reliable measure of the overall glycemic and insulin demand (Brand-Miller, Thomas et al. 2003).

The magnitude of the postprandial rise in plasma TRP/LNAA concentration was similar to the magnitude of LNAA update. The approximate percentage rise from baseline was greatest after the CHGI meal (23%), followed by the MHGI meal (17%) and the MLGI meal (7%; Figure 5.4). These findings are in accordance with previously published data (Lieberman, Caballero et al. 1986; Lyons and Truswell 1988; Wurtman,

Wurtman et al. 2003). Lyons & Truswell (1988) made the suggestion that the postprandial TRP/LNAA response will be greater after a high GI compared to a low GI food however GI testing was not completed. The present results further this claim and indicate the GL is indeed a more reliable indicator of postprandial plasma TRP/LNAA levels. In theory, if the MLGI meal was eaten as a double portion (~ 3826 kJ) it would have provided a substantially increased energy load, but the GI value will remain the same (Chapter 3). On the other hand, by doubling the CHO content (to 150 g), the GL would have increase nearly 1.5 fold (GL ~ 77), which would have surpassed the GL value for the MHGI meal. Therefore, this theoretical double-portion of the MLGI meal would likely result in a larger postprandial insulin demand and therefore also a greater rise in the plasma TRP/LNAA concentration. Thus, we suggest the GL may be valid as a tool to predict the postprandial TRP/LNAA response to mixed macronutrient meals, but this remains untested.

The availability of dietary TRP to the brain indicated by the plasma concentration of TPR/LNAA is known to increase central serotonin production in healthy individuals (Blum, Vered et al. 1992). Further, the findings from Lyons and Truswell (1998) indicate the postprandial rise in plasma TRP/LNAA after CHO based meals is similar when consumed in the morning and in the evening prior to bedtime. In a separate study, when the present CHGI meal was administered 4 h before the usual bedtime in healthy sleepers, sleep initiation was improved compared to an isoenergetic low GI version (Afaghi et al., 2007). It was proposed that the efficacy of the high GI CHO meal to improve sleep was mediated via increased serotonin production and would be indicated by an increased plasma TRP/LNAA level, although amino acid concentrations were not measured. The present data provide evidence to confirm the

suggested underlying mechanism demonstrating a marked rise in the TRP/LNAA after the CHGI meal. Although serotonin was not measured in the present study, insulin reached maximal levels from baseline between 45-60 min after meal consumption. The corresponding increases from basal levels after the CHGI meal (650%) and after the MHGI meal (450%) are consistent with published data to support increased serotonin production in healthy persons. Blum et al. (1992) reported a 461% peak rise in postprandial insulin and an approximate 4-fold increase in platelet poor serotonin after ingestion of a CHO rich snack (200 kCal). It may be speculated that the high insulin response observed for the CHGI meal and MHGI meals may similarly yield an increase in serotonin and therefore has the potential to alter sleep initiation.

This study is not without limitation. Subjective mood and alertness could have been assessed throughout the 4 h study period to provide more meaningful data on the effects of each meal on sleep initiation. The venepuncture procedure for blood collection was a major methodological limitation and 2 participants were unable to complete the procedure. Consequently, the biochemical data were limited by a low statistical power. Because of the time course of glycemic testing, it was not feasible to include additional samples. To minimise this risk in the future, blood collections could be made using an indwelling cannula. Finally, the participants were all healthy, young, male adults therefore it is unknown to what extent gender may influence the postprandial TRP/LNAA response to these meals. Recent evidence suggests the GL may be more relevant to women than men with respect to weight loss (McMillan-Price, Petocz et al. 2006), however, whether these findings relate to postprandial TRP/LNAA levels or changes in sleep behaviour remain untested.

In summary, the study results support a substantial dose-response effect of the GL of mixed macronutrient meals on the postprandial rise in the plasma TRP/LNAA concentration. It is likely the GL may be useful to predict the postprandial TRP/LNAA response to mixed macronutrient meals. Further testing is needed on the efficacy of the MHGI meal to improve sleep onset when served as an evening meal. Future studies should include postprandial measurement of serotonin to elucidate the link between the postprandial glycemic responses and sleep onset.

CHAPTER 6: Efficacy of a high glycemic index (GI) compared to an isoenergetic low GI mixed macronutrient meal to improve sleep quality in insomnia.

The enclosed study at the time of writing this thesis was presented as follows:

C.P Herrera, P. Ruell, H. O'Connor, C.M. Chow (2010). Influence of the glycemic load (GL) on subjective and objective measures of sleep quality in sleep initiation insomnia. *International Journal of Psychophysiology 77(3), p. 292.*

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ABSTRACT

Introduction: Insomnia is a pervasive sleep disturbance limiting sleep quality. There is evidence in healthy sleepers to suggest sleepiness will increase after ingestion of large carbohydrate (CHO) meals. The glycemic index (GI) of CHO is thought to play a role in sleep initiation given the availability of tryptophan to the brain is promoted after high GI foods. Evidence is lacking regarding the efficacy of the GI of regular meals to promote sleep initiation in participants with insomnia. This study evaluates the efficacy of a mixed macronutrient high GI (MHGI) meal compared to a mixed macronutrient low GI (MLGI) meal to improve sleep quality in participants with insomnia.

Methods: Subjective and PSG sleep were assessed on 2 consecutive nights in a repeated measures, single blind study. Participants (4 men, 4 women) meeting research diagnostic criteria for insomnia were randomised to either the MHGI or MLGI meal. Biochemical measurement of postprandial glucose, insulin, plasma tryptophan relative to other large neutral amino acids (TRP/LNAA), and platelet poor plasma serotonin (PPP-5HT) was completed and compared between meals using paired samples t-tests.

Results: The MHGI meal resulted in improved subjective, but not PSG sleep which showed large variability between nights and within individuals. The participants were more rested after the MHGI meal; these improvements were apparent in women only. The postprandial glucose response tended to be larger after the MHGI meal compared to the MLGI meal ($p = 0.057$) but there were no differences between insulin responses. The postprandial TRP/LNAA levels were approximately 3.5 fold greater after the MHGI meal compared to the MLGI meal and marginally significant ($p = 0.13$), but did not result in a postprandial change in PPP-5HT.

Discussion: This study demonstrates significant clinical improvement in subjective sleep quality after a mixed macronutrient high GI meal despite marginal statistical significance. The substantial variability in the night-to-night PSG data may have masked the influences of the meal response. Given the present findings we suggest that there is a physiological threshold in the postprandial plasma TRP/LNAA concentration that must be surpassed in order to promote measurable changes to serotonin and sleep behaviour. Future studies should elucidate more clearly the sex-differences underlying the impact of the GI on sleep behaviour.

Keywords: glycemic index, glycemic load, insomnia, tryptophan, serotonin

INTRODUCTION

Globally, insomnia is the most common sleep complaints across all stages of adulthood (NIH 2005) and is characterised by subjective dissatisfaction with the quantity, quality, or timing of sleep that is present on most nights of the week for at least 1 month (Morin and Espie 2003). Primary treatment for insomnia includes pharmacotherapy and cognitive behavioural therapy (CBT), however pharmaceuticals carry negative side effects (Ringdahl, Pereira et al. 2004) and CBT is associated with poor patient adherence (Ong, Kuo et al. 2008). Sleep hygiene education is often included with behavioural interventions, but there is insufficient evidence to assess the effectiveness as a single therapy (Morgenthaler, Kramer et al. 2006). One aim of sleep hygiene is to make patients more aware of how dietary practices may be either detrimental or beneficial for sleep.

Complementary and alternative medicine therapies have long been popular with those battling insomnia, but have limited scientific support (Pearson, Johnson et al. 2006). Treatment with the pharmaceutical grade l-tryptophan is well established and demonstrates a dose-response effect in its clinical efficacy. Remarkably, as little of 250 mg of tryptophan improves sleep in people with chronic insomnia, while a 1000 mg dose is associated with more consistent results (Hartmann and Spinweber 1979). On the other hand, the availability of dietary tryptophan (TRP) to the brain is dependent on its plasma concentration relative to other large neutral amino acids (TRP/LNAA) since each amino acid competes for a single transport molecule across the blood brain barrier (Wurtman 1988). A high glycemic index (GI) meal results in a marked postprandial

rise in the concentration of TRP/LNAA with a peak between 3-4 h (Lyons and Truswell 1988). In a study involving participants with insomnia, provision of a bedtime snack containing a CHO (glucose) and dietary sourced TRP (pumpkin seeds) improved self-reported (e.g., sleep diary) measures of sleep efficiency, total awake time after sleep onset, and sleep quality compared to a pre-treatment week (Hudson, Hudson et al. 2005). Recently, Afaghi et al. (2007) provided PSG data linking the GI to sleep initiation in healthy sleepers, but evidence is lacking regarding the effectiveness in persons with insomnia. These authors reported a 50% reduction in the SOL after a high GI CHO rich compared to a low GI CHO rich meal given 4 h prior to sleep (Afaghi, O'Connor et al. 2007). Although interesting, these studies do not provide sufficient guidance on the optimal and realistic meal composition needed to improve sleep. First, major differences in meal timing and the study population exist. Second, neither study included the necessary biochemical measures to provide a mechanistic explanation for such sleep improvements. Third, the findings of Afaghi et al. (2007) are limited in application given the high GI CHO rich meal was of considerably high glycemic load (GL) and therefore undesirable for regular use (Salmeron, Ascherio et al. 1997).

A dietary intervention resulting in a marked elevation of the postprandial plasma TRP/LNAA concentration potentially represents an accessible, cost-effective and low risk strategy for management insomnia but remains inadequately evaluated for this purpose. Therefore, this study aimed to evaluate the efficacy of a high GI mixed macronutrient (MHGI) meal compared to an isoenergetic low GI mixed macronutrient (MLGI) meal consumed 3 h before bedtime to improve subjective and objective sleep

quality in insomnia. In addition, the postprandial changes in the plasma TRP/LNAA concentration and serotonin production were assessed.

The following a priori hypotheses were formulated based on the variance in GI and GL, since a greater release of insulin should follow the MHGI meal: (1) the MHGI meal will result in greater glycemic, insulin, and amino acid responses than the MLGI meal (2) the MHGI meal will result in greater subjective post-sleep improvements, and (3) the MHGI meal will result in improved objective sleep measures indicated by PSG (i.e., shorter SOL and improved sleep efficiency). Sex differences will be explored where possible to elucidate the potential influence on the biochemical and sleep responses to the meals.

METHODS

Participants

Recruitment of participants with insomnia included flyers on university and community notice boards and a local newspaper advertisement. Ethical approval (Appendix C) was received from the Human Research Ethics Committee of The University of Sydney and participants provided written informed consent prior to participation (Appendix E). Inclusion criteria defined suitable participants as men and women between 18-55 y. An ability to attend overnight sleep studies on five occasions was also required and this limited the participant pool to residents living in the Sydney Metropolitan area. Personal interview and electronic mail was utilised to facilitate the administration of validated screening questionnaires to objectively determine the research diagnostic criteria (RDC) for primary insomnia following published guidelines (Edinger, Bonnet et al. 2004). The Pittsburgh Sleep Quality Index (PSQI) was used to determine the previous one-month average sleep onset latency, its weekly occurrence, and to assess the overall sleep quality (Buysse, Reynolds et al. 1989). The Insomnia Severity Index (ISI) was used to assess the level of weekly disturbance to SOL and the overall severity of insomnia symptoms (Bastien, Vallieres et al. 2001). The final RDC for sleep initiation insomnia was indicated by a self-reported poor sleep quality (PSQI > 6), a minimum of sub clinical insomnia (ISI > 11), and an SOL of at least 30 min occurring at least three times per week for duration of at least one month. Exclusion criteria included any current self-report of significant medical, psychiatric, or sleep disorders, food allergies, pregnancy, diabetes, or shift work (Appendix G).

Meals

Two previously designed isoenergetic mixed macronutrient meals of known composition were either high GI (MHGI) or low GI (MLGI) (see Chapter 5). Meals were consumed 3 h before individualised habitual bedtimes determined by a sleep diary. The timing of the meals was chosen to match the suspected peak postprandial TRP/LNAA concentration. Briefly, the MHGI meal provided 1916kJ (7.9g fat, 18.2 g protein, 75 g CHO) with GI (79) and GL (59) values. The MLGI meal provided 1913 kJ (7.9 g fat, 18.6 g protein, 75 g CHO) with GI (51) and GL (38). The dietary amino acid contribution of TRP (determined from chicken and rice ingredients) was identical for each meal at approximately 168 mg (Harada, Hirotani et al. 2007).

Study design and protocol

The study was a randomised single blind cross-over trial. The first night was designated a familiarisation period and data were not included in the subsequent analysis. This was to minimise the first night effect (FNE; Chapter 4) and to screen for the presence of sleep disorders (e.g., obstructive sleep apnoea and periodic limb movements) characterised by >5 or >15 events per hour of sleep (Redline, Budhiraja et al. 2007). Participants were randomly allocated to receive the MHGI and MLGI meals for two consecutive nights in a counterbalanced order. A two night PSG period was needed to account for the extreme night-to-night variability in sleep quality that is present in those with insomnia (Vallieres, Ivers et al. 2005). The period between sleep studies was variable (ranging from 0-28 days) to facilitate women participants being measured in the luteal phase of menstruation on each occasion. This was to avoid a known disturbed sleep pattern during the follicular phase (Lee, Shaver et al. 1990).

