A Pilot Investigation into the Effect of a Micronutrient Supplement on Symptoms of Insomnia in an Adult Population; A Multiple Baseline Design

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Abstract

Insomnia is a debilitating condition that can cause severe psychological distress and is frequently comorbid with other mental health conditions. Although psychological treatment is effective, it is hindered by cost and availability, while hypnotic sleep medications are recommended for time-limited use and can impair day-time functioning. For these reasons, investigation into alternative treatment options for sleep difficulties is necessary. The present study examined the effect of a multi-ingredient micronutrient formula called Daily Self Defense (revised formula of EMPowerplus) on adults suffering from insomnia; following a multiple baseline design. The final sample comprised 14 participants, aged 18 years or older, who were randomised into one of three baseline groups; ranging from one to three weeks in length. Following the baseline phase, participants took part in an open-label trial of Daily Self Defense for eight weeks; after which a three month follow-up was conducted. Although there was a trend towards a small improvement during baseline phase for some measures, there was much greater improvement during the intervention phase. Time series graphs and Modified Brinley plots revealed decreases in insomnia severity, depression, stress and anxiety for 10 out of 12 participants who completed the intervention phase. Cohen's *d,* and the percentage of participants showing reliable positive change, confirmed moderate or large effect sizes for all outcome variables. Furthermore, of 12 participants who completed the intervention phase, all were compliant with taking the capsules, and any side effects experienced were mild and transitory. This study provides evidence for the potential of micronutrient interventions in effectively treating insomnia in adults. It also indicates the need for future research utilising placebo-controlled designs as well as comparisons to current available treatments.

1. Introduction

1.1 Background to Insomnia

Chronic insomnia is a condition estimated to affect between 12 to 15% of the adult population, and as many as 50% of those in primary health care settings (Blampied & Bootzin, 2013). A national survey found that 13% of New Zealanders aged 20-59 years are affected by insomnia (O'Keeffe, Gander, Scott, & Scott, 2012), and that Maori are affected disproportionately with a prevalence of 19.1% compared with 8.9% for non-Maori. Insomnia is a debilitating condition that causes severe psychological distress, depression, physical and mental illness, impaired functioning and loss of focus and productivity (Bootzin & Epstein, 2011). As humans, sleep is vital to our functioning and well-being. The cognitive processes and neural activity that occur during sleep are necessary for appropriate brain growth and function, and a lack of quality sleep can play havoc with an individual's health, mood, emotions and energy. Good quality sleep, of an adequate length, is vital for the functioning and well-being of the human brain. Unfortunately, insomnia, or the inability to regularly obtain sleep of sufficient frequency, length and quality, is the most common sleep problem among adults and one of the most prevalent health problems faced in the 21st century (Ancoli-Israel & Roth, 1999). Its prevalence increases with age, and women are more likely to be affected than men , at a rate of 24% vs. 14% (Touitou, 2007).

The core complaints of individuals with insomnia are that they have difficulty initiating sleep, they wake often during the night, or they have poor sleep quality that is unrefreshing. Insomnia can last for episodes of several days, several weeks, or chronic episodes of months and years (Rothenberg, 1997). The Diagnostic Statistical Manual of Mental Disorders – Fifth Edition (APA, 2013) requires that insomnia is characterised by difficulty initiating or maintaining sleep, or early morning waking with the inability to return to sleep, that occurs at least three nights per weeks and has been present for at least three

months. This sleep disturbance (or associated daytime fatigue) causes clinically significant distress or impairment in social, occupational, or other important areas of functioning.

1.1.1 Aetiology of Insomnia. While the exact causes of an individual's insomnia may vary, the majority of research identifies heightened arousal, anxiety, worry and rumination, as being leading factors in the cause and maintenance of sleep disruption (Bootzin & Epstein, 2011). Daytime events, especially those that are emotionally stressful have been found to impact sleep quality and wellbeing. These are thought to do so through triggering cortical and emotional arousal which in turn disrupts sleep pattern, also shortening the amount of REM sleep, which is thought to play a role in regulating emotion (Wuyts et al., 2012). Several psychological and physiological aspects are thought to contribute to the onset and maintenance of insomnia; anxious-ruminative traits, life stressors, homeostasis weakening mechanisms, menopause, and biologic or genetic central nervous system hyper-arousal have all been identified as factors that can be involved (Basta, 2007).

The most widely accepted model for the development and maintenance of insomnia comes from the 3-P's model proposed by Spielman (1991), suggesting that three distinct elements contribute to the onset and course of insomnia. The model indicates that predisposing factors such as acquired or inherited characteristics can make an individual more susceptible to develop a sleeping disorder. It suggests that precipitating events or life stressors such as health problems, family or marital conflict, and work or school stress can trigger the onset of insomnia, and that dysfunctional attitudes and behaviours around sleep can perpetuate and maintain these sleep difficulties. According to this model, insomnia may become independent of its initial cause over time (Basta, 2007).

Traits such as hyper- arousal, or hereditary genetics can influence an individual's vulnerability for developing insomnia (Bootzin & Epstein, 2011). Genetic studies have

identified genes that are likely to be important in the regulation of circadian rhythms and henceforth influence the time of sleep onset and waking (Taheri & Mignot, 2002). Hyper-arousal, particularly cognitive hyper-arousal, has been identified as a particularly significant vulnerability factor, and those with insomnia have been found to have higher levels of cognitive arousal and feel less sleepy in the bedroom than those who report normal sleep (Robertson, Broomfield, & Espie, 2007).

Neuroimaging studies have found that individuals with insomnia show a pattern of increased subcortical brain activation during sleep, and decreased prefrontal cortical activation when awake (Nofzinger et al., 2004). In a study comparing individuals with insomnia to healthy participants, those with insomnia showed a greater global cerebral glucose metabolism during sleep and while awake. They also showed a smaller decrease in relative metabolism between waking to sleep states, and reduced relative metabolism in the prefrontal cortex when awake (Nofzinger et al., 2004). These differences may be due to a failure of arousal mechanisms in insomniacs which are responsible for the reduction in activity from waking to sleep states.

Changes to circadian rhythm and homeostatic processes have also been identified as potential causal mechanisms for sleep difficulties. These processes regulate sleep and wake cycles and a dysregulation to them may cause an imbalance in time spent awake and asleep. The circadian clock, which is located in the nucleus of the hypothalamus, uses environmental cues such as light, routine, food consumption and social interaction to regulate circadian rhythms. Sleep behaviours that are developed in response to insomnia, for example stimulating night time activities or food consumption, may interrupt the environmental cues the circadian clock requires to regulate a healthy sleep-wake cycle (Thacher, Pigeon, & Perlis, 2006).

Precipitating factors including family, work, school and ill health are all stressors that may affect an individual's sleep (Bootzin & Epstein, 2011). Highly stressful life events have been found to be closely associated with the onset of insomnia. The impact of these stressors is mediated by certain predisposing personality traits, individuals with insomnia (compared to controls) have been found to overall be more discontent (both as children and adults), rate their interpersonal relationships as less satisfying, have poorer self-concept, and have maladaptive coping skills for dealing with stress (Basta, 2007).

The way in which an individual responds to these stressors and to sleep difficulties themselves, in the form of rumination and worry, can cause an acute sleep problem to become a chronic condition. Dysfunctional or maladaptive cognitive processes, including negative cognitions around sleep, can both exacerbate and perpetuate insomnia symptoms (Belanger, Savard, & Morin, 2006). A significant feature of those who suffer from chronic insomnia is the frequent presence of intrusive and worrisome thinking, which is thought to both cause and maintain insomnia symptoms (Morin, Stone, Trinkle, Mercer, & Remsberg, 1993). A study examining the role of beliefs and attitudes about sleeping in 145 adults, found that those who suffered from insomnia endorsed stronger beliefs about the negative consequences of poor sleep, expressed more hopelessness in regards to fear of losing control over their sleep, and expressed more helplessness about its unpredictability. The findings suggest that some beliefs and attitudes towards sleep may be playing a role in the perpetuation of insomnia symptoms (Morin et al., 1993).

Behaviours around sleeping have also been found to play a role in the maintenance of sleep difficulties. Extended time in bed, irregular sleep-wake schedules, daytime napping, and non-sleep behaviours in the bedroom environment (using technology, working) have all been identified as possible factors that may contribute to and maintain insomnia (Bootzin & Rider, 1997).

1.1.2 Adverse effects on health. While there is still much to be understood about the nature of sleep processes, it is evident that the cognitive processes, neural activity and metabolism that occur during this state are vital to the healthy growth, nurture and maintenance of the brain (Hobson, 2005). Disturbed sleep, whether due to quality, timing or duration, can cause many adverse health concerns. The most obvious are fatigue and impaired cognitive function, but mood is also greatly affected (APA, 2013). Short sleep duration has is also a risk factor for increased body mass, metabolic dysfunction, type 2 diabetes and hypertension (O'Keeffe et al., 2012).

An individual not obtaining enough quality sleep suffers severe negative consequences in mood, cognitive function, memory, emotion regulation, attention, energy, productivity and physical health. When co-morbid with other psychological conditions (which it often is), insomnia has a strong impact on an individual's quality of life and on their health. Individuals with insomnia are likely to take more medications, need more healthcare resources, be absent from work due to illness more often, and have more work-related and motor vehicle accidents (Ancoli-Israel & Roth, 1999; O'Keeffe et al., 2012). In New Zealand, the estimated annual savings associated with effectively treating all people with insomnia between ages 20-59, is $21.8 million (O'Keeffe et al., 2012).

The processes of sleep and circadian rhythms determine daily physiological patterns and have an important impact on metabolic health (Depner, Stothard, & Wright, 2014). Insufficient sleep associated with insomnia can contribute to the dysregulation of the metabolic system and can contribute to weight gain, obesity and type 2 diabetes through altering the timing and quantity of food intake. Sleep deficiency associated with metabolic dysregulation can also lead to the disruption of energy balance, increase inflammation, impaired glucose tolerance and insulin sensitivity (Depner et al., 2014)

Sleep disturbance is so interrelated with a person's overall functioning that it is considered 'trans-diagnostic' in that it significantly increases the risk for co-morbid disorders, including anxiety and depression (Harvey, Eidelman, & Talbot, 2008; Sarsour et al., 2010). Insomnia is repeatedly found to occur with a range of psychological disorders, and those with insomnia report more day time symptoms of depression and anxiety than good sleep control subjects (Morin et al., 2006) .

Depression is over-represented among populations with sleep disorders (O'Keeffe et al., 2012). Even though sleep difficulties are a symptom of depression, insomnia or sleep problems sometimes appear prior to an episode of depression. There are bi-directional associations between insomnia and depression that can make it hard to differentiate cause and effect interactions between the two disorders (Franzen & Buysse, 2008). Studies using longitudinal data have shown that insomnia is a risk factor for developing depression and that the combination of insomnia and depression increases episode severity and duration of depression(Franzen & Buysse, 2008).

The impact of sleep disruption on mood has been found across many studies. In an epidemiological study conducted in Switzerland, subjects were interviewed six times over the course of 20 years, and it was found that those who had experienced insomnia for two weeks or longer had a higher risk for later developing depression (Franzen & Buysse, 2008).

In a pilot study examining the relationships between sleep deprivation on sleepiness, affect and psychomotor vigilance, sleep deprivation had a significant impact on all three domains (Franzen, Siegle, & Buysse, 2008).

Sarsour and colleagues (2010) conducted a study investigating the associations between insomnia severity and depression. Five hundred and forty one participants suffering non-restorative sleep were compared with 717 subjects who had never experienced sleep difficulties, and non-restorative sleep was found to significantly impact emotional function.

In a study investigating the impact of sleep deprivation on aspects of affective functioning in healthy adolescent and adults, it was found that lack of sleep resulted in less positive affect compared to rested controls, on nine out of twelve positive affect items. Participants who were deprived of sleep also reported a greater increase in anxiety during a catastrophizing task and rated the likelihood of potential catastrophes as more likely when sleep deprived compared to the rested condition (Talbot, McGlinchey, Kaplan, Dahl, & Harvey, 2010).

There is a close relationship between emotional and mental disorders, daily stress, and sleep disorders, which must be recognised to increase the effectiveness of interventions (Rothenberg, 1997). The frequency of sleep complaints in the general population, the variety of causes, and the significant overlap between the complaints of patients with sleep disorders and the complaints of patients with mental disorders, makes it an extremely important area of continuing research in the mental health field (Rothenberg, 1997).

1.2 Current Treatments

1.2.1 Behavioural and Psychological Interventions. Behavioural treatments for insomnia target appropriate sleep education, hygiene, and cognitive process that inhibit sleep. It is thought that sleep difficulties may arise because appropriate sleep hygiene behaviour or stimulus control have not been established (Blampied & Bootzin, 2013). Stimulis control therapy (SCT) is often used as a treatment for insomnia and involves ensuring that stimuli in the bedroom encourage sleep, rather than disrupt it; for example limiting use of internet, televisions or computers in the bedroom (Haynes & Bootzin, 2010).

Patients can benefit from many behavioural treatments including restructuring their bedtime habits and sleep-wake schedules, restricting time in bed, and relaxation training. A vast majority of insomnias can be reduced to some extent by adhering

to sleep hygiene rules such as keeping regular sleep hours, going to bed only when sleepy, exercising regularly later in the day, avoiding stimulants near bedtime, and minimising light, noise, and extremes in temperature in the bedroom (Rothenberg, 1997). In several sleep clinic settings, time and cost constraints ensue that clinicians are required to provide treatment suggestions for patients that they will see only for a few sessions, thus, many of their recommendations focus on sleep hygiene (Bootzin & Rider, 1997). Although sleep hygiene is an important factor in treatment and must not be over looked, there are limitations to what it can achieve. A meta-analysis of 59 treatment outcome studies, evaluating non-pharmacological treatments for insomnia, found that sleep hygiene as a single intervention was not effective, and that stimulus control and sleep restriction were the most effective behavioural treatments (Morin, Culbert, & Schwartz, 1994).

Sleep hygiene, education and stimulus control are regarded as necessary but not sufficient, in the effective treatment of sleep disorders. Other treatment techniques focus on reducing the individual's levels of stress and arousal, through relaxation, meditation and progressive muscle relaxation. Cognitive behaviour therapy (CBT) is used to target the maladaptive beliefs, mind-racing, and ruminative worry, thought to contribute to sleep difficulties (Haynes & Bootzin, 2010; Morin et al., 2006).

The role of emotional, behavioural and cognitive factors in insomnia is receiving increasing recognition and resulting in the increased use of CBT for the management of insomnia (Belanger et al., 2006). CBT for insomnia focuses on reducing sleep-incompatible behaviours, managing arousal, and restructuring sleep-related dysfunctional cognitions. CBT has been studied in extensive controlled evaluations and has become the treatment of choice among psychological treatments for insomnia (Morin et al., 2006). Controlled clinical trials show that 70-80% of patients with primary insomnia benefit from CBT intervention and that these improvements are sustained over time (Belanger et al., 2006; Morin, Colecchi, Stone,

Sood, & Brink, 1999; Morin et al., 1994; Morin, Hauri, et al., 1999; Murtagh & Greenwood, 1995).

Although cognitive behavioural interventions can produce effective and sustained results, they are largely self-directed treatment approaches and rely heavily on the individual to maintain practice of techniques and behaviours in their own home. Cognitive and behavioural therapies also rely on the individual's access to a clinical therapist which can be both costly and timely and, given the lack of publically funded therapy, it is not always available to all members of the population.

1.2.2 Pharmacological Interventions. While behavioural and cognitive approaches, when used together, can effectively treat insomnia, pharmacological treatment is still the most widely available form of treatment. A wide range of medications and non-prescription treatments are available that exhibit their effect through the action of sedation. The most commonly prescribed are those that affect the GABA neurotransmitter such as benzodiazepines which are anxiolytic, and newer "z" hypnotics such as zolpidem, zaleplon and zopiclone. These medications selectively affect the GABA neurotransmitter and have a hypnotic or sedating effect, or both (Bootzin & Epstein, 2011).

There are several limitations to the pharmacological treatment options available; hypnotics often cause daytime impairment and sedation due to their half-life, which interferes with a person's daily functioning, energy and mental clarity (Sateia & Nowell, 2004). Furthermore, benzodiazepines and hypnotics are only safe and effective for short term use, as after this a tolerance develops and the therapeutic effect of the drug wears off, benzodiazepines are also highly addictive and the user may find themselves suffering severe withdrawal upon discontinuation of the drug. Both classes of drug can cause a rebound effect with termination of use, often causing insomnia that worse than it was to start with (Sateia & Nowell, 2004). As well as significant side effects, there are limited trials demonstrating the

efficacy of these medications in successfully treating insomnia long term (Krystal et al., 2010).

Benzodiazepines and benzodiazepine-like agents (the 'Z' drugs) are the most widely used hypnotics (Touitou, 2007); however, their usefulness is limited to episodic insomnia of short duration (less than 4 weeks) as after this time their therapeutic effect wears off and with continued use, addiction can develop. They can cause serious adverse effects including daytime somnolence which increases the risk of road accidents and falls (Touitou, 2007).

Once a patient has been treated with a sleeping medication, it can be difficult for them to terminate use. Benzodiazepine withdrawal symptoms have been well documented and can often last as long as 4 or 5 weeks following discontinuation (Rothenberg, 1997). Withdrawal effects appear to be related to the dose taken and the length of time that the hypnotic is taken for, therefore long term treatment of insomnia with hypnotics is not appropriate (Rothenberg, 1997). Although the benzodiazepine-like "Z" drugs were once considered safer than benzodiazepines, the recent research has revealed similar negative effects (Brandt & Piechocki, 2013).

While hypnotic medication may be useful for short term use in conjunction with non-pharmacological interventions or to help break an acute cycle of sleeplessness and distress, behavioural strategies are more appropriate as long-term solutions (Rothenberg, 1997).

Although the pharmacological approach to treatment has remained the most commonly used for decades, the widespread concern about long-term effectiveness, habituation, tolerance and the potential complications of these medications (Sateia & Nowell, 2004) makes the search for other options most desirable. Given that insomnia is a distressing and debilitating condition, for which behavioural and cognitive therapies are not always funded or accessible, and psychopharmacological interventions are not safe as long term treatment options, it seems necessary to continue searching for safe, effective and accessible

treatment choices. One avenue that is worthy of exploration is the use of micronutrients as a treatment for insomnia. There is increasing scientific interest, both internationally and in NZ, in the use of complementary and alternative medicines (CAM) for the treatment of mental health conditions. In particular, micronutrient formulas have been receiving significant attention for their use in treating symptoms of psychiatric disorders, including bipolar disorder, anxiety, depression and ADHD. Even though almost 90 years of scientific literature is behind the importance of dietary nutrients for mental health (Kaplan et al. 2007), less is known about these micronutrient formulae than conventional medications.

1.3 Association between Micronutrients and Mental Health

It has long been known that nutrition plays an important part in mental health and wellness. As far back as antiquity this concept was recognised, demonstrated by Socrates famous quotation; "let food be thy medicine and medicine be thy food". In 1910, the People's Home Library, which provided in home medical guidance at a time in history when it was not always possible to visit the doctor, informed families that 'insanity' was in fact caused by inadequate or imperfect nutrition (Ritter, 1910). Although knowledge of the importance of nutrition in mental health dates back as far as the ancient Greeks, the introduction of psychiatric medications in the 1950's brought about a shift in research and treatment focus, towards a preference for drug based treatments and interventions. Our current health systems reflect this shift towards medication and drug based treatments, and in so doing, has lost sight of much of the evidence surrounding the importance of nutrients for optimum brain health and function.

Micronutrients, or vitamins and minerals, play an integral role in both physical and mental health. They are involved in many brain functions including the synthesis of neurotransmitters and the energy metabolism of nerve cells (Haller, 2005). They also play a

role in neuronal receptor binding of neurotransmitters, and help preserve the integrity of axons and their myelin sheaths (Haller, 2005). Although the brain accounts for only 2% of an adult's entire body weight, it accounts for 20% of its resting metabolic rate. The human brain has a limited ability to store nutrients, and cannot decrease its need for energy when the supply of nutrition is limited, therefore it needs an almost continuous supply of energy brought to it through the blood-brain barrier. B-vitamins support this energy supply to the brain through the metabolism of glucose (Haller, 2005).

Deficiencies in certain vitamins are known to be the cause of malfunction in the brain, and can result in a diverse range of neuropathology and neuropsychiatric symptoms (Bémeur, Montgomery, & Butterworth, 2011). They have the potential to cause changes in brain function and behaviour, and have even been found to influence personality and mood (Haller, 2005). The most common deficiency disorders are those involving the group B vitamins which can result in disorders such as Korsakoff syndrome and pellagra (Kaplan, Crawford, Field, & Simpson, 2007).

When we examine the complex role that each nutrient plays in integral brain functions, it makes sense that they have such on impact on our mental health and wellbeing. Kaplan and Colleagues (2007) provide a summary of the role each individual nutrient plays within brain processes. Vitamin B9 (folate) is involved in the synthesis of monoamine neurotransmitters and in serotonin and dopamine systems in the brain. It functions as a cofactor for enzymes that convert tryptophan into serotonin and can heighten serotonin function by slowing the destruction of brain tryptophan (Cousens, 2000). Vitamin B12 (Cobalamin) is involved in the synthesis of many neurotransmitters, as well as assisting to maintain myelin sheaths on nerves resulting in healthy nerve conductance (Hutto, 1997). Vitamin B1 (Thiamine) assists with the synthesis of acetylcholine, GABA and glutamate (Bell et al., 1992). Vitamin B6 (Pyridoxine) plays a basic role in the production of many

neurotransmitters and a deficiency can result in a reduction of brain production of serotonin (Kaplan et al., 2007).

Minerals also play an essential role in maintaining healthy brain function and mood. Calcium is an important cofactor for enzymes and assists with intracellular contact. An imbalance of calcium can result in anxiety, depression and cognitive dysfunction (Milne, 2000). Chromium plays a role in the metabolism of glucose and lipids and this may be responsible for its role in mood (Milne, 2000) . Iron is essential for the production of adenosine triphosphate (ATP), the energy source of mitochondria. Magnesium also assists in the production of ATP through metabolising carbohydrates and fats, as well as these functions, it is essential for more than 300 biochemical reactions in the body, including maintaining normal nerve function. Zinc is involved in the structure and regulation of gene expression and is the co-factor for over 200 enzymes, playing a role in virtually all aspects of metabolism (Milne, 2000). Selenium is an essential trace mineral which forms part of antioxidant enzymes that help to protect cells from the effect of free radicals (Kaplan et al., 2007).

1.4 Effect of Single Nutrient Interventions on Psychiatric Symptoms

Since the 1920's, most research that has been conducted on micronutrients has investigated the effect of single nutrient treatments *at a time* on psychiatric symptoms. Research has found a wide range of benefits using B-vitamin supplementation to treat psychiatric symptoms. In a case study where a patient was admitted to hospital with severe manic symptoms, complete normalisation of behaviour and mood was achieved after supplementation with vitamin B12, an effect that was maintained six months later with continued use of B12 (Goggans, 1984).

In a randomised controlled trial (RCT) conducted with patients with depression (n=24) or schizophrenia (n=17) deficient in folate, Godfrey et al. (1992) found that supplementation with 15mg folic acid (vitamin B9) daily, resulted in clinically significant improvement in the treatment group when compared to placebo after three and six months of treatment, and these group differences increased with time. Another RCT of 127 adults with depression taking fluoxetine, found that supplementation with folic acid greatly improved the therapeutic effects of fluoxetine (Coppen & Bailey, 2000).

Benton, Griffiths, and Haller (1997) conducted an RCT with 120 healthy female college students, and found that thiamine (vitamin B1) supplementation, when compared with placebo, was effective in improving mood, and that this effect was greater in those who had originally low level of thiamine. During the study, improvement in thiamine status was also associated with reports of feeling more "clearheaded, composed and energetic" (Benton et al., 1997).

In a case series of nine adults with schizophrenia and co-morbid depression, supplementation with pyridoxine (vitamin B6) was associated with markedly lower depression scores in two patients after four weeks of treatment (Shiloh, Weizman, Weizer, Dorfman-Etrog, & Munitz, 2001). A meta-analysis of nine RCT trials (n = 940) investigating the effect of pyridoxine on premenstrual syndrome in women found that supplementation with pyridoxine was more beneficial than placebo in reducing overall symptoms and particularly reduced symptoms of depression (Wyatt, Dimmock, Jones, & Shaughn O'Brien, 1999).

