

List of abbreviations

| | |
|----------------------------------|-------|
| Body mass index | BMI |
| Central nervous system | CNS |
| Corticotrophin-releasing hormone | CRH |
| Cognitive behaviour therapy | CBT |
| Electroencephalogram | EEG |
| Electromyogram | EMG |
| Electrooculogram | EOG |
| Epworth sleepiness sleep | ESS |
| Free fatty acids | FFA |
| Glycemic index | GI |
| Growth hormone | GH |
| Glycemic load | GL |
| Growth hormone releasing hormone | GHRH |
| Large neutral amino acids | LNAAs |
| L-tryptophan | L-Trp |
| Multiple Sleep Latency Test | MSLT |
| Non-rapid eye movement | NREM |
| Polyunsaturated fatty acids | PUFA |

| | |
|-----------------------------------|------|
| Rapid eye movement | REM |
| 6-sulfatoxymelatonin | 6-SM |
| Slow wave sleep | SWS |
| Tryptophan | Trp |
| Tryptophan –free amino acid drink | TFD |

Introduction

The present thesis explores the effect of macronutrients on sleep in healthy male subjects, specifically the glycemic index (GI) of carbohydrates and the Atkins' diet. For the latter, also monitored were the behavioural responses and dream recall in response to this diet in the short-term.

The thesis consists of a literature review section (Chapter 1), which introduces the importance of sleep, sleep-inducing factors, meal macronutrients in relation to behavioural effects and sleep pattern, highlighting limitations of existing published studies. The chapter also considers the effects of food components on blood biochemical changes. Chapter 2 outlines the general methodology used in this thesis. Chapter 3 reports the results of the study entitled "High-glycemic-index carbohydrate meals shorten sleep onset". This work appears in the *American Journal of Clinical Nutrition* 2007; 85:426-30. Chapter 4 reports the findings on the effect of the Atkins' diet on sleep and Chapter 5 its effect on behavioral responses. The works contained in these chapters have been reported at the Annual Australasian Sleep Conference. Chapter 6 provides a general discussion, recommendations and conclusion related to the outcomes of the thesis investigations.

The Appendix section contains questionnaires (food and sleep diaries, medical evaluation), recruitment flyers, consent forms, participant information statements, meal plans (Control mixed meal, the Atkins'

meals, high-GI and low-GI), subjective rating scales (visual analogue, Epworth Sleepiness Scale), the Atkins' diet related symptom questionnaire and ethics approval letters.

Chapter 1

LITERATURE REVIEW

Literature review

1. Sleep

Sleep is vital to life in all species (Douglas 2002) and on the average adults human require 7-8 h of sleep each night (Stephoe et al 2006). There are significant impaired cognitive functions and feelings of unwellness in sleep deprived individuals (Krueger et al 1999). Sleep may be characterized by brain electrical activity recorded from the cortex. The electroencephalogram (EEG) (Voderholzer et al 1998) is characterized by the amplitude and frequency of the brain waves (Ringdahl et al 2004). Sleep includes two basic sub-phases recognized in all mammals and birds. They are the non-rapid eye movement (NREM) sleep and rapid eye movement (REM) sleep. NREM sleep consists of stages 1-4, where each stage has a clearly defined electrical frequency and amplitude recorded by EEG. Sleep is entered usually from stage 1 representing the transition from wake-to-sleep. Stage 1 lasts only between 1-7 minutes with a predominance of theta waves in the EEG ranging from 2-7 Hz (Carskadon & Dement 1994; Muzur 2005). Stage 2, which is considered by some researchers as “true” sleep, is entered when either sleep spindles (12-14 Hz) or K-complexes (large amplitude, ≤ 2 Hz) first appear. These

sleep structures reflect neural process in the thalamus (Muzur 2005). There is evidence that shows the involvement of stage 2 in the thermoregulatory process which maintains normal sleep (Muzur 2005). From stage 2, sleep becomes progressively deeper. Stages 3 and 4, slow wave or delta sleep (SWS), in which the body's core temperature is reduced, are needed for cerebral restoration (Muzur 2005). Stages 3 and 4 differ only in the delta quantity (Muzur 2005). A reduction in the percentage of stage 3 and 4 is considered a decline in sleep quality (Ringdahl et al 2004). REM sleep may be response to NREM sleep by restoring optimal core temperature when it falls to a critically low level (Muzur 2005). During REM sleep the EEG pattern is of low amplitude and mixed frequency with beta, alpha, and theta waves, with low or absent muscles tonus and marked by episodes of ocular saccades, which are related to saw-tooth-like waves (Muzur 2005). The EEG manifestations and metabolic characteristics of REM sleep and waking are very similar (Muzur 2005). REM sleep is, therefore, often known as "paradoxical" sleep.

Sleep, a restorative process, is homeostatically regulated (Muzur 2005). Homeostasis infers that sleep rebound would occur following sleep deprivation. Progressive enhancement in the incidence of sleep rebound

following longer *vs.* shorter sleep deprivation supports the idea that the presence of an accumulated substrate causes the organism to fall asleep (Muzur 2005).

In the restorative theory, sleep serves as a restorative process where tissue restoration or protein synthesis is greatest and sleep allows brain metabolic restoration (Oswald & Corner 1980; Shapiro et al 1984). However, this theory has not been fully supported. The compatible view with restoration theory suggests that sleep alters information processing and sleep serves to preserve the information as well as the process of storage of new information obtained through experience and environment. Sleep increases and facilitates synaptic activity and efficacy for storing obtained information. Exposing neural systems to visual/auditory/tactile activities increases the secretion of growth factors, which may ease synaptic stimulation within groups of neurons that alter information processing (input-output) during sleep. This theory also proposes that substrates resulted from synaptic activity possibly facilitate sleep (Krueger et al 1999).

A more recent theory considers the common statement of “being tired” as “neural fatigue” (Muzur 2005). This is a condition resulting from the

visual/auditory/tactile activities the central nervous system was exposed to during the previous period of waking, that is the activity of perception (Muzur 2005). In this theory sleep serves as a means of recovery from “neural fatigue” (Muzur 2005). One theory suggests that perceptual overloads (useless perceptions), which are not stored in the brain and are removed during sleep affect the amplitude and/or frequency of delta waves

2. Dreaming

Sleeping and dreaming are two cognitive functions that are not necessarily a connected process (Muzur 2005). Different theories have been suggested to explain dreaming, including the “activation/synthesis model”, “cognitive model” and “cortical activity” theories (Manica 1995).

The neurophysiologists discuss dreaming from the biologists’ viewpoint. Their “activation/synthesis” model supports the idea that the same mechanism that affect REM sleep production also generates dreams (Manica 1995). They suggest that cholinergic neurons in the brain which

maintain REM sleep can randomly generate sensory information (visual, auditory, tactile, etc.). This information can be processed by the forebrain and compared with long term memory's resource and is then transformed into experience and dreams. This model considers involvement of various transmitters and metabolic changes in activating memory and dreaming (Manica 1995).