Sleep variables from PSG were scored by an expert in sleep physiology (CMC) blinded to all conditions.

On all occasions participants arrived to the research laboratory within 15 min of their scheduled meal time. Participants were provided time to relax and were free to ambulate between the sleep laboratory, toilet, and research kitchen where a TV was made available. Participants were monitored by the primary investigator and were not permitted to sleep prior to testing.

Biochemical measurements (blood collections) were completed just prior to meal consumption (baseline) and at 60, 120, and 180 min intervals on the both testing night in each meal condition. Due to budgetary constraints analysis was completed on the second night only. The second night was chosen to minimise potentially unwanted daytime meal variability (Clark, Gardiner et al. 2006). It was predicted participants would better adhere to the participant instructions on the second night. Blood was collected from an indwelling cannula placed into a forearm vein that was removed just after the last collection. The sampling line was kept patent using 0.9% saline in between collections. A 2 mL sample was collected into a lithium heparin tube and immediately centrifuged at 2,500 g for 10 min at 4°C. The supernatant was stored at -80°C for determination of plasma amino acids by high phase liquid chromatography (HPLC, Shimadzu). Analysis was completed in duplicate according to published guidelines (Huq, Thompson et al. 1993). On a separate occasion, the sample was thawed and determination of blood glucose enzymatically using a Thermo Electron kid (Noble Park, Vic, Australia; cat no TR15103) and insulin using an Alpco ELISA kit (Alpco Diagnostics, Salem, NH, USA; cat no 80-INSHU-E01). A separate 4 mL

sample was collected into an EDTA tube and immediately centrifuged at room temperature at 200 g for 10 min. The resulting platelet rich plasma was spun at 4,500 g for 10 min. The supernatant was stored at -80°C for determination of platelet poor plasma serotonin (PPP-5HT) using a commercially available enzyme immunoassay kit (IBL, Hamburg, Germany, RE59121). It has been shown that food consumption influences PPP-5HT (Blum, Vered et al. 1992). Moreover, PPP-5HT levels correlate well with changes in central serotonergic processes (Yan, Urano et al. 1993). To compare glucose, insulin, and TRP/LNAA responses between meals the area under the curve (AUC) within the study period (180 min) was calculated according to published guidelines (Brouns, Bjorck et al. 2005).

Subjective and PSG sleep measures

Participants completed a pre-sleep questionnaire (Appendix G) consisting of three individual 10cm visual analogue scales (VAS) used to assess subjective levels of postprandial satiety, sleepiness (both at baseline, 1, 2, and 3 h) and meal palatability immediately after the meal consumption only. Anchor terms were as follows: satiety 0 = still hungry, 10 = completely full; sleepiness 0 = completely awake, 10 = very sleepy; palatability 0 = poor taste, 10 = good taste).

PSG sleep parameters were measured using a digital polygraph system (Compumedics W- or E-series, Compumedics Pty Ltd, Australia) with the following protocol: EEG (C4, 01, A1, A2), EOG (LOC, ROC), and EMG (masseter) according to published guidelines (Iber, Ancoli-Israel et al. 2007). A grounding and reference electrode was placed at the Fpz and Pz position. Electrodes were applied within the last half hour closest to bedtime at which point the participants were required to remain in the sleep

laboratory. The following PSG variables were computed: sleep onset latency (SOL), total sleep time (TST), sleep efficiency (SE, time asleep/TST x 100), time awake after sleep onset (WASO), non-rapid eye movement sleep stage 1 (NREM1), stage 2 (NREM2), stage 3 (NREM3), rapid eye movement sleep latency (REML), and REM sleep (REMS). SOL was further defined as the first occurrence of any stage of sleep from lights off which was marked one minute after physiological calibrations. PSG sleep variables were examined by an expert in sleep physiology (CMC) blinded to all conditions.

Participants were permitted to sleep in self-selected body positions and could void in a nearby toilet if required. The sleep environment was maintained between 21-23^{o}C with complete darkness and free of windows. Participants retired to bed *ad libitum* scheduling their desired sleep time to replicate habitual sleep practises. After awakening, sleep recording was ceased and the electrodes were removed.

Participants completed a researcher designed post-sleep questionnaire (Appendix G) each morning after PSG testing to assess subjective sleep parameters similar to those obtained on the sleep diary, including SOL, number of nocturnal awakenings, WASO, and TST. Participants were also asked to rate the quality of sleep compared to home on a five point Likert scale (much worse, worse, about the same, better, much better) and to describe the extent the sleep quality was perceived as (1) refreshing (2) restless (3) difficult to fall asleep and (4) how rested they feel this morning (not at all, somewhat, average, very, extremely). A 10cm VAS scale was also used to assess a perceived feeling of sleepiness (0 = completely awake, 10 = very sleepy).

Participants were offered a standard breakfast (cereal and milk) before leaving the sleep laboratory. Participants were instructed prior to all test nights to maintain regular daytime routines but to refrain from napping, planned exercise (other than usual walking/commuting), alcohol, excessive caffeine (> 1 cup), and to remain nil by mouth (except water) within 3 h of the MHGI or MLGI dinner meal. All subjects complied with experimental conditions to the extent of our questionnaires.

Statistical Analysis

The sample size was calculated based on an effect size obtained for SOL (1.3) powered at 85% and alpha of 5% in a study comparing a high and low GI CHO meal given 4 h prior to sleep in healthy sleepers (Afaghi, O'Connor et al. 2007). It was estimated that a modestly large effect size of 0.85 (Cohen 1988) and a calculated sample size of 15 (Faul, Erdfelder et al. 2007) were appropriate to test the *a priori* hypotheses regarding meal differences. Data were tested for normality before statistical procedures were preformed using SPSS version 16 (SPSS Inc, Cary, NC, USA). Data are reported means ± standard deviation (SD). Data obtained for sleepiness, hunger, and palatability by VAS and subjective sleep measures were analysed by paired samples t-test to identify meal difference. Paired samples t tests were also used to compare the averaged night 1 and night 2 PSG data between meals. A repeated measures analysis of variance (ANOVA) was used to identify differences between individual night PSG data; within subjects factors were meal (MHGI or MLGI) and night (night 1 or night 2); gender was a between subjects factor. A separate ANOVA with *a priori* contrasts was used to examine the postprandial meal effects on all biochemical measures; within subjects factors were meal (MHGI or MLGI) and time (0, 60, 120, 180 min). In addition, a paired samples t-tests was used to identify differences between meals on the AUC data (glucose, insulin, and TRP/LNAA). Independent samples t-tests were used to identify all gender differences. Significance was met at $p < 0.05$ level.

RESULTS

A total of 14 participants volunteered to take part in the study. Three volunteers failed to complete the RDC questionnaires and 2 were unwilling to complete PSG procedures. One participant was excluded due to significant periodic limb movements. A total of 8 (4 men, 4 women) participants were randomised to the MHGI or MLGI meal and completed all dietary and PSG procedures. Individual participant characteristics, ISI and PSQI scores, and the relative energy load (EL) of the test meals is summarised in Table 6.1. The EL was calculated using an averaged energy value between the MHGI and MLGI meals (~ 1915 kJ) and individual participant BMI ($kg.m^{-2)}$. Mean EL of the test meals was significantly higher for women (32.0 ± 4.1 $kJ.kg^{-1}$) than men (25.4 ± 3.7 $kJ.kg^{-1}$, p = 0.03). The BMI ($kg.m^{-2}$) was slightly higher for men (23.5 ± 2.1) than women (20.7 ± 0.96, p = 0.06).

Table 6.1. Participant characteristics, research criteria, and relative energetic load (EL) of the test meals in study participants

Sex/Age	BMI ($kg.m^{-2}$)	ISI	PSQI	EL ($kJ.kg^{-1}$)
M/32	22.9	11	9	28.2
M/32	24.9	14	14	21.8
M/31	25.4	28	17	22.5
M/28	20.8	15	8	29.0
F/20	22.2	11	9	27.0
F/30	20.1	13	10	34.5
F/39	20.7	23	16	36.1
F/29	20.1	22	11	34.2

M, male; F, female; BMI, body mass index. ISI, Insomnia Severity Index; PSQI, Pittsburgh Sleep Quality Index; EL, energy load. Threshold scores for research diagnostic criteria for insomnia were ISI (>8) and PSQI (>6).

Meal palatability and subjective ratings of postprandial satiety and sleepiness

Palatability ratings were similar on all study nights and were identical between the MHGI (7.7 ± 1.5 cm) and MLGI (7.8 ± 1.4 cm, p = 0.92) meals, indicating an overall good taste; there were no sex differences. Subjective ratings of postprandial satiety were similar immediately after meal consumption (averaged data at baseline, MHGI: 8.1 ± 1.5 cm; MLGI 7.6 ± 1.9 cm, p = 0.26) and throughout the study period, indicating participants were not overly hungry at bedtime (averaged data at 180 min, MHGI: 5.7 ± 1.9 cm; MLGI: 5.3 ± 2.6 cm, p = 0.54). Sex differences were present after the MLGI meal on the second night. Women tended to report greater ratings for satiety immediately after meal consumption (women: 9.1 ± 1.3cm; men 7.9 ± 1.6 cm; p = 0.06) and at bedtime (180 min, women: 7.4 ± 1.6 cm; men 4.1 ± 2.2 cm; p = 0.06). Subjective ratings on postprandial sleepiness indicated levels were similar on all study nights immediately after meal consumption (averaged data at baseline, MHGI: 3.2 ± 1.6 cm; MLGI: 3.1 ± 1.9 cm; p = 0.69) and were greatest at bedtime (averaged data at 180 min, MHGI 6.6 ± 1.9 cm; MLGI 6.2 ± 2.4 cm; p = 0.50), indicating sleepiness increased across the study period. There were no differences between meals, nights, or across sexes.

Subjective post-sleep variables

There were no significant differences in subjective post-sleep variables across nights therefore averaged data are presented. Participants reported feeling more rested after the MHGI meal (2.8 ± 0.8 cm) than the MLGI meal (2.3 ± 0.9 cm; $p = 0.01$) on the 5-point Likert scale corresponding to "average" and "somewhat", respectively. Women were more rested after the MHGI meal (3.0 ± 0.5 cm) than the MLGI meal (2.3 ± 1.0 cm, $p < 0.05$) meal but the ratings in men were not statistically different (MHGI: 2.6 ± 1.1 cm; MLGI: 2.3 ± 0.9 cm; $p = 0.20$). There were no other differences in subjective post-sleep variables.

PSG sleep variables

Table 6.2 summarises the effects of the meals on the sleep variables averaged across nights. There were no significant differences in PSG sleep variables between meal conditions, however, there tended to be less EEG arousals (lower AI) after the MHGI compared to the MLGI meal ($p = 0.06$), however significant sex differences were present. The AI difference between meals in women was statistically significant ($p < 0.05$) but in men it was not ($p = 0.60$). Overall, men had a higher AI than women after both the MHGI meal ($p < 0.001$) and the MLGI meal ($p < 0.05$). Other marginal sex differences included the tendency for a shorter SOL in women compared to men after the MHGI meal ($p = 0.06$). There was a tendency in men to have more light sleep (NREM1) after the MLGI meal compared to the MHGI meal ($p = 0.07$) but no differences observed in women ($p = 0.75$). SWS tended to be greater in females after the MLGI meal compared to the MHGI meal ($p = 0.06$) but no differences were observed in men ($p = 0.24$).