Lecithin (a form of phosphatidylcholine) was found, in a small RCT (within-subject crossover, n=6) to produce greater symptom remission than placebo for five patients (Cohen, Lipinski, & Altesman, 1982).

Research had also examined the effect of individual minerals on mental health (Kaplan et al., 2007). Thys-Jacobs, Starkey, Bernstein, and Tian (1998), found that in a RCT of 466 women with moderate to severe premenstrual syndrome, calcium carbonate reduced total symptoms by 48% compared with baseline, and was more beneficial than placebo. By the third month of treatment all four symptoms factors, negative affect, water retention, food cravings and pain, were significantly reduced.

Chromium, which plays an important role in the metabolism of glucose and lipids (Milne, 2000), has been used in several studies for its mood regulation properties. In a case series of five patients with dysthymic disorder, supplementation with chromium was found to produce symptom remission in all five patients (McLeod, Gaynes, & Golden, 1999). The authors subsequently conducted another case series, this time with eight patients with refractory mood disorders, and again found symptom remission in all patients (McLeod & Golden, 2000). In both studies, single-blind trials on several patients with alternative supplements confirmed the specificity of response to chromium. The authors hypothesise that the antidepressant effect of chromium may be due to its enhancement of insulin utilisation, which in turn increases the level of tryptophan available in the central nervous system (McLeod & Golden, 2000). An RCT (n = 15) of adults with atypical depression found that chromium produced significant symptom remission in 70% of the treatment group, compared with 0% in the placebo group (Davidson, Abraham, Connor, & McLeod, 2003).

Magnesium has been investigated as a therapy for the treatment of manic symptoms in bipolar disorder. Ten patients with severe and treatment-resistant mania were given intravenous magnesium sulphate in combination with their existing medications. Seven of the ten patients showed marked clinical improvement in symptoms, and doses of their current medications were able to be reduced after magnesium supplementation (Heiden et al., 1999). In an RCT of 20 adults with manic symptoms, magnesium was used in conjunction with

verapamil and compared to a placebo group who received verapamil and a placebo. Magnesium plus verapamil was found to produce significant improvement in manic symptoms, while placebo did not (Giannini, Nakoneczie, Melemis, Ventresco, & Condon, 2000). In another study, nine female patients with rapid-cycling bipolar disorder were treated in an open label trial using either a magnesium compound or lithium for a period of up to 32 weeks (Chouinard, Beauclair, Geiser, & Etienne, 1990). The magnesium was found to have clinical effects equivalent to lithium and seven of the nine patients showed a significant positive response.

Benton and Cook (1991) conducted an RCT with crossover design on 50 healthy adults. They found that supplementation with 100 mg selenium daily for five weeks was associated with significantly improved mood and was more effective than placebo.

Historically, the trend in nutrient therapy has tended to examine or test a single nutrient for efficacy in treatment. This rationale closely follows the principle of the medical model; that one problem should be solved by one ingredient or drug, with some promising, albeit modest, results. However, as our bodies are complex systems where multiple chemical reactions involving a wide range of micronutrients are continuously occurring, it makes more sense physiologically for us as humans to use multi nutrient supplementation to address malfunctions (Mertz, 1994).

Through the process of evolution we have come to require a wide range of nutrients in combination and in specific balance. With such complex brains it is important that we eat a varied diet to maximise the range of nutrients we are taking in. The way nutrients present naturally, in the form of food, combines several nutrients together, that often enhance each other, for example if calcium is taken with a small amount of magnesium and vitamin D the absorption is improved. In fact it is possible for treatments using single nutrients to cause an

imbalance and create deficiencies in other nutrients, for example if folate is ingested without vitamin B12 it can create a B12 deficiency in the body (Mertz, 1994).

In the last decade, a significant shift has been made in the research that is being published in major medical journals indicating the importance of using complex multi-nutrient formulas for treatment of mental health concerns. Where previously this multi-nutrient therapy was thought of as "confounded" and imprecise research, attitudes are starting to shift as it becomes obvious that to treat issues in an organ as physiologically complex as the brain, it is more beneficial to use a broad-spectrum supplementation approach (Kaplan & Leung, 2011). Studies are increasingly using treatments containing multiple ingredients, containing between 6-36 different nutrients (Kaplan & Leung, 2011), this research will now be discussed.

1.5 Effect of Combined Micronutrient Interventions on Psychiatric Symptoms

Recently micronutrients have been receiving increasing attention, both in New Zealand and internationally, as a treatment for supporting brain function and optimum mental health. During the 20th century, most nutrient interventions contained a single nutrient until the work of Bell et al. (1992), who found that a combination of vitamins B1, B2 and B6 enhanced the therapeutic effect of tricyclic antidepressants, in a study of 14 geriatric patients suffering from depression.

During the 21st century, the number of studies using broad-spectrum micronutrient formulas has increased significantly, with many studies examining formulas containing between 6-36 ingredients (Kaplan & Leung, 2011). Due to the complex nature of the human brain and body, this move towards multi-nutrient formulas makes physiological sense. In 1994, Walter Mertz drew attention to the fact that the 'one disease – one nutrient' concept was out of date, and more recently Burford-Mason (2009) stated that to treat health

issues with one vitamin at a time "breaks the basic laws of physiology". The author explains that no vitamin works alone, and instead works in conjunction with all the other vitamins, minerals, amino acids and essential fats. Approximately 40 essential nutrients are required to regulate and repair body tissue and continue the innumerable bodily processes that maintain our health, and a deficiency in any one of these can cause illness (Burford-Mason, 2009).

1.5.1 Depressed mood. Benton and colleagues (1995) explored the idea that supplementation with vitamins may influence mood. One hundred and twenty nine healthy adults took either a vitamin supplement containing nine vitamins, or a placebo, for one year. Female participants reported significantly improved mood at the end of 12 months, indicated by feeling "more agreeable", more composed and reporting better mental health. Although there was no significant change in mood or mental health in the male participants, they also reported feeling "more agreeable" by the end of the study (Benton et al., 1995).

Heseker et al. (1992) conducted an RCT with 1081 healthy men between the ages of 17-29 years. At baseline, those men who had chronic nutrient deficiencies were found to have increased irritability, nervousness, depression and fear. For eight weeks, the participants were given a formula containing the recommended daily allowance (RDA) of vitamins B1, B2, B6, B9, B12, vitamin C and vitamin E. By the end of eight weeks, clinically significant improvements in mood were seen in those who had identified nutrient deficiency at baseline (Heseker et al., 1992).

Harris and colleagues (2011) also investigated the association between mood and nutrient deficiency. They conducted an RCT of 50 healthy men between 50-69 years and supplemented the treatment group with a multi-nutrient formulation containing vitamins, minerals, antioxidants and herbal extracts. Compared with the placebo group there was a significant reduction in the overall score on a measure of depression, anxiety and stress, and

an improvement in alertness and overall daily functioning in the treatment group (Harris et al., 2011).

In an RCT of 459 healthy Guatemalan women (aged 15-49 years), Nguyen and colleagues (2009) compared the impact of weekly versus daily combinations of micronutrient supplementation on depressed mood. The supplement formula used, which contained folic acid, iron, zinc and vitamin B12, was taken either daily or weekly (in varied doses) for a total of 12 weeks. At baseline 49.3% of the sample had depression scores above clinical cut-off, and by the end of the trial depression scores were lower in all treatment groups with a reduced total of 37.7% meeting clinical cut-off for depression (Nguyen et al., 2009).

While the research discussed above involved non-clinical populations, the impact of multi-nutrient formulations on clinically unwell patients has also been researched. Gariballa and Forster (2007) conducted an RCT with 225 hospitalised acutely ill older patients where the treatment group received an oral nutritional supplement, designed to provide 100% of the reference nutrient intake of vitamins and mineral for a healthy older person. After six months of treatment, there were statistically significant reductions in depression ratings in the supplemented group and these were significantly more beneficial than placebo.

In another RCT, selenium, vitamin C and folate were administered to 73 elderly patients in a nursing home to examine their effect on mood (Gosney, Hammond, Shenkin, & Allsup, 2008). Measures of anxiety and depression were taken at baseline, and again after eight weeks of micronutrient supplementation. At the conclusion of eight weeks, no significant effect on anxiety level was found, but for those patients who entered the trial depressed, there was a clinically significant reduction in depression scores for the supplemented group, but not in the placebo group.

1.5.2 Bipolar Disorder. All trials investigating the effect of micronutrient formulas on symptoms of bipolar disorder have been conducted using one product only, EMPowerplus (Truehope, AB, Canada). This micronutrient formula contains 36 ingredients; a mix of vitamins, minerals and amino acids. It has now become the most researched multi-ingredient formula on the market, with 25 current publications, and more under review (Rucklidge & Kaplan, 2013).

Kaplan and Colleagues (2001) investigated the therapeutic benefit of EMPowerplus on symptoms of bipolar disorder in 11 adults. After six months of treatment, symptom reduction ranged from 55 – 66% on the treatment measures which included the Hamilton Rating Scale for Depression (HAM-D), the Young Mania Rating Scale (YMRS), and the Brief Psychiatric Rating Scale. The need for psychotropic medications also decreased by more than 50% for those who completed the six month trial.

A year later, using the same micronutrient formula (EMPowerplus), Kaplan, Crawford, Gardner, and Farrelly (2002) examined the effect of the treatment on two medication free boys, who were eight and twelve years of age. The children were both deemed as having mood liability and explosive rage, and one of the boys had atypical obsessive-compulsive disorder while the other child had pervasive developmental delay. Both children benefitted from the micronutrient intervention. An ABAB design revealed that mood, angry outbursts and obsessional symptoms improved when initially treated with the supplement, returned when the children ceased taking the supplement, and again remitted upon reintroduction of the supplement (Kaplan et al., 2002).

Popper (2001) conducted an open label trial investigating the effect of EMPowerplus in 22 children and adults with bipolar disorder. The treatment resulted in clinical improvement in symptoms for 19 of the 22 patients, and of the 15 taking medication when starting the trial, 11 were stable on the nutrient formula only by the end of the trial.

In an open label trial using EMPowerplus to treat symptoms of bipolar disorder, Simmons (2003) found clinically significant improvements in 16 out of 19 adults taking the formula. Thirteen of the adults in the study, who had been taking medication prior to starting the nutrient formula, where able to remain stable while taking only the nutrients.

In a database analysis of 358 adults with bipolar, it was found that treatment with EMPowerplus over a six month period, led to a 45% decrease in symptoms (Gately, 2009). A linear regression analysis revealed that symptom decrease was associated with a decrease in medications and an increase in dosage of the nutrient formula.

Rucklidge, Gately, and Kaplan (2010) conducted a database analysis on 120 children and adolescents with bipolar taking EMPowerplus. After six months, a significant decrease in symptoms from baseline was evident, with 46% of the group showing a greater than 50% improvement. These improvements were found to be similar in those who had comorbid ADHD.

In an open label trial by Frazier and colleagues (2012), ten children treated with EMPowerplus showed a decrease in symptoms of bipolar. Intent-to-treat analysis showed a 37% decrease in depression scores and a 45% decrease in mania scores. The seven children who completed the full 6.5 months of the study demonstrated clinically significant decreases in scores of both depression and mania.

1.5.3 Anxiety and Stress. Over the past decade there has been a number of trials examining the impact of high dose B vitamin formulas on stress and anxiety (Rucklidge & Kaplan, 2013). Five randomised controlled trials have been conducted using high dose B vitamin interventions, and all report treatment benefit. Carroll, Ring, Suter, and Willemsen (2000) examined the effect of a multivitamin and mineral supplement (Berocca) on psychological wellbeing in 80 healthy males. The double-blind randomised-control trial

found that relative to placebo, treatment with Berocca was associated with both consistent and statistically significant reductions in anxiety and perceived stress. Those in the treatment group also rated themselves as less tired and more able to concentrate by the end of the four week trial (Carroll et al., 2000).

Schlebusch et al. (2000) conducted an RCT to assess the effects of a vitamin and mineral formula (Berocca Calmag) on stress in a sample of South Africans. Three hundred patients with predetermined high stress levels were recruited from two centres, and were randomly assigned to either a placebo or treatment group. After 30 days, both groups demonstrated significant reductions in all measures, but the treatment group showed greater reduction in all measures of stress, with this beneficial effect increasing throughout the day.

In another RCT, Kennedy et al. (2010) assessed the effect of a high dose B-complex vitamin and mineral formula (Berocca®) on mood and cognition. Two hundred and fifteen healthy male adults completed measures of mood, stress, general health and cognitive performance, at baseline, and again after 33 days of treatment. The results showed that treatment with the multi-vitamin and mineral formula led to significant improvements in perceived stress, vigour, general health and cognitive performance compared with placebo group.

Stough and colleagues (2011) examined the effect that a high dose vitamin B complex had on mood and psychological strain that was associated with chronic work stress. They conducted a three month long double-blind randomised controlled trial, in which sixty participants were assigned to a group receiving Blackmore's executive B Active™, a group receiving a sustained release version of the vitamin B formula, or a placebo. No differences were found between the two treatment groups, and both treatment groups reported significantly lower personal strain and a reduction in confusion and depressed/dejected mood

after the 12 weeks compared with placebo. No effect was found of the treatment on state anxiety or depression measures.

In an RCT, Rucklidge et al. (2012) compared two micronutrient formulas, Berocca™ and CNE™ (equivalent to EMPowerplus), to assess their impact on emotions and stress experienced after a 6.3 earthquake in Christchurch, New Zealand. Ninety one adults who were experiencing heightened anxiety or stress two to three months after the earthquake were randomised to receive Berocca™, CNE™ low dose, or CNE™ high dose for 28 days. During this time, they were monitored weekly using online questionnaires. All treatment groups were found to demonstrate significant declines in psychological symptoms, with the CNE™ treatment groups showing a greater reduction in intrusive thoughts compared with Berocca™. The CNE™ high dose group reported greater improvement in mood, anxiety and energy, with twice as many reporting "much" to "very much" improved, and were five times more likely to continue taking the formula after the trial than the Berocca™ group.

In an open label trial by Gruenwald, Graubaum, and Harde (2002), the authors evaluated a probiotic multivitamin formula in 42 adults suffering from stress or exhaustion. The treatment was taken daily with breakfast for a total of 6 months, and was found to be associated with an overall 40.7% improvement in stress by the end of the trial. In addition to this, participants showed significant improvements in other areas related to stress, including decreased fatigue, a 29% decrease in occurrence of infections, and a decrease of 91% in gastrointestinal discomforts.

In a recent study, researchers were struck with a very challenging situation, where a 7.1 magnitude earthquake took place in the midst of a trial assessing a micronutrient treatment for Attention-Deficit-Hyperactivity Disorder (ADHD). This natural event presented a rare opportunity to investigate whether individuals with ADHD taking the nutrient supplement were more emotionally resilient post-earthquake than individuals with ADHD

who were not taking the supplement. Rucklidge, Johnstone, Harrison, and Boggis (2011) assessed 33 adults with ADHD using a measure of depression, anxiety and stress, pre-earthquake, and again post-earthquake. Seventeen adults were not taking micronutrients at the time the earthquake occurred, and 16 were, creating a control group and a treatment group. At one week post-earthquake there were no significant between group differences; however, by two weeks post-earthquake, the treatment group reported significantly less anxiety and stress than the control group, and these differences could not be explained by other variables (Rucklidge, Johnstone, et al., 2011).

Long and Benton (2013) examined the effect of supplementation with multivitamins and minerals and/or docosahexaenoic acid (DHA) on laboratory-based measures of stress impulsivity and aggression. Using a double-blind randomised trial, 202 adult males with no history of impulsivity or aggression were treated with either a micronutrient formula (Centrum) and DHA, DHA and a placebo, Centrum and a placebo, or placebo and placebo. After 3 months it was demonstrated that those taking micronutrients had decreased perceived stress compared with other groups, but no significant change was found on measures of aggression or impulsivity.

A case study of an 18 year old male with Obsessive Compulsive disorder (OCD) was conducted using the micronutrient formula EMPowerplus. Prior to the trial, the young man had undergone one year of cognitive behavioural therapy (CBT) which had reduced his OCD symptoms from a severe level to moderate. However, within a year his anxiety had increased back into the severe range and he had developed major depression. Rucklidge (2009) used an ABAB study design to examine the effect of the micronutrient formula on his OCD symptoms. After 8 weeks of taking EMPowerplus his mood had become stable, his anxiety was reduced and his obsessions were in remission. After discontinuation of the nutrients for an 8 week period his improvements regressed and his symptoms returned. With the

reintroduction of the formula it was found that again his symptoms improved (Rucklidge, 2009).

1.5.4 Attention Deficit Hyperactivity Disorder. In a study conducted by Harding, Judah, and Gant (2003), twenty children with attention deficit hyperactivity disorder (ADHD) were given either Methylphenidate (Ritalin) or a dietary supplements for four weeks and their outcomes on neurocognitive tasks were compared. The dietary supplement contained a mix of vitamins, minerals phytonutrients, amino acids, essential fatty acids, phospholipids and probiotics, designed to treat ADHD biochemical risk factors. Both groups of children demonstrated significant improvements on the outcome measures, which were essentially identical in both groups, suggesting that the nutrient supplement was of equal efficacy in treating symptoms of ADHD as methylphenidate.

In another study, the effect of the nutrient supplement, EMPowerplus, on children with psychiatric symptoms was explored. Kaplan, Fisher, Crawford, Field, and Kolb (2004) used an open label case series design, over 16 weeks, to examine the effect of the supplement on 11 children with mood and behaviour problems, including five children with ADHD. Significant improvement was found on seven out of eight Child Behaviour Checklist scales (CBCL), including measures of attention and significant improvement in mood.

Rucklidge, Taylor, and Whitehead (2011) also explored the effect of the EMPowerplus micronutrient formula on symptoms of ADHD. They conducted an eight week open label trial where they gave the formula to 14 adults with both ADHD and severe mood dysregulation. Improvements across all outcome measures were found including measures of inattention and hyperactivity, impulsivity, depression, anxiety, stress and quality of life, all with medium to very large effect sizes. The study included a natural two month extension and at follow up, those who had continued to take the nutrient formula showed maintenance of

changes or increased improvements, while those who had stopped taking the nutrients had regressed towards baseline.

Most recently, a double-blind randomised controlled trial investigated the effect of supplementation with EMPowerplus nutrient formula 80 adults with ADHD. Rucklidge and colleagues (2014) randomised 80 adults into either a micronutrient or placebo group, where they received the intervention for a total of eight weeks. Intent-to-treat analysis demonstrated significant between-group differences favouring the micronutrient group on both self and observer ADHD rating scales. Although clinician rating scale results were not significantly different, they rated those in the micronutrient treatment group as more improved globally and on ADHD symptoms, than those taking placebo. Further, for those with moderate or severe depression scores at baseline, there was a greater improvement in mood in the nutrient group than placebo.

1.5.5 Autism. Another area where micronutrient interventions have shown promising preliminary results is in the area of autistic spectrum disorders. Adams and Holloway (2004) conducted a randomised, double blind placebo controlled trial, where they investigated the effect of a vitamin and mineral supplement on children with autistic spectrum disorder (ASD). The supplement was a liquid suspension called Spectrum Support™ II, and contained 15 vitamins, 11 minerals and 3 amino acids. 20 children with ASD, between the ages of three and eight years, were randomised into either an active or placebo group, and received their assigned intervention for a total of three months. Using a global, parent-rated scale, the study found significant group differences in measures of sleep and gastrointestinal problems, in favour of the nutrient intervention.

Another study, conducted by L. Mehl-Madrona, B. Leung, C. Kennedy, S. Paul, and B. J. Kaplan (2010), was interested in the self-injurious behaviour (SIB), aggression and

tantrums that often accompany ASD. The study used the naturally occurring situation of some parent's preference for a non-pharmaceutical approach, and treated this group of 44 children with the EMPowerplus micronutrient formula. This group was matched with 44 similar children whose families had selected conventional treatments who formed the medication group. During the trial, both groups significantly improved on the Childhood Autism Rating Scale and the Childhood Psychiatric Rating Scale, as well as showing significant decreases in total Aberrant Behaviour Checklist scores, with the micronutrient group showing a significantly greater improvement on this scale than the medication group. Compared with medication, the intensity of SIB was found to be lower in the micronutrient group by the end of the study, and improvement on the Clinical Global Impressions scale was higher. Other advantages noted for treatment by micronutrient compared with medication were: lower activity level, less social withdrawal, less anger, better spontaneity with the examiner, less irritability, lower intensity SIB, markedly fewer adverse events and less weight gain. In contrast, the advantages of medication intervention were recorded as being covered by insurance, fewer pills and less frequent dosing.

Adams and colleagues (2011) examined the effect of a mineral and vitamin formulation (Syndion™) in adults and children with autism. A randomised, double-blind, placebo controlled trial was conducted over a three month period where 141 participants were administered either the nutrient supplement or a placebo, and pre and post symptoms of autism were measured. During the trial, blood results of those in the supplement group showed an improvement in the levels of many vitamins, minerals and biomarkers towards normal levels. This group showed significantly greater improvements than placebo on the Parental Global Impressions-Revised (PGI-R), as well as on measures of hyperactivity, tantrums and receptive language. Regression analysis indicated that degree of improvement

on the PGI-R was strongly associated with several biomarkers, with biotin and vitamin K being the most significant.

1.6 Hypotheses

A review of this micronutrient literature suggests that mood disorders, anxiety, stress, ADHD and autistic spectrum disorder can, in some cases, be managed with a mixture of biologically active minerals and vitamins, without the use of other traditional psychopharmacological interventions. Positive results have been reported in over 25 publications, in multiple settings, with many types of designs and analysis, adding further support to the notion that micronutrients may influence mental health function.

This study plans to extend the use of micronutrients in its treatment of psychiatric symptoms, to decipher if it also has a positive effect on the symptoms of insomnia. As such, this is a pilot trial to determine whether there is indeed an effect, and if a broad spectrum multi-nutrient formula, EMPowerplus, is associated with improved sleep pattern and quality compared with a no treatment baseline period. Due to previous research demonstrating a reduction in stress, anxiety and ruminative thinking from micronutrient supplementation, and anecdotal reports of improved sleep quality, it is hypothesised that treatment with the micronutrient supplement will lead to a reduction in insomnia symptom severity, and a decrease in depression, anxiety and stress scores.

The effect of the nutrient formula on symptom severity, sleep onset, sleep length, night waking, sleep efficiency and subjective feeling of restedness, will be assessed using single case methodology. As this is a pilot study, it does not require large numbers of participants, its main objective is to detect if there is indeed an effect of micronutrients on insomnia symptoms. This will be established by taking multiple baseline measures of the participants sleep patterns, and then monitoring changes during treatment with EMP+.

It is hypothesised that thus study will find the following:

1. The micronutrient intervention will be associated with improvements in insomnia symptom severity as measured by the Pittsburgh Insomnia Rating Scale.
2. The micronutrient intervention will be associated with improvements in sleep onset latency, frequency of night waking, total sleep duration, sleep efficiency, and subjective feeling of restedness as reported in daily sleep diaries.
3. The micronutrient intervention will be associated with improvements in depression, anxiety and stress, as measured by the DASS.
4. Improvements will be maintained over a three month follow-up period for those participants who remain on the intervention.

2. Method

2.1 Participants

Participants were recruited in Christchurch, New Zealand, between January 2013 and November 2013 using advertising on The University of Canterbury campus, internet health forums, and through community health services. From 51 people who completed the screening questionnaire, 14 adults with symptoms of insomnia participated in the current study. Of the 51 people who fully completed the online screening questionnaire, 20 met the required cut-off, meaning their insomnia symptoms were severe enough to allow them to participate in the study (see inclusion criteria). All people who completed the screening questionnaire who did not meet criteria for the study were sent a link to a list of sleep support services in the Canterbury area. Every person who met the screening cut-off and all other eligibility criteria was contacted via email and offered an appointment to discuss participation.

Three people who completed online screening and were eligible for the study did not reply to the offer of participation. Two participants who attended the initial baseline appointments withdrew from the study before treatment had commenced due to external life stressors and an inability to commit to the time frame of the study. One participant was excluded from the study during baseline phase due to exclusion criteria.

All study procedures were approved by the Human Ethics Committee at the University of Canterbury. The trial was registered retrospectively with the Australian New Zealand Clinical Trials Registry (ACTRN12613000364774).