In contrast to the neurobiologists' theory, the cognitive model of dreaming considers dreams as a symbolic process of explaining, interpreting, and reorganizing the information stored in the memory during waking periods. The cerebral and cognitive structure developed during ontogenesis organizes this symbolic process (Manica 1995).

According to a new theory, dreaming is a process occurring in the cortical region when traces of memory are fixed and stored as a result of the cholinergic release of acetylcholine (Muzur 2005) on waking. Dreaming is thought to be a process of elimination of the perceptive activity of the preceding period of wakefulness. However, the dream content is a poor reflection of the activities of the wakeful period (Muzur 2005).

Dreams are often recalled following awakening from REM sleep. However, dreams have also been reported following awakening from NREM sleep (Muzur 2005). Dream reports from NREM sleep which mainly originated from stage 2, are recalled at up to 75% of awakening, but during REM sleep there is 80-90% recall (Muzur 2005). Dream production and recall are affected by sleep stages or cycles (Cipolli et al 2004). It has been demonstrated that there is a positive relation between dream recall frequency and the frequency of nocturnal awakening of healthy subjects (Cory et al 1975; Halliday 1988).

3. Neurochemical regulation of sleep

Various hormones particularly neuropeptides and steroids influence sleep regulation. Among neuropeptides growth hormone releasing hormone (GHRH), ghrelin, and galanin promote sleep, whereas corticotrophin-releasing hormone (CRH) and somatostatin diminish NREM sleep and may enhance REM sleep. The interaction of GHRH and CRH has a major role in sleep regulation. GHRH is released during the first half of the night and stimulates growth hormone (GH) release and SWS appearance around sleep onset. CRH in contrast is released during the second half of

the night and affects cortisol release and REM sleep appearance in the morning hours (Steiger 2007). The CRH release thus dictates the cortisol circadian rhythm with a dip that coincides with the GH peak, but a rise in the morning. However, acutely administered cortisol increases SWS and reduces REM sleep. These changes in sleep are likely due to negative feedback inhibition of endogenous CRH (Friess 2004, Bohlhalter 1997), which causes a shift in the ratio of CRH and GHRH towards the latter (Steiger 2002). Melatonin, another neuropeptide, is released based on the light-dark cycle and increases sleep propensity and promotes sleep consolidation (Dijk & Cajochen 1997; Steiger 2007). Its secretion is considered as a marker of circadian rhythm (Ohashi et al 1999) and is sensitive to day light or artificial bright light exposure (Ohashi et al 1999; Schernhammer et al 2001; Herljevic et al 2005). Figure 1 shows the circadian rhythm variations of melatonin, cortisol and GH (Knutsson et al 1997; Bogdan et al 2001).

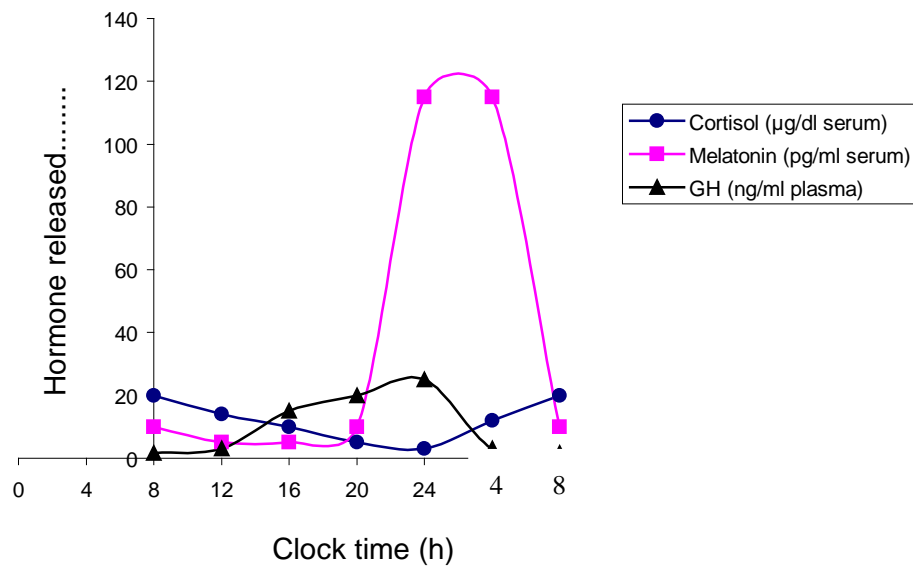


FIGURE 1. Comparison of melatonin, cortisol and GH release during the night

Growth hormone: GH is released from the anterior pituitary gland and its release is associated with SWS sleep (Merimee & Rabin 1973). Many factors stimulate the secretion of GH. These include hypoglycemia, stress, exercise, and protein depletion as well as a number of inhibitors such as obesity, elevation of free fatty acids (FFA) levels, and glucocorticoids (Merimee & Rabin 1973).

The glucose concentrations at which hypoglycemic symptoms appear and at which GH is released are 3.1 ± 0.3 and 3.7 ± 0.4 mM for normal

subjects and controlled type-2 diabetes, respectively (Spyer et al 2000). Changes in the EEG of diabetic, and healthy subjects occur at an hypoglycemia threshold of ≤ 3 mM (Pramming et al 1988) and ≤ 2.6 (Harrad et al 1985), respectively.

Cortisol: Cortisol, the adrenal glucocorticoid hormone, released from hypothalamic pituitary-adrenal, affects human sleep, behaviour and mood (Gibson et al 1999). Physiological cortisol levels appears to contribute to REM sleep maintenance and administration of cortisol increases SWS through feedback inhibition of CRH (Steiger 2007). Alteration to the circadian rhythm of cortisol may affect the sleep pattern (Gibson et al 1999). An inverse schedule of meal timing alters its circadian rhythm (Gibson et al 1999). During the period of Ramadan, an Islamic religious intermittent fasting, fasting influences the distribution of cortisol, with peaks in both the early morning and evening (Sliman et al 1993) and increased during the night. There was a reduced amplitude of the cortisol rhythm (Roky et al 2004). Cortisol secretion rhythmicity changes could be one factor for reduction of REM sleep during Ramadan (Roky et al 2001). Cortisol levels increases following ingestion of a high protein meal. Ingested protein (30% of energy intake) increases salivary cortisol,

whereas 5% protein in the meal does not alter cortisol levels (Gibson et al 1999). The mean cortisol levels were greater after high protein compared to low protein meals at 30 and 60 minutes after, but not 120 minutes after the meal. Women with poorer psychological well-being demonstrated greater release of cortisol after a high protein meal (Gibson et al 1999).