Table 6.2. Effects of the MHGI and MLGI meals on averaged PSG sleep variables in participants with insomnia

PSG variable	MHGI	MLGI	Meal difference
SOL (min)	**28.3 ± 19.2**	**28.1 ± 18.1**	
Men	37.3 ± 17.6[b]	33.6 ± 16.2	
Women	19.4 ± 17.3	24.3 ± 19.8	
REML (min)	**99.9 ± 42.5**	**103.7 ± 36.2**	
Men	107.0 ± 46.3	102.3 ± 32.2	
Women	92.8 ± 40.0	104.6 ± 41.9	
SE (%)	**86.8 ± 7.5**	**88.2 ± 5.6**	
Men	84.2 ± 7.0	86.4 ± 6.0	
Women	89.4 ± 7.4	89.9 ± 4.9	
TST (min)	**452.9 ± 45**	**473.2 ± 50.2**	**p = 0.09**
Men	441.8 ± 44.9	470.7 ± 52.5	
Women	464.1 ± 45.1	475.6 ± 51.3	
WASO (min)	**40.6 ± 31.6**	**36.0 ± 29.8**	
Men	44.9 ± 31.3	40.0 ± 34.9	
Women	36.3 ± 33.3	31.9 ± 25.4	
NREM1 (%)	**2.4 ± 1.1**	**2.7 ± 1.5**	
Men	2.5 ± 0.8	3.2 ± 1.6	p = 0.07
Women	2.3 ± 1.3	2.3 ± 1.3	
NREM2 (%)	**52.3** ± 7.7	**50.4 ± 7**	
Men	51.2 ± 9.5	50.4 ± 8.7	
Women	53.5 ± 5.7	50.4 ± 5.4	
SWS (%)	**20.0** ± 42	**20.6 ± 4.4**	
Men	20.9 ± 4.6	18.9 ± 5.2	
Women	19.1 ± 3.8	22.2 ± 2.9	p = 0.06
NREMS (%)	**74.8** ± 5.0	**73.7 ± 5.9**	
Men	74.6 ± 5.6	72.6 ± 5.3	
Women	74.9 ± 4.4	74.9 ± 6.5	
REMS (%)	**25.3** ± 4.9	**26.3 ± 5.9**	
Men	25.5 ± 5.6	27.4 ± 5.3	
Women	25.1 ± 4.4	25.1 ± 6.6	
AI (no./h)	**8.1 ± 2.7**	**9.2 ± 3.1**	**p = 0.06**
Men	8.1 ± 2.7[a]	9.2 ± 3.1[a]	
Women	6.0 ± 1.2	7.7 ± 2.0	p < 0.01

Values are mean ± sd **.**

Bold values represent entire sample (n = 8), non-bold for each sex (4 men, 4 women)

Comparison between sexes; [a] ($p < 0.05$) or [b] ($p \leq 0.10$)

Table 6.3 summarises the effects of the meals on the sleep variables over both nights. There was a trend for a shorter SOL on the first compared to the second night after both the MHGI and the MLGI meals for all participants (bolded value; F, night, (1,6) = 5.6, p = 0.06). Figure 6.1 shows the objective PSG values compared to the post-sleep subjective ratings for SOL on individual nights after the MHGI and MLGI meals. Women tended to have a shorter SOL on each night compared to men but these differences were not statistically significant. There was a delayed REML on the first compared to the second night for both men and women, but this was differential by meal and sex (F, meal x night x sex (1,6) = 6.3, p = 0.05). Women had a delayed REML on the first compared to the second night after the MHGI meal whereas REML in men was delayed after the MLGI across nights; however these differences were not statistically significant (in women: p = 0.16; in men; p = 0.41). Light sleep (NREM1) tended to be greater on the first compared to the second night in women but not men (F, night by sex (1,6) = 5.1, p = 0.06) in whom no differences were observed between meals (MHGI: p = 0.26; MLGI: p = 0.14). In men, SWS on night 1 was greater than night 2 after the MHGI meal but was similar in women (F, night x sex (1,6) = 6.8, p = 0.04). Men had a similar AI on each night and these were significantly higher than for women (F, meal x night x sex (1,6) = 9.5, p = 0.02). Women had a greater AI on the first night compared to the second night after the MLGI ($p < 0.05$).

Table 6.3. Effects of the MHGI and MLGI meals on individual night PSG sleep variables in participants with insomnia

Sleep variables	MHGI Night 1	MHGI Night 2	MLGI Night 1	MLGI Night 2
SOL (min)	**24 ± 15.3**[#]	**32.7 ± 22.6**	**22.7 ± 18**[#]	**35.2 ± 17**
Men	30.3 ± 14.9	44.2 ± 19.3	27.0 ± 17.9	40.1 ± 13.3
Women	17.8 ± 14.5	21.1 ± 21.6	18.3 ± 19.6	30.3 ± 20.9
REML (min)	**111.4 ± 49.3**	**88.3 ± 33.6**	**108.2 ± 34.9**	**99.1 ± 39.2**
Men	107.1 ± 57.9	106.9 ± 40.7	115.6 ±26.7	89.9 ± 35.6
Women	115.8 ± 47.6	69.8 ± 7.2	100.8 ± 44.4	108.3 ± 45.7
SE (%)	**86.5 ± 8.9**	**87.2 ± 6.3**	**89.8 ± 5.3**	**86.5 ± 5.7**
Men	85.2 ± 8.7	83.3 ± 6.0	88.8 ± 4.7	84.1 ± 6.8
Women	87.8 ± 10.2	91.1 ± 4.0	90.8 ± 6.4	89.0 ± 3.5
TST (min)	**448.6 ± 48.8**	**457.3 ± 43.7**	**480.6 ± 46.7**	**465.8 ± 55.6**
Men	448.5 ± 50.8	435.0 ± 44.7	482.4 ± 55.1	459.0 ± 55.0
Women	448.8 ± 54.6	479.5 ± 45.2	478.8 ± 45.2	472.5 ± 63.7
WASO (min)	**45.9 ± 43.0**	**35.3 ± 14.8**	**34 ± 23.9**	**37.9 ± 36.2**
Men	47.1 ± 45.6	42.6 ± 14.3	33.6 ± 23.5	46.4 ± 46.6
Women	44.8 ± 47.3	27.9 ± 12.7	34.4 ± 27.8	29.5 ± 26.7
NREM1 (%)	**2.5 ± 1.3**	**2.3 ± 0.8**	**2.9 ± 1.4**	**2.5 ± 1.7**
Men	2.4 ± 0.99	2.6 ± 0.78	3.1 ± 1.7	3.3 ± 1.7
Women	2.7 ± 1.7	2.1 ±0.92	2.8 ± 1.2	1.8 ± 1.4
NREM2 (%)	**51.4 ± 7.2**	**53.3 ± 8.4**	**51.0 ± 7.7**	**50.0 ± 6.7**
Men	49.3 ± 9.1	53.1 ± 10.9	51.6 ± 10.1	49.3 ± 8.6
Women	53.5 ± 5.4	53.5 ± 6.9	50.4 ± 6.0	50.5 ± 5.6
SWS (%)	**21.3 ± 4.3**	**18.7 ± 3.9**	**20.8 ± 5.4**	**20.3 ± 3.5**
Men	23.6 ± 3.8*	18.2 ± 3.9	19.7 ± 7.3	18.1 ± 2.5
Women	19.0 ± 3.7	19.1 ± 4.4	21.9 ± 3.2	22.5 ± 3.1
NREMS (%)	**75.2 ± 4.2**	**74.3 ± 5.8**	**74.8 ± 5.3**	**72.7 ± 6.7**
Men	75.3 ± 4.8	74.0 ± 7.0	74.4 ± 3.1	70.7 ± 6.9
Women	75.1 ± 4.1	74.7 ± 5.4	75.1 ± 7.7	74.7 ± 6.6
REMS (%)	**24.9 ± 4.1**	**25.7 ± 5.8**	**25.3 ± 5.3**	**27.3 ± 6.64**
Men	25.0 ± 4.8	26.1 ± 7.0	25.6 ±3.1	29.3 ± 6.9
Women	24.9 ± 4.0	25.4 ± 5.4	25.0 ± 7.5	25.3 ± 6.7
AI (no. h^{-1})	**8.3 ± 3.1**	**8.0 ± 2.4**	**9.2 ± 3.1**	**9.2 ± 3.4**
Men	10.7 ± 2.2 [a]	9.9 ± 1.5 [a]	10.3 ± 3.6 [a]	11.3 ± 3.6 [a]
Women	5.9 ± 1.5	6.0 ± 1.1	8.1 ± 2.6*	7.2 ± 1.6

Values are mean ± sd

Bold values represent entire sample (n=8), non-bold for each sex (4 men, 4 women)

Comparison between nights; * ($p < 0.05$) or # ($p \leq 0.10$); between sexes; [a] ($p < 0.05$) or [b] ($p \leq 0.10$)

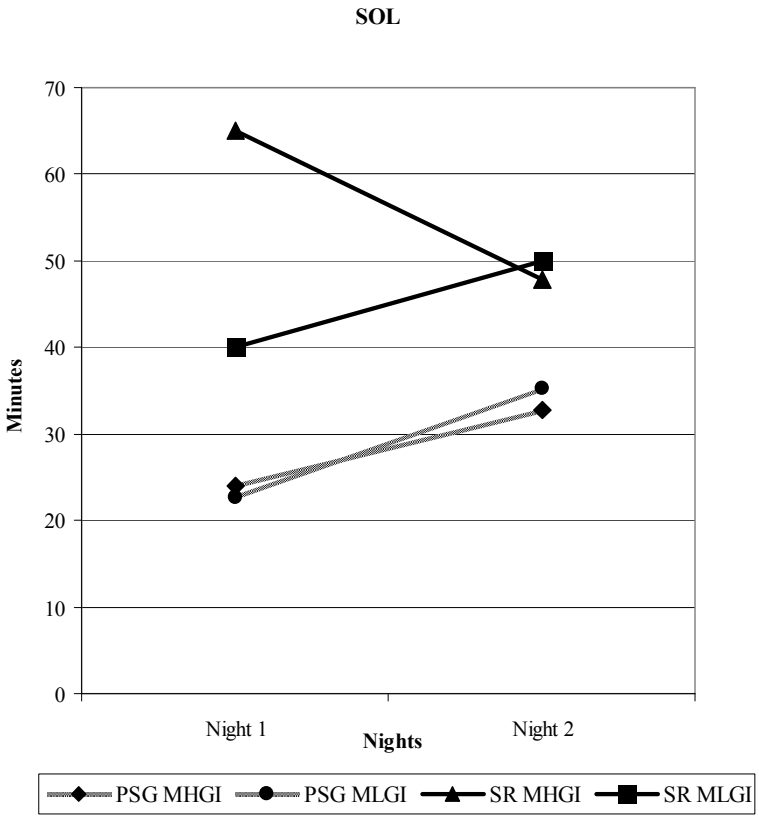

Figure 6.1. Effects of the MHGI and MLGI meals on objective and subjective sleep onset latency (SOL) in participants with insomnia. The PSG SOL was shorter on the first night compared to the second in each meal condition ($p = 0.056$). The PSG values were lower than subjective ratings. Abbreviations: PSG, polysomnography; MHGI, mixed macronutrient high GI; MLGI, mixed macronutrient low GI; SR, subjective rating.

Postprandial change in biochemical measures

The postprandial glucose and insulin responses are shown in Figure 6.2 (n = 6, 3 men, 3 women); two participants were not willing to complete the procedure. The glucose response (AUC) tended to be greater after the MGHI meal (179.0 ± 115.0) compared to the MLGI meal (71.3 ± 66.4, p = 0.06). There was no significant difference in the postprandial rise in insulin levels between meals (MHGI: 1536.3 ± 1574.2; MLGI: 1529.3 ± 1515.3).

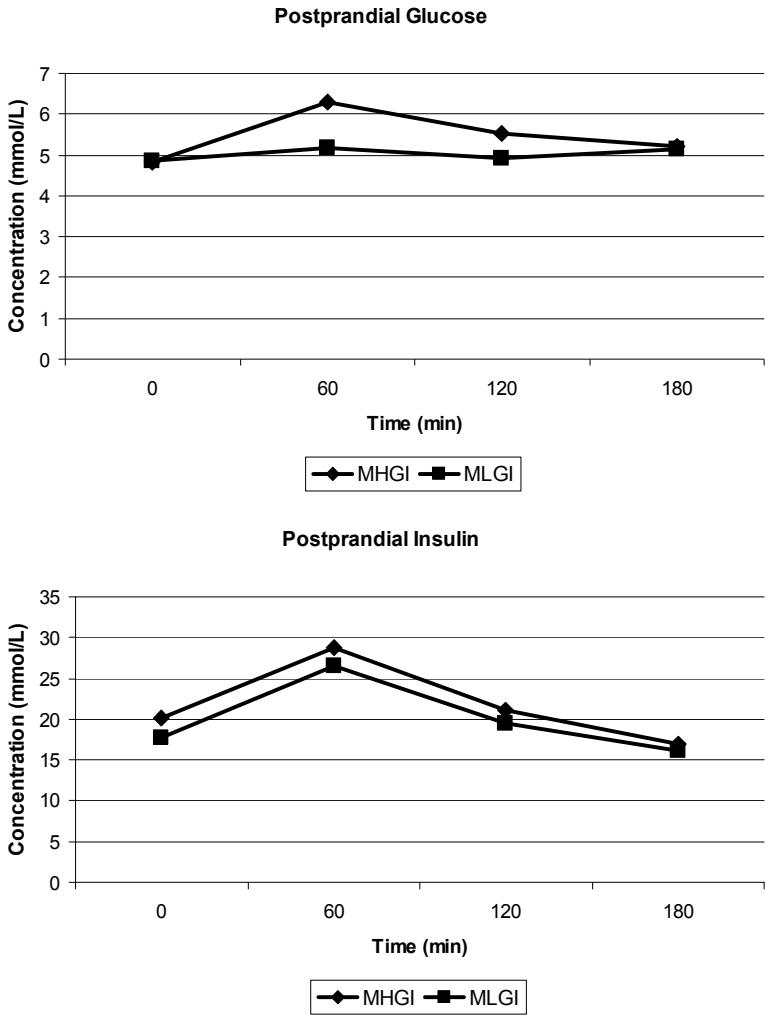

Figure 6.2. Postprandial glucose and insulin responses after the MHGI and MLGI meals in participants with insomnia. The glucose response (AUC) tended to be greater after the MHGI meal compared to the MLGI meal (p = 0.06) but the insulin responses were not statistically different. MHGI, mixed macronutrient high GI; MLGI, mixed macronutrient low GI.

The postprandial TRP/LNAA response is shown in Figure 6.3 (n = 4, 1 man, 3 women); two participants were unwilling to undergo the procedure. Both meals elicited a significant postprandial change (time; $F(3,9) = 8.8$, $p < 0.01$), however there was a brief decline at 60 min followed by an increase to 180 min. The maximum percentage rise from baseline occurred at 3 h postprandially after the MHGI (17%) and the MLGI meals (8%, both $p < 0.05$). The AUC after the MHGI (1.73 ± 1.3) was approximately 3.5-fold greater than after the MLGI meal (0.50 ± 0.5) and this difference was marginally significant (paired t-test, $p = 0.13$). Table 6.4 indicates the postprandial plasma TRP/LNAA concentrations.