2.1.1 Inclusion and Exclusion Criteria. To be invited to participate in the trial, participants were firstly required to meet diagnostic criteria for insomnia as measured by the

Pittsburgh Insomnia Symptom Questionnaire (PISQ). Secondly the severity of their insomnia was measured using the Pittsburgh Insomnia Rating Scale (PIRS) and this score was used to determine eligibility for participation in the trial.

The Pittsburgh Insomnia Rating Scale (PIRS) is a 65 item self- report scale, used to measure severity of insomnia in clinical trials (Moul DE., 2002), and which may be particularly useful for tracking the impact of an intervention (Veqar Zubia, 2014). Interpretations of total scores on the PIRS state that a total score of 0 (lowest possible score) is "good", and a total score of 195 (highest possible score) is "bad" (Moul DE., 2002; Veqar Zubia, 2014). As no further indication is given in the literature as to a suggested clinical cut-off for the 65 item scale, the Likert scale used within the measure was used as an indication to interpret total scores. The PIRS uses a 4 point Likert scale to rate each of 65 questions about symptoms of insomnia; with 0 indicating "not at all bothered", 1 indicating "slightly bothered", 2 indicating "moderately bothered" and 3 indicating "severely bothered". These ratings were used to determine overall total scores corresponding to those not affected by insomnia symptoms, those slightly affected by insomnia symptoms, those moderately affected by insomnia symptoms, and those severely insomnia symptoms. In order to investigate whether the treatment intervention (micronutrients) did indeed have an impact, a conservative cut-off score was chosen at the mid-point between "slight" insomnia symptoms (total score = 65) and "moderate" insomnia symptoms (total score = 130). This mid-point fell at 97.5 and therefore it was determined that participants would have to meet a total PIRS score of 98 or above to be affected by symptoms of insomnia to the degree required to measure impact of the intervention in the trial. Only those with a PIRS total score of 98 or above at screening were invited to participate in the study. Participants were not required to have a pre-existing diagnosis of insomnia, and were not excluded for having a comorbid psychiatric diagnosis. Only those who were not currently taking a psychiatric or sleep

medication, or who had been medication free for at least 4 weeks, were considered for the trial.

Other inclusion criteria included:

1) Participants had to be over 18 years of age
2) Participants had to be able to travel to the University Campus for four appointments during the trial
3) Participants had to be able to swallow six capsules per day as well as complete the daily and weekly measures required

Other exclusion criteria included:

1) Any participant with sleep apnoea
2) Any participant who was pregnant or breastfeeding
3) Any individual with a child under two years of age
4) Any participant with a neurological disorder involving brain or other central function (e.g. MS, narcolepsy, epilepsy)
5) Any participant with a serious medical condition for which major medical intervention was anticipated during the trial
6) Participants who took an oral antibiotic in the previous six weeks were excluded temporarily.
7) Any participant judged clinically to be at serious risk for suicide or violence in the opinion of the researchers.

These criteria resulted in the exclusion of one participant during the baseline phase as mentioned previously. This participant was clinically judged by the researchers to be at

serious risk for suicide and was referred to the University Health Centre to be clinically assessed.

2.1.2 Final Sample. Of the 14 adults who participated in the study, 12 participants completed the entire trial. One participant chose to drop out of the study after four weeks of treatment due to no perceived benefit. Another participant dropped out after three days of treatment due to experiencing side effects. Due to reported life stressors, two of the twelve participants who completed the entire trial, failed to consistently complete measures throughout the trial making analysis of their results on some measures invalid. Figure 1 provides a visual diagram of the flow of participants in and out of the study. The mean age of participants at data collection was 36.64 (SD = 13.24, range 21–58). Twelve participants identified as being of New Zealand European ethnicity, one identified as being of Indian ethnicity and one as "other". The most common category for occupation status of participants was: student (nine participants), the other occupations listed where: learning skills advisor, business owner, artist, vet nurse and importer.

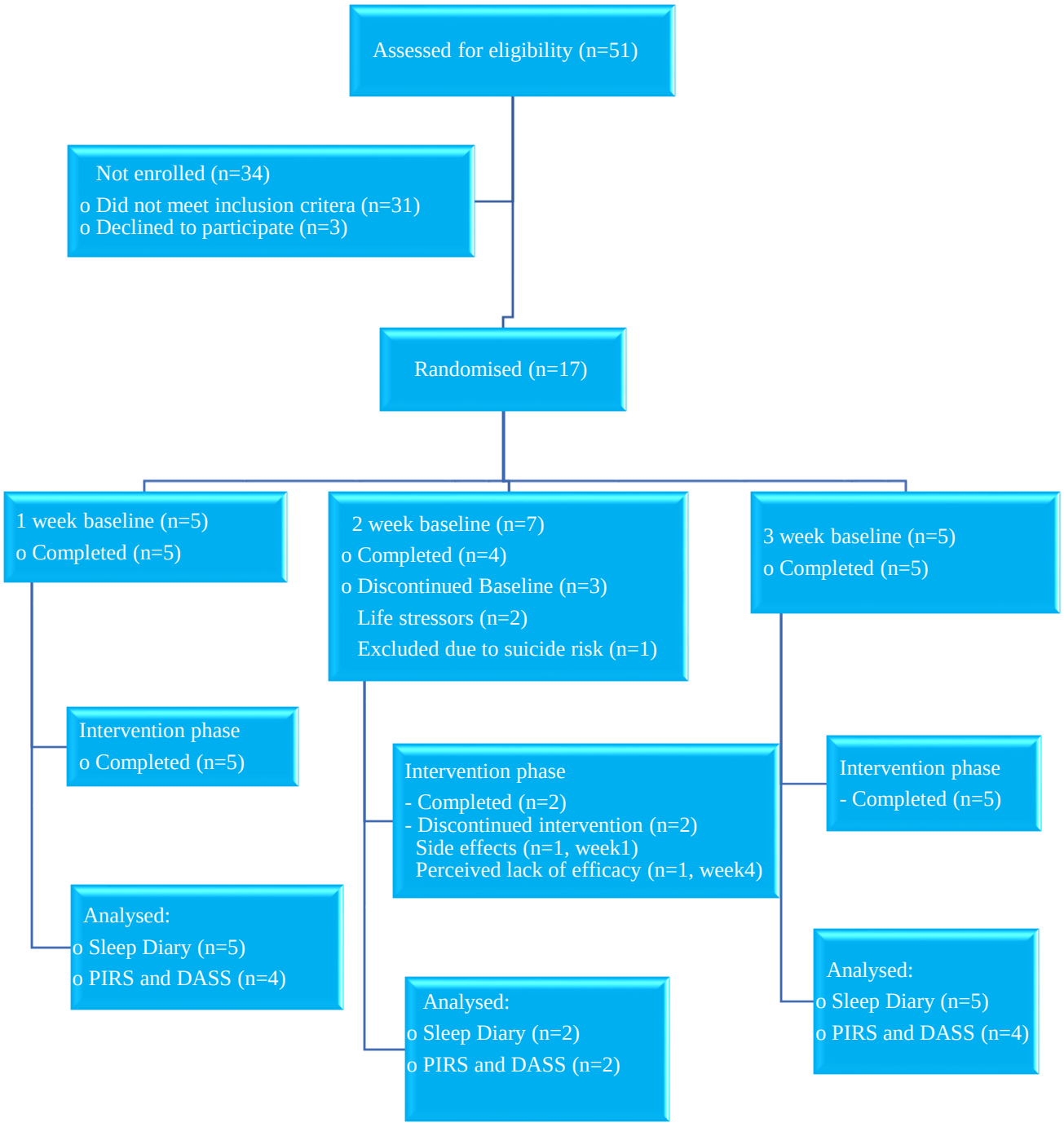


Figure 1. Consort Flow Diagram.

2.2 Measures

2.2.1 Measurements of Demographic Variables. *History questionnaire.* This questionnaire was used to assess demographic variables including the participant's ethnicity, occupation, household income, physical health, psychiatric health, previous or current medication, antibiotic use history and other important medical information.

2.2.2 Self-rated Dependent Measures. *Pittsburgh Insomnia Symptom Questionnaire (PISQ)* The PISQ is a 13-item self-report instrument designed to establish a clinically relevant case definition of insomnia, that is consistent with widely used insomnia criteria (Okun et al., 2009). PISQ questions are based on the American Psychiatric Association's fourth edition of the Diagnostic and Statistical Manual-IV criteria (American Psychiatric Association, 2013) for primary insomnia, and are consistent with the American Academy of Sleep Medicine's Research Diagnostic Criteria. PISQ items contain multiple choices on an ordinal scale which assess the presence, frequency and severity of the complaint. For example, a question "During the past month have you had difficulty falling asleep?" includes choices ranging from 0 = never to 5 = always. In an assessment of the psychometric properties of the PISQ it was found to identify 9.8% of the sample as meeting criteria for insomnia which is a number consistent with established diagnostic rates (Okun et al., 2009). Reliability of the scale was established with Cronbach's α ($\alpha = 0.89$), and the scale had a high specificity (> 90%) (Okun et al., 2009).

Pittsburgh Insomnia Rating Scale (PIRS). The PIRS is a 65 item self-report instrument designed to assess severity of insomnia. The scale is widely used in clinical practice (Veqar Zubia, 2014), and findings suggest that PIRS has excellent internal consistency, test-retest reliability and good validity (Moul DE., 2002; Veqar Zubia, 2014).

Answers are indicated on a 4-point Likert scale; 0 = “Not at all bothered”, 1 = “Slightly bothered”, 2 = “Moderately bothered”, 3 = “Severely bothered”, and Scores can range from 0 to 195. For the purpose of this study a cut-off point for severity was set at the mid-point between “slight” insomnia symptoms (total score 65) and “moderate” insomnia symptoms (total score 130) resulting in a cut off score of 98 to have insomnia symptoms deemed severe enough to participate in the study. The test- retest reliability of the PISQ has been found to be good (r=0.90) (Moul DE., 2002) and internal consistency was found to be excellent; Cronbach’s α (α = 0.93) (Veqar Zubia, 2014).

Depression Anxiety and Stress Scale (DASS). The DASS is a 42 item questionnaire consisting of 3 self-report scales designed to measure negative emotional states of depression anxiety and stress (Lovibond & Lovibond, 1995). The scale uses “over the past week” questions to measure current symptoms of depression, anxiety and stress. The anxiety subscale shows good correlation of 0.81 with the Beck Anxiety Inventory and the depression subscale shows moderate correlation of 0.74 with the Beck Depression Inventory (Lovibond & Lovibond, 1995). Internal consistency has been found to be favourable with Cronbach’s α = 0.96, 0.89 and 0.93 for depression, anxiety and stress respectively (Brown, Chorpita, Korotitsch, & Barlow, 1997).

The Expanded Consensus Sleep Diary for morning (CSD-M). The CSD-M is an expanded version of the Consensus sleep diary, an original collaboration by insomnia experts and potential users, to create a standard sleep diary for insomnia aiming to facilitate accurate comparisons across studies. The CSD-M includes additional items about early morning awakenings, estimated total time asleep, Likert scale rating for refreshing quality of sleep, napping/dozing, alcohol, caffeine, and medication use. (Carney et al., 2012).

2.3 Design and Procedures

Participants who were interested in the study first completed an online screening process from which eligible participants were invited to participate in the study. The study followed a multiple baseline design, whereby participants were randomly assigned to a baseline phase of one, two or three weeks, followed by an eight week treatment phase. During both baseline and treatment phases, daily sleep diaries were completed by the participants, as well as weekly online questionnaires consisting of the PIRS and DASS.

During the intervention phase participants visited the University of Canterbury to meet with the researcher at both four weeks, and eight weeks of intervention. The follow up phase of the study involved each participant completing the PIRS and DASS online, three months after completing the treatment phase. All participants were monitored by a clinical psychology student under the supervision of a registered Clinical Psychologist.

2.3.1 Screening Phase. Individuals who were interested in the trial visited the link provided on the advertisements which guided them to the Mental Health and Nutrition Research Group's website, www.menthalhealthandnutrition.co.nz. Here they completed the online screening questionnaire which determined eligibility for the study. Those who did not meet criteria for the study were emailed by the researcher with an explanation as to why they were excluded, and were provided with a link (also through the Mental Health and Nutrition website) to a list of sleep support services within the Canterbury area. Those who met the criteria for participation in the study were emailed and invited to meet with the researcher at the University of Canterbury to further discuss the study and participation.

During the initial appointment each participant was provided with a detailed information sheet explaining the study. Copies of the information sheet and consent form provided at this meeting can be found in Appendix A. The study was verbally explained in

detail to each participant including the aims, limitations of confidentiality and requirements of participating in the study, and participants were given ample opportunity to discuss and ask questions about any aspect of the study they wished. Participants were informed that participation in the study was entirely voluntary and that at any time they could withdraw from the trial, without any negative impact on their future health care. Participants were offered the option of taking time to consider participation before contacting the researcher at a later date. Informed consent was obtained from the participant.

2.3.2 Baseline Phase. After consent was obtained, each participant was assigned an identification number in consecutive order from 1 to 14 depending on the order in which they entered the study. They were also asked to open an envelope containing their baseline length. Baseline lengths had previously been randomly assigned to identification numbers and the researcher was blinded as to what they were. Daily sleep diaries of adequate length for the participants baseline phase were given along with instructions for completing the diary. Participants were also informed that the online questionnaire would be emailed to the participant to complete at the end of each week of participation. A copy of the daily sleep diary, with instructions, can be found in Appendix B. A second appointment was made for the participant to meet with the researcher at the end of their assigned baseline phase to pick up further sleep diaries and collect the micronutrient capsules. At each visit to the University the participant was provided with a $10 petrol voucher to compensate travelling costs.

After completing their baseline phase, each participant met again with the researcher to hand in their completed baseline sleep diaries, and collect enough new sleep diaries and micronutrient capsules for the first four weeks of intervention. Participants were instructed to take three capsules with breakfast and three capsules with lunch to avoid side effects and minimise the risk of an energising effect too late in the day. The participant was advised to

contact the researcher immediately if they experienced any adverse side effects from the micronutrients.

2.3.3 Intervention Phase. Daily Self Defense (DSD) is a newer version of EMPowerplus (EMP+), a broad spectrum micronutrient formula containing 16 minerals, 14 vitamins, 3 amino acids and 3 antioxidants. An ingredient list for DSD can be found in Appendix C. Participants took six capsules daily during the study, often dividing the dose into two, three capsule doses. They were informed of the importance of taking the nutrients with plenty of food and water to minimise any side effects. DSD was provided to the participants free of cost for the duration of the study, and if participants wished to continue taking the supplement during the follow up period they were provided with another bottle. Participants were also provided with contact details for purchasing the product within New Zealand if they wished to continue using the supplement after completion of the trial.

Participants were seen at four weeks and eight weeks after final baseline appointment. At the four week appointment, the first four weeks of daily sleep diaries was collected and the participant was provided with the second four weeks' worth of diaries, as well as a second bottle of micronutrients (enough for the remaining four weeks). They were given a chance to discuss the trial with the researcher and were asked about any changes they had noticed. Participants also completed weekly questionnaires throughout the treatment phase which asked about side effects and changes in mental health or insomnia symptoms.

At eight weeks of treatment participants finished the trial. During this appointment the daily sleep diaries were collected, and the participants were asked how they felt the micronutrients had gone for them, and if they had noticed any changes. The participant was thanked for their involvement in the study and reminded that the researcher would be in

contact in approximately three months' time to send them an online follow-up questionnaire to review their mental health and sleep symptoms.

2.3.4 Follow- up Phase. Approximately three months after completing the intervention phase participants were emailed an online follow up questionnaire to complete. This questionnaire included the DASS and PIRS as well as asking whether the participant was still taking the nutrient formula and reasons why they did or did not chose to continue.

2.4 Statistical Analysis

The primary outcome measures were the PIRS and the Consensus daily sleep dairy. The PIRS provides information about the severity of a broad range of insomnia symptoms, while the daily sleep diary provides measures of sleep onset latency, frequency of night waking, total sleep duration, sleep efficiency, and feeling of restedness upon waking. Individual changes across baseline and intervention phases are presented for the daily sleep diary variables using time series graphs, and Percentage Exceeding the Median (PEM), is used to describe effect size (Ma, 2006). Modified Brinley plots are used to analyse individual changes over time for the PIRS and then for the secondary outcome measures (Depression, Anxiety, and Stress scores from DASS). Reliable Change is calculated for each measure using the procedures of Jacobson and Truax (1991), as well as Cohen's *d* effect sizes.

3. Results

The results of the intervention, assessed using the primary and secondary outcome measures, are presented in the following sections. Primary outcome measures included data from the daily sleep diaries and the PIRS, while secondary outcome measures include the DASS data. Individual changes across baseline and intervention phases, as reported in daily sleep diaries, are presented first, using time series graphs[1] following the conventions of the multiple-baseline design. Modified Brinley plots were used in multiple-baseline format to analyze individual changes in the primary PIRS measure. Modified Brinley plots were also used to analyse changes over time in Depression, Anxiety, and Stress scores (DASS) and to summarise the outcomes of the study across several measures.

Demographic characteristics of the final sample are presented in Table 1 below.

Table 1: Demographic Characteristics of Final Sample

Characteristic	Number	Mean	Standard deviation	Percentage
Sex				
Male	2			14
Female	12			86
Age in years (mean)		37.64	13.24	
Ethnic group				
NZ European	12			86
Indian	1			7
Other	1			7

[1] All graphs created using Sigma Plot 12.5 (Systat Software Inc.)

3.1 Safety and Adherence

The adverse effects that were reported by participants during the study were mild and transitory, and were able to be remedied by reducing the dose of capsules, or by simply waiting until the symptoms passed without any explicit action. This was true for all but one participant, who decided to withdraw from participation in the study due to side effects, as shown in Figure 1.

Four out of fourteen (29%) participants experienced side effects which may have been related to the intervention. One participant experienced a mild stomach upset for the first few days of taking the capsules which then ceased. One participant reported a very dry mouth at the beginning of the fourth week of taking the capsules which ceased by the end of the week. Another participant reported experiencing an increase in intensity of sensations such as sights and sounds during the seventh week of taking the capsules, which was remedied by reducing the dose to three capsules per day. A fourth participant reported headache, reflux, mild nausea and dizziness after starting the intervention. This participant decided to withdraw from the study during the first week of taking the capsules due to the side effects experienced (Refer to Figure 1).

Table 2: Treatment-emergent Adverse Effects Reported by Participants

Participant #	Adverse effect	Time occurred	Action taken
7	Gastrointestinal disturbance	Week1 Intervention	None. Symptoms passed on their own
10	Dry Mouth Headache	Week 4 Intervention	None. Symptoms passed on their own
11	Sensitivity to light and sound	Week 7 Intervention	Reduced dose to 3 capsules per day. No further problems
5	Headache Reflux Nausea Dizziness	Week 1 Intervention	Participant chose to withdraw from the study

3.2 Compliance

Of the thirteen participants who continued with the intervention phase, all (100%) were compliant in terms of adherence to the treatment protocol. This involved taking the recommended micronutrient dose (six capsules) daily, without missing a significant number of doses, which was defined as a compliance rate of greater than 80%, or no more than 67 missed capsules across the intervention phase. Of the thirteen compliant participants, eight reported perfect compliance, two participants reported a total of three missed capsules, one reported a total of 27 missed capsules, and one participant reported a total of 30 missed capsules.

3.3 Sleep Diary data: Time Series Multiple-baseline Analysis

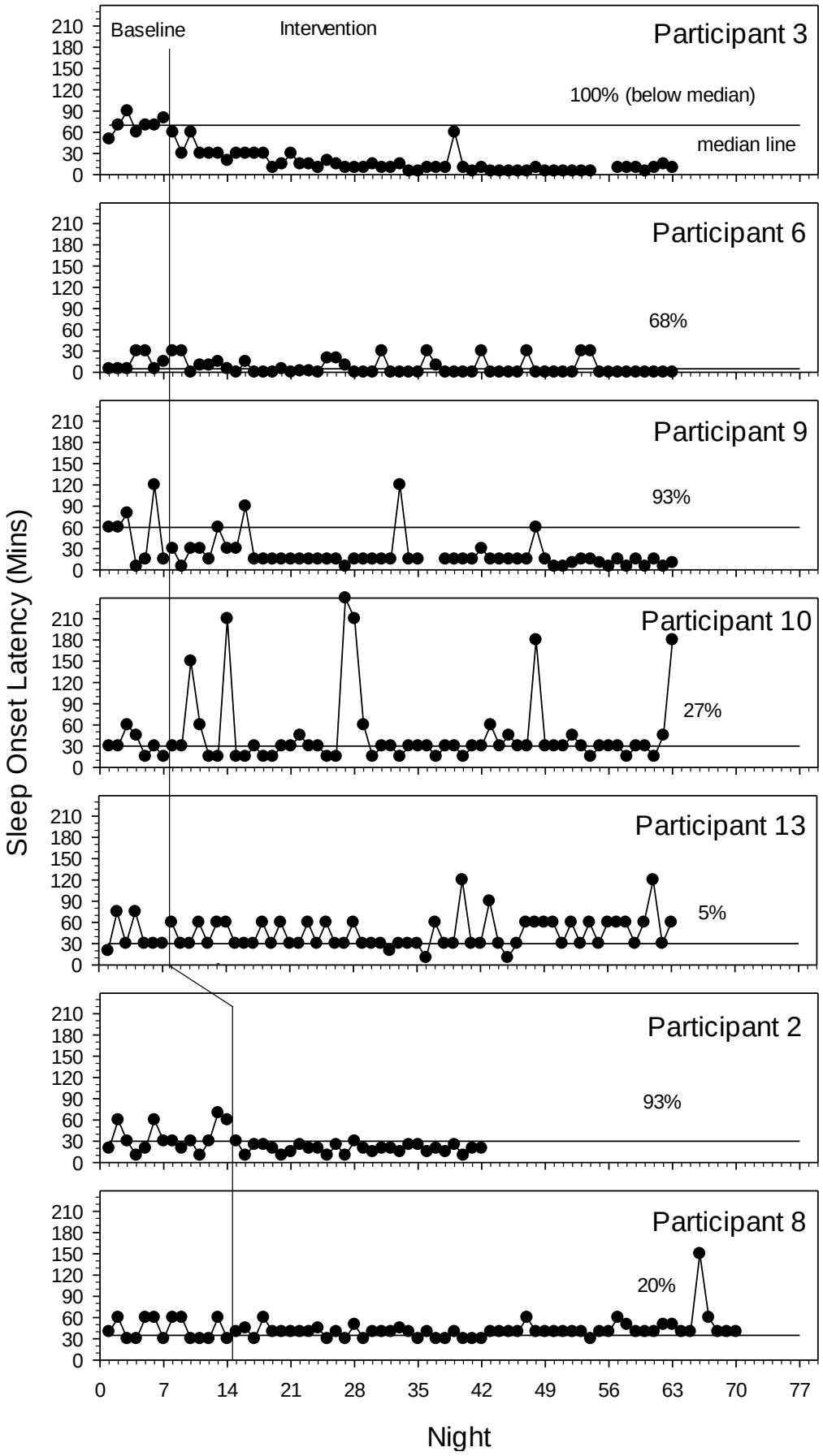
In this section, data from the sleep diaries is presented as time series graphs following the conventions for the multiple-baseline design. Data from Participants 5 and 12 are not included in these graphs as Participant 5 withdrew participation in the study during the first week of intervention, and Participant 12 did not complete their daily sleep diaries. On each graph the middle or median value from baseline phase has been extended to form a median line through the intervention phase, and the percentage of intervention data points exceeding the median (in direction of desired change) is noted. This Percentage Exceeding the Median (PEM), is used to describe effect size in single-case research (Ma, 2006). A PEM in the range of 70-90% is reported to indicate a moderate treatment effect, while a PEM >90% indicates a large treatment effect (Ma, 2009).

Information from the sleep diaries was used to calculate each participant's nightly sleep onset latency, frequency of night waking, total time spent asleep, sleep efficiency score ($SE = \left(\frac{Time\ asleep}{Time\ in\ bed}\right)$x100), and subjective feeling of restedness on waking. These data are presented in a multiple-baseline across-participants format in Figures 2 to 6.