Melatonin: Melatonin secretion from the pineal gland, located at the midline of brain, has a strong circadian rhythm. The secretion of melatonin occurs at the onset of darkness and is suppressed by light. Melatonin level peaks between midnight and 0200 h and with the lowest levels occurring in the afternoon (Bogdan et al 2001). A positive relationship has been observed between a high endogenous melatonin levels and sleep quality (Morris et al 1990; Haimov et al 1994). In aging, which is associated with poor sleep quality (Karacan et al 1976; Dement et al 1982; Lugaresi et al 1983; Gislason & Almqvist 1987; Webb 1989) lower melatonin levels have been reported (Iguchi et al 1982; Touitou et al 1984; Sack et al 1986; Waldhauser et al 1988; Van Coevorden et al 1991; Ferrari et al 1995; Hajak et al 1996). Lower melatonin levels are also observed in aged insomniacs when compared with normal controls (Haimov et al 1994; Garfinkel et al 1995; Hajak et al 1995; Attenburrow

et al 1996). However, in one study, which consisted of a large sample of good sleepers (n = 52) and insomniacs (n = 56) aged 55-80 years, no significant difference in total melatonin production was observed between the two groups (Lushington et al 1998). A review of different studies reveals an hypnotic effect of exogenously administered melatonin during the day when melatonin level is low (Dollins et al 1994; Tzischinsky & Lavie 1994). Administration of exogenous melatonin to patients suffering from melatonin deficiency made them feel sleepy (Haimov et al 1993), and facilitated sleep onset and shortened sleep latency to stage 2 (Haimov et al 1993; Haimov et al 1995). Melatonin administered to disabled children with severe sleep disorders, and to blind children with circadian rhythm disorders improved sleep (Pillar et al 2000). Side-effects associated with melatonin administration are headache and an odd taste in the mouth (Ellis et al 1996). However, only a minority of patients regard those negative effects. In patients suffering from chronic primary insomnia, nocturnal plasma melatonin levels tend to be higher in the evening and were significantly lower during the middle of the night (Hajak et al 1995). Since melatonin is released during the dark period of the light-dark cycle in both nocturnal as well as diurnal animals, it cannot be directly related to sleep (Ellis et al 1996). Low circulating melatonin levels are likely to be associated with sleep

disruption through elevated core body temperature (Dawson & Encel 1993; Dijk & Cajochen 1997). It is suggested that melatonin exerts a hypnotic effect through the thermoregulatory mechanism by lowering core body temperature (Dijk & Cajochen 1997). Administration of melatonin shortens the sleep onset latency and increases the percentage of stage 2 by suppressing the normal diurnal rise of core-body temperature (Muzur 2005) via increased blood flow in distal skin regions and loss of heat (Cajochen et al 2003). However, endogenous melatonin release did not cause a fall in core temperature (Cajochen et al 2003). The metabolite of melatonin found in the urine, 6-sulfatoxymelatonin (6-SM), is highly stable and it correlates strongly with plasma melatonin (Zimmermann et al 1993).

4. Sleep difficulties

Sleep difficulties represent a major community health problem. Sleep complaints include difficulty falling asleep, waking during the night, and early morning awakening (Owens & Matthews 1998). Sleep difficulties are twice as common among women as men. The quality of sleep is altered during the menstrual cycle in pre-menopausal women and during

pregnancy. Sleep disturbances increase around the forties in women, and anxiety and depression may contribute to an increased risk for sleep disturbances (Owens & Matthews 1998). Depressed subjects suffer from difficulties in falling sleep, frequent nocturnal awakening, early morning awakening, reduction of SWS and increased REM sleep (Reimann et al 2001). The antidepressant effects of many depression drugs are associated with REM sleep suppression (Mayers & Baldwin 2005). Circadian rhythm disorders (e.g. shiftwork) and sleep apnea also impact on the sleep pattern. The daytime sleep of shift-workers shows shorter sleep latency and total sleep duration, more intermittent wakefulness and a more uniform REM sleep distribution than night-sleep (Kerkhof & van Vianen 1999). Sleep breathing disorders, in particular, obstructive sleep apnoea, is associated with poor sleep quality, characterized by sleep fragmentation and abnormal sleep pattern (Loredo et al 2001).

Insomnia is defined as a subjective dissatisfaction with the quantity, quality or timing of sleep. These sleep complaints must occur at least three times a week for at least one month to meet the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) criteria (Association 1994). The International Classification of Sleep Disorder considers insomnia as a dyssomnia, which includes difficulties initiating or

maintaining sleep (insomnia) and excessive sleepiness (hypersomnia) (Ringdahl et al 2004).

The prevalence of insomnia is high. Approximately 15% of Americans have long-term sleep difficulties (Ringdahl et al 2004), and 42% of healthy, middle-aged, women of varying menopausal status reported some type of sleep disturbance (Owens & Matthews 1998). In an Australian study of the general population over 21 years old, the prevalence ranged from 13% to 20%. Sleep complaints included “wake up early and unable to return to sleep” and “not having enough sleep. (Lack et al 1988).

Insomnia negatively impacts on mental performance. Impaired cognition is associated with increased motor vehicle accidents. Indeed, 16-20% of motor vehicle accidents on highways were related to driver sleepiness and fatigue (Johns & Hocking 1997). In Australia the direct and indirect costs of sleep disorders including health costs, work and motor vehicle accidents and productivity losses is estimated to amount to USD 4524 million per year, which is 0.8% of the Australian gross domestic product. The annual nonfinancial costs of the burden of disease is estimated at USD 2970 million or 1.4% of the total burden of disease in Australia

(Hillman et al 2006).

There are two treatment strategies for insomnia: pharmacological and non-pharmacological (Ringdahl et al 2004). Non-pharmacologic treatments for insomnia are considered effective if they reduce sleep onset latency or increase total sleep time by 30 minutes (Smith et al 2002). The most effective non-pharmacological treatments are cognitive behavior therapy (CBT); exercises; herbal medicine and improving sleep hygiene. CBT consists of stimulus control therapy, relaxation therapy, paradoxical intention therapy, imagery training, sleep restriction and temporal control therapy. CBT reduced the sleep onset latency by 54% in insomniacs compared to 16% with relaxation therapy and 12% with placebo therapy (Ringdahl et al 2004). Pharmacological treatment for insomnia which are commonly used include; benzodiazepines (BDZs), L-Trp, melatonin, zolpidem, zaleplon, and zopicone (Ringdahl et al 2004).

In stimulus control therapy that is effective for sleep-onset insomnia, the patient is instructed to get out of bed and leave bedroom during extended awakening. The patient then returns to bed only when feels very sleepy, repeating the cycle as often as needed through the night. Patients avoid sleep-incompatible behaviors in the bed/bedroom (reading or watching

television); and eliminate daytime napping (Edinger et al 2001).