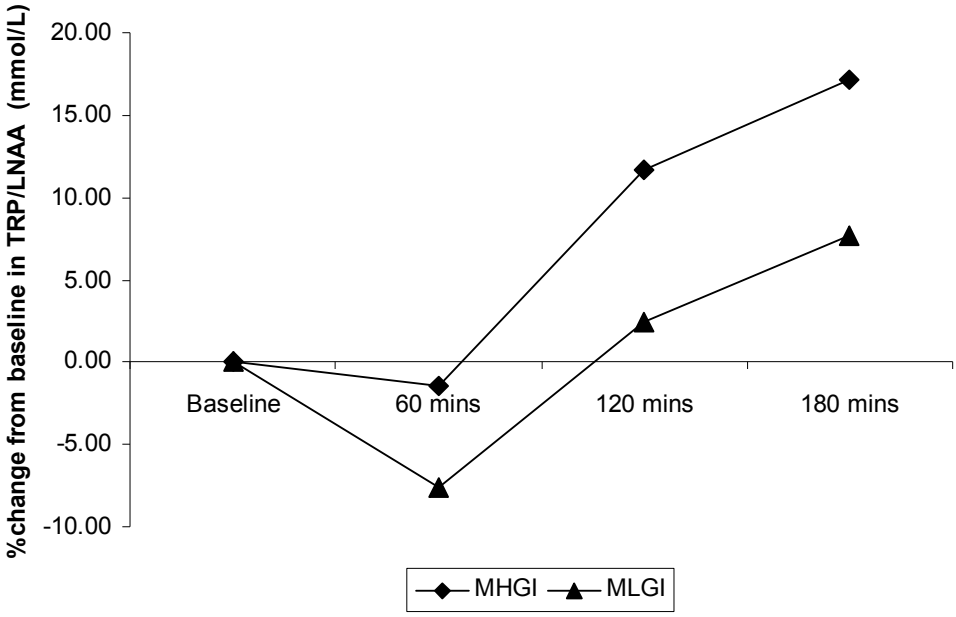

Figure 6.3. Effects of the MHGI and MLGI meals on the postprandial TRP/LNAA levels in participants with insomnia. The TRP/LNAA levels were maximal at bedtime (3 h postprandially) reaching a peak percentage rise from baseline after the MHGI (17%) and MLGI (8%). The area under the curve (AUC) after the MHGI meal was nearly 3.5-fold higher than the MLGI meal but this was not statistically significant (paired samples t-test, $p = 0.06$). Abbreviations: MHGI, mixed macronutrient high GI; MLGI, mixed macronutrient low GI.

Table 6.4. Mean TRP/LNAA concentration after the MHGI and MLGI meals in participants with insomnia

Time	MHGI (mmol/L)	MLGI (mmol/L)
0, baseline	0.1023 ± 0.012	0.1098 ± 0.015
60 min	0.1011 ± 0.022	0.1045 ± 0.016
120 min	0.1148 ± 0.028	0.1117 ± 0.008
180 min	0.1200 ± 0.017	0.1117 ± 0.012

Values are mean ± sd. MHGI; mixed macronutrient high GI; MLGI, mixed macronutrient low GI

Platelet Poor Plasma Serotonin (PPP-5HT)

There was no significant postprandial change in PPP-5HT concentrations (n = 4, 1 man, 3 women); data were erroneous for four participants due to platelet contamination (Yan 1993). The levels were similar between meals at all time points: baseline MHGI (8.7 ± 1.7 $ng.ml^{-1}$) and MLGI (8.8 ± 1.8 $ng.ml^{-1}$); 60 min HGI (9.3 ± 1.5 $ng.ml^{-1}$) and MLGI (8.9 ±1.3 $ng.ml^{-1}$); 120 min MHGI (8.3 ±1.7 $ng.ml^{-1}$) and LGI (8.2 ±1.0 $ng.ml^{-1}$); and 180 min MHGI (7.7 ±1.8 $ng.ml^{-1}$) and MLGI (8.6 ± 0.9 $ng.ml^{-1}$).

DISCUSSION

To our knowledge this is the first study to compare the effects of a high and low GI mixed macronutrient meal on subjective and PSG sleep in participants meeting RDC for insomnia. The primary aim was to demonstrate empirical efficacy for the MHGI meal to improve sleep onset and sleep efficiency. In addition, the influence of the GI of mixed macronutrient meals on postprandial TRP/LNAA and PPP-5HT concentrations were investigated to explore the possible mechanisms underpinning observed sleep changes. The pre-sleep ratings for meal palatability, satiety, and postprandial sleepiness confirm the MHGI and MLGI meals are suitable for use as an evening meal and represent a practical, cost-effective strategy to study the effects of the GI and GL on sleep. Subjective sleep improvement was present after the MHGI meal and this was especially apparent for women. Overall, there were no differences in the averaged PSG sleep data between meals, however women showed relative improvements (i.e., shorter SOL) compared to men. The individual night PSG sleep data demonstrated significant variability among all participants. Evidence to support the mechanisms underlying improvements to sleep after the MHGI meal were limited to the postprandial increase in TRP/LNAA given there were no changes in postprandial PPP-5HT levels.

The MHGI and MLGI were designed to deliver approximately 1915 kJ (~ 457 kCal), but to vary in their glycemic responses. The meals were provided as an evening meal and served at 3 h prior to individual habitual bedtimes. The participants rated both meals similarly indicating both were highly palatable and favoured a good taste. Subjective ratings for satiety immediately after meal consumption were similar in both meals and across nights. As would be expected, postprandial satiety declined and

reached lowest levels at bedtime (3 h), but no single rating was below a score of approximately 4 cm on the 10-cm VAS. These findings indicate the meals provided sufficient energy to the participants and extreme hunger was clearly obviated up to bedtime. To the extent of the study questionnaires, only two subjects (both men) provided anecdotal evidence for perceived hunger. Indeed, the energetic load (kJ/kg) was significantly greater in women compared to men indicating the MHGI and MLGI meal size was larger for women. It is likely this difference contributed to the subjective sleep improvements in women only.

Post-sleep assessment of sleep quality indicated women, but not men reported a more restful sleep after the MHGI meal. This sex-different is consistent with published data. In response to a high CHO, low fat meal given throughout the working day, women tended to report greater postprandial sleepiness than men (Wells and Read 1996; Wells, Read et al. 1998). Moreover, in women, a decline in alertness is prominent for those eating larger sized-meals compared with a more habitual sized lunch meals (Smith, Ralph et al. 1991). Unfortunately, the measurement of satiety by VAS may not have been sufficiently sensitive to detect meaningful changes to hunger. In men, brain imaging data show greater activation in the prefrontal cortex and paralimbic areas in response to hunger that may be perceived as greater anxiety and stress (Del Parigi, Chen et al. 2002). Increased cortical activation is known to perpetuate disturbed sleep quality (Vgontzas, Bixler et al. 2001). Indeed, the post-sleep assessment of WASO was not remarkable between meals. Thus, the subjective sleep measurements alone were not sensitive to detect the effects of hunger during the sleep period.

The average PSG data after the MHGI also did not indicate significant sleep improvement however sex differences were apparent corroborating the subjective sleep improvements after the MHGI meal in women but not men. There was a strong tendency in women to have a shorter SOL for after the MHGI meal (19.4 min) compared to men (37.3 min). Further, women, but not men, had a shorter SOL after the MHGI meal compared to the MLGI meal (24.3 min) but this difference was not statistically significant. On the other hand, men experienced a poor sleep quality after both the MHGI and MLGI meals. This was characterised by a prolonged SOL, reduced sleep efficiency, increased WASO, more light sleep (NREM1), and more sleep fragmentation (AI) compared to women (Table 6.2). A recent meta-analysis reports a clear female predominance for insomnia however the factors (Zhang and Wing 2006). Nearly 55% of insomniacs report having recent psychosocial stressor (Specchio, Prudenzano et al. 2004). Unfortunately, we did not formally assess anxiety or mood states. Also, the period between studies was variable, especially between men and women. Thus, a possible explanation for the present findings may be related to differences in underlying psychopathology. In addition, the individual night PSG data demonstrate significant night-to-night variability, especially among values for SOL and REML (Table 6.3). The SOL for most participants was shorter on the first night compared to the second night in each meal condition. The data in both sexes (bolded value) on REML show a tendency for a delayed REML on the first night compared to the second, but this response was variable in men and women (Table 6.3). It has known that variability in sleep pattern is a characteristic inherent of insomnia (Vallieres, Ivers et al. 2005). Indeed, using a single PSG study may miss the bigger picture of the insomnia complaint (Edinger, Marsh et al. 1991). Thus, different data

sets may have been reported had we not conducted sleep studies on two consecutive nights.

Noteworthy, is the measurement of both subjective and PSG sleep as it affords important clinical inference. Figure 6.1 shows the PSG SOL values on individual nights in comparison to subjective SOL values. The PSG values were considerably lower than the subjective ratings for SOL on each study night. Further, the self-reported SOL after the MHGI meal shows a decline from night 1 to night 2 and therefore does not follow with PSG values. These findings demonstrate contrasting subjective and objective measures of sleep and are consistent with the presence of psychophysiological insomnia (Perlis, Giles et al. 1997). A greater cortical activation has been observed in patients with psychophysiological insomnia compared to good sleepers (Bastien, St-Jean et al. 2008). Thus, the meal effects would have been further masked by difficulty initiating normal sleep processes.

The biochemical responses observed after the MHGI and MLGI meals provide reasonable evidence for a high GI meal to promote sleep quality despite a small sample size. There was a significantly greater postprandial glucose response after the MHGI meal compared to the MLGI meal but insulin release was not different between meals (Figure 6.2). The postprandial TRP/LNAA level peaked at bedtime (3 h postprandially) for both meals (Figure 6.3). The AUC data further indicate TRP/LNAAA levels after the MHGI meal were approximately 3.5-fold higher than the MLGI meal although this difference was only marginally significant. The magnitude of the percentage increase in plasma TRP/LNAA after the MHGI meal (17%) was nearly identical to a previous study using the same meals in healthy volunteers after an

overnight fast (Chapter 5). In addition, the previous study demonstrated a greater TRP/LNAA response after a high GI CHO (CHGI) meal which has previously been shown to improve SOL in compared to an isoenergetic low GI CHO meal in healthy sleepers (Afaghi et al. 2007). Whilst the TRP/LNAA response after the CHGI meal (23%) was only marginally higher than the MHGI meal, this meal did not improve sleep initiation in participants with insomnia. These findings raise several factors regarding the efficacy of the MHGI meal to improve sleep. First, the GL of the MHGI meal was not sufficiently high to elicit a postprandial TRP/LNAA level similar to the CHGI meal (Chapter 5). We may speculate that the relative increase after the CHGI meal reflects a maximum physiological response to typical mixed macronutrient meals. Thus, it is likely that a specific physiological threshold may exist that corresponds to a sufficient TRP/LNAA level that would promote 5HT production and measurable changes in sleep behaviour. Theoretically, this physiological threshold may have been surpassed in women given the greater energetic load, however the present study was underpowered to appropriately identify this sex-specific response and we therefore caution over generalisation. Moreover, the study populations were different between these studies. Thus, it remains largely unknown whether such a threshold would be similar for both healthy and poor sleepers.

Despite the study limitations previous discussed it should also be noted that control for daytime meals may be warranted as it is known the synthesis and release of neurotransmitters are influenced by their nutrient precursors (Wurtman, Hefti et al. 1981). Second, postprandial 5HT concentration may have been further influenced by poor pharmacokinetics of ingested dietary TRP. Provision of vitamin B3 and B6 may

increase the conversion rate of TRP to 5HT and meet dietary requirements (Young 1991).

In summary, subjective sleep was improved after a high GI compared to an isoenergetic low GI mixed macronutrient evening meal provided 3 hr prior to the usual bedtime. Given the sleep period inherent to insomnia demonstrates clear night-to-night variability, a long-term prospective dietary intervention using validated subjective sleep measures represents a cost-effective strategy to further test the efficacy for regular consumption of a high GI mixed macronutrient meal to improve sleep quality.

CHAPTER 7 – Conclusions

Sleep propensity increases with progression of the day. At dark onset the release of melatonin increases sleep pressure culminating in a rapid sleep onset in regular sleepers. The transition between sleep-wake states reflects complex activity in many neuronal regions. For those with insomnia, heightened psychophysiological process deters sleep despite a high propensity. The relationship between the GI and sleep lies in the insulin-mediated availability of the amino acid tryptophan relative to other large neutral amino acids (TRP/LNAA), where available TRP is readily converted into serotonin (5HT). Recently, Afaghi et al (2007) showed that a high GI CHO rich meal given 4 h before bedtime shortens the sleep onset latency (SOL) compared to an isoenergetic low GI meal in healthy sleepers. These findings form the basis of this thesis.