Sleep-onset latency: Figure 2 presents sleep-onset latency measured in minutes. In Baseline, Participants 7 and 14 showed no evidence of difficulty initiating sleep and therefore it was not possible to detect a treatment effect with this measure. The other participants all reported some degree of delayed sleep onset. Note that the criterion for clinical severity of sleep onset latency is 30 minutes or more, occurring on three nights per week (American Psychiatric Association, 2000). Against this criterion, Participant 6 showed only marginal difficulty with sleep onset on some nights, but all remaining participants met this criterion, showing evidence of difficulty with sleep onset. Notably, Participants 3 and 4 had no nights during baseline where sleep onset was less than 30 minutes. Participants 1, 2, 3, 4, 6, 8, 10, and 13 all show relatively stable or increasing (worsening) patterns of sleep onset latency during baseline, while Participants 9 and 11 showed some improvement during this phase, which reduces the ability to detect a treatment effect for these cases.

When the intervention began, sleep onset latencies for Participants 1, 2 and 3 immediately decreased and continued to improve across the intervention phase, with each participant showing a percentage of data points exceeding the baseline median (PEM) of 95%, 93% and 100% respectively, indicative of a large effect size. A delayed treatment effect can be seen for Participants 4 and 9; with both showing a fairly stable pattern of sleep onset scores of 30 minutes or less in the final two weeks of intervention. Despite this delay in response, Participant 9's PEM still showed a large effect size; however, Participant 4's PEM was 64%, reflecting the delay in treatment effect.

Participant 8 and Participant 11 show a small treatment effect evident in the reduction of variability in sleep onset scores. No treatment effect was observed for Participants 10 and 13.


Baseline
Intervention
Participant 3
100% (below median)
median line
Participant 6
68%
Participant 9
93%
Participant 10
27%
Participant 13
5%
Participant 2
93%
Participant 8
20%
Sleep Onset Latency (Mins)
Night
0
7
14
21
28
35
42
49
56
63
70
77
0
30
60
90
120
150
180
210

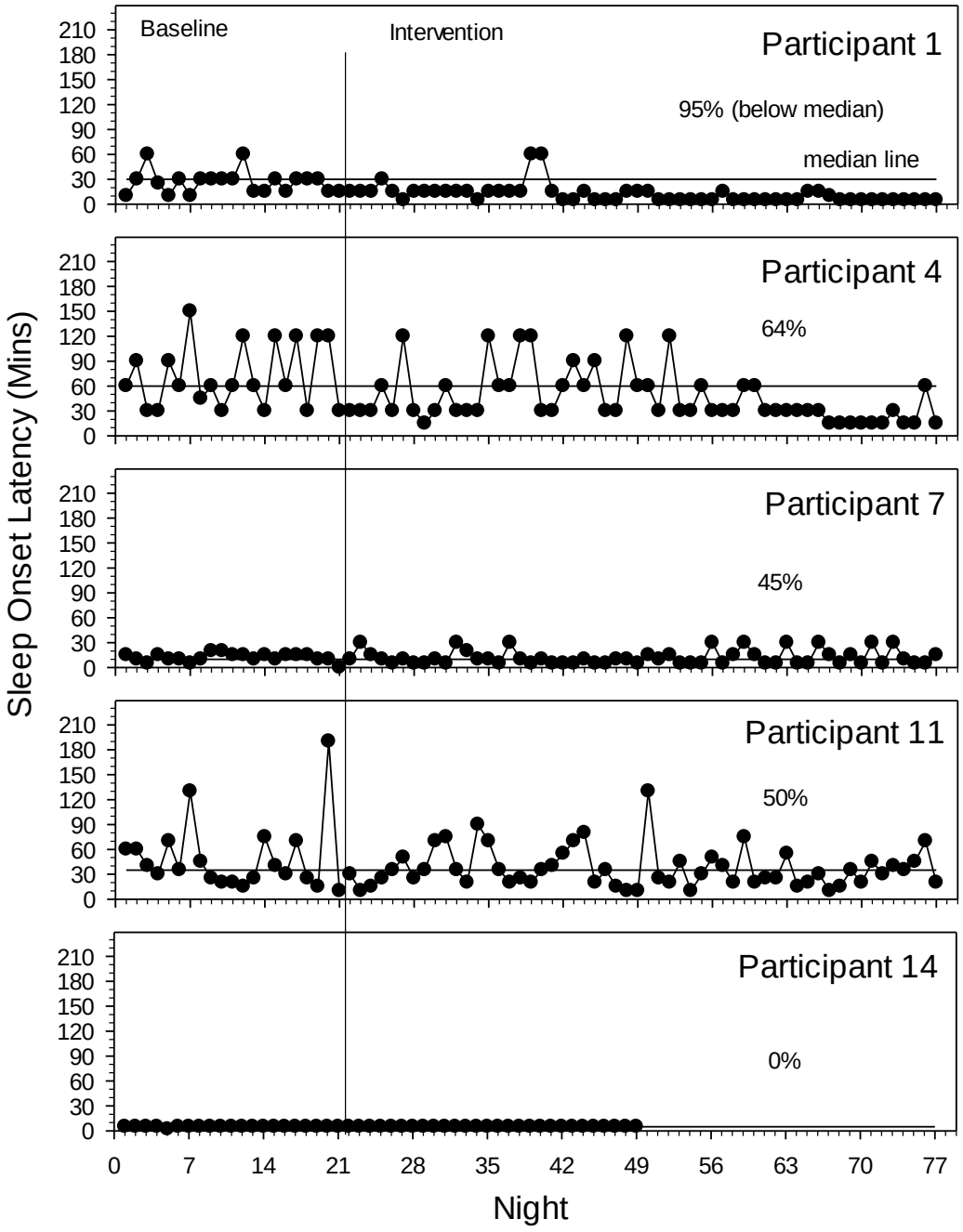
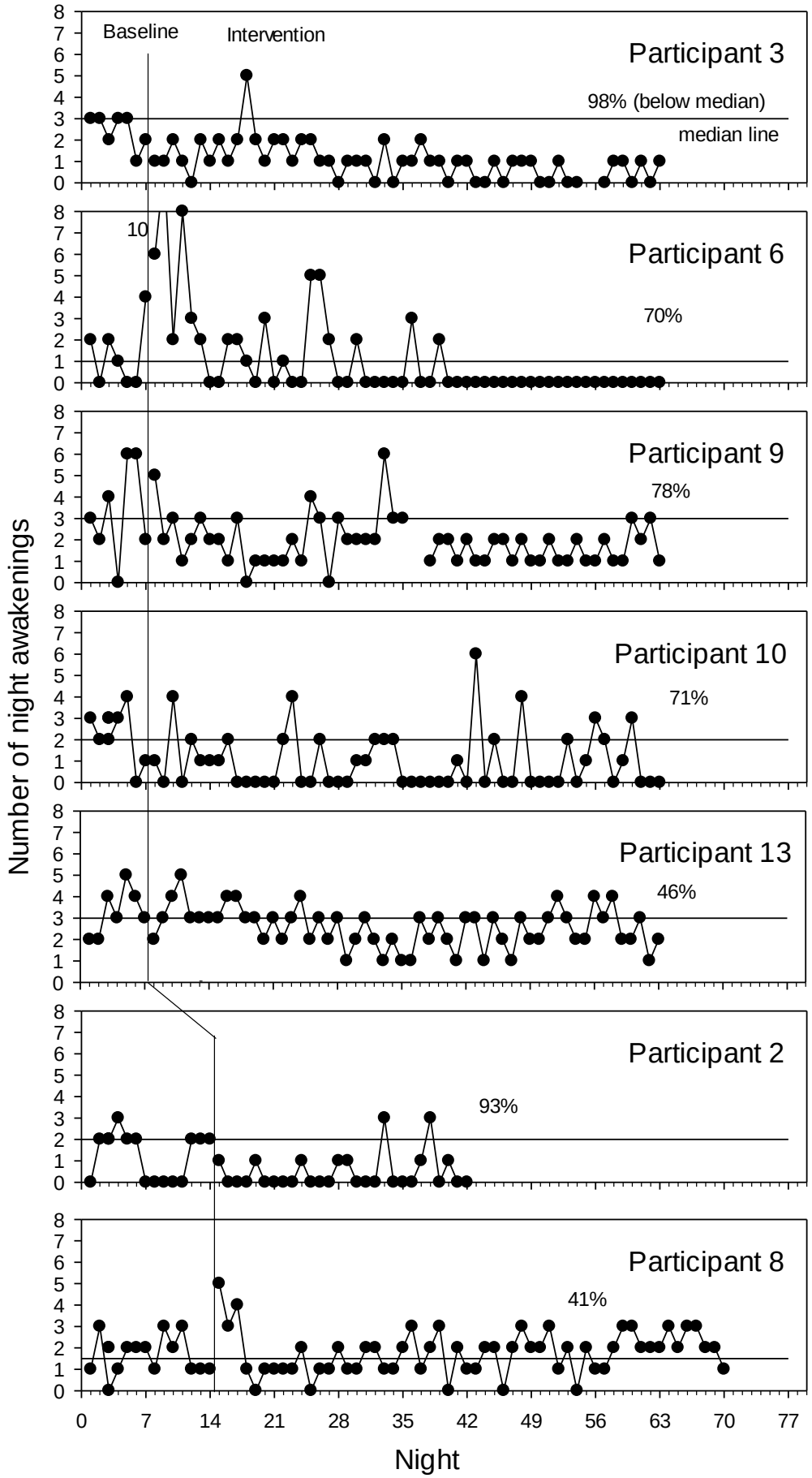


Figure 2: **Times series of each participant's nightly sleep onset latency (in minutes) as reported in daily sleep diaries. The horizontal line shows the baseline median and the percent of nights in intervention below the median is reported as a percentage for each participant.**

Frequency of night waking: The DSM-IV(American Psychiatric Association, 2000) criteria for insomnia define a difficulty maintaining sleep, or "multiple night wakings", as waking three or more times per night, at least three nights per week. Against this criterion, Participants 3, 4, 9, 10, 13, and 14 show evidence of difficulty with night waking (Figure 3). While the remaining participants all reported some degree of night waking, they did not meet criterion for this particular sleep difficulty. It must be noted that for these participants their problem may have been in their inability to initiate sleep once woken, rather than the frequency of night waking per se. The majority of participants showed stable or worsening (increasing) patterns of waking during baseline, while Participant 10 showed some improvement during this phase, which reduces the ability to determine a treatment effect for this case.

When the intervention began, waking frequency for Participants 2, 7, and 14 immediately decreased, and they maintained this improvement across the intervention phase, reflected in PEM effect sizes of 93%, 98% and 82% respectively. Participants 3, 6, and 9 showed a delayed pattern of responding to the intervention, with frequency of waking reducing more obviously after four weeks of treatment. Despite this delay, Participant 3's PEM (98%) still indicated a large effect size, and Participant 6 and 9's PEM indicated a moderate effect size (70% and 78% respectively). Participants 1, 4 and 13 showed evidence of a slight treatment effect towards the end of the intervention phase with a slight reduction in waking frequency. No treatment effect was observed for Participants 8, 10 and 11.


Baseline
Intervention
Participant 3
98% (below median)
median line
Participant 6
70%
Participant 9
78%
Participant 10
71%
Participant 13
46%
Participant 2
93%
Participant 8
41%
Number of night awakenings
Night
0
7
14
21
28
35
42
49
56
63
70
77

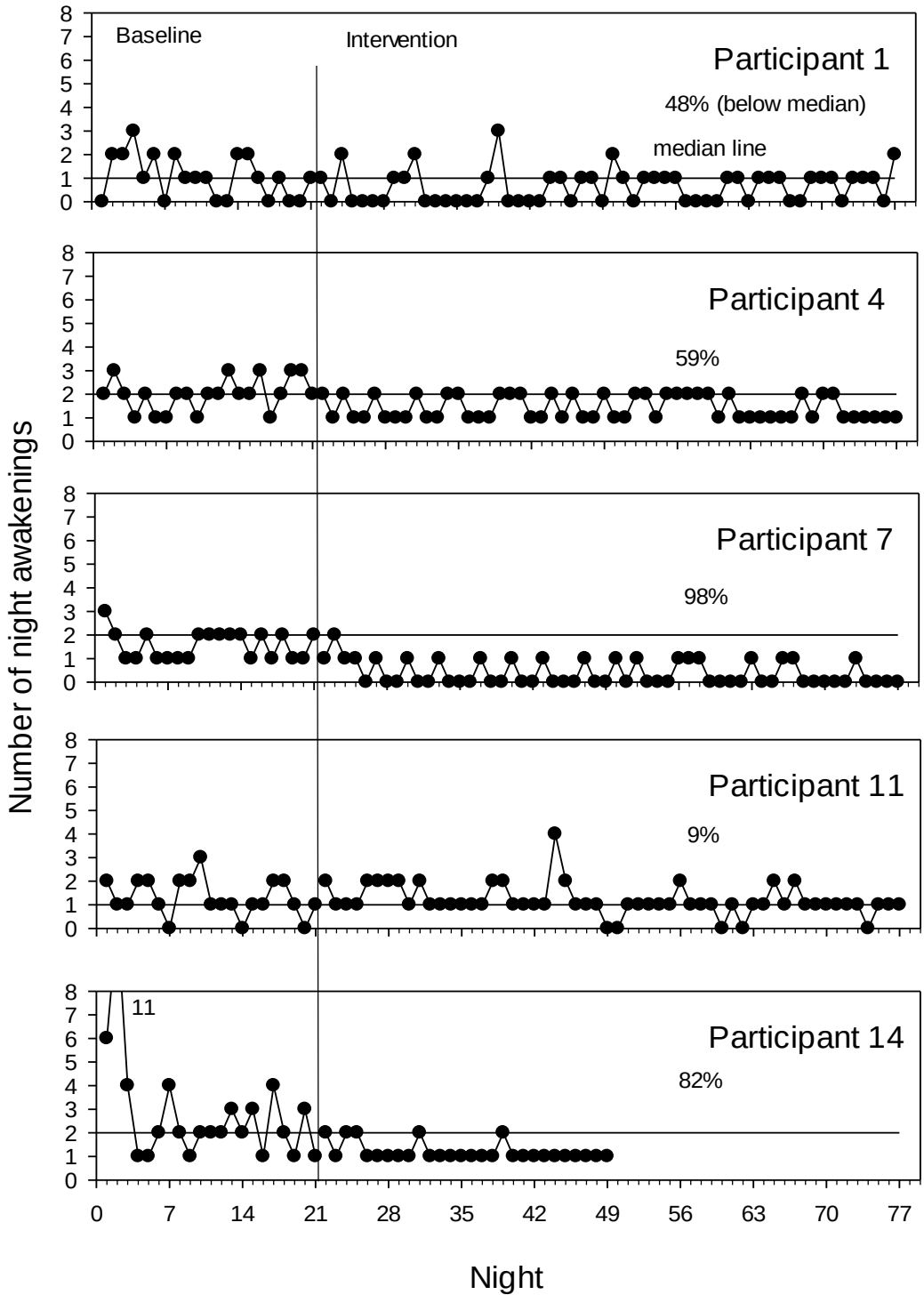


Figure 3: **Time series of each participant's frequency of night waking as measured by daily sleep diaries. Median lines and PEM as for Figure 2.**

Total time asleep: Sleep duration is reported in Figure 4 as a percentage of 8 hours to most succinctly capture the wide range of data for this measure and to clearly display each person's deviation from the 8-hour ideal. In baseline, all participants, with the exception of Participant 2, had total nightly sleep of less than 8 hours on the majority of nights. Participants 1, 3, 6, 8, 10 and 14 showed fairly stable patterns of total sleep; with most participants reporting a median sleep durations of approximately 80% of 8 hours total sleep (i.e., 6.4 hours). Participants 9 and 13 showed some improvement during baseline phase which reduces the ability to determine a treatment effect for these cases. Participants 2, 4 and 11 showed large variability in baseline scores with no clear trend.

The treatment effect can be discussed in two ways for this set of graphs, firstly through comparing each participant's percentage of nights per week, with a total sleep time of 8 hours of more (during both baseline with intervention phase), and secondly by examining their median hours asleep in baseline and then in the treatment phase. The PEM effect size is also considered for each participant irrespective of their baseline median being below, at or above 8 hours of sleep.

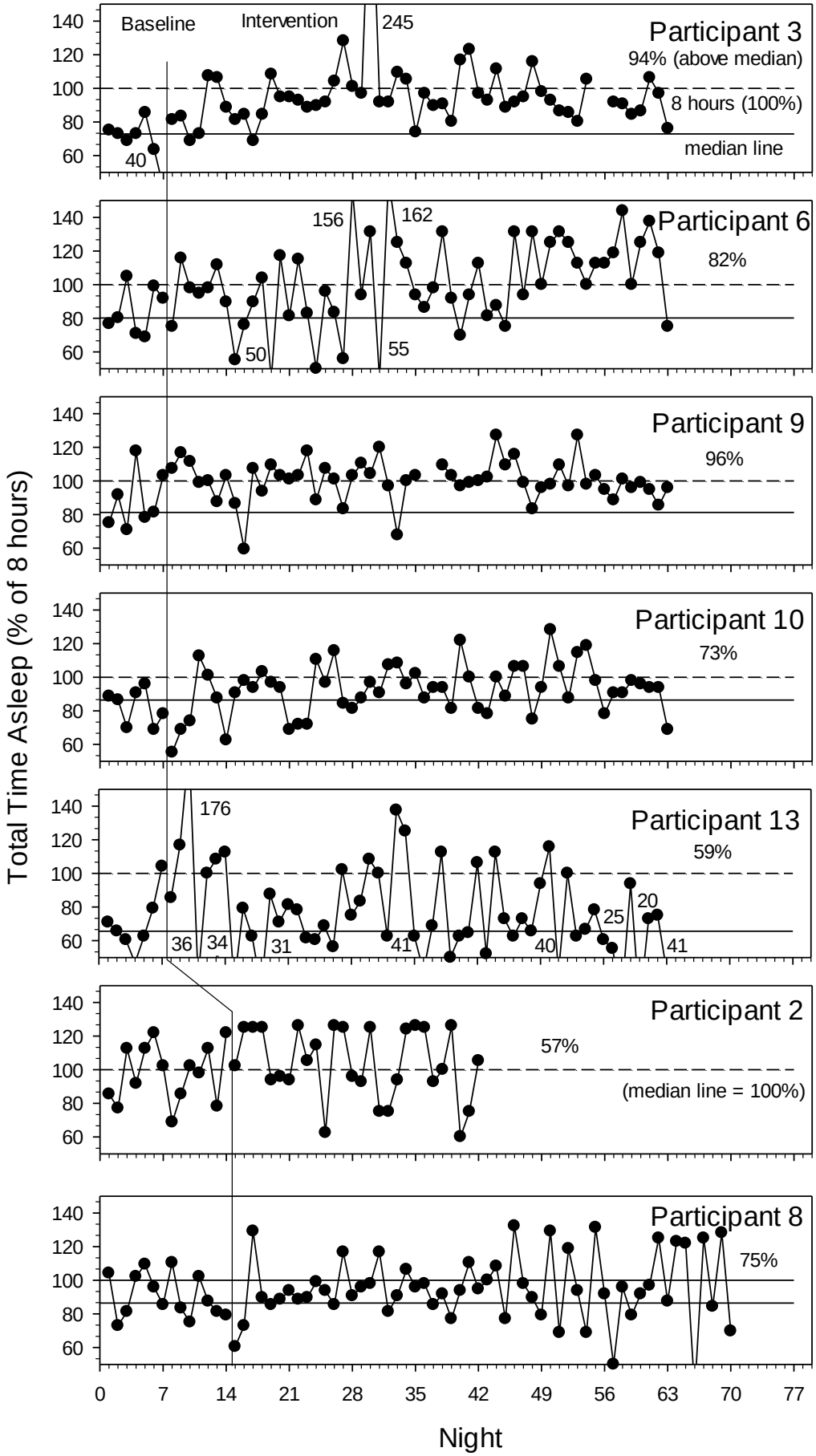
When the intervention began, total time asleep for Participants 1, 3, 4, 7, 9, and 14 immediately increased and continued to improve across the remainder of the intervention phase. Participant 1 had 0% of nights per week with a total of eight hours or more sleep during baseline, which increased to 32% of nights per week during intervention, and a PEM of 82% indicated a moderate effect size. Participant 3 also had 0% nights of eight hours or more total sleep during baseline, and this increased to 27% of nights per week during intervention, and a PEM of 94% indicating a large effect. Participant 4 had a total sleep time of ≥8hours on 24% of nights per week during baseline, which increased to 52% of nights per week during intervention, and a PEM of 88% indicating a moderate effect. Participant 7 slept for ≥8hours on 33% of nights during baseline, which increased to 91% of nights during

intervention, reflected in a large effect size (PEM = 93%). Participant 9 had a total sleep time of ≥8hours on 29% of nights during baseline, which rose to 59% per week during intervention, and a PEM of 96% indicated a large treatment effect. Participant 14 had 0% of nights where they slept for ≥8hrs during baseline, which increased to 61% of nights during intervention, and a PEM of 100% indicating a large effect.

Participants 6 and 10 showed a delayed treatment effect that became more evident in the second half of the intervention. Participant 6 was getting ≥8hours sleep on 29% of nights during baseline and this improved to 50% of nights in intervention. Participant 10 was getting 0% of ≥8hours sleep during baseline, which increased to 30% of nights per week in intervention. Both Participants showed a moderate treatment effect with a PEM of 82% and 73% respectively.

Participant 8 appears to show two phases of response during intervention; firstly there appears to be an immediate treatment effect that lasted the first 4 weeks, which is followed by an increased variability in scores and disappearance of a treatment effect during the second four weeks of treatment.

Participant 11 showed a small treatment effect through the reduction of very short total sleeps. Participants 2, 13 showed no treatment effect for this variable.