Relaxation therapy is based on the observation that hyperarousal interferes with sleep. Some methods such as progressive muscle relaxation and autogenic training seek to reduce somatic arousal (e.g. muscle tension/release relaxation technique), whereas others, such as imagery training or meditation, focus primarily on cognitive arousal (e.g., intrusive thoughts). Relaxation is the most frequently used treatment for insomnia and it has been found helpful with younger adults, although its therapeutic effect is of a lesser degree compared to stimulus control or sleep restriction (Morin et al 1999).

Sleep restriction therapy consists of curtailing the amount of time spent in bed to the actual amount of sleep. For example, if a subject reports sleeping an average of 6h per night out of 9-10h spent in bed, the initial prescribed sleep window (i.e. from initial bedtime to final arising time) would be 6h. The allowable time in bed is increased by 15-20 minutes for a given week when sleep efficiency (total sleep/ time in bed x 100%) exceeds 85%, decreased by the same amount of time when sleep efficiency is <80%, and kept stable when sleep efficiency fell between 80-85%. Adjustment is made according to the subject's acceptance and

willingness to comply with the prescribed regimen. A sleep window is never smaller than 4h (Morin et al 1993).

With paradoxical intention therapy, the patient confronts their fear of sleeplessness by staying awake. This decreases concern about the consequences of lack of sleep and decreases anxiety about falling asleep.

Patients who undergo cognitive therapy do not necessarily improve their sleep parameters, but they have increased satisfaction with their sleep patterns (Simon & VonKorff 1997). Temporal control measures recommend a constant time of waking with minimal daytime napping (Spielman et al 1987; Friedman et al 1991).

5. Effects of macronutrients on sleep and behavior

There is evidence to suggest a link between feeding and sleeping, as it has been shown in animals that when feeding stops, nonfeeding activities of exploring, rest, or sleep follow. A few hours after lunch people feel less alert, tired and perform less well than they do prior to lunch (Wells & Read 1996). An acute change in mood and reduction in alertness have

been reported post-lunch and breakfast (Lloyd et al 1994; Lloyd et al 1996; Wells et al 1997).

Several studies have investigated the effect of ingested meals at different times of day on postprandial sleepiness (Wells & Read 1996; Wells et al 1997; Wells et al 1998). In a study, 16 subjects were each studied on two occasions. On each occasion subjects ingested high fat/low carbohydrate (energy ratio 54:41) and low fat/ high carbohydrate (energy ratio 7:88) meals 4 h apart in a counter-balanced order during the day. To control for the effect of the circadian rhythm, one group ingested the meals 2 h later than the other group (Wells et al 1998). Postprandial sleepiness was assessed using the Multiple Sleep Latency Test (MSLT), a series of five 20 min sleep periods that monitored how quickly a subject fell asleep. The MSLT results indicated that the subjects were significantly sleepier at 1.5 h after both the meals than before the meals. However, no significant differences in sleepiness were observed between the two meals (Wells et al 1998). When the Akerstedt EEG sleepiness test was applied, When the Akerstedt EEG sleepiness test was applied, an increased sleepiness (increased power density in the alpha and theta bands of the EEG) was observed 3 h and 20 min after ingestion of either meal. The Akerstedt test required that the subject sat on a chair and looking straight ahead and focusing

for 5 min on an object on a wall 1 m from them to observe changes in EEG. (Wells et al 1998).

High carbohydrate meals and sleep: Carbohydrate is a common macronutrient found in diets. Several studies have demonstrated the effects of carbohydrate ingestion on sleep. In one study, SWS percentage and duration were significantly reduced following a high carbohydrate /low-fat diet compared to a low carbohydrate /high fat diet, whereas REM sleep duration was significantly increased in the same subjects (Phillips et al 1975).

In comparing the effects of high carbohydrate (glucose, and potato sources) with low carbohydrate (crisp bread source) on sleep it was found that the high carbohydrate meal significantly reduced stage 4 sleep and significantly increased REM sleep in the first half of the night (Porter & Horn 1981). However, in the sleep polysomnography of an afternoon nap after ingestion of either a high-fat, a high-carbohydrate, or a mixed meal, no significant difference was observed for sleep onset latency or sleep stages for any of the test meals (Orr et al 1997). These studies showed that macronutrients effect on sleep will be significant when ingested before bedtime.

A study in the newborn investigated the effect of feeds containing 10% glucose + Trp or 5% glucose + valine or routine formula “Similac” (Yogman & Zeiset 1983). The authors reported that infants fed glucose + Trp entered active sleep and quiet sleep significantly sooner compared to the group fed Similac. The valine group entered active sleep and quiet sleep significantly later compared to the Similac group. These findings suggest that variations in the composition of the diet may influence sleep behaviour in newborns (Yogman & Zeiset 1983). Table 1 summarises the findings on carbohydrate diets on sleep, implicating the influence of carbohydrate on sleep indices before bedtime.

TABLE 1

Effect of diets on SWS and REM sleep in healthy subjects

| Author | Diet | SOL | SWS | REM sleep |
|--------------------|--|-----|---|--|
| Philip et al 1975 | low carbohydrate /high fat vs. High carbohydrate/low fat- polysomnography over night | ns | increase (sig. p < .05) | decrease (sig. p < .05) |
| Porter & Horn 1981 | low carbohydrate vs. high carbohydrate- polysomnography over night | ns | increase (sig. p < .05) | decrease (sig. p < .05) |
| Orr et al 1997 | High carbohydrate meal vs. mixed meal or high fat–afternoon nap study | ns | | |
| Yogman et al 1983 | 10% glucose+Trp vs. routine formula “similac”-infant study | | entered quiet sleep sooner (sig. p < .05) | entered active sleep sooner (sig. p < .05) |

Glycemic index (GI) and glycemic load (GL) of carbohydrates: The GI determines the rise in blood glucose level after eating a food compared with a standard blood glucose curve obtained following ingestion of glucose or white bread in the same subject. This index varies between 20 for fructose and 100 for glucose. Both the amount and source of carbohydrate influence the glycemic response, but the GI provides a measure of carbohydrate quality not quantity given the same amount of carbohydrate (Foster-Powell et al 2002). The GL represents the total of glycemic response (amount and source of carbohydrate) to a food or meal and is calculated by $GI (\%) \times \text{grams of carbohydrate per serving}$ (Foster-Powell et al 2002).