The primary aim of this thesis was to evaluate the efficacy of a high GI mixed macronutrient (MHGI) meal to improve sleep quality compared to an isoenergetic low GI mixed macronutrient (MLGI) meal. Sleep quality was measured in the laboratory using subjective and PSG measures in participants with insomnia. In addition, biochemical analysis was performed to measure the postprandial TRP/LNAA and 5HT concentrations. The new findings from this thesis can be encapsulated as follows: (1) a meta-analysis confirms the first night effect (FNE) in healthy sleepers and provides an estimate for the observed changes in 9 PSG sleep variables across the first two consecutively recorded nights; (2) the postprandial plasma TRP/LNAA concentration demonstrates a dose-response to the glycemic load (GL) of a meal; (3) the postprandial TRP/LNAA response to the mixed macronutrient meal was identical in healthy

individuals (Chapter 5) and participants with insomnia (Chapter 6); and (4) preliminary findings show that the MHGI meal improved subjective sleep quality, especially in women.

The findings from Chapter 4 provide novel advancement to aid in PSG study designs. This study provided the first quantitative review of the FNE in 24 studies involving 421 healthy subjects over 4 decades. Notably, there was poor clinical homogeneity in studies involving participants with insomnia and depression. Therefore, a qualitative review was provided. It was determined that the final study (Chapter 6) should perform PSG recordings on 2 consecutive nights under each meal condition.

The presented work in Chapters 5 provides valuable biochemical data to support the underlying mechanisms promoting sleep initiation after high GI meals. Notably, this study demonstrated the postprandial change in TRP/LNAA concentration was not proportional to the GI but instead is likely mediated by the GL. The TRP/LNAA response was 3.5 fold greater after the MHGI meal compared to the MLGI meal but both were attenuated compared to the carbohydrate only high GI (CHGI) meal. This CHGI meal was replicated from the study from Afaghi et al (2007). Given the CHGI meal provided substantial energy (kJ) and a large CHO load (~ 140 g) compared to the mixed macronutrient meals, it is likely the TRP/LNAA response after the CHGI meal reflects a maximum physiological response. Further, the TRP/LNAA response after the MHGI meal was only marginally reduced compared to the CHGI meal (Chapter 5). Thus, this study provided sufficient indication that the MHGI meal may promote sleep onset similar to the CHGI meal.

The therapeutic impact of a high GI mixed macronutrient meal to improve sleep onset was marginal. The findings in Chapter 6 indicate the MHGI meal improves subjective sleep quality when provided as a regular evening meal 3 h prior to bedtime but only to women with insomnia. Remarkably, the postprandial increase in plasma TRP/LNAA concentration after the mixed macronutrient meal was identical in healthy participants (Chapter 5) and in the participants with insomnia (Chapter 6). However, despite an elevated TRP/LNAA response, especially after the MHGI meal, platelet poor plasma (PPP) 5HT concentration remained unchanged. Further studies are required to evaluate a GL dose-response on 5HT concentration in order to confirm the mechanisms underlying improved sleep after high GI meals.

Comparison to previous findings

The MHGI and MLGI meals were provided as an evening meal replacement at 3 h prior to individualised habitual bedtimes. This was done to match the measured peak postprandial TRP/LNAA level (Chapter 5). The only other study to measure TRP/LNAA levels in the evening was the study by Lyons & Truswell (1998). In this study, the increase in TRP/LNAA concentration was nearly identical when carbohydrate meals were provided after an overnight fast and in the evening between 1800 and 1830 h (Lyons and Truswell 1988). Whilst the present results mimic this previous finding, there may be underlying circadian modulation of the individual glucose and insulin responses.

The diurnal cortisol rhythm may mediate the diurnal variation in carbohydrate tolerance (Van Cauter, Shapiro et al. 1992). Under experimental conditions, the postprandial glucose response measured after a mixed meal (43% CHO) was greater in the evening rather in the morning, yet this was not associated with a commensurate insulin secretion (Van Cauter, Shapiro et al. 1992). In addition, this meal (500 kCal) was provided at 2000 h and 'light out' was performed using a fixed scheduled at 2300 h, which is nearly identical with respect to the meal size and meal timing in the present study. Circadian changes such as low cortisol levels in the evening are associated with increased insulin sensitivity (Van Cauter, Shapiro et al. 1992). In the present studies we did not measure cortisol levels and therefore its relationship to insulin sensitivity remains unknown. Further, the biological consequences of experimental sleep loss in healthy participants cannot be applied to primary insomnia (Riemann, Klein et al. 2002). However, it is likely the incoherence among the glucose and insulin responses among the insomnia population presented in Chapter 6 is due to elevated cortisol

secretion (Riemann, Kloepfer et al. 2009)and disturbed endocrine function (Buckley and Schatzberg 2005) Indeed, in the present study (Chapter 6) there was a postprandial decline in insulin just after meal consumption in three participants.

Further study on the relationship between circadian rhythmicity (cortisol), feeding behaviour, and the metabolic responses leading to increased availability of TRP is needed, especially in primary insomnia. These findings could provide future evidence for dietary intake to be used as a therapy for other abnormal circadian rhythm disorders such as jet-lag and shift work.

Another difference in meal timing exists between the current study and that of Afaghi et al. (2007) in which the CHO high GI meal was provided both at 4 h and 1 h prior to bedtime. In their study, the CHO high GI meal (replicated here as CHGI) resulted in a significant reduction to SOL both at 1 h and 4 h compared to a low GI CHO meal given at 4 h prior to bedtime only. Unfortunately, we did not measure 5HT levels after the CHGI meal in the current project. In the rat, levels of 5HT and its metabolite, 5-hydroxyindolacetic acid (5-HIAA), increase as soon as the beginning of meal consumption (Orosco and Nicolaidis 1992). However, 5HT function in humans may also be measured indirectly through changes in melatonin concentration since 5HT is the intermediary product to melatonin (Zimmermann, McDougle et al. 1993). Remarkably, Afaghi et al. (2007) reported the evening collection of urinary 6-hydroxymelatonin (6-SM), a metabolite of melatonin, showed no significant difference between the CHGI meal at 4 h, the CHGI meal at 1h or the low GI CHO meal at 1 h before bedtime (Afaghi, O'Connor et al. 2007). At least in the animal model, only carbohydrate ingestion, which stimulates pancreatic insulin secretion and not protein or

fat mobilisation, could reliably account for both synthesis and release of hypothalamic 5HT (Orosco and Gerozissis 2001). Thus, these findings together indicate that additional evidence is needed to elucidate whether the relative improvements to sleep after a high GI meal are indeed mediated solely by increased 5HT pathways.

Alternative hypothesis

Support for the hypothesis of a direct central mechanism leading to postprandial sleepiness after CHO ingestion exists. Activity in the hypothalamus involving the hypocretin-orexin system (orexins) may act as a "gate" to allow sleep or increase mutually exclusive behaviour, such as feeding (Burdakov, Alexopoulos et al. 2005). Orexins are a specialised group of glucose sensing neurons (GSN) that exhibit specific inhibitory and excitatory activity depending on extracellular levels of glucose in the brain. In the animal model, low extracellular glucose levels act to inhibit GSN containing orexins, thereby resulting in increased locomotion and food-seeking behaviour. Alternatively, high extracellular glucose levels promote excitation of GSN containing melanin-concentrating hormone, resulting in increased sleepiness. Basal levels in the hypothalamus appear to be about 1.4 mmol/L in the rat brain (de Vries, Arseneau et al. 2003). Generally, extracellular concentrations of glucose in the brain are 10-30% lower than levels in the blood (Routh 2002; de Vries, Arseneau et al. 2003; Levin, Routh et al. 2004). Importantly, brain glucose concentration follows closely to changes in the plasma (Silver and Erecinska 1994). Given typical meal to meal fluctuations in blood glucose are between 4-8 mmol/L, this corresponds to levels in the brain at approximately 1 and 2.5 mmol/L which may result in a physiological stimulus for sleep (Burdakov, Luckman et al. 2005). Remarkably, the findings presented in this thesis support this proposed mechanism.

First, it was reported that postprandial sleepiness measured during the day occurred as early as 30-60 min after ingestion of CHO compared to baseline conditions (Wells, Read et al. 1997; Wells, Read et al. 1998). Indeed, Afaghi et al. (2007) also reported a shorter SOL in healthy sleepers after the high GI CHO meal (CHGI) given 1 h before bedtime compared to the low GI CHO meal given 4 h before bedtime. Remarkably, the corresponding blood glucose level after the CHGI meal (9 mmol/L, Chapter 5) was nearly identical to the level originally reported (Afaghi, O'Connor et al. 2007). Theoretically, the corresponding blood glucose levels in the brain after the CHGI meal between 0.9 – 2.7 mmol/L, may have been sufficient to stimulate central GSN activity thereby promoting sleep, especially between 30 – 60 min when peak glucose occurred. Moreover, the peak glucose response of the CHGI meal was significantly higher than the MHGI meal, both in healthy participants (~7.8 mmol/L, Chapter 5) and in participants with insomnia (~6.5 mmol/L, Chapter 6). Thus, if the central mechanism is validated, it would appear the MHGI meal did not provide the necessary postprandial glucose response sufficient to change sleep behaviour, especially in the final study. Consistent with our previous suggestion, it appears that the GL of mixed meals may be a useful marker to predict the acute glucose and insulin response and therefore postprandial sleepiness.

In consideration of the alternative hypothesis suggesting a direct central mechanism for the relative sleep improvements after CHO ingestion, a high GI snack eaten at approximately 45 min to 1 h prior to bedtime may be a more useful strategy to improve sleep quality. This type of intervention could be delivered in a small energetic load and therefore would obviate potential weight gain or other negative metabolic effects of regular consumption of high GL evening meals.

Limitations of the project

Several factors contributed to a low statistical power in the experimental chapters. First, Chapter 5 used a venepuncture procedure for blood collection thus limiting the data as participants were unwilling to undergo the procedure. The blood collection procedure was change in Chapter 6 and an indwelling cannula was used. Unfortunately 5HT data were lost due to platelet contamination as PPP 5HT sample preparation is considerably difficult (Maria, Ribeiro et al. 2004). Second, both experimental studies were repeated measures studies involving six days (Chapter 5), however, this time requirement cannot be avoided by GI testing. Further, the final study course was five nights (Chapter6) and participant payment was minimal due to financial constraints. In addition, the PSG procedure in the final study period was appropriate in view of the findings from the meta-analysis of the FNE (Chapter 4). Thus, despite recruitment of 14 mixed-gender participants, the final participant group consisted of only eight persons and was mixed 50% by gender. Given the present findings future studies should aim to exploit the interaction between meal size and the sex-differences among the efficacy of the mixed macronutrient high GI meal to improve sleep quality.

A separate methodological concern relates to the recruitment of participants with insomnia in the final study. Despite each participant meeting the research diagnostic criteria for insomnia, the participants were not matched for difficulties in maintaining sleep. Further, the threshold ISI was arbitrarily chosen. A more rigorous RDC for insomnia is needed in view of the clinical heterogeneity of the participants. It can be noted here that part of the *a* priori hypothesis was in fact a lower glycemic response of the MHGI compared to the CHGI meal. Thus, a group of healthy sleepers was not tested given there is little room for sleep improvement (Bonnet and Arand 2003).

Directions for further research

Further studies are needed to expose the relationship between the GI and GL of mixed macronutrient meals and sleep quality. A dietary intervention using similar meals varying in GI or GL would provide data to substantiate a dose-response effect on factors included in this thesis; notable the postprandial TRP/LNAA response, 5HT synthesis and/or release, and sleep improvements.

The screening questionnaire used for participant recruitment also provided detail on the habitual meal timing. These data indicate, at least for some participants, the MHGI and MLGI meals were provided closer to bedtime than the usual evening meal. Indeed, food acts as a zeitgeber in humans for circadian rhythmicity (Krauchi, Cajochen et al. 2002). Thus, a long-term study is needed to evaluate the efficacy of a high GI or high GL meal as an evening meal replacement, especially in participants with insomnia who demonstrate significant night-to-night variability in sleep quality. A minimum of a 1 or 2 weeks would be necessary to overcome the conditioned and maladaptive behaviours contributing to this reduced quality of sleep.

Finally, it is likely PSG studies in the laboratory are not required to evaluate treatment efficacy in primary insomnia. Instead, actigraphy and/or sleep diaries could be employed as these are both valid and reliable forms of assessment of insomnia (Morin 2004) and for other circadian rhythm disorders (Buysse, Ancoli-Israel et al. 2006).

REFERENCES

Aber, W. R., A. J. Block, et al. (1989). "Consistency of respiratory measurements from night to night during the sleep of elderly men." Chest **96**(4): 747-51.

Adam, K. and I. Oswald (1983). "Protein synthesis, bodily renewal and the sleep-wake cycle." Clin Sci (Lond) **65**(6): 561-7.

Afaghi, A., H. O'Connor, et al. (2007). "High-glycemic-index carbohydrate meals shorten sleep onset." Am J Clin Nutr **85**(2): 426-30.

Afaghi, A., H. O'Connor, et al. (2008). "Acute effects of the very low carbohydrate diet on sleep indices." Nutr Neurosci **11**(4): 146-54.

Agnew, H. W., Jr., W. B. Webb, et al. (1966). "The first night effect: an EEG study of sleep." Psychophysiology **2**(3): 263-6.

America, U. S. o. (2005). Dietary guidelines for Americans. H. a. H. Services and Agriculture. Washington, D.C., US Government Printing Office.

Ansseau, M., D. J. Kupfer, et al. (1985). "Internight Variability of Rem Latency in Major Depression - Implications for the Use of Rem Latency as a Biological Correlate." Biological Psychiatry **20**(5): 489-505.