Baseline
Intervention
Participant 3
94% (above median)
8 hours (100%)
median line
245
40
Participant 6
82%
156
162
50
55
Participant 9
96%
Participant 10
73%
Participant 13
59%
176
36
34
31
41
40
25
20
41
Participant 2
57%
(median line = 100%)
Participant 8
75%
Total Time Asleep (% of 8 hours)
Night
0
7
14
21
28
35
42
49
56
63
70
77
60
80
100
120
140

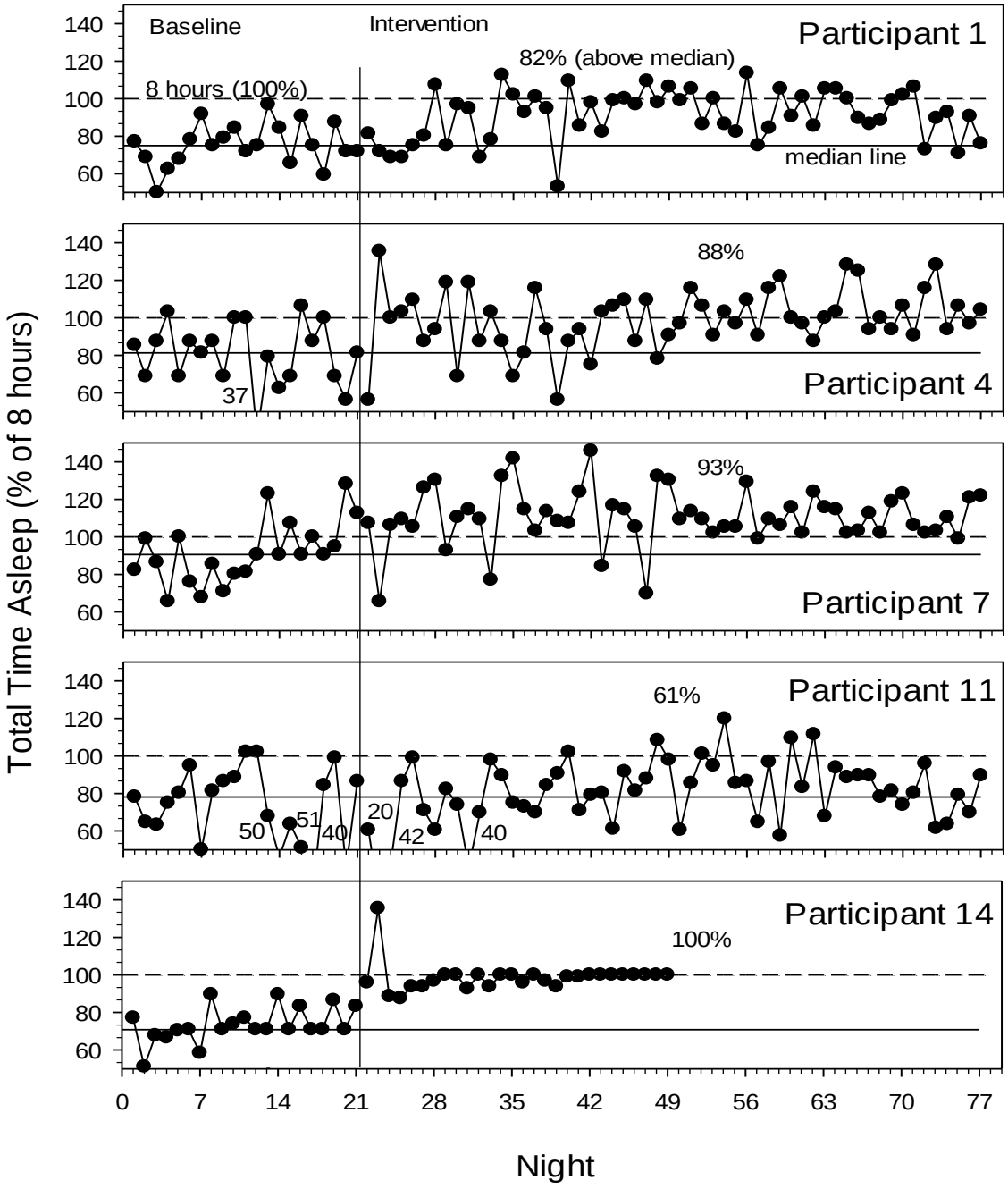
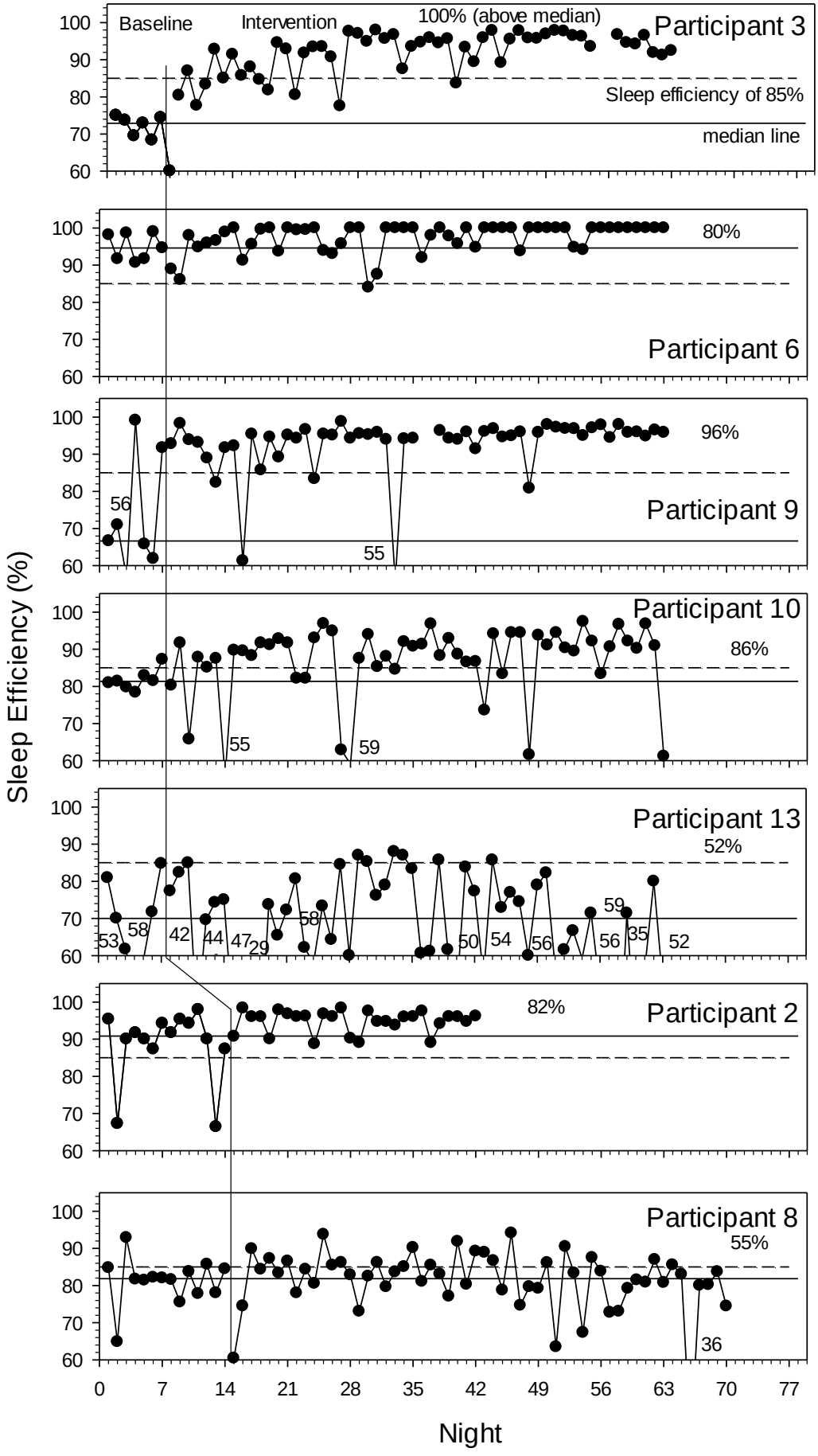


Figure 4: **Time series of each participant's total nightly sleep duration as measured by daily sleep diaries. Numbers on the graphs report the value of data points that are off-scale. Median lines and percentages are as for Figure 2.**

Sleep efficiency: A sleep efficiency score below 85% is thought to indicate problematic or abnormal sleep (A. J. S. Spielman, P; Thorpy, M.J, 1987). Against this criterion all participants, except 1, 2 and 6, showed a baseline median efficiency score that was in the clinical range (Figure 5). Participants 1, 2, 3, 4, 8, 10, 11, and 14 showed stable or worsening patterns of sleep efficiency during baseline, while Participants 7, 9, and 13 showed some evidence of improvement which may affect the ability to determine a treatment effect for these cases.

When intervention began Participants 1, 2, 3, 4, 7, 9, and 14 showed an immediate increase in sleep efficiency scores and this improvement was maintained or continued to increase across the rest of the intervention phase. PEM scores indicate that all of these participants showed a large effect size with the exception of Participant 2 who showed a moderate effect size.

Participant 6 showed a moderate treatment effect (PEM 80%) even though baseline scores already showed sleep efficiency above 85%. Participant 10 showed a moderate treatment effect (PEM 86%) and a general trend of improvement with some outlying low scores, and Participant 11 showed some reduction in variability of scores. Participants 13 and 8 showed no evidence of a treatment effect, and in fact Participant 8 appeared to be getting worse on this measure.


Baseline
Intervention
100% (above median)
Participant 3
Sleep efficiency of 85%
median line
80%
Participant 6
96%
Participant 9
56
55
Participant 10
86%
55
59
Participant 13
52%
53
58
42
44
47
29
58
50
54
56
56
59
35
52
82%
Participant 2
Participant 8
55%
36
Sleep Efficiency (%)
Night
0
7
14
21
28
35
42
49
56
63
70
77
60
70
80
90
100

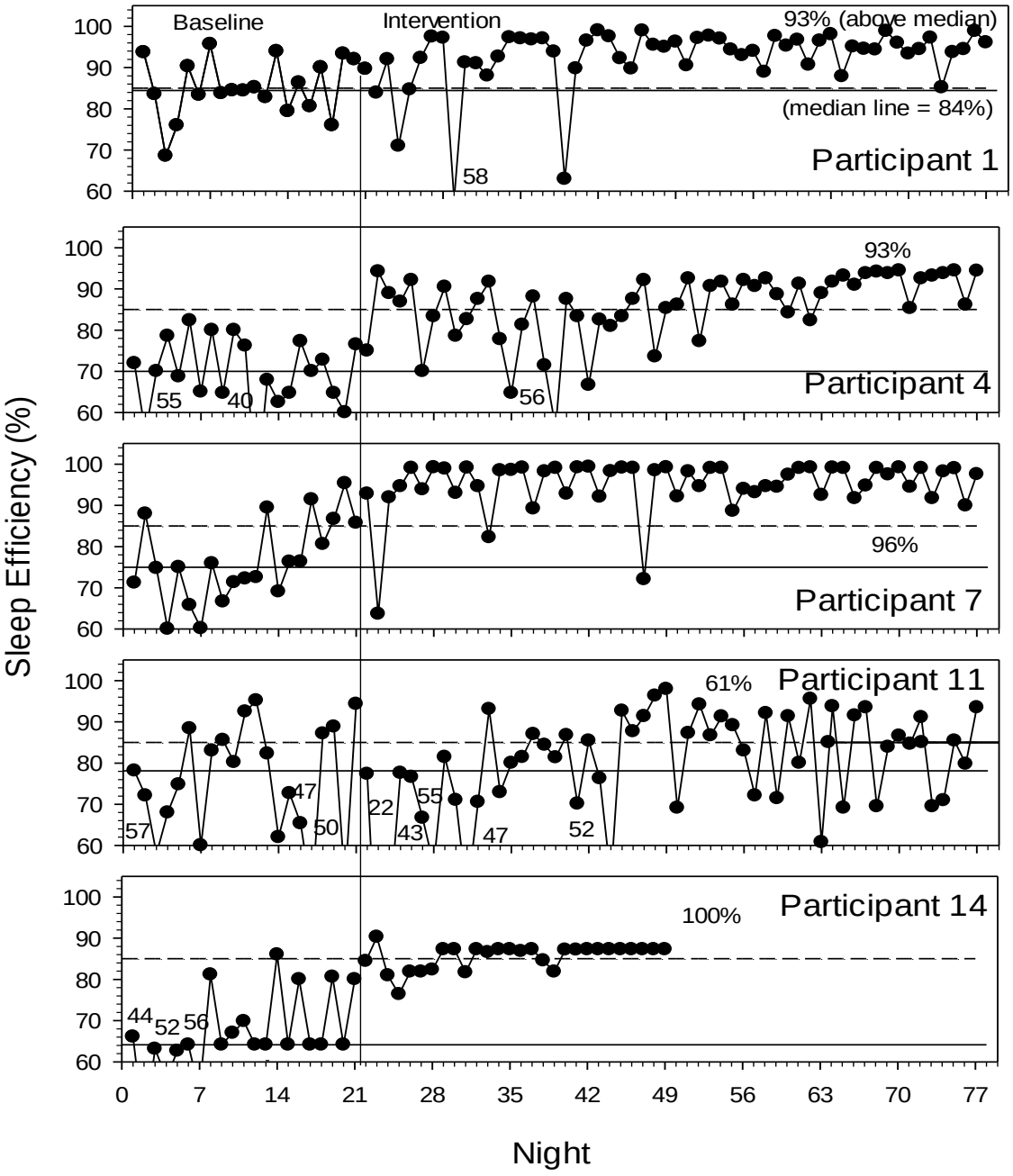
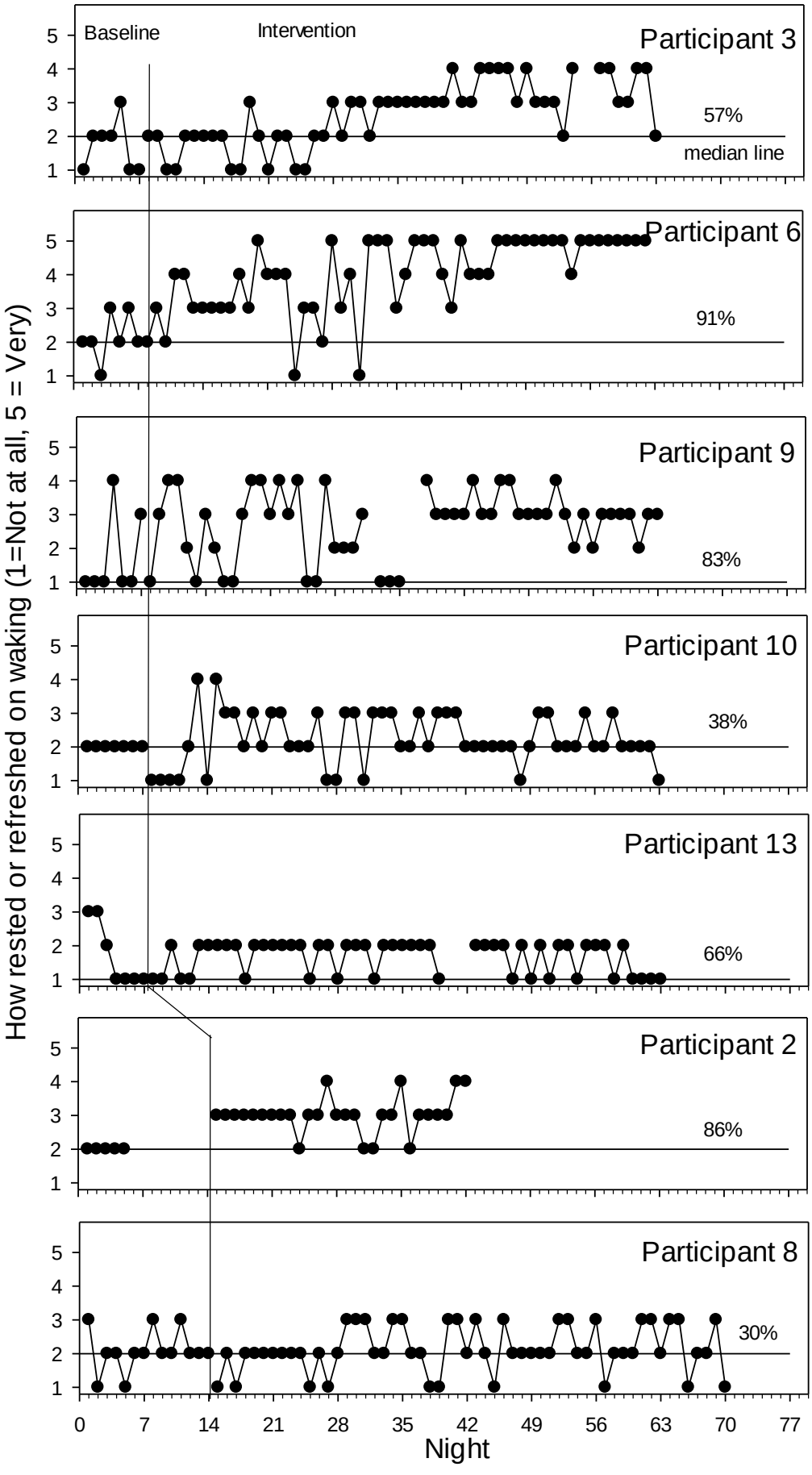


Figure 5: **Time series of each participant's nightly sleep efficiency score as measured by daily sleep diaries. Numbers on the graphs report data points that are off the scale. Median lines and percentages as for Figure 2.**

Subjective feeling of restedness: The majority of participants showed stable or worsening (decreasing) patterns of restedness during baseline, although Participant 2 was missing some baseline data which makes accurate interpretation of this phase difficult (Figure 6). Participant 7 showed some improvement during baseline which reduces the ability to determine a treatment effect for this case.

When intervention began, Participants 1, 2, 6, 7 and 14 showed an immediate improvement in feelings of restedness which were sustained or continued to improve for the remainder of the intervention. PEM scores indicate that Participants 1, 2 and 7 had a moderate effect size, while Participants 6 and 14 had a large effect.

Participants 3 and 9 showed a delayed treatment effect which became evident by week 4 of the intervention, and while Participant 9's PEM still indicated a moderate effect size, the delay in treatment effect means that the PEM does not reflect this for Participant 3. Participants 4 and 10 showed small treatment effects with an increased number of higher ratings. Participants 8, 11 and 13 showed no treatment effect on the restedness measure.


Baseline
Intervention
Participant 3
57%
median line
Participant 6
91%
Participant 9
83%
Participant 10
38%
Participant 13
66%
Participant 2
86%
Participant 8
30%
How rested or refreshed on waking (1=Not at all, 5 = Very)
Night
0
7
14
21
28
35
42
49
56
63
70
77

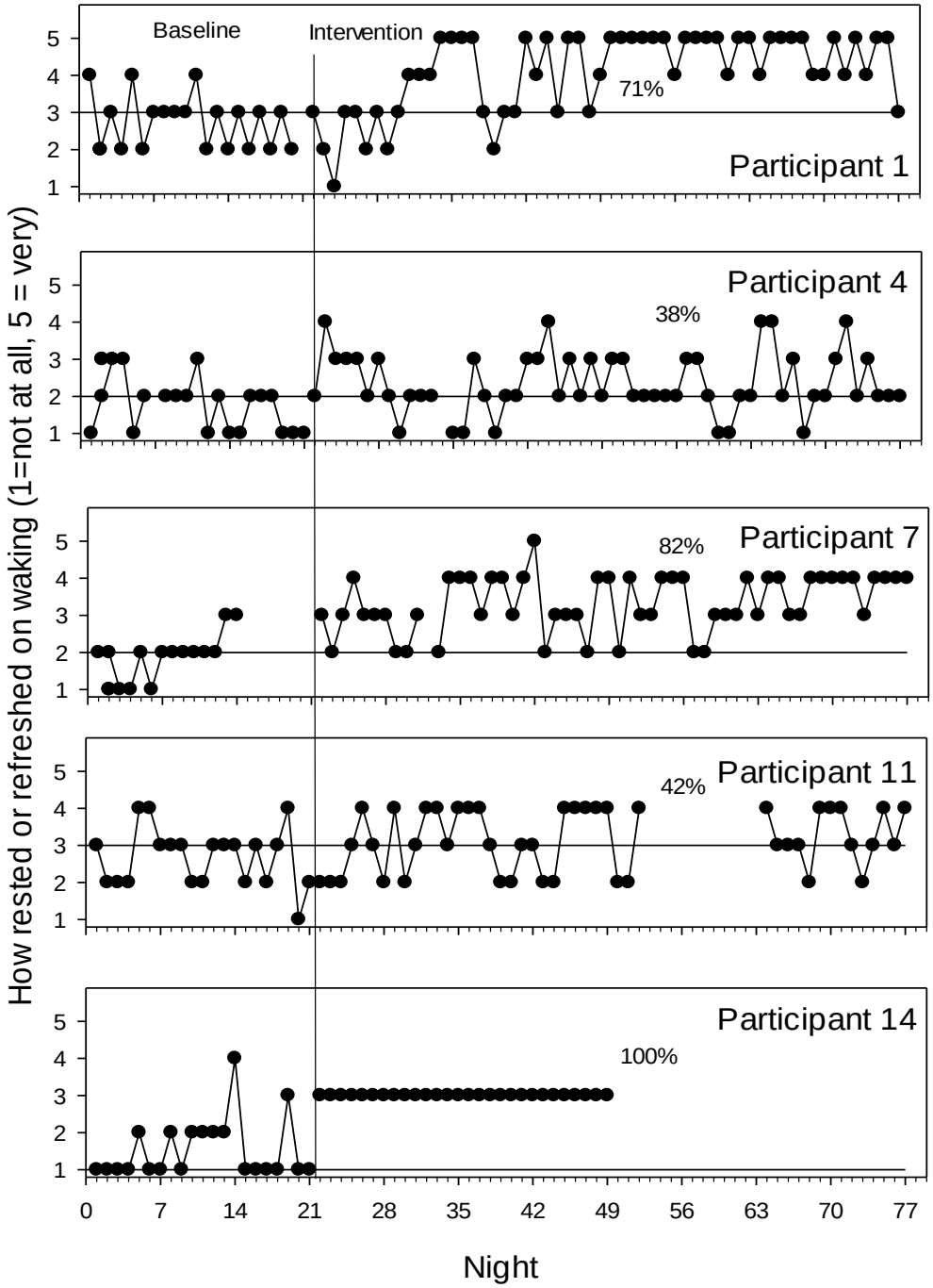


Figure 6: **Time series of each participant's daily feeling of restedness upon waking as measured by daily sleep diaries. Median lines and percentages as for Figure 2.**

3.3.1 Summary of Time Series Data. In summary, six participants (Participants 1, 2, 3, 6, 7, and 14) showed a moderate or large treatment effect on four out of the five nightly variables measured, while Participant 9 showed a moderate or large treatment effect on all five variables. Participants 4, 8 and 10 showed moderate or large treatment effect in some of the variables, and Participants 11 and 13 showed no treatment effect on any variable.

Table 3. Summary of Effect Sizes using PEM for Sleep Diary Data

	Variable				
Participant #	Sleep onset latency	Frequency of night waking	Total sleep time	Sleep efficiency	Feeling of Restedness
1	✓✓		✓	✓✓	✓
2	✓✓	✓✓		✓	✓
3	✓✓	✓✓	✓✓	✓	
4			✓	✓✓	
6		✓	✓	✓	✓✓
7		✓✓	✓✓	✓✓	✓
8			✓		
9	✓✓	✓	✓✓	✓✓	✓
10		✓	✓	✓	
11					
13					
14	✓		✓✓	✓✓	✓✓

Effect Size using PEM:

Small or none = Blank

Moderate = ✓

Large = ✓✓

3.4 Change over Time in Insomnia, Depression, Anxiety, and Stress

The following sections will present data for the ten participants who completed PIRS and DASS measures throughout the intervention phase, using modified Brinley plots (Blampied, 2007, 2014). These plots were first introduced by Brinley (1965) who used a scatter plot in an innovative way to present group mean data from different cognitive experiments. Brinley measured cognitive performance speed of groups of young and elderly

participants on several different tasks. Mean performance values from each group were plotted on a scatterplot to enable comparison of results for the different cognitive tasks for each age group. Brinley plots have since been used to display systematic effects of various categorical variables on mean performance in a range of psychological experiments (Blampied, 2014); for a recent example see Dye, Green, and Bavelier (2009).

Modified Brinley plots use data from individuals rather than group means (Blampied, 2007, 2014; Jacobson, Follete, & Revenstorf, 1984; Jacobson & Truax, 1991; Sobell, Sobell, & Gavin, 1995; Stunkard & Penick, 1979). Each data point represents an individual's measurement made at time 1 (T1), plotted on the X axis against a measurement on the same variable, taken at a later time (Tx), which is plotted on the Y axis. If there is no change of scores from T1 to Tx, and the axis have the same origin and scale, then all data points will lie along the 45º diagonal line; however, if the intervention has had a systematic effect on the participants, then this effect will be evident by movement of the plotted points either above or below the diagonal line, depending on whether it is an improvement or deterioration (Blampied, 2014).

Modified Brinley plots are particularly suitable for the analysis of outcome research of psychological therapies, and have been said to be especially useful for single-case research as they show the effect of an intervention on a group, while also preserving each individual's identity in the display (Blampied, 2007). Interpretation can be assisted by adding lines of clinical cut-off for the measure, both at T1 and Tx, (Jacobson, et al., 1984; Jacobson & Truax, 1991), and means, confidence intervals, and other measures of variance, and effect sizes can also be included (Blampied, 2014).

Data for the current study are presented as individual participant's scores on a measure at a particular time point, plotted against their score on the same measure at another specified time point. As noted above, a treatment effect is evident when scores deviate from

the 45º diagonal line. For both the PIRS and DASS, data points that fall below the line show a decrease in score and therefore an improvement on this variable, while data points that fall above the line show an increase in score and therefore deterioration. Arrows displayed on the graphs show this directional change that is indicative of improvement. The crosses on each graph represent the group mean for each time point, and in some graphs Cohen's $d_{(within)}$ effect sizes are also displayed.