GI and insulin response: Insulin secretion occurs in response to changes in blood glucose level to maintain glucose homeostasis (Evans-Molina et al 2007). Its secretion increases more following the ingestion of a high carbohydrate meal than after a high fat or high protein meal (Wells et al 1997; Stevenson et al 2005). Insulin secretion can be affected by the quality (source) and quantity of carbohydrate ingested (Foster-Powell et al 2002). The GI is influenced by the physical form of a food, by processing and by fat content of the food, which reduces GI through

delayed gastric emptying (Truswell 1992). Grains high in amylose have been reported to generate lower glycaemic and insulin responses than those low in amylose (high GI) (Goddard et al 1984; Behall et al 1988; Behall & Howe 1995; Holt & Brand-Miller 1995). It has been demonstrated that quick cooking rice produces greater plasma glucose and insulin response when compared with normal rice and low amylose puffed rice cake has a significantly higher plasma glucose and insulin response than a high amylose rice cake (Holt & Brand-Miller 1995).

The effect of blood insulin level on sleepiness has not been fully investigated in humans. In animal studies infusions of insulin and glucose but not glucose alone, increased the total sleep time over a 24 h period, and increased REM sleep duration during the light period of the light/dark cycle (Danguir & Nicolaidis 1980). Others have found a reduction in REM sleep during the first 5 h of an 8-h light period (Schubert & Makhoulf 1993). In rats that were employed for antibody induction against peripheral insulin, an increased sleep onset latency and disturbed sleep in the light period and increased sleep in the dark period have been reported (Wells et al 1997). Injection of significant doses of insulin in rats led to increased brain serotonin, which was associated with convulsions. The authors suggested that the elevated brain serotonin may

have been related to abnormal neuronal activity and not changes in brain Trp, a precursor for serotonin synthesis (Fernstron & Wurtman 1971).

Trp and GI: Trp is an essential amino acid. Its uptake into the brain has an important influence on sleep. Trp is a precursor of serotonin, a sleep-inducing agent. Its ratio to the other large neutral amino acids (LNAAs, valine, isoleucine, leucine, tyrosine and phenylalanine) (Trp:LNAA) determines its ease of entry into the brain. A high GI meal potentiates the increase in this ratio. As the insulin level increases, it promotes the muscle uptake of LNAAs (Lieberman et al 1986). As the plasma level of LNNAs falls, the plasma Trp:LNAA increases, favouring Trp entry into the brain for conversion to serotonin (Wurtman & Wurtman 1995).

The protein content of a meal has a direct effect on the level of plasma Trp, since Trp is present in small quantity in proteins compared to the other LNAAs. Thus, an increased protein percentage in a meal will reduce Trp:LNAA and influence the level of brain serotonin (Fernstron & Wurtman 1972; Young 1991). It has been suggested that a protein levels as low as 4% in a carbohydrate meal sufficiently blocks the elevation of Trp:LNAA in the plasma (Teff et al 1989). In contrast, a carbohydrate to protein ratio of 13:1 (80.5% carbohydrate, 6% protein, 13.5% fat) in a

high carbohydrate meal significantly enhanced plasma Trp:LNAA compared to before the meal (Wurtman et al 2003). It has been demonstrated that in a balanced diet the proportion of carbohydrate to protein that will neither raise nor lower plasma LNAA is 5:1 (67% carbohydrate, 12% pro, 21% fat) in humans (Berry et al 1991). Increasing or decreasing this ratio impacts on the Trp level and its ease of entry into the brain (Berry et al 1991; Wurtman et al 2003). Thus, meals can change plasma Trp:LNAA (Fernstron & Wurtman 1972; Martin-Du Pan et al 1982; Maher et al 1984; Lieberman et al 1986; Layons & Truswell 1988; Wurtman et al 2003) significantly, depending on the carbohydrate to protein ratio in a meal (Layons & Truswell 1988) and the GL of a carbohydrate meal. The Trp:LNAA ratio increases significantly after higher doses of glucose compared to low doses (Martin-Du Pan et al 1982).

Two studies found that carbohydrate ingestion in the morning raised the plasma Trp:LNAA significantly more than when it was ingested in the afternoon (Ashley et al 1982; Ashley et al 1985). However, Lyons and colleagues reported that the increased plasma Trp:LNAA ratio after a high carbohydrate meal was not dependent on the time of the day (Lyons & Truswell 1988).

In view of the importance of Trp as a precursor for serotonin, there was a significant interest in manipulating the level of Trp and its observed effect on sleep. Plasma Trp depletion following ingestion of a Trp free amino acid drink (TFD) results in a dramatic reduction (up to 85%) in plasma Trp and brain serotonin metabolism (Bhatti et al 1998; Voderholzer et al 1998; Arnulf et al 2002; Evan et al 2002). The experimental effects of Trp depletion on sleep varied. Reduced or unchanged REM sleep latency, increased or unchanged REM sleep percentage and increased REM density have been reported (Bhatti et al 1998; Moore et al 1998; Voderholzer et al 1998; Arnulf et al 2002).

The relation between ingestion of a protein and carbohydrate meal and synthesis and turnover of brain serotonin was observed in rats (Wurtman et al 1981). High doses of Trp rapidly enhanced the serotonin level in the brain, and changes of dietary Trp over several weeks were associated with parallel changes in brain serotonin. In rats injected with insulin or that ingested a carbohydrate diet there was an increased level of plasma and brain Trp and serotonin in the brain (Fernstron & Wurtman 1971). It was found that the carbohydrate to protein ratio of 7:1 (86% carbohydrate and 12% protein) in a high carbohydrate meal significantly increased

platelet-poor plasma serotonin by 4.47 fold, while a 70% protein-rich meal significantly reduced serotonin to 28% of the initial values (Blum et al 1992). Serotonin release is also involved in behaviour such as control of mood, aggressiveness, and depression (Young 1991; Wurtman & Wurtman 1995; Benton 2002), and carbohydrate meals can affect mood and behaviour in humans (Young 1991; Wurtman & Wurtman 1995).

Carbohydrate, mood and behaviour: It is well known that foods have a direct influence on overall mood and behaviour. Chocolate makes us feel better (Wurtman & Wurtman 1995). High carbohydrate meals reduce alertness and decrease pain perception (Spring 1984). Drinking coffee makes us more alert. Postprandial mood and sleepiness possibly are responses mediated by nutrients in the small intestine (Murray et al 1993; Wells et al 1995). Drowsiness is a common phenomenon reported by subjects following food ingestion. Several studies have reported postprandial changes in subjective rating of sleepiness. The consistent finding among these studies is that the subjective ratings of sleepiness are increased following a carbohydrate or fat-rich meal, but not after a macronutrient-balanced or high protein meal (Spring et al 1987).