Ansseau, M., D. J. Kupfer, et al. (1985). "Paradoxical Shortening of Rem Latency on 1st Recording Night in Major Depressive Disorder - Clinical and Polysomnographic Correlates." Biological Psychiatry **20**(2): 135-145.

Antonijevic, I. A., H. Murck, et al. (2000). "NPY promotes sleep and inhibits ACTH and cortisol release in young men." Neurophamacology **39**: 1474-81.

Ashley, D. V., D. V. Barclay, et al. (1982). "Plasma amino acid responses in humans to evening meals of differing nutritional composition." American Journal of Clinical Nutrition **36**(1): 143-53.

Ashley, D. V., R. Liardon, et al. (1985). "Breakfast meal composition influences plasma tryptophan to large neutral amino acids ratios of healthy lean young men." J Neural Transm **63**: 271-283.

Australia, C. o. (2003). Dietary Guidelines for Australian Adults. NHMRC. Canberra, AusInfo.

Bastien, C. H., G. St-Jean, et al. (2008). "Chronic psychophysiological insomnia: hyperarousal and/or inhibition deficits? An ERPs investigation." Sleep **31**(6): 887-98.

Bastien, C. H., A. Vallieres, et al. (2001). "Validation of the insomnia severity index as an outcome measure for insomnia research." Sleep Med Rev **2**: 297-307.

Bax, L., L.-M. Yu, et al. (2006). "Development and validation of MIX: comprehensive free software for meta-analysis of causal research data." BMC Medical Research Methodology **6**(50).

Bax, L., L. Yu, et al. (2008). "MIX: comprehensive free software for meta-analysis of causal research data. Version 1.7." from http://mix-for-meta-analysis.info.

Benca, R., W. Obermeyer, et al. (1992). "Sleep and Psychiatric Disorders." Arch Gen Psychiatry **49**: 651-668.

Berlin, I., D. Warot, et al. (1993). "Comparison of the effects of zolpidem and triazolam on memory function, psychomotor performances, and postural sway in healthy-subjects." Journal of Clinical Psychopharmacology **13**(2): 100-106.

Bermudez, F. F., Forbes, J. M., & Injidi, M. H. (1983). "Involvment of melatonin and thyroid hormones in the control of sleep, food intake and energy metabolism in the domestic fowl." J. Physiol. **337**: 19-27.

Berry, E. M., J. H. Growdon, et al. (1991). "A balanced carbohydrate: protein diet in the management of Parkinson's disease." Neurology **41**: 1295-97.

Blum, I., Y. Vered, et al. (1992). "The influence of meal composition on plasma serotonin and norepinephrine concentration." Metabolism **41**(2): 137-40.

Bogdan, A., B. Bouchareb, et al. (2001). "Ramadan fasting alters endocrine and neuroendocrine circadian patterns. Meal-time as a synchronizer in humans?" Life Sciences **68**: 1601-1615.

Bolla, K. I., S. R. Lesage, et al. (2008). "Sleep disturbance in heavy marijuana users." Sleep **31**(6): 901-8.

Bonnet, M. H. and D. L. Arand (2003). "Situational insomnia: consistency, predictors, and outcomes." Sleep **26**(8): 1029-36.

Borbely, A. A. and P. Achermann (1999). "Sleep homeostasis and models of sleep regulation." J Biol Rhythms **14**(6): 557-68.

Brand-Miller, J. (2009). "Home of the Glycemic Index and Glycemic Load." Retrieved December 2009, from www.glycemicindex.com.

Brand-Miller, J. C., S. H. A. Holt, et al. (2003). "Reply to R Mendosa." Am J Clin Nutr **77**(4): 994-a-995.

Brand-Miller, J. C., M. Thomas, et al. (2003). "Physiological validation of the concept of glycemic load in lean young adults." J Nutr **133**(9): 2728-32.

Brezinova, V. and I. Oswald (1972). "Sleep After a Bedtime Beverage." British Medical Journal **2**: 431-433.

Brouns, F., I. Bjorck, et al. (2005). "Glycaemic index methodology." Nutrition Research Reviews **18**(01): 145-171.

Brunner, D. P., M. Magdalena, et al. (1994). "Changes in sleep and sleep electroencephalogram during pregnancy." Sleep **17**(7): 576-582.

Brzezinski, A., M. G. Vangel, et al. (2005). "Effects of exogenous melatonin on sleep: a meta-analysis." Sleep Med Rev **9**(1): 41-50.

Buckley, T. M. and A. F. Schatzberg (2005). "On the interactions of the hypothalamic-pituitary-adrenal (HPA) axis and sleep: normal HPA axis activity and circadian rhythm, exemplary sleep disorders." J Clin Endocrinol Metab **90**(5): 3106-14.

Burdakov, D., H. Alexopoulos, et al. (2005). "Metabolic state signalling through central hypocretin/orexin neurons." Journal of Cellular & Molecular Medicine **9**(4): 795-803.

Burdakov, D., S. M. Luckman, et al. (2005). "Glucose-sensing neurons of the hypothalamus." Philosophical Transactions of the Royal Society of London - Series B: Biological Sciences **360**(1464): 2227-35.

Buysse, D. J., S. Ancoli-Israel, et al. (2006). "Recommendations for a standard research assessment of insomnia." Sleep **29**(9): 1155-1173.

Buysse, D. J., C. F. Reynolds, 3rd, et al. (1989). "The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research." Psychiatry Res **28**(2): 193-213.

Caballero, B., N. Finer, et al. (1988). "Plasma amino acids and insulin levels in obesity: response to carbohydrate intake and tryptophan supplements." Metabolism **37**(7): 672-6.

Chen, C. N., R. S. Kalucy, et al. (1974). "Plasma tryptophan and sheep." British Medical Journal **4**(5944): 564-6.

Clark, C. A., J. Gardiner, et al. (2006). "Effects of breakfast meal composition on second meal metabolic responses in adults with Type 2 diabetes mellitus." Eur J Clin Nutr **60**(9): 1122-9.

Clausen, J., E. Sersen, et al. (1974). "Variability of sleep measures in normal subjects." Psychophysiology **11**(4): 509-516.

Coates, T. J., J. M. George, et al. (1981). "First night effects in good sleepers and sleep-maintenance insomniacs when recorded at home." Sleep **4**(3): 293-8.

Coble, P., R. J. McPartland, et al. (1974). "Is there a first night effect? (a revisit)." Biological Psychiatry **9**(2): 215-9.

Cohen, J. (1988). Statistical power analysis for the behavioral sciences Hillsdale, NJ, Lawrence Erlbaum.

Cooke, A. R. (1975). "Progress in gastroenterology. Control of gastric emptying and motility." Gastroenterology **68**: 804-16.

Craig, A. and E. Richardson (1989). "Effects of experimental and habitual lunch-size on performance, arousal, hunger, and mood." International Archives of Occupational and Environmental Health **61**(5): 313-319.

Curcio, G., M. Ferrara, et al. (2003). "Effect of total sleep deprivation on the landmarks of stage 2 sleep." Clin Neurophysiol **114**(12): 2279-85.

Curcio, G., M. Ferrara, et al. (2004). "Paradoxes of the first-night effect: a quantitative analysis of antero-posterior EEG topography." Clinical Neurophysiology **115**(5): 1178-1188.

Daley, M., C. M. Morin, et al. (2009). "The economic burden of insomnia: direct and indirect costs for individuals with insomnia syndrome, insomnia symptoms, and good sleepers." Sleep **32**(1): 55-64.

Dawson, D. and N. Encel (1993). "Melatonin and sleep in humans." J Pineal Res **15**: 1-12.

De Gennaro, L., M. Ferrara, et al. (2002). "Are polysomnographic measures of sleep correlated to alexithymia?: A study on laboratory-adapted sleepers." Journal of Psychosomatic Research **53**(6): 1091-1095.

de Vries, M. G., L. M. Arseneau, et al. (2003). "Extracellular glucose in rat ventromedial hypothalamus during acute and recurrent hypoglycemia." Diabetes **52**: 2767-2773.

Del Parigi, A., K. Chen, et al. (2002). "Sex differences in the human brain's response to hunger and satiation." Am J Clin Nutr **75**(6): 1017-22.

Dement, W. and N. Kleitman (1957). "Cyclic variations in EEG during sleep and their relation to eye movements, body motility, and dreaming." Electroencephalography & Clinical Neurophysiology **9**: 673-690.

Dijk, D. J. and C. Cajochen (1997). "Melatonin and the circadian regulation of sleep initiation, consolidation, structure, and sleep EEG." Journal of Biological Rhythms **12**(6): 627-635.

Edinger, J. D., M. H. Bonnet, et al. (2004). "Derivation of research diagnostic criteria for insomnia: report of an American Academy of Sleep Medicine Work Group." Sleep **27**(8): 1567-96.

Edinger, J. D., A. I. Fins, et al. (1997). "Sleep in the laboratory and sleep at home: comparisons of older insomniacs and normal sleepers." Sleep **20**(12): 1119-26.

Edinger, J. D., G. R. Marsh, et al. (1991). "Sleep variability across consecutive nights of home monitoring in older mixed DIMS patients." Sleep **14**(1): 13-7.

Egger, M., D. S. Smith, et al. (1997). "Bias in meta-analysis detected by a simple, graphical test." BMJ(315): 629-34.

Egger, M., G. D. Smith, et al., Eds. (2001). Systematic Reviews in Health Care: Meta-analysis in context. London, BMJ Publishing Group.

Ercan, N., M. C. Gannon, et al. (1994). "Effect of added fat on the plasma glucose and insulin response to ingested potato given in various combinations as two meals in normal individuals." Diabetes Care **17**(12): 1453-9.

Erman, M. K., D. Loewy, et al. (2004). "Comparison of temazepam 7.5 mg with temazepam 15 mg for the treatment of transient insomnia." Curr Med Res Opin **20**(4): 441-9.

Faul, F., E. Erdfelder, et al. (2007). "G*Power 3: A flexible statistical power analysis programfor the social, behavioral, and biomedical sciences." Behavior Research Methods **39**: 175-197.

Feige, B., S. Scaal, et al. (2007). "Sleep electroencephalographic spectral power after withdrawal from alcohol in alcohol-dependent patients." Alcohol Clin Exp Res **31**(1): 19-27.

Fernstron, J. and R. Wurtman (1972). "Brain serotonin content: physiological regulation by plasma neutral amino acids." Science **178**: 414-416.

Flint, A., A. Raben, et al. (2000). "Reproducibility, power and validity of Visual Analogue Scales in assessment of appetite sensations in single test meal studies." Int J Obes (Lond) **24**: 38-48.

Foster-Powell, K., S. H. Holt, et al. (2002). "International table of glycemic index and glycemic load." Am J Clin Nutr **76**: 5-56.

Furukawa, T., C. Barbui, et al. (2006). "Imputing missing standard deviations in meta-analyses can provide accurate results." Journal of Clinical Epidemiology **59**(1): 7-10.

Gibson, E. L., S. Checkley, et al. (1999). "Increased salivary cortisol reliably induced by a protein-rich midday meal." Psychosomatic medicine **61**: 214-224.

Gustavsen, I., J. G. Brarnness, et al. (2008). "Road traffic accident risk related to prescriptions of the hypnotics zopiclone, zolpidem, flunitrazepam and nitrazepam." Sleep Medicine **9**(8): 818-822.

Hamilton, M. (1960). "A rating scale for depression." J Neurol Neurosurg Psychiatry **23**: 56-62.

Harada, T., M. Hirotani, et al. (2007). "Correlation between breakfast tryptophan content and morningness-eveningness in Japanese Infants and Students Aged 0-15 yrs." J Physiol Anthropol **26**(2): 201-207.

Harnish, M., S. Greenleaf, et al. (1998). "A Comparison of Feeding to Cephalic Stimulation on Postprandial Sleepiness." Physiol Behav **64**(1): 93-96.

Harsh, J., J. Peszka, et al. (2000). "Night-time sleep and daytime sleepiness in narcolepsy." Journal of Sleep Research **9**(3): 309-16.

Hartmann, E. and C. L. Spinweber (1979). "Sleep induced by L-tryptophan: effect of dosages within the normal dietary intake." J Nerv Ment Dis **167**(8): 497-99.

Hatoum, H. T., S. X. Kong, et al. (1998). "Insomnia, health-related quality of life and healthcare resource consumption. A study of managed-care organisation enrollees." Pharmacoeconomics. **14**(6): 629-37.

Hauri, P. J. and E. M. Olmstead (1989). "Reverse first night effect in insomnia." Sleep **12**(2): 97-105.

Hedges, L. and I. Olkin (1985). Statistical methods for meta-analysis. Orlando, Fl, Academic Press.

Heitkemper, M., M. Jarrett, et al. (2005). "Subjective and objective sleep indices in women with irritable bowel syndrome." Neurogastroenterology & Motility **17**(4): 523-30.

Henny, P. and B. E. Jones (2008). "Projections from basal forebrain to prefrontal cortex comprise cholinergic, GABAergic and glutamatergic inputs to pyramidal cells or interneurons." Eur J Neurosci **27**(3): 654-70.

Higgins, J. P. and S. G. Thompson (2002). "Quantifying heterogeneity in a meta-analysis." Stat Med **21**(11): 1539-58.