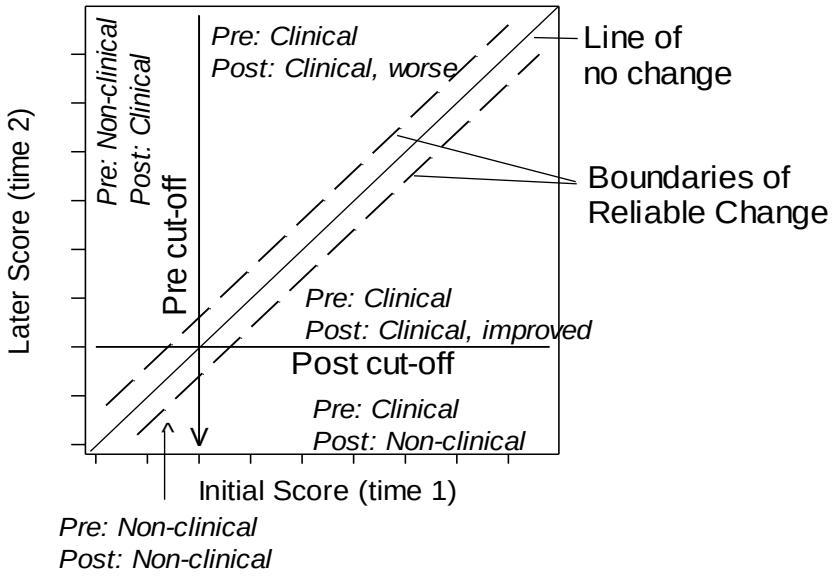


***Figure 7.*Clinical interpretation of a Modified Brinley plot where improvement is shown by a reduction in score (see the arrowhead on the vertical pre cut-off line). The solid diagonal line is the line of no effect, and the dashed diagonal lines show the Reliable Change boundaries where Reliable Change = +/- 1.96xSE_M (after Truax & Jacobson, 1991)**

3.4.1 Changes in PIRS over Time in Multiple Baseline Format. Figure 8 shows the effect of the micronutrient intervention on the primary measure of insomnia severity, the Pittsburgh Insomnia Rating Scale (PIRS). Consistent with the multiple baseline design, participants received baseline lengths of one week, two weeks or three weeks, and their initial screening score was treated as an additional baseline score. Each participant's weekly PIRS

score is plotted against their initial screening score, thus showing change over time relative to initial screening. As noted above, in the absence of any change over time, all data points would lie on or closely about the diagonal line. Increases in PIRS scores at any time point are shown by points above the line, and decreases by points below the line. The bold dashed line in the figure demarcates baseline weeks from subsequent treatment weeks. The data points show little dispersion on the X-axis because (a) participants were selected to be above a clinical cut-off of 98 on the PIRS, and (b) because each individual's point in the plot is referenced to their initial screening score, and is constant across graph panels. Treatment Week 4 (the mid-point of the treatment phase) and the last week of treatment (Week 8) are then shown in plots to the right of the baseline plots. These weeks were chosen to (a) allow for any delayed treatment effect to emerge (Week 4) and (b) to show the effect of treatment after full exposure to the micronutrients (Week 8).

The top line of plots present data for the group with one week baseline. The initial plot, presenting initial PIRS score versus Baseline 1, shows that two participants had a small decrease in insomnia rating during baseline, while the other two showed a slight increase. The middle line of plots present data for the group with two weeks baseline. The initial two plots presenting initial PIRS versus Baseline 1 and Baseline 2, show a slight deterioration in the first week of baseline, and a move back towards the line of no change during the second week of baseline. The third line of plots presents data for the group with three weeks baseline. The first three plots compare initial PIRS with Baseline 1, Baseline 2 and Baseline 3 respectively, and show that three Participants show a slight improvement during baseline weeks, while one Participant shows an increase in insomnia severity. Overall, to a large extent these baselines were stable across the weeks, although there was a trend for the group with a three week baseline to show slight improvement during this phase.

The mid-point plots compare each participant's initial PIRS score with their score after four weeks of intervention. For the first group, the plot shows that by the mid-point of intervention, all four participants have shown a decrease in insomnia severity, in the second group both participants have also shown an improvement, and this is replicated in the third group where all four participants have also shown a decrease in insomnia severity. The reductions in PIRS scores shown in this week are consistently larger than any reductions observed in any baseline week. The general trend across plots is that a treatment effect is able to be detected after four weeks of micronutrient intervention.

The Treatment Week 8 plots compare each participant's initial PIRS score with their score at the end of the treatment phase. For the first group we can see that three out of four participants have shown further improvement, while one participant is showing some deterioration towards baseline. In the second group one participant has sustained their improvement at mid-point, and the other's data point is missing for this time point. All participants in the third group showed further improvement relative to Week 4. Overall, the majority of participants were showing a treatment effect by mid-point and this treatment effect had increased by the end of the intervention phase.

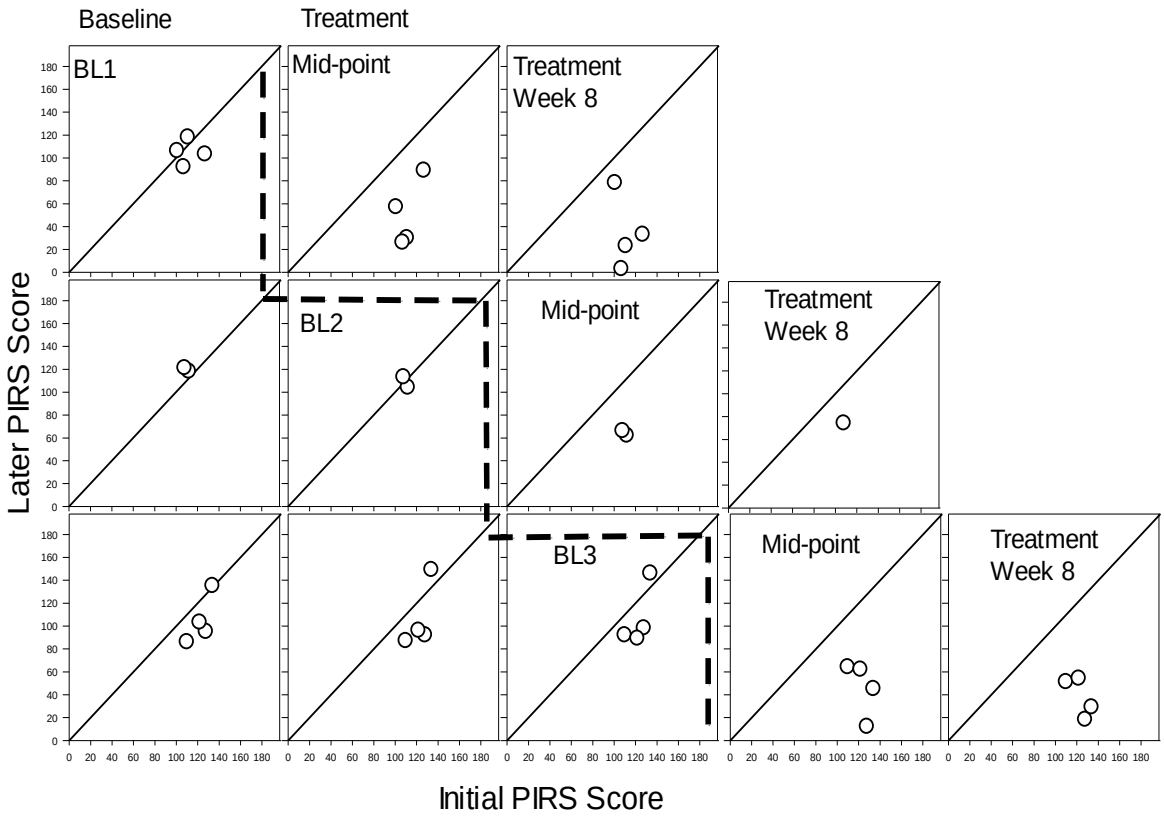


Figure 8. **Modified Brinley plots showing the effect of a micronutrient intervention on insomnia severity in a multiple baseline format as measured by the PIRS. The bold dashed line separates baseline phases (on the left) from treatment phases on the right. Data from each week of baseline are shown for all participants, then from the mid-point of treatment (Week 4) and then from the last week of treatment (Week 8). The diagonal line is the line of no change over time; improvement is indicated by points below the line. [NB: Ns for the 1-week and 3-week baseline group = 4 throughout. One participant ceased recording data during the treatment phase for the 2-week group, so there is a data point for only one person at Treatment Week 8.]**

In summary, Figure 8 shows a trend for relatively stable baselines across weeks, with a slight tendency for the third group to show slight improvement in baseline phase. By the mid-point of intervention all participants are showing a treatment effect, and by the end of intervention the majority of participants show a larger effect.

3.4.2 Plots of Weekly Changes in PIRS over Treatment Phase. Figure 8 examined changes in PIRS scores for Treatment Weeks 4 and 8 relative to initial screening score, and examined the stability of scores across baselines of different lengths and the replication of the treatment effects across the different baseline-length groups. Figure 9 shows the effect of the micronutrient intervention on insomnia as rated by the PIRS, referencing each individual's weekly score against their mean score in baseline, again using modified Brinley plots. Given the evidence from Figure 8 that baseline scores were relatively stable, individual's mean baseline scores can be reported with confidence that they accurately, but conservatively, represent baseline performance (for a fuller discussion of the issues arising in selecting baseline reference values, see Blampied, 2014). Mean baseline scores were computed participant by participant, by combining the initial screening score with all subsequent baseline points. The number of scores averaged thus depended on which baseline length condition the participant was assigned to (1, 2 or 3 weeks). In Figure 9 the bold cross on each graph panel shows the overall mean for the week plotted against the overall mean baseline score, and these means were used to compute Cohen's $d_{(within)}$ Effect Size for each week. Cohen's *d* was computed using the formula $\frac{Mean(baseline)-Mean(week)}{SDaverage}$ taken from Cumming (2012), such that improvement in insomnia symptoms is related to a positive ES.

The initial plot presenting mean of Baseline versus Intervention Week 1 shows that approximately one third of participants showed no change, one third showed a small improvement, and the final third are already showing a moderate improvement in insomnia

severity. Cohen's *d* for this plot (1.04) indicates that a large treatment effect is already evident. Over the next three plots all participants show a decrease in scores and appear to split into two clusters, one cluster showing small to moderate improvement, and the other showing moderate to large improvement. As the weeks progress, the effect size continues to increase in magnitude (e.g. *d*= 2.92 at Week 4). These trends can be seen across the remaining four plots and by Week 8 the mean of scores has dropped from 109.8 at baseline to 40.33, with an effect size of *d*= 3.42 which is more than three times the effect size at Week 1.

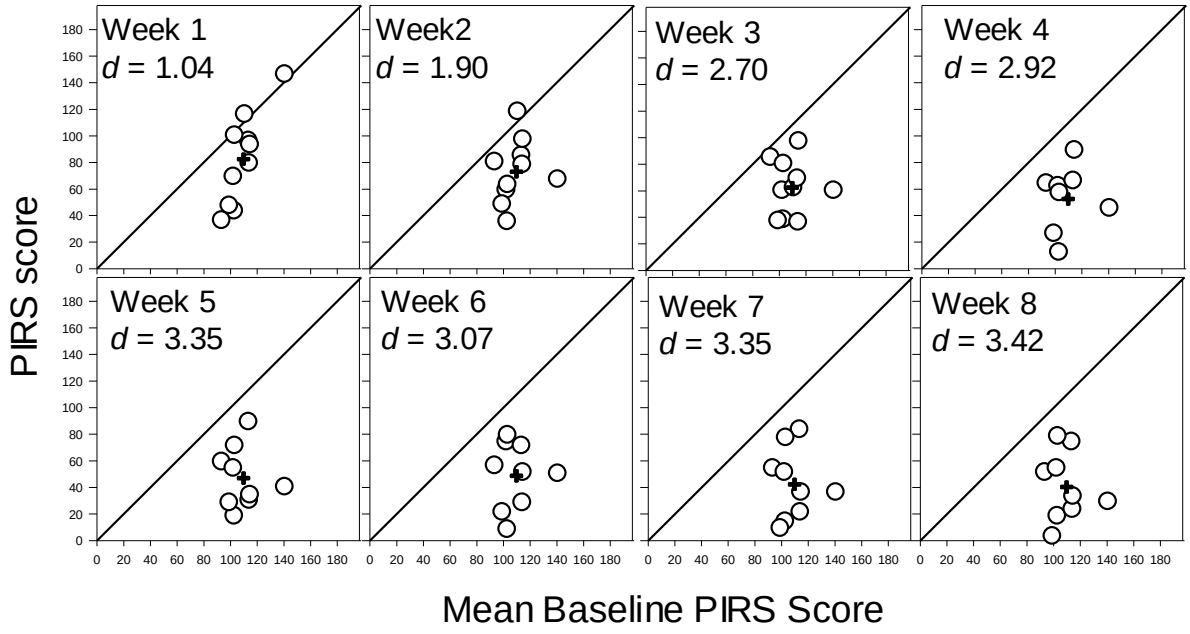


Figure 9. **Modified Brinley plots showing the effect of micronutrient intervention on insomnia severity week by week, as measured by the PIRS. The cross shows the composite mean for the week plotted against the composite baseline mean. Cohen's *d* Effect Size is also shown week by week. [NB: Weeks 1 – 3, n = 10, Weeks 4 – 8 n = 9. A data point is missing for Participant 3 at Week 4]**

3.4.3 Summary of Findings from Primary Outcome Measures. Primary outcome measures included data from the daily sleep diaries and the PIRS. Individual changes across baseline and intervention phases, as reported in daily sleep diaries, were presented using time series graphs following the conventions of the multiple-baseline design. These graphs showed that seven out of twelve participants (58.3%) showed a moderate or large treatment effect, on at least four of the five variables measured. Three of the twelve participants (25%) showed a moderate or large treatment effect on some of the variables, and only two participants (16%) showed no treatment effect. Overall, ten out of twelve participants, or 83%, showed a treatment response to the intervention as measured by the daily sleep diaries.

Modified Brinley plots were used in multiple-baseline format to analyse individual changes in the PIRS measure. Figure 8, which compares each participant's initial score, with final baseline, Treatment Week 4 and Treatment Week 8, shows a trend for relatively stable baselines across weeks, with a treatment effect evident for all participants at Week 4, and a larger effect for most participants by Week 8. This trend is replicated in Figure 9, which shows a treatment effect at Week 1 of intervention (d= 1.04), that increases in magnitude with each passing week. By the end of treatment (Week 8), the group mean of scores has dropped from 109.8 at Baseline, to 40.33, with an effect size of d= 3.42, a very large effect.

3.4.4 Changes over Time in DASS; Depression, Anxiety, and Stress. The DASS subscales of Depression, Anxiety, and Stress were considered secondary measures in this study, and were included to assess any general changes in psychological wellbeing concurrent with changes in the primary sleep and insomnia measures**.** Figures 10, 11 and 12 show the effect of the micronutrient intervention on depression, anxiety and stress, as measured by the DASS, referencing each individual's weekly score against their mean score in baseline, again using modified Brinley plots. The bold cross on each graph panel shows the overall mean for the week, plotted against the overall mean baseline score. As with the PIRS

data, mean baseline scores were computed participant by participant, by combining the initial screening score with all subsequent baseline points. Also as for the PIRS, reductions in DASS scores are indicative of improvement.

Depression: In Figure 10 the initial plot presenting means of Baseline versus Intervention Week 1 shows that the majority of participants started to report small improvements in depression levels in the first week, relative to their baseline levels. The next three plots show that three participants are showing a moderate to large improvement, three are still only showing very slight improvement, and two have actually gotten worse. By the mid-point (Week 4), the treatment effect size is moderate (d = 0.64). Over the remaining four plots, all participants show improvement and by Week 8 the mean of the Depression scores has dropped from 10.46 at Baseline to 3.56, with a large effect size of d = 1.46.

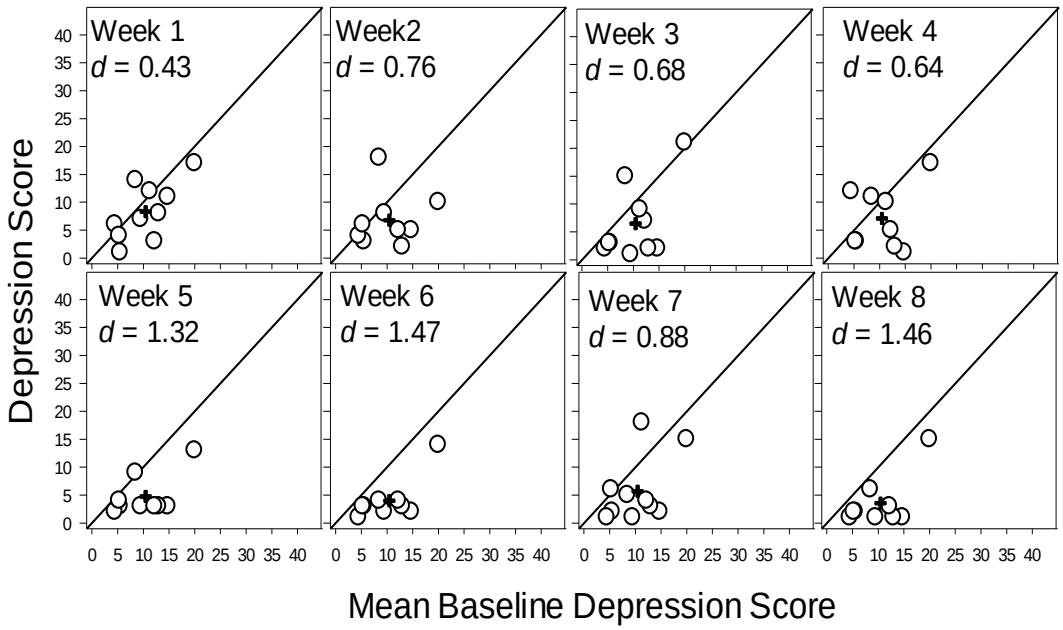


Figure 10: Modified Brinley plots showing weekly effect of micronutrient intervention on mood as measured by the Depression Subscale of the DASS. Cross marks the mean for each week relative to the overall Baseline mean.

Anxiety: In Figure 11 the initial plot presenting means of Baseline versus Intervention Week 1 levels of Anxiety shows that already the majority of participants reported an improvement in anxiety in Week 1 relative to Baseline, and already a large treatment effect is evident (d = 0.89). Over the next three plots all except one participant continued to improve; by the end of treatment (Week 8) all participants showed further improvement, the group mean dropped from 6.58 at Baseline, to 1.33, and the effect size increased in magnitude (d= 1.68 at Week 8).

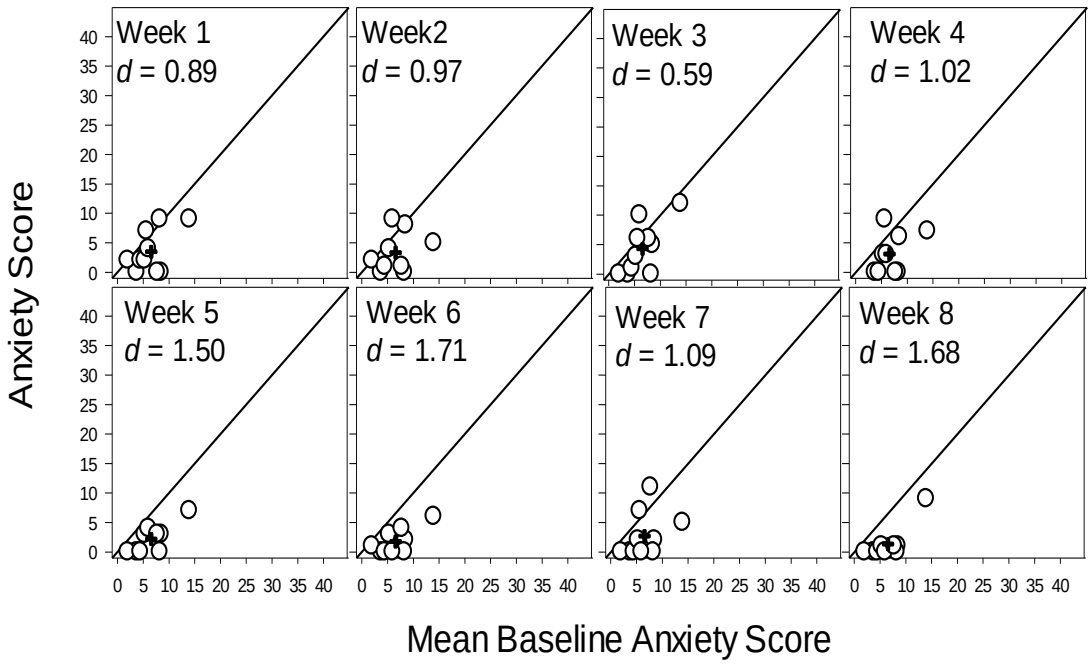


Figure 11. Modified Brinley plots showing weekly effect of micronutrient intervention on anxiety as measured by the Anxiety Subscale of the DASS. Cross marks the mean for each week relative to the overall Baseline mean.

Stress: In Figure 12 the initial plot presenting means of Baseline versus Intervention Week 1 shows that a majority of participants showed small or moderate improvement, and one participant shows particularly large improvement in reported stress levels by the end of the first week of treatment. Over the course of the next three plots all except one participant continued to improve and the effect size continued to increase. By the mid-point of therapy (Week 4) *d*= 2.16, and by the end of eight weeks of intervention all participants showed a moderate to large improvement, with the group mean decreasing from 17.43 at Baseline, to 6.33 at Week 8. The effect size continued to increase and by the end of intervention *d*= 2.85, a large effect.

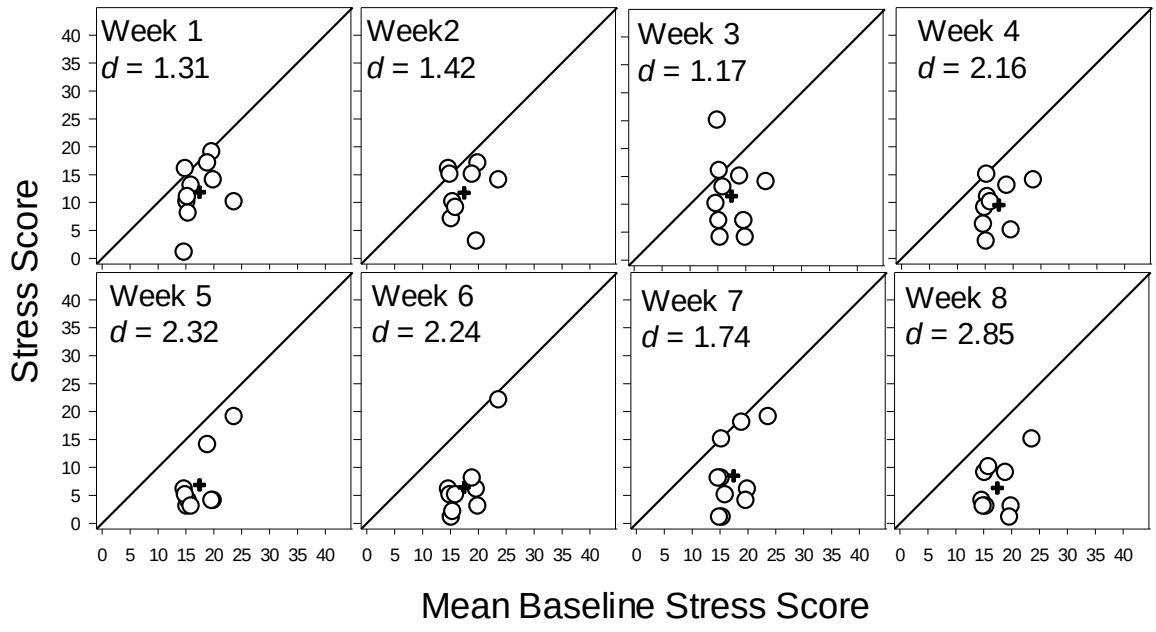


Figure 12. Modified Brinley plots showing the weekly effect of micronutrient intervention on stress as measure by the Stress Subscale of the DASS. Cross marks the mean for each week relative to the overall Baseline mean.

3.4.5 Summary of the Effect of the Intervention on Insomnia (PIRS) and Depression, Anxiety, and Stress (DASS) at Baseline, 8 Weeks, and Follow-up.

Figure 13 provides a summary of the effect of the micronutrient intervention on insomnia severity, depression, anxiety and stress as measured by the PIRS and the DASS. Figure 13 plots initial scores for each measure against final baseline (i.e., Baseline week 1, or 2, or 3, depending on the baseline assigned), treatment Week 8, and follow up scores for each participant. The far right hand plot compares treatment Week 8 scores with scores at 3 months follow up to examine stability of treatment gains over time (after Stunkard & Pennick, 1979), and as 8 of the 9 participants who completed follow-up measures had ceased to take the micronutrient this becomes a natural reversal design, in so far as one compares baseline, treatment, and follow-up phases.

Further features are employed in Figure 13 to aid in summarising the findings of the study. As noted previously, in the absence of any change over time, all data points would lie on the diagonal line. Increases in scores at any time point are shown by points above the diagonal line, and decreases by points below this line. Bold horizontal and vertical lines indicate the clinical cut-off for the DASS (i.e., Depression = 13, Anxiety = 10, and Stress – 18; Lovibond & Lovibond, 1995) , and the screening cut-off for the PIRS (i.e., 98). Reliable change boundaries are shown using dotted lines and the arrowheads show the direction of therapeutic change. Reliable Change was calculated for each measure using the procedures of Jacobson and Truax (1991). Data points lying below -1.96xSE (SE = Standard Error of Measurement) can be considered as showing Reliable positive change (RC+), those lying above +1.96xSE as showing reliable deterioration (RC-) and those between the boundaries as being indeterminate (RCo) (see Blampied, 2014).