Serotonin release, stimulated by carbohydrate not only is involved in sleep onset, but also control of mood, depression and accelerates the onset of satiety (Wurtman & Wurtman 1995). Conversely, decreases in brain serotonin resulting from a low-carbohydrate weight loss diets block this response (Wurtman & Wurtman 1995). Many patients prefer to eat carbohydrate-rich meals to feel well (Wurtman & Wurtman 1995). It has been demonstrated that low levels of blood glucose in a glucose tolerance test was associated with greater aggressiveness, antisocial personality and a strong craving for sugar (Benton 2002) reflecting the high level of insulin secretion in these subjects (Virkkunen & Narvanem 1987). Evidence indicates that depression and seasonality of mood disorders are related to inadequate serotonin release (Wurtman & Wurtman 1995; Capiello et al 1996). Several studies demonstrated that in rapid Trp depletion tests, impairment of mood was seen in healthy subjects with mood changes being significantly more evident in those with a family history of affective disorders (Young et al 1985; Ellenbogen et al 1996; Klaassen et al 1999; Evan et al 2002).

The influence of macronutrients on mood has been demonstrated after meal ingestion. There were no significant differences in postprandial performance tests (tasks related to cognitive, visual, free recall, and

reaction time) between four iso-caloric dietary conditions of low fat-high carbohydrate, medium fat-medium carbohydrate, high fat-low carbohydrate and no breakfast. However, mood significantly improved after the ingestion low fat-high carbohydrate breakfast containing 9.5% protein (Lloyd et al 1996). In a similar study in which subjects were given low fat-high carbohydrate midday meal, they reported longer reaction time, were drowsier, muddled and less cheerful than when they had been given a medium fat-medium carbohydrate meal. The observed effects were unlikely to have been due to an increased serotonin release because the low fat-high carbohydrate meal contained 15% protein, which blocked serotonin secretion (Lloyd et al 1994).

High fat meals, mood and sleepiness: A high fat meal stimulates insulin secretion without altering the plasma glucose response. High fat meals in fact suppress the plasma glucose response (Wells et al 1997; Stevenson et al 2005). There is a dominant effect of fat on mood, fatigue and drowsiness compared to that of carbohydrates. A lower-energy-high fat meal produced a greater decline in postprandial mood than the higher-energy-low-fat meal (Wells & Read 1996). The significant effects of a higher fat to carbohydrate ratio on mood has been demonstrated (Lloyd et

al 1994). An isocaloric replacement of fat for carbohydrate has a greater effect on postprandial drowsiness (Wells et al 1995). However, this increased drowsiness was not related to the serotonin level. An increase in plasma fatty acids did not promote extracellular hypothalamic serotonin (Orosco & Gerozissis 2001). In animal studies a significant decrease in extracellular hypothalamic serotonin was observed in response to ingestion of lard or vegetable margarine enriched with saturated fatty acids. This was not evident after the ingestion of polyunsaturated fatty acids (PUFA) oils such as sunflowers and olive oil (Orosco et al 2002).

High fat meals may induce sleepiness as a result of cholecystokinin (CCK) release. CCK is a polypeptide hormone secreted by the duodenum cells in the gastrointestinal tract in response to ingested fat, protein and to a much lesser extent carbohydrate (Wells et al 1997). A relationship between the lassitude and release of CCK after the ingestion of both high fat-low carbohydrate and low fat-high carbohydrate meals has been reported. The high fat midday meal produced significantly higher CCK, greater fatigue rating, and more sleepiness 3 h after the meal when compared to a high carbohydrate meal containing 7% protein (Wells et al 1997). These findings have been confirmed in a study where CCK

infusion induced sedation in both humans and animals independent of insulin release (Fara et al 1969; Stacher et al 1979; Kapas et al 1991), A high fat-low carbohydrate and low fat-high carbohydrate lunch produced drowsiness, feelings of uncertainty and confusion when compared to a medium fat-medium carbohydrate lunch (Lloyd et al 1994). A low-carbohydrate high-fat diet given long-term to trained athletes produced adverse responses causing feelings of depression, increased tension, anger, and decreased vigor compared to a high carbohydrate-low fat or medium carbohydrate -medium fat diet (Keith et al 1991).

Decreased mood is demonstrated after fat ingestion. The suggested mechanisms for this include release of CCK (Wells & Read 1996) and the satiating potency of fat, which decreased extracellular hypothalamic serotonin (Orosco et al 2002). The timing of meals affects postprandial mood and behaviour. The high fat-low carbohydrate meal ingested in the morning exerted a greater depression on alertness and mood 3 h after the meal (but no effect 1 h after the meal) in comparison to other type of meals (Wells & Read 1996). This difference was not observed when meal was ingested at midday (Wells & Read 1996). Circadian body temperature dip and its associated decreased alertness and mood (Monk 2005) may be a confounding factor in food studies evaluating their effect on sleep. For

example, sleepiness following a midday meal may be linked to the circadian post-lunch dip (Wells et al 1998; Monk 2005) rather than the composition of the meals per se. Similarly, sleepiness observed following a meal ingested close to bedtime may be related to subjective feeling of sleepiness at the subjects' usual bedtime (Folkard & Barton 1993). On the other hand, meals that are administered during the forbidden zone may not alter sleepiness due to high alertness during this period (Folkard & Barton 1993).

High protein meals, mood, and behavior: Limited studies have examined the effect of high protein meals on sleepiness and mood. However, both high protein (55%) and high carbohydrate (55%) lunch meals containing the same amount of fat (30%) produce similar postprandial effects of lassitude, with feelings of feebleness and slow mental performance (Smith et al 1988). A high carbohydrate meal releases insulin and a high protein meal also releases insulin through CCK secretion. Both systemic CCK and insulin have been implicated in the mediation of postprandial sleepiness (Kapas et al 1991; Kapas et al 1993). A high protein meal lowers the plasma Trp ratio and probably serotonin release (Wurtman et al 2003).

6. The Atkins' diet

The diet was introduced by Dr. Atkins in 1976 (Atkins 1992). The Atkins' diet consisted of high protein-high fat and low carbohydrate. The diet is popular because of its efficacy in weight reduction in the short-term (Foster et al 2003). The Atkins' diet induces a state of ketosis and promotes lipid oxidation (Foster et al 2003). Despite unrestricted protein and fat in the diet, there is a decrease in the energy intake induced by the Atkins diet. The mechanism responsible for the decreased energy intake may be related to the monotony or simplicity of the diet and changes in plasma or central satiety factors (Foster et al 2003). By-products of this diet include β -hydroxybutyrate, acetoacetate, and acetone (Kossoff 2004), together known as ketones

A diet similar to the Atkins' diet is the ketogenic diet. It restricts carbohydrate intake with only 5-10 g of carbohydrate per day. The remainder of the energy comes from long-chain triglycerides. The ketogenic diet includes 80% fat, 15% protein, and 5% carbohydrate, whereas the Atkins diet contains 60% fat, 30% protein, and 10% carbohydrate (Kossoff 2004). This amount of fat in a diet is higher than that recommended by the "The National Cholesterol Education

Program”, which suggests that the total daily fat intake should not exceed 25-35% of total calories (Tapper-Gardzina et al 2002).