Higgins, J. P., S. G. Thompson, et al. (2003). "Measuring inconsistency in meta-analysis." BMJ(327): 557-560.

Hillman, D. R., F. Fanzca, et al. (2006). "The economic cost of sleep disorders." Sleep **29**(3): 299-305.

Hudson, C., S. Hudson, et al. (2005). "Protein source trptophan versus pharmaceutical grade tryptophan as an efficacious treatment for chronic insomnia." Nutritional Neurosciences **8**(2): 121-127.

Huq, F., M. Thompson, et al. (1993). "Changes in serum amino acid concentrations during prolonged endurance running." Jpn J Physiol **43**(6): 797-807.

Iber, C., S. Ancoli-Israel, et al. (2007). The AASM Manual for the Scoring of Sleep and Associated Events: Rules, Terminology and Technical Specifications, 1st ed. Westchester, Illinois, American Academy of Sleep Medicine.

Jan, J. E., H. Espezel, et al. (1994). "The treatment of sleep disorders with melatonin." Dev Med Child Neurol **36**: 97-107.

Jasper, H. H. (1958). "The ten-twenty electrode system of the International Federation. ." Electroencephalogr Clin Neurophysiol **10**: 370-375.

Jenkins, D. J. A., C. W. C. Kendall, et al. (2000). "Viscous and nonviscous fibres, nonabsorbable and low glycaemic index carbohydrates, blood lipids and coronary heart disease." Current Opinion in Lipidology **11**(1): 49-56.

Johns, M. and C. Dore (1978). "Sleep at home and in the sleep laboratory: Disturbance by recording procedures." Ergonomics **21**(5): 325-330.

Jones, B. (2003). "Arousal Systems." Frontiers in Bioscience **8**: s438-451.

Kajimura, N., M. Kato, et al. (1998). "A polysomnographic study of sleep patterns in normal humans with low- or high-anxiety personality traits." Psychiatry & Clinical Neurosciences **52**(3): 317-20.

Katz, D. A. and C. A. McHorney (2002). "The relationship between insomnia and health-related quality of life in patients with chronic illness." J Fam Pract. **51**(3): 229-35.

Kayumov, L. (1994). "Human adaptation to hypoxia during night sleep." Homeostasis in Health and Disease **35**(6): 274-276.

Kennaway, D. J. and A. Voultsios (1998). "Circadian rhythm of free melatonin in human plasma." J Clin Endoc Metab **83**(3): 1013-1015.

Knutson, K. L., K. Spiegel, et al. (2007). "The metabolic consequences of sleep deprivation.[see comment]." Sleep Medicine Reviews **11**(3): 163-78.

Knutson, K. L. and F. W. Turek (2006). "The U-shaped association between sleep and health: The 2 peaks do not mean the same thing - Comment on Patel SR; Malhotra A; Gottlieb DJ et al. correlates of long sleep duration. SLEEP 2006;9(7): 881-889." Sleep **29**(7): 878-879.

Knutsson, U., J. Dahlgren, et al. (1997). "Circadian cortusol rhythms in healthy boys and girls: relationship with age, growth, body composition, and pubertal development." Journal of Clinical Endocrinology and Metabolism **82**: 536-540.

Kobayashi, I., J. M. Boarts, et al. (2007). "Polysomnographiclly measured sleep abnormalities in PTSD: A meta-analytic review." Psychophysiology **44**: 660-669.

Kovac, J., J. Husse, et al. (2009). "A time to fast, a time to feast: The crosstalk between metabolism and the circadian clock." Molecules and Cells **28**(2): 75-80.

Krauchi, K., C. Cajochen, et al. (2002). "Alteration of internal circadian phase relationships after morning versus evening carbohydrate-rich meals in humans." Journal of Biological Rhythms **17**(4): 364-376.

Kupfer, D. J., E. Frank, et al. (1989). "Eeg Sleep in Young Depressives - 1st and 2nd Night Effects." Biological Psychiatry **25**(1): 87-97.

Kupfer, D. J., B. L. Weiss, et al. (1974). "First night effect revisited: a clinical note." Journal of Nervous & Mental Disease **159**(3): 205-9.

Kushida, C. A., M. R. Littner, et al. (2005). "Practice parameters for the indications for polysomnography and related procedures: an update for 2005." Sleep **28**(4): 499-521.

Kwan, R. M., S. Thomas, et al. (1986). "Effects of a low carbohydrate isoenergetic diet on sleep behavior and pulmonary functions in healthy female adult humans." Journal of Nutrition **116**(12): 2393-402.

Lacey, J. H., P. Stanley, et al. (1978). "The immediate effects of intravenous specific nutrients on EEG sleep." Electroencephalography & Clinical Neurophysiology **44**(3): 275-80.

Lack, L., W. Miller, et al. (1988). "A Study of Sleeping Difficulties in An Australian Population." Community Health Studies **7**(2): 200-207.

Lawton, C. L., J. K. Wales, et al. (1995). "Serotoninergic manipulation, meal-induced satiety and eating pattern: effect of fluoxetine in obese female subjects." Obes Res **3**(4): 345-56.

Le Bon, O., P. Minner, et al. (2003). "First-night effect in the chronic fatigue syndrome." Psychiatry Research **120**(2): 191-199.

Le Bon, O., L. Staner, et al. (2001). "The first-night effect may last more than one night." Journal of Psychiatric Research **35**(3): 165-172.

Leathwood, P. and P. Pollet (1982/83). "Diet-Induced mood changes in normal populations." J Psychiatr Res **17**(2): 147-154.

Lee, K., J. Shaver, et al. (1990). "Sleep patterns related to menstrual cycle phase and premenstrual affective symptoms." Sleep **13**(5): 403-9.

Levin, B. E., V. H. Routh, et al. (2004). "Neuronal glucosensing: what do we know after 50 years?" Diabetes **53**: 2521-2528.

Lieberman, H. R., B. Caballero, et al. (1986). "The composition of lunch determines afternoon plasma tryptophan ratio in humans." J Neural Transm **65**: 211-17.

Livesey, G. (2005). "Low-glycaemic diets and health: implications for obesity." Proc Nutr Soc **64**(1): 105-13.

Lloyd, H., P. Rogers, et al. (1996). "Acute Effects on Mood and Cognitive Performance of Breakfasts Differing in Fat and Carbohydrate Content." Appetite **27**: 151-164.

Lloyd, H. M., M. W. Green, et al. (1994). "Mood and cognitive performance effects of isocaloric lunches differing in fat and carbohydrate content." Physiol Behav **56**(1): 51-57.

Lorenzo, J.-L. and M.-J. Barbanoj (2002). "Variability of sleep parameters across multiple laboratory sessions in healthy young subjects: the "very first night effect"." Psychophysiology **39**(4): 409-13.

Lyons, P. M. and A. S. Truswell (1988). "Serotonin precursor influenced by type of carbohydrate meal in healthy adults." Am J Clin Nutr **47**(3): 433-9.

Maria, T. M., C. A. F. Ribeiro, et al. (2004). "Influence of the sample preparation method on the serotonin determination in plasma and platelets." Biomedical Chromatography **18**(9): 739-744.

Marzec, M. L., L. M. Selwa, et al. (2005). "Analysis of the first night effect and sleep parameters in medically refractory epilepsy patients." Sleep Medicine **6**(3): 277-80.

McGinty, D. J. and M. B. Sternman (1968). "Sleep suppression after basal forebrain lesions in the cat." Science **160**: 1253-1255.

McMenamy, R. and J. L. Oncley (1958). "The specific binding of L-tryptophan to serum albumin." J Biol Chem **233**(6): 1436-47.

McMillan-Price, J., P. Petocz, et al. (2006). "Comparison of 4 diets of varying glycemic load on weight loss and cardiovascular risk reduction in overweight and obese young adults: a randomized controlled trial." Arch Intern Med **166**(14): 1466-75.

Meltzer, E. O. (1990). "Performance effects of antihistamines." J Allergy Clin Immunol **86**(4 Pt 2): 613-9.

Mendels, J. and D. R. Hawkins (1967). "Sleep laboratory adaptation in normal subjects and depressed patients ("first night effect")." Electroencephalography & Clinical Neurophysiology **22**(6): 556-8.

Merimee, T. J. and D. Rabin (1973). "Progress in endocrinology and metabolism." Metabolism **22**: 1235.

Milross, M. A., A. J. Piper, et al. (2002). "Night-to-night variability in sleep in cystic fibrosis." Sleep Medicine **3**(3): 213-219.

Monk, T. H. (1987). "SUBJECTIVE RATINGS OF SLEEPINESS - THE UNDERLYING CIRCADIAN MECHANISMS." Sleep **10**(4): 343-353.

Monk, T. H. (2005). "The post-lunch dip in performance." Clinics in Sports Medicine **24**(2): e15-23.

Moore, R. Y. and R. Silver (1998). "Suprachiasmatic nucleus organization." Chronobiology International **15**(5): 475-487.

Morgenthaler, T., M. Kramer, et al. (2006). "Practice parameters for the psychological and behavioral treatment of insomnia: an update. An american academy of sleep medicine report." Sleep **29**(11): 1415-9.

Morin, C. M. (2004). "Insomnia treatment: taking a broader perspective on efficacy and cost-effectiveness issues." Sleep Med Rev **8**(1): 3-6.

Morin, C. M. and C. A. Espie (2003). Insomnia: a clinical guide to assessment and treatment. New York, Springer.

Morin, C. M., M. LeBlanc, et al. (2006). "Epidemiology of insomnia: Prevalence, self-help treatments, consultations, and determinants of help-seeking behaviors." Sleep Medicine **7**(2): 123-130.

Moruzzi, G. (1964). "Reticular Influences on the Eeg." Electroencephalogr Clin Neurophysiol **16**: 2-17.

Mosko, S. S., M. J. Dickel, et al. (1988). "Night-to-night variability in sleep apnea and sleep-related periodic leg movements in the elderly." Sleep **11**(4): 340-8.

Murray, B. E., K. A. Clarke, et al. (1993). "The effect in rats of intestinal lipid on post prandial activity measured by Dopller shift rader." Pro Nutr Soc **52**: 359A.

NIH (2005). "National Institutes of health state of the science conference statement." Sleep **28**(9): 1049-1052.

Nowell, P. D., S. Mazumdar, et al. (1997). "Benzodiazepines and zolpidem for chronic insomnia - A meta-analysis of treatment efficacy." Jama-Journal of the American Medical Association **278**(24): 2170-2177.

Ohashi, Y., N. Okamoto, et al. (1999). "Daily rhythm of serum melatonin levels and effect of light exposure in patients with dementia of the Alzhemer's type." Biol Psychiatry **45**: 1646-52.

Ohayon, M. M., M. A. Carsadon, et al. (2004). "Meta-Analysis of qualitative sleep parameters from childhood to old age in healthy individuals: Developing normative sleep values across the human lifespan." Sleep **27**(7): 1255-73.

Olson, L. G. (1996). "A community survey of insomnia in Newcastle." Aust N Z J Public Health **20**(6): 655-7.

Ong, J. C., T. F. Kuo, et al. (2008). "Who is at risk for dropout from group cognitive-behavior therapy for insomnia?" J Psychosom Res **64**(4): 419-25.

Opperman, A. M., C. S. Venter, et al. (2004). "Meta-analysis of the health effects of using the glycaemic index in meal-planning." Br J Nutr **92**(3): 367-81.

Orosco, M. and K. Gerozissis (2001). "Macronutrient-induced cascade of events leading to parallel changes in hypothalamic serotonin and insulin." Neuroscience and Biobehavioral Reviews **25**: 167-174.

Orosco, M. and S. Nicolaidis (1992). "Spontaneous feeding-related monoaminergic changes in the rostromedial hypothalamus revealed by microdialysis." Physiol Behav **52**: 1015-1019.

Orosco, M., C. Rouch, et al. (2002). "Behavioral responses to ingestion of different sources of fat. Involvement of serotonin?" Behavioural Brain Research **132**(1): 103-109.

Orr, W., G. Shadid, et al. (1997). "Meal Composition and Its Effect on Postprandial Sleepiness." Physiol Behav **62**(4): 709-712.

Oswald, I. and M. Corner (1980). "Sleep as a restorative process:human clues." Progress in Brain Research **46**: 170-172.

Pai, M., M. McCulloch, et al. (2004). "Systematic reviews and meta-analysis: An illustrated, step-by-step guide." The National Medical Journal of India **17**: 86-95.

Pawlak, D. B., C. B. Ebbeling, et al. (2002). "Should obese patients be counselled to follow a low-glycaemic index diet? Yes." Obes Rev **3**(4): 235-43.

Pearson, N. J., L. L. Johnson, et al. (2006). "Insomnia, trouble sleeping, and complementary and alternative medicine - Analysis of the 2002 National Health Interview Survey data." Archives of Internal Medicine **166**(16): 1775-1782.

Perlis, M. L., D. E. Giles, et al. (1997). "Psychophysiological insomnia: the behavioural model and a neurocognitive perspective." J Sleep Res **6**(3): 179-88.

Phillips, F., C. N. Chen, et al. (1975). "Isocaloric diet changes and electroencephalographic sleep." Lancet **2**(7938): 723-5.

Porter, J. M. and J. A. Horne (1981). "Bed-time food supplements and sleep: effects of different carbohydrate levels." Electroencephalography & Clinical Neurophysiology **51**(4): 426-33.