PIRS: The intial plots compares intital scores with scores at the last baseline measure. Participants are clustered very closely to around the diagonal line with some scores slightly above and some slightly below, indicating that scores across baseline were relatively stable. The second plot, comparing initial scores with treatment Week 8 shows a dramatic movement of participants down and away from the diagonal line (in the direction of therapeutic change) showing a large treament effect (d= 4.08). All participants are now below the initial cut-off score for entering the study, and all particpants sit in the RC+ zoneshowing that this treatment effect is unlikely (p< .05) to be due to measurement error. No participant showed either deterioration (RC-) or was indeterminate (RCo). The third plot compares intital scores with the 3 month follow up, and even though all bar one participant had discontinued use of micronutrients at this stage, the participants are still maintaining some improvement, although not as dramatic as Week 8. The final plot which compares treatment Week 8 to 3 month follow up shows that almost all participants have shown no change or have deteriorated in the months between the end of treatment and follow-up. This was to be expected due to the fact that only one participant conintued using the micronutrient intervention after Week 8 (the participant represented by filled dot).

DASS: The initial plots comparing scores at initial screening with scores at end of baseline shows that mostly participants show little change across these measures. In the Depression scale, two show improvement and one shows deterioration, and in the Anxiety scale one shows some improvement while the rest are clustered very closely about the line of no change. In the Stress scale one participant shows improvement and one shows deterioration. Overall most show little or no change across baseline and are relatively stable in baseline. The second column of plots comparing initital score with treatment Week 8 show dramatic movement of the points down and away from the diagonal line in the direction of

therapeutic change. The majority of participants show a reduction in depression, anxiety and stress scores across the intervention phase, with large effect sizes of *d*= 1.37, *d*= 1.48 and *d*= 2.52 respectively. The percentage showing Reliable Change is also large for both depression and stress (67% and 78%), while it is moderate for anxiety (56%). The third row of plots comparing initial score with 3 month follow up shows a trend of deterioration back towards baseline levels, with some participants worse than at intial screening. Notably, the participant who continued with the micronutrient intervention (the filled dot in Figure 13) is one, but not the only one of those still showing a treatment effect. The final row of plots compares scores at treatment Week 8 with those at follow up, creating a natural reversal design. Across subscales, all participants showed little or no change, or a deterioration from treatment Week 8.

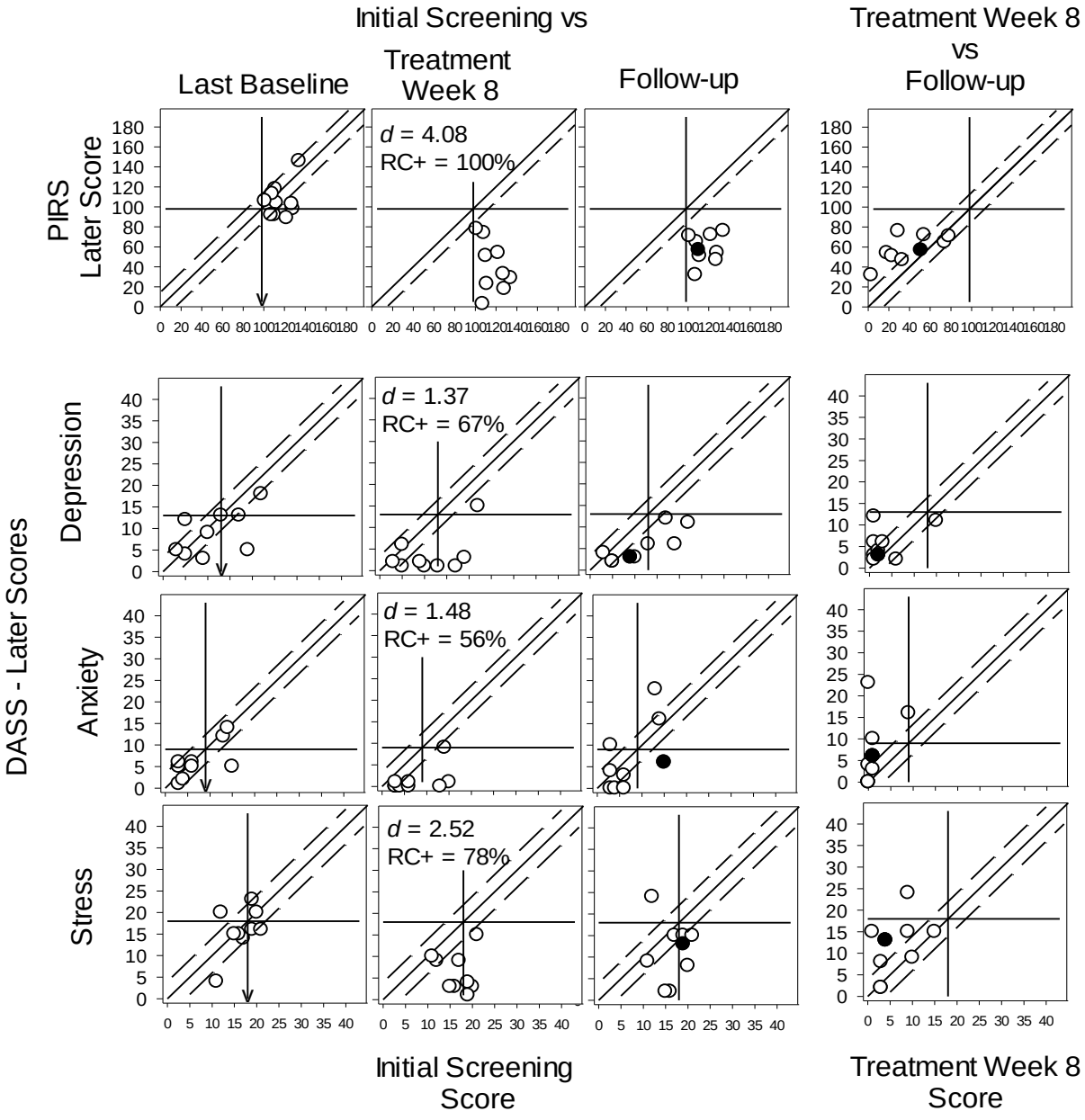


Figure 13: A summary Modified Brinley plot graph summarising PIRS and DASS data over baseline, treatment and follow-up. The filled dot marks the participant who continued to take micronutrients through follow-up. Reliable change boundaries are shown using dotted lines, and RC+ shows the percentage of participants showing Reliable positive change.

4. Discussion

4.1 Summary of Findings

This study investigated an innovative approach, using a multi-ingredient micronutrient intervention for the treatment of insomnia in adults. The results showed that treatment with micronutrients resulted in clinically significant decreases in severity of insomnia symptoms, and reduced stress, anxiety and depression levels, amongst a sample of adults who were suffering from chronic insomnia. This study is the first to use a multi-ingredient micronutrient to treat insomnia in adults. The findings add a new element to the current literature that examines micronutrient interventions for mental health concerns, and the positive result is consistent with previous studies which have found that micronutrients can significantly improve mood, and reduce levels of anxiety and stress. The current study found there were significant improvements with the introduction of micronutrient intervention, for all dependent variables, as demonstrated by time series analysis and modified Brinley plots. There is considerable agreement that in clinical outcome research, information on individual change is of key importance (Blampied, 2014; Jacobson et al., 1984; Sobell et al., 1995; Stunkard & Penick, 1979), and modified Brinley plots are particularly useful in this respect, as they show the effect of an intervention on a group, while also preserving each individual's identity in the display (Blampied, 2007).

Fourteen individuals participated in the trial, one participant withdrew following the first three days of the intervention phase and another following week four of the intervention phase. The participants recruited reflected a group of adults who were experiencing moderate to severe levels of insomnia, measured using the Pittsburgh Insomnia Rating Scale.

The hypotheses were generally supported by the results of the study. The primary hypothesis predicted that micronutrient intervention would be associated with reduced severity of

insomnia symptoms. This hypothesis was supported such that all participants showed a decrease in insomnia severity during intervention phase, and by the end of treatment (Week 8) the group mean PIRS score had dropped from 109.8 at baseline, to 40.33, with a large effect size of $d = 3.42$.

Reliable change was also calculated for each the PIRS and DASS measures (Jacobson & Truax, 1991), and the percentage of participants showing reliable positive change (%RC+) was used as another measure of effect size. By Week 8 of treatment, all participants, or 100%, were in the reliable positive change zone for the PIRS, indicating that this treatment effect is unlikely ($p < .05$) to be due to measurement error. Reporting the percentage showing positive reliable change is complimentary to Cohen's *d* as it says how many participants changed positively to a degree plausibly larger than measurement error, while being silent about the size of the change. On the other hand, *d* measures the size of the mean change, scaled by the standard deviation, and using both gives more information than either effect size alone.

The second hypothesis, that improvement in insomnia symptoms would be endorsed by daily sleep diaries, was also supported. The diaries showed that during the intervention phase, five out of twelve (42%) participants showed a moderate or large treatment effect on measures of sleep onset latency, six out of twelve (50%) showed a moderate or large treatment effect on frequency of night waking, and nine out of twelve (75%) participants showed a moderate or large treatment effect on total sleep duration. Daily sleep diary data also confirmed that nine out of 12 (75%) participants also showed a moderate or large treatment effect on measures of sleep efficiency, and six out of twelve (50%) showed a moderate or large treatment effect on feelings of restedness upon waking.

Although there was a tendency for a small improvement on some measures during baseline, the majority of baseline measures showed predominantly stable baseline phases, followed by much steeper improvement during the intervention phase. This improvement was seen across multiple dependent measures and by the majority of participants, demonstrating a compelling treatment effect, above that of trends towards regression to the mean.

The third hypothesis, that micronutrient intervention would be associated with reduction in levels of stress, anxiety and depression, was also supported. Out of the ten participants who completed the DASS measures, all experienced a reduction in depression rating during treatment. By the end of treatment Week 8, the group mean depression score had dropped from 10.46 at baseline to 3.56, with a large effect size of $d = 1.46$, and the percentage showing Reliable Change was also large at 67%.

All participants also showed a reduction in anxiety, and the group baseline mean of 6.58 dropped to 1.33 by end of treatment, with $d = 0.89$, a large treatment effect, and a moderate percentage showing Reliable Change of 56%.

Further, all participants showed a moderate to large improvement in stress levels, with the group baseline mean of 17.43 reducing to 6.33 by Week 8. A large treatment effect was also detected for this variable and by treatment Week 8, $d = 2.85$, and a large percentage (78%) showed reliable positive change.

Some quotes from participants illustrate the experience of the intervention in a qualitative way. At the end of the intervention phase, one participant stated “I have noticed a huge difference, when I wake up I can go back to sleep rather than staying awake, and I wake up feeling great”, another participant stated “I have never slept this well”. Another participant commented “I don’t think I have ever slept better, my main issue has always been getting to sleep, it took a long time, sometimes hours, now (end of intervention) it takes a maximum of

30 minutes, and normally less", the same participant also said "I have better quality sleep, I'm less worried about sleep and I wake up with better energy levels". Another participant stated "The nutrients started working after about two weeks and made a great difference, I'm not waking up nearly as much, and lots of nights I'm not waking up at all".

Intervention with the micronutrient supplement was associated with mild side effects in four out of fourteen participants. One participant experienced a mild stomach upset for the first few days of taking the capsules which then passed. One participant reported a very dry mouth at the beginning of their fourth week taking the capsules which had passed by the end of the week. Another participant reported experiencing an increase in intensity of sensations such as sights and sounds during the seventh week of intervention, although they were unsure if this was related to the capsules. A fourth participant reported headache, reflux, mild nausea and dizziness after starting the intervention. This participant decided to withdraw from the study during the first week of taking the capsules due to the side effects experienced.

Of the twelve participants who completed the entire intervention phase, all (100%) were compliant in terms of adherence to the treatment protocol. This involved taking the recommended micronutrient dose (six capsules) daily, without missing a significant number of doses, which was defined as a compliance rate of greater than 80%, or no more than 67 missed capsules across the intervention phase. Of the twelve compliant participants, eight reported perfect compliance, two participants reported a total of 3 missed capsules, one reported a total of 27 missed capsules, and one participant reported a total of 30 missed capsules.

The final hypothesis, that improvements would be maintained or increased over the follow up period for those who chose to stay on the micronutrient intervention was difficult to examine due to the fact that despite significant improvement during intervention phase for

many participants, only one participant continued to take the capsules after treatment Week 8. Reasons stated for not continuing included cost, access, life stress. The two participants, who did not experience a treatment effect during the study, stated that they had discontinued the micronutrients as they had not found them beneficial.

The follow-up period, therefore, became a natural reversal design. The one participant who remained on the intervention showed little or no further improvement between end of intervention phase and 3 month follow-up, while the majority of other participants showed some deterioration back towards baseline.

At the three month follow up, a participant who had stopped taking the nutrients reported “I'm a little more restless during the night and it is slightly harder to get to sleep.” another said “my sleep wasn’t as good two weeks after stopping the supplement”. Another participant commented “I am much more anxious than I was during the period that I was taking the micronutrients, I am going to go back to taking them as I believe they greatly improved my quality of life."

The novel findings of the current study build on previous research demonstrating the positive effect of micronutrient intervention with broad spectrum micronutrients in those with mental health problems. They support and replicate findings from a number of other studies which have demonstrated the therapeutic effect of micronutrients (specifically EMPowerplus) on mental health problems such as bipolar disorder, OCD, ADHD, stress and anxiety (Kaplan et al., 2007; Kaplan et al., 2002; Kaplan et al., 2004; Kaplan & Leung, 2011; Kaplan et al., 2001; Lewis Mehl-Madrona, Brenda Leung, Carla Kennedy, Sarah Paul, & Bonnie J. Kaplan, 2010; Popper, 2001; Rucklidge, 2009; Rucklidge et al., 2012; Rucklidge et al., 2014; Rucklidge et al., 2010; Rucklidge & Harrison, 2010; Rucklidge, Harrison, & Johnstone,

2011; Rucklidge, Johnstone, et al., 2011; Rucklidge & Kaplan, 2013; Rucklidge, Taylor, et al., 2011).

This research has demonstrated a positive treatment response when using micronutrients for the treatment of insomnia, and showed that this intervention has a minimal side effect profile (i.e., side effects were mild and transitory) when compared with pharmacological treatments, and continues to exhibit a treatment effect over an extended period. Hypnotic medications, used to treat insomnia, frequently cause daytime impairment and sedation due to their half-life, and are only safe for short term use (Sateia & Nowell, 2004). Their usefulness is limited to insomnia of short duration (less than four weeks) as after this time their therapeutic effect wears off and with continued use, addiction can develop (Touitou, 2007). Furthermore, withdrawal effects of hypnotic medications have been well documented and can last as long as five weeks following discontinuation. Given that withdrawal appears to be related the dose taken and the length of time that the hypnotic is taken for, long term treatment with hypnotics is not appropriate (Rothenberg, 1997). The minimal side effect profile and long term effectiveness of the micronutrient intervention are therefore particularly notable as they provide an effective and non-addictive alternative to the pharmacological treatments currently available.

Other research that has investigated the impact of multi-ingredient micronutrient formulas (not EMP+) on mental health conditions has also found a positive treatment response. It is challenging to compare these studies to each other and to those which use EMP+, due to the varying formulas and doses used. However, the overall findings from the current study suggest a strong potential for the use of multi-ingredient micronutrient formulas, including EMP+, in the treatment of sleep difficulties, anxiety, stress and depressed mood in adults.

The exact mechanism through which micronutrient formulas produce a treatment effect is not yet known. Kaplan and colleagues (2007) have discussed four possible conceptual frameworks that could explain how micronutrients might act within the brain to treat the symptoms of mental health disorders. These models are discussed by the authors as being compatible rather mutually exclusive or exhaustive, and may explain co-existing pathways through which micronutrients exhibit their therapeutic influence on mental health.

The first model suggests that unstable mood may be the manifestation of inborn errors of metabolism, which can affect brain function through their influence on enzyme and coenzyme reactions (Ames, 2004). These inborn errors can diminish the binding affinity for a coenzyme by a known enzyme, which then leads to a lower rate of metabolic activity. Ames and colleagues (2002), investigated human genetic diseases that are associated with this type of inborn error and found that in most cases, symptoms could be corrected by treatment with additional cofactors (micronutrients). It is therefore plausible, that, in some cases of mental illness, inborn errors of metabolism may slow metabolic activity involving neurotransmitters, contributing to the development of psychiatric symptoms. Treatment with additional micronutrients may serve to facilitate this metabolic activity, thereby enhancing neurotransmitter activity within the brain and leading to the resolution of mental health symptoms (Kaplan & Leung, 2011).

The second conceptual framework explains how psychiatric symptoms could occur as a product of deficient methylation reactions (Kaplan et al., 2007). Methylation, or adding a methyl group to a molecule, is integral to many brain processes, especially those involved in the synthesis of neurotransmitters. Given that micronutrients are a vital ingredient in methylation, the authors suggest that micronutrient treatment may enhance the methylation process of vital brain enzymes, thus enhancing synthesis of neurotransmitters (Kaplan et al., 2007).

Another theory is that psychiatric symptoms are the result of alternations of gene expression, caused by nutrient deficiency. It has been well established that the level of available nutrients can alter the expression of genes, and in this case they may be responsible for the expression of genes involved in mental health disorders, many of which have been found to have a genetic component (Kaplan et al., 2007; Kaplan & Leung, 2011). Ames's (2010) triage hypothesis proposes that nature ensures our survival by altering metabolism within the body when the availability of micronutrients becomes limited. Nutrients are redirected towards those processes most essential for our immediate survival, albeit at the expense of the body's long term health, and this can lead to long term degenerative disorders. Perhaps mental health conditions reflect long term suboptimal levels of micronutrients necessary for brain function, resulting in malfunctioning brain processes and deterioration in mental health (Kaplan et al., 2007).

Impairment in mitochondrial function has also been implicated as an underlying contributor to a variety of neurological and psychiatric disorders (Kaplan & Leung, 2011). Elevated levels of oxidative stress can contribute to neuronal damage, and it is possible that psychiatric symptoms may be the manifestation of deficiencies in micronutrients that are needed to remedy this oxidative stress.

While the exact causes of an individual's insomnia are thought to vary, the majority of research identifies heightened arousal, anxiety, worry and rumination, as being leading factors in the cause and maintenance of sleep disruption (Bootzin & Epstein, 2011). Events that are emotionally stressful have been found to impact sleep quality and wellbeing, and are believed to do so through triggering cortical and emotional arousal (Wuyts et al., 2012). Several psychological and physiological aspects can contribute to the onset and maintenance of insomnia. Anxious-ruminative personality traits, life stressors, homeostasis weakening mechanisms, and biologic or genetic central nervous system hyper-arousal have all been

identified as factors that can be involved (Basta, 2007). It is therefore plausible that through the correction of inborn errors of metabolism, enhancement of the synthesis and activity of neurotransmitters, and the continuous availability of an abundance of micronutrients, issues of biologic or central nervous system hyper-arousal may be amended, and the brains ability to process emotional stress may be enhanced.

These theories and models propose pathways through which micronutrient interventions may exhibit their therapeutic effect on mental health symptoms. As mentioned above, these mechanisms are likely to be interrelated and there is much overlap that occurs between them. Overall, the theories indicate that some individuals may require higher levels of available micronutrients than others, and that perhaps some require levels higher than are obtainable through diet.

Depletion of nutrients in the food supply should also be considered. Research shows that the levels of minerals and trace elements in fruits and vegetables have been diminishing over the past 50 years (Ekholm et al., 2007; Mayer, 1997), and the modern Western diet is typically dominated by refined, processed carbohydrates, with limited amounts of fresh produce (Cordain et al., 2005). Thus, it is likely that many Westerners are consuming suboptimal levels of micronutrients, and are therefore vulnerable to the problems described in the models above. It may be that certain individuals are particularly vulnerable to these nutritional depletions in food due to different biochemical needs (Rucklidge, Taylor, et al., 2011). The literature is demonstrating that supplementation with multi-ingredient micronutrient formulas can remedy these deficiencies and may be a promising and innovative way forward in the treatment of mental health conditions.

4.2 Limitations

One limitation of this study was the open label nature of the trial and the lack of placebo control. Both the participant and researcher were aware that they were in active intervention phase and this can make both parties susceptible to expectancy effects. While we cannot rule out a placebo effect, there are many reasons that it is unlikely to have explained the therapeutic effects; for example, most participants showed some delay in treatment response after beginning the nutrient intervention, and even those who showed an initial improvement mostly showed only a small effect which increased in size the longer the individual was taking the micronutrients. A placebo effect would be expected to occur immediately, rather than gradually growing in magnitude over the weeks. Participants were also informed several times prior to beginning the study that this was an experimental treatment and that the researcher did not know if it would be effective. Many of the participants had trialled other treatments in the past, including natural over-the-counter remedies, none of which they found effective in the long term, which makes it unlikely that it would only be with this intervention that they experienced a placebo effect. So while in this case placebo effects cannot be ruled out, there are a number of reasons why they are unlikely to be responsible for the therapeutic effect found.

This study is a pilot trial, and the promising results bring to attention the need for future placebo-controlled research into the effect of micronutrient intervention for insomnia. Initially, direct replication with a larger sample would be useful to establish the reliability, and replicability of the research findings. Randomised controlled trials, reversal trials and effectiveness trials (where the intervention is tested in a clinical environment) could all be used to systematically establish the replicability of the effect found in the study. Generality could be explored through systematic replication, where various attributes of the participants

(age, gender), or of the treatment (dosage, length of intervention) are systematically varied (Blampied, 2013), and the effect on the treatment outcome is examined.

Spontaneous remission of symptoms must be considered when interpreting the results of the study. However, given that a majority of participants experienced a therapeutic effect despite experiencing chronic insomnia, it is unlikely that spontaneous remission is responsible for the positive effect observed. Also, baseline data showed the baseline phase to be relatively stable, with a steep improvement during the intervention phase, making spontaneous remission of symptoms an unlikely to explanation.

Participation in research trials involves exposure to regular therapeutic input, including contact with the therapist, assessment of symptoms, assistance provided to ensure compliance, and empathic responses. It must be considered that contact with the researcher may have contributed to the effects seen in this study. However, it is unlikely that this therapist effect could have accounted for the compelling treatment effect seen in the study, contact with researcher was minimal and only occurred at three points during the study (the beginning, middle and end). All measures were completed online or filled out at home in sleep diary format, limiting the amount of contact with the researcher. Furthermore, appointments were focused purely on maintaining compliance, checking for side effects, and administering new diaries and capsules. No psychological sleep intervention or advice was given.

4.3 Feasibility

With any novel treatment approach, feasibility is an important consideration. An important factor, that became an issue of feasibility during this study, was participant compliance with completing measures. Some participants struggled with remembering to

complete their daily sleep diaries and weekly online measures, and in future, more effective strategies for reminding participants to complete measures would be essential. During the current study email prompts were used; however, the success of this strategy relied on participants consistently checking their emails, and so perhaps in future daily text reminders would be more effective.

Side effects caused by the intervention must also be noted as impacting the feasibility of the study. Four participants (28%) experienced side-effects that may have been associated with the intervention, and while only one participant experienced side effects that were not transient, the effects caused the participant to cease participation in the study.

One of the biggest feasibility considerations that arose during this study was the financial commitment of continuing the micronutrient capsules at the end of the intervention phase. Although the majority of participants reported improvements during the intervention phase, all except one stopped taking the nutrients at the completion of trial. Many named cost and availability as the main reasons preventing them continuing with the intervention. Future research should investigate if therapeutic gains achieved with the relatively expensive micronutrient formula used here might be maintained if participants switched to less expensive over-the-counter vitamin and mineral preparations, or perhaps made more extensive changes in their diet to enhance the nutritional value of their food.

Challenges for the researchers included arranging suitable appointment times with participants that were not during work hours, and that accommodated those who had young children. Another challenge was ensuring that participants filled out measures within the required time frame. Communication via phone, text and email was useful in this respect, however, in future it would be recommended that text be used more predominantly as email was not always found to be a reliable or efficient way to contact participants.