Both the Atkins’ diet and the ketogenic diet cause constipation, halitosis and ketosis (Kossoff 2004). Other side effects that are relatively less common are kidney stones, growth inhibition (Kossoff 2004), hyperlipidaemia (Tapper-Gardzina et al 2002; Kossoff 2004), insulin resistance, and risk of other chronic conditions and some cancers (Tapper-Gardzina et al 2002; Bravata et al 2003). The diets also affects behaviour, mood and causes hypoglycemia (Atkins 1992). Hypoglycemia is defined as blood glucose level < 3.5 mM (Bolli 1998). However, sleep pattern changes such as slowing of the EEG pattern with increasing SWS has been reported only during severe hypoglycemia (Howorka et al 1996), and a glucose threshold of ≤ 2.6 mM was required for slow wave changes (Harrad et al 1985).

The ketogenic diet administered to adolescents with morbid obesity ($>200\%$ of ideal body weight) for 20 weeks resulted in a significant weight loss. Weight reduction in these individuals, in whom SWS was augmented and REM sleep diminished, led to a normalization in their sleep pattern (Willi et al 1998). These sleep changes have also been

observed in weight loss and starvation (MacFadyen et al 1973). Additionally, the ketogenic diet has been administered to patients with narcolepsy. These subjects went on the ketogenic diet for 8 weeks and showed a significant improvements on the Narcolepsy Symptom Status Questionnaire-Total score, but not in Epworth Sleepiness Scale (ESS) and Stanford Sleepiness Scale scores (Husain et al 2004).

7. Summary and discussions

The literature indicated that macronutrients have an influence on sleep and behavioral responses. In particular, a carbohydrate meal suppresses SWS, increases REM sleep and serves to enhance mood through an increase in serotonin levels. It is noted that high GI carbohydrate has the ability to increase serotonin through its insulin response. It is possible that the hypnotic effect of carbohydrate may be mediated by serotonin. However, the direct effect of a high GI carbohydrate meal on sleep has not been investigated and it represents the work undertaken in this thesis. Since the proportion of protein in a carbohydrate meal has a negative influence on serotonin synthesis, we have included minimal protein content in the GI carbohydrate meals used in this investigation to

minimise its confounding effects on sleep.

The fat component of a diet increases subjective sleepiness. This may be mediated by the CCK released from the intestine. Proteins also stimulate the release of CCK. The popular Atkins' diet is effective in weight reduction despite a number of side effects such as halitosis, constipation and ketosis. The diet, with a high fat, high protein and restricted carbohydrate content has not been evaluated for its effects on sleep or systematically documented for its effects on mood, fatigue or sleepiness. Thus, this thesis examined its effects on sleep patterns and behavioural responses.

8. Hypotheses

Study 1 (chapter 3):

Hypothesis

It was hypothesised that carbohydrate-based high-GI compared with low-GI meals ingested 4 h before bedtime would improve sleep quality and that the timing of the high-GI meal (4 h compared with 1 h) before bedtime would also influence sleep quality.

Rational and Significance: If it can be shown that an early evening high

GI carbohydrate meal compared to a low GI meal improves the quality of sleep, then subjects with poor sleep could be advised to consume an early high GI meal prior to bed. If it can be demonstrated that 4 h prior to bed is an appropriate time for a high GI meal, which influences the quality of sleep of normal subjects, then the manipulation of the evening meal composition and timing could potentially offer a convenient approach to promote sleep for subjects with sleep disturbance (e.g. insomnia).

Study 2 (chapter 4 and 5):

Hypothesis

1. The Atkins diet in the short-term has an effect on the sleep pattern of healthy subjects compared to a normal mixed meal.
2. The Atkins diet in the short-term affects mood, fatigue and sleepiness of healthy subjects compared to a normal mixed meal.

Rational and Significance: The Atkins' diet has been used widely for weight loss for the past 40 years, but no study has addressed its effects on sleep, mood and fatigue. The current study provides new empirical data that will add to the existing knowledge in this field.

Chapter 2

METHODOLOGY

Methodology

1. Research design and methods

Study design:

“High-glycemic-index (GI) carbohydrate meals shorten sleep onset”

This study employed a randomized, cross-over repeated measures design in healthy good sleepers.

“Effect of the Atkins diet on sleep pattern, behavior and dream recall of healthy good sleepers”

This study employed a repeated measures design on sleep, behaviour and dream recall in healthy good sleepers.

Estimation of daily energy requirement: 3-day Food diary:

Prior to meal plans, the daily energy requirement of potential participants were first estimated via a 3-day food recall exercise. For this purpose, 5 subjects, aged 18-25 y, 71.8 ± 6.6 kg, who engaged in moderate physical activity) were recruited. On the basis of their 3-day food diary, the daily

energy requirement was calculated as 10450 ± 1020 kJ (Appendix 1.1). Accordingly, for subjects weighing between 65-75 kg, the test meals were calculated to contain 3212 kJ (768 kcal) for the GI study, whereas it included 4563 kJ (1090 kcal) for the Atkins' diet (Appendix 5.1, 5.2, 5.3).

The Recruitment Process:

Flyers were placed on notice boards on the Cumberland campus of Sydney University and in the local news papers. The flyer contained brief information about the study.

Following ethics approval by the University of Sydney Humans Ethics committee, healthy subjects were screened via a telephone interview and by using a medical questionnaire prior to recruitment.

Inclusion criteria:

- Volunteers 18-35 y
- Sedentary males, BMI 20-27 kg/m²
- Non-smoker
- Willing to eat the meals provided in the study
- Regular sleeper (evaluated through a 2-week sleep diary).

Exclusion criteria:

- Insomnia, depression, sleep disorders, nocturnal eating and active people (vigorous exercise, 3 times or more per week)
- Vigorous exercise 24 h prior to a sleep study
- Consumption of >20 g (2 standard alcoholic drinks/d on a regular basis)
- Use of sedative medication
- Evidence of chronic illness (severe medical, psychiatric or nutritional disorders), cardiorespiratory diseases, gastro-esophageal reflux
- Evidence of food allergies or intolerances
- Excessive caffeine consumption of more than 400 mg a day and smokers.
- Circadian disorders (frequent naps, shift work).

General protocol:

Subjects recruited for the study completed a medical evaluation questionnaire (Appendix 1.2), a 2-week sleep diary (Appendix 1.3), consent forms (Appendix 3.1 & 3.2) and participants' information statements (Appendix 4.1 & 4.2). All subjects underwent a familiarization night, where a full polysomnography was conducted for the purpose of adaptation and screening for sleep-related disorders (details to follow in chapter). Sleep studies were conducted at the Delta Sleep Research Unit, located on the Cumberland campus in Lidcombe,

The University of Sydney. The room temperature was set on 22°C and subjects wore their usual sleep wear during all test nights. The lighting (600 Lux) in the sleep laboratory remained the same throughout all studies. The bedrooms in the laboratory were kept dark during the sleep period.