Radulian, G., E. Rusu, et al. (2009). "Metabolic effects of low glycaemic index diets." Nutr J **8**(5): 5.

Rahemtulla, Z., C. Baldwin, et al. (2005). "The palatability of milk-based and non-milk-based nutritional supplements in gastrointestinal cancer and the effect of chemotherapy." Clinical Nutrition **24**(6): 1029-1037.

Rechtschaffen, A. and A. Kales (1968). A manual of standardized terminology, techniques and scoring system for sleep stages of human subjects. Washington, DC, USA Government Printing Office.

Rechtschaffen, A. and P. Verdone (1964). "Amount of Dreaming: Effect of Incentive, Adaptation to Laboratory, and Individual Differences." Perceptual & Motor Skills **19**: 947-58.

Redline, S., R. Budhiraja, et al. (2007). "The scoring of respiratory events in sleep: reliability and validity." J Clin Sleep Med **3**(2): 169-200.

Redline, S., T. Tosteson, et al. (1991). "Measurement of sleep-related breathing disturbances in epidemiologic studies. Assessment of the validity and reproducibility of a portable monitoring device." Chest **100**(5): 1281-6.

Reynolds, C. F., T. F. Newton, et al. (1982). "Electroencephalographic Sleep Findings in Depressed Outpatients." Psychiatry Research **6**(1): 65-75.

Riedel, B. W., C. F. Winfield, et al. (2001). "First night effect and reverse first night effect in older adults with primary insomnia: does anxiety play a role?" Sleep Medicine **2**(2): 125-133.

Riemann, D., T. Klein, et al. (2002). "Nocturnal cortisol and melatonin secretion in primary insomnia." Psychiatry Research **113**(1-2): 17-27.

Riemann, D., C. Kloepfer, et al. (2009). "Functional and structural brain alterations in insomnia: implications for pathophysiology." Eur J Neurosci **29**(9): 1754-60.

Ringdahl, E. N., S. L. Pereira, et al. (2004). "Treatment of primary insomnia." J Am Board Fam Pract **17**(3): 212-9.

Robertson, M. D., R. A. Henderson, et al. (2002). "Extended effects of evening meal carbohydrate-to-fat ratio on fasting and postprandial substrate metabolism." Am J Clin Nutr **75**(3): 505-510.

Roehrs, T., G. Vogel, et al. (1990). "Dose effects of temazepam in transient insomnia." Arzneimittelforschung **40**(8): 859-62.

Rosenberg, R., J. Caron, et al. (2005). "An assessment of the efficacy and safety of eszopiclone in the treatment of transient insomnia in healthy adults." Sleep Medicine **6**(1): 15-22.

Ross, R. J., W. A. Ball, et al. (1999). "Rapid eye movement sleep changes during the adaptation night in combat veterans with posttraumatic stress disorder." Biological Psychiatry **45**(7): 938-941.

Rotenberg, V. S., J. Hadjez, et al. (1997). "First night effect in depression: New data and a new approach." Biological Psychiatry **42**(4): 267-274.

Rotenberg, V. S., L. Kayumov, et al. (1997). "REM sleep in depressed patients: different attempts to achieve adaptation." Journal of Psychosomatic Research **42**(6): 565-75.

Roth, T. (2004). "Characteristics and determinants of normal sleep." J Clin Psychiatry **65 Suppl 16**: 8-11.

Routh, V. H. (2002). "Glucose-sensing neurons: are they physiologically relevant?" Physiol Behav **76**: 403-413.

Sakurai, T. (2005). "Roles of orexin/hypocretin in regulation of sleep/wakefulness and energy homeostasis." Sleep Medicine Reviews **9**(4): 231-241.

Saletu, B., G. Klosch, et al. (1996). "First-night-effects on generalized anxiety disorder (GAD)-based insomnia: Laboratory versus home sleep recordings." Sleep **19**(9): 691-697.

Salmeron, J., A. Ascherio, et al. (1997). "Dietary figer, glycemic load, and risk of NIDDM in men." Diabetes Care **20**: 545-50.

Saper, C. B., G. Cano, et al. (2005). "Homeostatic, circadian, and emotional regulation of sleep." J Comp Neurol **493**(1): 92-8.

Saper, C. B., T. C. Chou, et al. (2001). "The sleep switch: hypothalamic control of sleep and wakefulness." Trends in Neurosciences **24**(12): 726-731.

Sateia, M. J. and P. D. Nowell (2004). "Insomnia." Lancet **364**(9449): 1959-1973.

Schmidt, H. S. and R. Kaelbling (1968). "Laboratory adaptation effect on sleep patterns: A comparison of six consecutive nights." Psychophysiology **5**(2): 213.

Schmidt, H. S. and R. Kaelbling (1971). "The differential laboratory adaptation of sleep parameters." Biological Psychiatry **3**(1): 33-45.

Schwartz, G. J., P. R. McHugh, et al. (1993). "Gastric loads and cholecystokin synergistically stimulate rate gastric vagal afferents." American Journal of Physiology **265**(4): R872-R876.

Sforza, E. and J. Haba-Rubio (2005). "Night-to-night variability in periodic leg movements in patients with restless legs syndrome." Sleep Medicine **6**(3): 259-267.

Sharpley, A. L., R. A. Solomon, et al. (1988). "Evaluation of first night effect using ambulatory monitoring and automatic sleep stage analysis." Sleep **11**(3): 273-6.

Siegel, J. M. (2004). "The neurotransmitters of sleep." J Clin Psychiatry **65**: 4-7.

Silver, I. A. and M. Erecinska (1994). "Extracellular glucose concentration in mammalian brain: continuous monitoring of changes during increased neuronal

activity and upon limitation in oxygen supply in normo-, hypo-, and hyperglycemic animals." J Neurosci **14**: 5068-5076.

Smith, A., S. Leekam, et al. (1988). "The influence of meal composition on post-lunch changes in performance efficiency and mood." Appetite **10**(3): 195-203.

Smith, A., A. Ralph, et al. (1991). "Influences of meal size on post-lunch changes in performance efficiency, mood, and cardiovascular function." Appetite **16**(2): 85-91.

Specchio, L. M., M. P. Prudenzano, et al. (2004). "Insomnia, quality of life and psychopathological features." Brain Research Bulletin **63**(5): 385-391.

Spiegel, K., R. Leproult, et al. (1999). "Impact of sleep debt on metabolic and endocrine function." Lancet **345**: 1435 - 9.

Spring, B. (1984). "Recent research on the behavioral effects of tryptophan and carbohydrate." Nutr Health **3**(1-2): 55-67.

Spring, B., O. Maller, et al. (1982/83). "Effects of Protein and Carbohydrate Meals on Mood and Performance: Interactions with Sex and Age." J Psychiatr Res **17**(2): 155-167.

Steiger, A. (2007). "Neurochemical regulation of sleep." Journal of Psychiatric Research **41**: 537-522.

Steiger, A. and F. Holsboer (1997). "Neuropeptides and human sleep." Sleep **20**: 1038-52.

Stepanski, E., T. Roehrs, et al. (1981). "Readaptation to the Laboratory in Long-Term Sleep Studies." Bulletin of the Psychonomic Society **17**(5): 224-226.

Suetsugi, M., Y. Mizuki, et al. (2007). "The effect of placebo administration on the first-night effect in healthy young volunteers." Progress in Neuro-Psychopharmacology & Biological Psychiatry **31**(4): 839-47.

Taheri, S., L. Lin, et al. (2004). "Short sleep duration is associated with reduced leptin, elevated ghrelin, and increased body mass index." PLoS Medicine / Public Library of Science **1**(3): e62.

Tamaki, M., H. Nittono, et al. (2005). "Examination of the first-night effect during the sleep-onset period." Sleep **28**(2): 195-202.

Thase, M. E., C. F. Reynolds, 3rd, et al. (1987). "Do nocturnal penile tumescence recordings alter electroencephalographic sleep?" Sleep **10**(5): 486-90.

Thorburn, A. W., J. C. Brand, et al. (1986). "The glycaemic index of foods." Med J Aust **144**(11): 580-2.

Toussaint, M., R. Luthringer, et al. (1998). "Changes in EEG power density during sleep adaptation in depressed inpatients (first-night effect)." Journal of Sleep Research **7**(SUPPL. 2): 279.

Toussaint, M., R. Luthringer, et al. (1995). "First-night effect in normal subjects and psychiatric inpatients." Sleep **18**(6): 463-9.

Toussaint, M., R. Luthringer, et al. (1997). "Changes in EEG power density during sleep laboratory adaptation." Sleep **20**(12): 1201-7.

Toussaint, M., R. Luthringer, et al. (2000). "Changes in EEG power density during sleep laboratory adaptation in depressed inpatients." Biological Psychiatry **47**(7): 626-33.

Vallieres, A., H. Ivers, et al. (2005). "Variability and predicability in sleep patterns of chronic insomniacs." Journal of Sleep Research **14**(447-453).

Van Cauter, E., E. T. Shapiro, et al. (1992). "Circadian modulation of glucose and insulin responses to meals: relationship to cortisol rhythm." Am J Physiol Endocrinol Metab **262**(4): E467-475.

van Straten, A., P. Cuijpers, et al. (2009). "Self-help treatment for insomnia through television and book: a randomized trial." Patient Educ Couns **74**(1): 29-34.

Vgontzas, A. N., E. O. Bixler, et al. (2001). "Chronic insomnia is associated with nyctohemeral activation of the hypothalamic-pituitary-adrenal axis: clinical implications." J Clin Endocrinol Metab **86**(8): 3787-94.

Wauquier, A., B. van Sweden, et al. (1991). "Ambulatory first night sleep effect recording in the elderly." Behavioural Brain Research **42**(1): 7-11.

Webb, W. B. and S. S. Campbell (1979). "The first night effect revisited with age as a variable." Waking & Sleeping **3**(4): 319-24.

Weikel, J. C., A. Wichniak, et al. (2003). "Ghrelin promotes slow-wave sleep in human." American Journal of Physiology **284**: E407-15.

Wells, A. and N. Read (1994). "Influences of Fat, Energy, and Time of Day on Mood and Performance." Physiol Behav **59**(6): 1069-1076.

Wells, A., N. Read, et al. (1998). "Effects of meals on objective and subjective measures of daytime sleepiness." J Appl Physiol **84**(2): 507-515.

Wells, A. S. and N. W. Read (1995). "Influences of dietary and intraduodenal lipid on alertness, mood, and sustained concentration." British Journal of Nutrition **74**(1): 115-23.

Wells, A. S. and N. W. Read (1996). "Influences of fat, energy, and time of day on mood and performance." Physiol Behav **59**(6): 1069-1079.

Wells, A. S., N. W. Read, et al. (1997). "Influences of fat and carbohydrate on postprandial sleepiness, mood, and hormones." Physiol Behav **61**(5): 679-86.

Wohlgemuth, W. K., J. D. Edinger, et al. (1999). "How many nights are enough? The short-term stability of sleep parameters in elderly insomniacs and normal sleepers." Psychophysiology **36**(2): 233-244.

Woodward, S. H., D. L. Bliwise, et al. (1996). "First night effects in post-traumatic stress disorder inpatients." Sleep **19**(4): 312-7.

Wu, M. F. and L. N. Boehmer (2004). "Activity of the dorsal raphe cells across the sleep-wake cycle and during cataplexy in narcoleptic dogs." J Physiol **554**: 202-215.

Wurtman, R. J. (1988). "Effects of their nutrient precursors on the synthesis and release of serotonin, the catecholamines, and acetylcholine: implications for behavioral disorders." Clin Neuropharmacol **11 Suppl 1**: S187-93.

Wurtman, R. J., F. Hefti, et al. (1981). "Precursor control of neurotransmitter synthesis." Pharmacol Rev. **32**: 315-335.

Wurtman, R. J. and J. J. Wurtman (1995). "Brain serotonin, carbohydrate-craving, obesity and depression." Obes Res **3 Suppl 4**: 477S-480S.

Wurtman, R. J., J. J. Wurtman, et al. (2003). "Effects of normal meals rich in carbohydrates or proteins on plasma tryptophan and tyrosine ratios." Am J Clin Nutr **77**(1): 128-32.

Yan, D., T. Urano, et al. (1993). "Correlation between serotonergic measures in cerebrospinal fluid and blood of subhuman primate." Life Sci **52**(8): 745-9.

Young, S. N. (1991). "Some effects of dietary components (amino acids, carbohydrate, folic acids) on brain serotonin synthesis, mood, and behavior." Can J Physiol Pharmacol **69**: 893-903.

Zammit, G. K., A. Kolevzon, et al. (1995). "Postprandial sleep in healthy men." Sleep **18**(4): 229-31.

Zhang, B. and Y. K. Wing (2006). "Sex differences in insomnia: a meta-analysis." Sleep **29**(1): 85-93.

Zimmermann, R. C., C. J. McDougle, et al. (1993). "Urinary 6-hydroxymelatonin sulfate as a measure of melatonin secretion during acute tryptophan depletion." Psychoneuroendocrinology **18**(8): 567-78.

Zucconi, M., L. Ferini-Strambi, et al. (1996). "Structured psychiatric interview and ambulatory sleep monitoring in young psychophysiological insomniacs." J Clin Psychiatry **57**(8): 364-70.

APPENDICES