2. Likert Scales

These scales were used to assess meal pleasantness, hunger and fullness and sleepiness (Appendix 6.1 – 6.3). The Likert scale was in several intervals with two extreme descriptors for each variable of interest (Likert 1932).

Pleasantness of the meal:

Pleasantness was rated on a 5-point scale from zero ‘not at all pleasant’ to +4 ‘extremely pleasant’ (Appendix 6.1). This scale was administered following ingestion of the high and low GI meals.

Hunger and fullness:

Hunger and fullness were rated on a 5 - point scale from zero ‘very

hungry' to +4 'very full'. This scale was administered after the test meals and at bedtime (Appendix 6.2).

Sleepiness:

The subjective feeling of sleepiness was rated on a four - point scale from zero 'not at all sleepy' to +3 'very sleepy' (Appendix 6.3). Subjects assessed their sleepiness at ½, 1, 2, 3, and 4 hours after each test meal.

3. Polysomnography

Sleep scoring:

Option for electroencephalogram (EEG) signal filtering was applied where required at an EEG low pass of 30 Hz, a high pass of 0.5 Hz, and a Notch filter at 50 Hz prior to sleep scoring. Sleep studies were scored at 30-s epochs according to the standard scoring criteria (Rechtschaffen & Kales 1968) by an expert sleep physiologist who was blinded to the treatments. Sleep recordings were evaluated for variables of sleep onset latency (SOL); rapid eye movement latency (ROL); sleep efficiency (SE); arousal index (no./h); sleep stages 1, 2, 3, and 4; non-rapid eye movement (NREM); rapid eye movement (REM); total sleep time (TST) and total wake time (TWT). Based on S-series Replay V2 User Guide

(Compumedics 1999), the following definitions were applied to evaluate sleep recording.

Sleep Onset Latency (SOL): The time from lights out to the start of the first three consecutive sleep epochs.

Rapid Eye Movement Latency (REM): The time from sleep onset to the first REM epoch.

Sleep Efficiency (SE): $100 \times (\text{TST} \div \text{Time available for sleep})$.

Start of Sleep Period: The start time of the first three consecutive sleep epochs.

End of Sleep Period: The end time of the last sleep epoch.

Total number of awakenings: Sum of the number of awakenings. The arousal index refers to the number of scored arousals (all types) per hour of study time.

Stage 1 Sleep (S1): The sum of all stage 1 sleep time within the sleep period.

Stage 2 Sleep (S2): The sum of all Stage 2 sleep time within the sleep period.

Stage 3 Sleep (S3): The sum of all stage 3 sleep time within the sleep period.

Stage 4 Sleep (S4): The sum all stage 4 sleep time within the sleep period.

Non Rapid Eyes Movement Sleep (NREM): The sum of all REM time within the sleep period.

REM Sleep: The sum of all REM sleep time within the sleep period.

Total Sleep Time (TST): The sum of REM and NREM time within the sleep period.

Total Time Awake During Sleep Period: The total time of awake epochs within the sleep period.

Total Sleep Period (TSP): End of sleep period – Start of sleep period.

Artifact (unscorable): Total time scored as artifact.

Electrode placement:

Electrical signals obtained from the scalp, eye movements and from the chin muscles enable objective measurement and scoring of sleep stages and arousal from sleep. A standard International 10-20 electrode placement (Rechtschaffen & Kales 1968) was applied at 30 minutes prior to bedtime. The electrodes occupied the temporal C3/A2 position (Figure 1.1) and occipital O2/A1 position. The scalp was prepared first using an abrasive gel, Nuprep, to exfoliate the skin and then an alcohol wipe (Medi-Swab 70% Isopropyl alcohol) to clean off skin oil. A conductive electrode gel (Signal-gel, Parker) was applied to the scalp. The electrode cups were filled with EC2 (Austro-Med Inc, GRASS) electrode cream, and secured on to the scalp by way of a gauze square. The left electrooculogram (EOG) electrode was placed 1 cm below and 1 cm laterally to the outer canthus of the left eye, and the right EOG was placed 1cm superior and 1 cm laterally to the outer canthus of the right eye by the way of adhesive electrodes (Figure 1.2). Two adhesive electrodes were attached underneath the chin in the location of the left and right *depression anguliori* muscles to record chin electromyogram (EMG). The ground electrode was attached on forehead. All electrode leads were collected at the signal box and connected to respective amplifiers and then to the Compumedics S-series Sleep system

(Compumedics Ltd, Melbourne, Australia).

Respiratory and leg movement signals:

Respiratory signals (airflow and respiratory effort) were measured using a thermistor probe (airflow sensor) and abdominal and thoracic inductive respiratory bands respectively. Oxygen saturation was measured via a Pulse Oximeter. Respiratory events were scored according to the ASDA (American Sleep Disorders Association 1992) guidelines. These measurements of respiratory and leg movements signals were conducted only on the familiarization night for exclusion of subjects with sleep breathing disorders and restless legs respectively.

Sleep Recording and Monitoring:

On study nights, sleep EEG, EOG and EMG only were recorded. During each sleep study, prior to lights out, a biological calibration was conducted to check for signal quality and artifacts. An impedance check (<10K) ensured that electrical signals fell within physiological range. All sleep parameters were recorded continuously from lights out at subjects usual bedtime until spontaneous waking (sleep *ad libitum*).

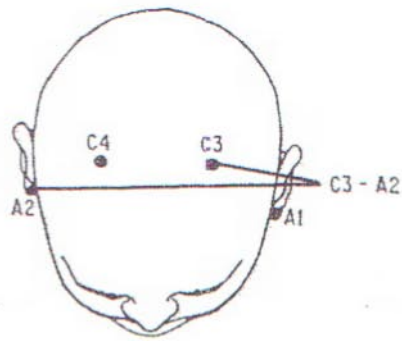


FIGURE 1.1. 10%-20% electrode placement system

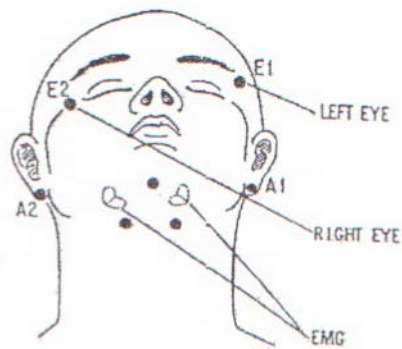


FIGURE 1.2. EOG and EMG electrode placement