4.4 Further Research

As many people seek alternative treatments for mental health difficulties because of the limited availability and financial constraints of psychotherapeutic interventions, and the side effects and long term impact of medications, further options for treating these difficulties deserve exploration (Rucklidge & Kaplan, 2013).

As with pharmaceuticals, the current micronutrient research has not yet documented their long-term safety over years and decades (Rucklidge & Kaplan, 2013), and further research should establish the longer term safety and efficacy of multi-ingredient formulas. Trialling longer intervention phases using groups at varying doses would be useful to investigate under which conditions the therapeutic effect plateaus, and to determine the dose-response relationship. It is certainly plausible that the optimum therapeutic dose varies between individuals, and perhaps some would respond better at a higher dose than used in the current study.

Further research needs to replicate the current findings to establish the reliability of the therapeutic effect, and would benefit from the use of placebo-controlled trials to account for the possibility of placebo or expectancy effects. It would also be interesting to conduct a trial directly comparing micronutrient intervention with current psychotherapeutic and pharmacologic interventions for insomnia.

Authors Rucklidge and Kaplan (2013) anticipate that a healthy gastrointestinal system will be considered essential in the future for maximising the treatment response to nutrient interventions. The health of the gut has been identified as impacting significantly on the absorption of nutrients, and it may be that an individual's gut flora influences their ability to respond to the treatment. Administering participants with probiotics before and during

micronutrient intervention may provide a way to improve response rate and magnitude, and would be a useful avenue to explore.

4.5 Conclusion

In conclusion, the results of the current study provide evidence that multi-ingredient micronutrient formulas are a promising treatment option for adults suffering from symptoms of insomnia. The findings give additional support to research indicating that micronutrient treatments are associated with improvements in psychiatric symptoms and overall functioning. Further placebo controlled research trials, that establish replicability, appear warranted. It is imperative that safe and effective treatment options are available, and is essential that researchers and clinicians are willing to investigate new and promising areas to provide clients with the most beneficial treatment options. Future research investigating the effect of multi-ingredient micronutrient formulas on mental health symptoms may provide further support for the use of micronutrients in this area. As knowledge is gained about the mechanisms through which these formulas exert their therapeutic effect, and absorption and dosage is further investigated, it may be possible to further optimise the treatment response rate, and assist in providing the best possible client care.

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6. Appendices

Appendix A: Participant Information Sheet and Consent Form

UNIVERSITY OF CANTERBURY
Te Whare Wānanga o Waitaha
CHRISTCHURCH NEW ZEALAND

INFORMATION SHEET: 31st October 2012

Title of research project: Investigation into the effect of a nutritional supplement on symptoms of insomnia in an adult population; pilot study using multiple baseline design.

Principal Investigator: Joanna Lothian
(Joanna.Lothian@pg.canterbury.ac.nz)

Other investigators: Assoc Prof Julia Rucklidge (Julia.Rucklidge@canterbury.ac.nz), Assoc Prof Neville Blampied

What is the purpose of the study?
This study is interested in investigating the impact of a micronutrient formula on symptoms of insomnia, including onset, duration and quality of sleep. The supplement we are studying is called Daily Self Defence (Revised version of EMPowerplus), and it contains 48 vitamins, minerals and amino acids. In previous research, these micronutrients have been found to reduce levels of stress, anxiety and intrusive thoughts in participants, and have anecdotally been reported to improve sleep. You have been invited to participate in the study as you completed the online eligibility screen and were rated as having insomnia symptoms of a moderate or severe quality. You are eligible for this study as you are not currently taking psychiatric or sleep medications. Approximately 15 adults will be invited to take part in this study.

Background
It has been proposed that some vitamins and minerals might help people with a variety of psychological concerns such as stress, anxiety, poor attention and low mood. There have been anecdotal reports of improved sleep from participants of these studies but impact on sleep has not been systematically assessed and researched. It is plausible that if the micronutrients reduce stress, anxiety and intrusive thoughts, they may also improve symptoms of insomnia. When a new idea such as this comes along, it is best to first study it in a variety of people (referred to as case series), to determine that there is an effect.

What would I have to do?
Potential participants will first be directed to a website, www.mentalhealthandnutrition.co.nz, that will provide them with more information about the study and will ask them some brief screening questions, collect demographical information, complete some questionnaires on insomnia symptoms, severity, and levels of depression, anxiety and stress, and provide contact details. This initial screening process should take approximately half an hour. On submission of the information, eligible participants will be contacted and invited to come to the university to meet with the researcher, sign consent forms, and receive the nutrients and sleep diaries. The first meeting with the researcher will take up to an hour.
Once it has been established that you are eligible to participate, you will be randomized to one of three groups: 1) Baseline length of 1 week, then 8 weeks taking 6 DSD pills per day,

2) Baseline length of 2 weeks, then 8 weeks of taking 6 DSD pills per day, 3) Baseline length of 3 weeks, then 8 weeks of taking 6 DSD pills per day.

During the baseline period and the 8 week treatment period when taking the capsules, you will be asked to complete a daily sleep diary which measures several aspects of your sleep pattern, as well as adherence with pill taking. Completing the sleep diary should take approximately 5 minutes each morning. You will also be asked to complete two online-questionnaires, on a weekly basis. These questionnaires will take approximately half an hour in total. These will assess your stress, anxiety and depression levels, and the severity of the insomnia symptoms you are experiencing. We will follow up with you three months following the end of the study regardless of whether you continued to take micronutrients and ask you again to complete these questionnaires.

During the 8 week trial when taking the capsules, you will have two appointments with the primary investigator, first at the end of 4 weeks of treatment, and again at the completion of the final week of treatment with the micronutrients. At your appointments, you will complete the weekly questionnaires for that week, and be able to speak to the investigator about any concerns or questions you have. The appointments will take no longer than an hour.

If you must take an antibiotic or antifungal agent orally for a health problem, it may be necessary for you to withdraw from the study for the time you complete the course of the drug. This is because antibiotics and antifungal drugs can interfere with the absorption of this nutrient supplement.

You will be asked to *not* try any alternative medicines or other forms of therapy until you have completed your involvement in this study. In addition, you are strongly encouraged to completely avoid alcohol, marijuana, caffeine, nicotine, and street drugs through the study as these substances may decrease the potential benefits of the treatment. At your appointments, and in your sleep diary, you will be asked to estimate your use of these substances.

Members of all cultures will be encouraged to participate in the study. Respect for Maori customs and traditions are of the highest priority and if necessary, home visits with a cultural advisor can be conducted. The researchers are available to discuss the research with the whanau to assist in developing their understanding of sleep disorders and how they can impact on Te taha hinengaro (mental wellbeing), whanaungatanga (family relationships), taha wairua (spiritual wellbeing) and taha tinana (physical wellbeing).

What are the risks?

Although we have no reason to suspect that this supplement can harm a physically healthy individual in any way, we will monitor you throughout the trial by asking you whether you are experiencing side effects or other changes to your physical or mental health.

This type of supplement has been used by many people for many years without any unpleasant results reported. More recently, investigators in Canada have published a research paper outlining the safety of the micronutrients being studied. Data were assembled from all the known published and unpublished studies for the complex formula with the largest amount of published research in mental health. Biological safety data from 144 children and adults were available from six sources: there were no occurrences of clinically meaningful negative outcomes/effects or abnormal blood tests that could be attributed to toxicity. In our trials conducted here at Canterbury, we have assessed to date over 100 participants taking micronutrients for up to 4 months. There were no abnormal blood results that concluded that these micronutrients were having an adverse effect on liver

and kidney function. Further, any side effects reported by this sample were temporary and mild.

The most common 'side effects' are that previously-experienced constipation has been relieved and that the patient is sleeping better; i.e., positive side effects rather than adverse events. Other side effects that are reported by people taking micronutrients are headaches and stomach aches, although they are typically mild and transitory. These difficulties can be avoided by taking capsules **on a full stomach,** and so we suggest you *always take your capsules with food and plenty of water.* We will review side effects weekly and make a referral to a medical practitioner if necessary. We are happy to provide you with copies of the studies that have been done to date on EMP+.

Micronutrients have the potential to interact with other medicines or drugs so you should avoid taking other medicines whilst on this treatment. For this reason, we are only including individuals in the study who *are not being concurrently treated for their illness using prescribed medications*. With respect to whether to take other medications, such as over-the-counter medications to treat colds, flu, stomach upset and sleep problems, because they may interact with the micronutrients, you should first discuss with us or your pharmacist before use. Pain killers such as Aspirin, Nurofen, Brufen or Voltaren (the NSAIDs or non-steroidal anti-inflammatory drugs) should be avoided whilst on the micronutrients as they can affect the ability of your blood to clot, and hence stop bleeding from a cut, in a similar way to some of the ingredients of DSD. So for example, if you needed a pain killer for a headache, it would be safer for you to take Paracetamol or Panadol than Nurofen whilst on DSD.

For safety reasons if you are, or become pregnant, *you will have to withdraw from the study.* Pregnancy should be avoided while taking the supplements. Further, we advise that during the trial, if relevant, you use appropriate contraception.

Will I benefit if I take part?

There may or may not be a direct medical benefit to you. Your insomnia symptoms may be improved during the study, and your anxiety, stress and depression levels may decrease, but there is no guarantee that this research will help you. The information we obtain from this study may help us to provide better treatments in the future for patients suffering from insomnia.

Do I have to participate?

If you decide not to participate in this study, or if you decide part-way through that you want to stop, you are certainly free to do so. This decision will not influence your ongoing health care in any way. Similarly, the study's investigators might choose to end your participation in the study at any time for any reason. If new information becomes available that might affect your willingness to participate in the study, you will be informed as soon as possible.

Will I be paid for participating, or do I have to pay for anything?

Arrangements will be made with each individual participant to ensure that your transportation costs to the university (to meet with the investigator) are covered. The capsules that you will take during the study will be provided at no cost.

Will my records be kept private?

All information about you that is collected in this study will be held in the strictest confidence. The only people who will have access to the information are the study investigators. The information will be stored in a locked filing cabinet in a locked room that only the investigators will have access to. We are very careful in dealing with confidential information; you can feel assured that all information you disclose concerning yourself and your family will be kept in a confidential file which will be kept locked at all times. This data will be stored for 10 years after collection. With your permission, data from this study may be used

in future related studies, which have been given ethical approval from a Health and Disability Ethics Committee. All information will be kept as group data. Therefore, forms will be coded and names removed such that you cannot be identified. Confidentiality will be respected and no material which could personally identify you will be used in any reports on this study. However, in cases where we are concerned about your safety or the safety of others, we may decide to breach confidentiality.

The results of the tests described above will be used for research purposes only in the context of this study. We are happy to discuss any of the results found from the present study with you and discuss the questionnaires upon the completion of your participation. We would need your permission and signed consent to send these test scores to another professional involved in your care.

What happens after the study?

If you feel you have benefited at the end of the trial, and want to continue taking the supplement, it is commercially available. We can provide you with the contact information so that you can continue to obtain it.

If you have any queries or concerns regarding your rights as a participant in the study you can contact an independent health and disability advocate. This is a free service provided under the Health and Disability Commissioner Act. Telephone (NZ Wide) 0800 555 050, Free Fax (NZ wide): 0800 2787 7678 (0800 2 SUPPORT), Email (NZ wide) advocacy@hdc.org.nz. You can also contact Joanna Lothian, the principle investigator of this study at joanna.lothian@pg.canterbury.ac.nz or Dr. Julia Rucklidge, the supervisor of this research, on 364-2987 ext7959, should you have any questions or concerns about this research. The Human Ethics committee at the University of Canterbury has reviewed and approved this study. We have also consulted with The Maori Consultation Group at the University of Canterbury.

UNIVERSITY OF CANTERBURY
Te Whare Wānanga o Waitaha
CHRISTCHURCH NEW ZEALAND

CONSENT FORM

Title of research project: Investigation into the effect of a micronutrient supplement on insomnia symptoms in adults; pilot study, multiple baseline design.

Principal Investigator: Joanna Lothian,

Other Investigators: Assoc Prof Julia Rucklidge, Assoc Prof Neville Blampied

I have read and I understand the information sheet dated 31st October 2012 for volunteers taking part in the study designed to assess the impact of a micronutrient formula on symptoms of insomnia. I have had the opportunity to discuss this study. I am satisfied with the answers I have been given. I understand that taking part in this study is voluntary (my choice) and that I may withdraw from the study at any time and this will in no way affect my continuing health care. I also understand that I may withdraw any information already provided.

I understand that my participation in this study is confidential and that no material which could identify me will be used in any reports on this study. I understand that the treatment, or investigation, will be stopped if it should appear harmful to me. I understand the compensation provisions for this study. I have had time to consider whether to take part. I know who to contact if I have any side effects to the study, or if anything occurs which I would consider a reason to withdraw from the study. I know who to contact if I have any questions about the study.

I wish to receive a copy of the results YES/NO

Participants should be advised that a significant delay may occur between data collection and publication of the results.

I agree to my GP or other current provider being informed of my participation in this study/the results of my participation in this study. YES/NO

I consent to being contacted 3 months after completion of the study regardless of whether I chose to continue to take the micronutrients and at that point I can choose whether to complete questionnaires. YES/NO

I consent to my name being placed in a separate database so that I can be contacted in the future should there be other studies for me to participate in with the understanding that I can choose whether to participate in such studies or not.

YES/NO

I consent to the use of my data for future related studies, which have been given ethical approval from a Health and Disability Ethics Committee YES/NO

I hereby consent to participate.

Signed:		Date:	
Printed name:			
Signature of person who gained consent:			
Address for results:			

The person who may be contacted about the research is:

Principle Investigator: Joanna Lothian, joanna.lothian@pg.canterbury.ac.nz

Or: Assoc Prof Julia Rucklidge, Principal Investigator, 364-2987 ext 7959

A signed copy of this consent form has been given to you to keep for your records and reference. Ingredients of Daily Self Defence attached.

Ingredients of Daily Self Defense (W) (Revised formula of EMPowerplus)

Ingredient	Amount in 1 capsule		Amount/Serving (3 capsules)		Amount/Day (6 capsules)	
Vitamin A (as retinyl palmitate)	666.7	IU	2000.0	IU	4000.0	IU
Vitamin C (as ascorbic acid)	50.0	mg	150.0	mg	300.0	mg
Vitamin D (as cholecalciferol)	500.0	IU	1500.0	IU	3000.0	IU
Vitamin E (as d-alpha tocopheryl succinate)	43.7	IU	131.0	IU	261.9	IU
Vitamin K_1 (as phylloquinone)	15.0	mcg	45.0	mcg	90.0	mcg
Vitamin K_2 (as menaquinone-7)	5.0	mcg	15.0	mcg	30.0	mcg
Thiamin (as thiamin mononitrate)	10.0	mg	30.0	mg	60.0	mg
Riboflavin	2.7	mg	8.0	mg	16.0	mg
Niacin (as niacinamide)	8.7	mg	26.0	mg	52.0	mg
Vitamin B_6 (as pyridoxine hydrochloride)	10.0	mg	30.0	mg	60.0	mg
Folate (as folic acid)	66.7	mcg	200.0	mcg	400.0	mcg
Folate (as L-methylfolate calcium)	66.7	mcg	200.0	mcg	400.0	mcg
Vitamin B_{12} (as methylcobalamin)	66.7	mcg	200.0	mcg	400.0	mcg
Biotin	63.0	mcg	189.0	mcg	378.0	mcg
Pantothenic acid (as d-calcium pantothenate)	5.0	mg	15.0	mg	30.0	mg
Calcium (as chelate)	81.0	mg	242.9	mg	485.8	mg
Iron (as chelate)	3.0	mg	9.0	mg	18.0	mg
Phosphorus (as chelate)	51.5	mg	154.6	mg	309.1	mg
Iodine (as chelate)	40.0	mcg	120.0	mcg	240.0	mcg
Magnesium (as chelate)	36.8	mg	110.4	mg	220.8	mg
Zinc (as chelate)	2.9	mg	8.8	mg	17.7	mg
Selenium (as chelate)	12.5	mcg	37.5	mcg	75.0	mcg
Copper (as chelate)	0.4	mg	1.3	mg	2.6	mg
Manganese (as chelate)	0.6	mg	1.8	mg	3.5	mg
Chromium (as chelate)	38.3	mcg	114.9	mcg	229.8	mcg
Molybdenum (as chelate)	8.8	mcg	26.4	mcg	52.8	mcg
Potassium (as chelate)	14.7	mg	44.2	mg	88.3	mg
Choline bitartrate	28.7	mg	86.1	mg	172.2	mg
Shilajit	12.5	mg	37.5	mg	75.0	mg
Spirulina	11.0	mg	33.0	mg	66.0	mg
Larch arabinogalactan	11.0	mg	33.0	mg	66.0	mg
Inositol	9.6	mg	28.7	mg	57.4	mg
Rhodiola rosea root extract	6.7	mg	20.0	mg	40.0	mg
Astragalus root extract	6.0	mg	18.0	mg	36.0	mg
Royal jelly 3X	4.0	mg	12.0	mg	24.0	mg
Grape seed extract	2.4	mg	7.2	mg	14.4	mg
Ginkgo biloba leaf extract	1.9	mg	5.8	mg	11.5	mg
Germanium sesquioxide (as chelate)	1.3	mg	3.8	mg	7.6	mg
Boron (as chelate)	147.2	mcg	441.6	mcg	883.2	mcg
Vanadium (as chelate)	73.2	mcg	219.7	mcg	439.4	mcg
Lithium orotate (as chelate)	61.4	mcg	184.1	mcg	368.2	mcg
Nickel (as chelate)	1.8	mcg	5.5	mcg	11.0	mcg
Cellulose	55.2	mg	165.6	mg	331.2	mg
Glycine	45.0	mg	135.0	mg	270.0	mg
Citric acid	26.8	mg	80.4	mg	160.9	mg
Magnesium stearate	24.0	mg	72.0	mg	144.0	mg
Silicon dioxide	20.0	mg	60.0	mg	120.0	mg

Appendix B: Daily Sleep Diary with Instructions

WEEKLY SLEEP DIARY **Name:**

Today's Date	**Example** 4/5/11				
1. What time did you get into bed?	10:15pm				
2. What time did you try to go to sleep?	11:30pm				
3. How long did it take you to fall asleep?	55 min				
4. How many times did you wake up, not counting your final awakening?	6 times				
5. In total, how long did these awakenings last?	2 hours, 5 min				
6. What time was your final awakening?	6:35am				
7. After your final awakening, how long did you spend in bed trying to sleep?	45 min				
8. Did you wake up earlier than planned?	Yes				
9. If yes, how much earlier?	1 hour				
10. What time did you get out of bed for the day?	7:20am				
11. In total, how long did you sleep?	4 hours 10min				
12. How would you rate the quality of your sleep?	○ Very poor • Poor ○ Fair ○ Good ○ Very good	○ Very poor ○ Poor ○ Fair ○ Good ○ Very good	○ Very poor ○ Poor ○ Fair ○ Good ○ Very good	○ Very poor ○ Poor ○ Fair ○ Good ○ Very good	○ Very poor ○ Poor ○ Fair ○ Good ○ Very good

Today's Date	Example: 4/5/11				
13. How rested or refreshed did you feel when you woke up for the day?	○ Not at all • Slightly ○ Medium amount ○ Well rested ○ Very well rested	○ Not at all ○ Slightly ○ Medium amount ○ Well rested ○ Very well rested	○ Not at all ○ Slightly ○ Medium amount ○ Well rested ○ Very well rested	○ Not at all ○ Slightly ○ Medium amount ○ Well rested ○ Very well rested	○ Not at all ○ Slightly ○ Medium amount ○ Well rested ○ Very well rested
14. How many times did you nap or doze?	2 times				
15. In total, how long did you nap or doze?	1hour ,10min				
16. How many (standard) drinks containing alcohol did you have?	2 drinks				
17. What time was your last drink?	5:30pm				
18. How many caffeinated drinks (coffee, tea, soda, energy drinks) did you have? Please list drinks.	3 drinks 1x espresso coffee 1x instant coffee 1x Green tea				
19. What time was your last caffeinated drink?	3:30pm				
20. Did you take any over-the- counter- OR prescription medication? **If so, list medication(s), dose, and time taken**	Yes Relaxo-herb, 50mg, 11pm Panadol, 500mg, 8pm				
21. Did you take the full six capsules of Daily Self Defense? **If not, how many did you take?**	Yes N/A				
22. Any further comments or information you would like to add?	I have a cold.				

General Instructions for completing your Sleep Diary

What is a Sleep Diary? A sleep diary is designed to gather information about your daily sleep pattern.

How often and when do I fill out the sleep diary? It is necessary for you to complete your sleep diary **every day.** If possible, the sleep diary should be completed within one hour of getting out of bed in the morning.

What should I do if I miss a day? If you forget to fill in the diary or are unable to finish it, leave the diary blank for that day.

What if something unusual affects my sleep or how I feel in the daytime? If your sleep or daytime functioning is affected by some unusual event (such as an illness, or an emergency) you may make brief notes on your diary.

What do the words "bed" and "day" mean on the diary? This diary can be used for people who are awake or asleep at unusual times. In the sleep diary, the word "day" is the time when you choose or are required to be awake. The term "bed" means the place where you usually sleep.

Will answering these questions about my sleep keep me awake? This is not usually a problem. You should not worry about giving exact times. And you should not watch the clock. Just give your best estimate.

Sleep Diary Item Instructions

Use the guide below to clarify what is being asked for each item of the Sleep Diary.

Date: Write the date of the morning you are filling out the diary

1. *What time did you get into bed?* Write the time that you got into bed. This may not be the time you began "trying" to fall asleep.
2. *What time did you try to go to sleep?* Record the time that you began "trying" to fall asleep.
3. *How long did it take you to fall asleep?* Beginning at the time you wrote in question 2, how long did it take you to fall asleep.
4. *How many times did you wake up, not counting your final awakening?* How many times did you wake up between the time you first fell asleep and your final awakening?
5. *In total, how long did these awakenings last?* What was the total time you were awake between the time you first fell asleep and your final awakening. For example, if you woke 3 times for 20 minutes, 35 minutes, and 15 minutes, add them all up (20+35+15= 70 min or 1 hr and 10 min).

6a. *What time was your final awakening?* Record the last time you woke up in the morning.

6b. *After your final awakening, how long did you spend in bed trying to sleep?* After the last time you woke up (item 6a), how many minutes did you spend in bed trying to sleep? For example, if you woke up at 8am, but continued to try and sleep until 9am, record 1 hour.

6c. *Did you wake up earlier than you planned?* If you woke up or were awakened earlier than you planned, check yes. If you woke up at your planned time, check no.

6d. *If yes, how much earlier?* If you answered "yes" to question 6c, write the number of minutes you woke up earlier than you had planned on waking up. For example, if you woke up 15 minutes before the alarm went off, record 15 minutes here.

7. *What time did you get out of bed for the day?* What time did you get out of bed with no further attempt at sleeping? This may be different from your final awakening time (e.g. you may have woken at 6:35am but did not get out of bed to start your day until 7:20am)
8. *In total, how long did you sleep?* This should just be your best estimate, based on when you went to bed and woke up, how long it took you to fall asleep, and how long you were awake in the night. You do not need to calculate this by adding and subtracting; just give your best estimate.
9. *How would you rate your quality of sleep?* "Sleep Quality" is your sense of whether your sleep was good or poor.
10. *How restful or refreshed did you feel when you woke up for the day?* This refers to how you felt after you were done sleeping for the night, during the first minutes that you were awake.

11a. How many times did you nap or doze? A nap is a time you decided to sleep during the day, whether in bed or not in bed. "Dozing" is a time you may have nodded off for a few minutes, without meaning to, such as while watching TV. Count all the times you napped or dozed at any time form when you first got out of bed in the morning until you got into bed again at night.

11b. *In total, how long did you nap or doze for?* Estimate the total amount of time you spent napping or dozing in hours and minutes. For instance if you napped twice, for 30 minutes and once for 60 minutes, and dozed for 10 minutes, you would answer "1 hour 40 minutes." If you did not nap or doze, write N/A" (not applicable).

12a. *How many drinks containing alcohol did you have?* Enter the number of alcoholic drinks you had the previous day, where 1 drink is defined as one 12 oz beer (can), one standard glass of wine, or one shot of liquor.

12b. *What time was your last drink?* If you had an alcoholic drink yesterday, enter the time of day in hours and minutes of your last drink. If you did not have a drink write "N/A" (not applicable).

13a. *How many caffeinated drinks (coffee, tea, soda, energy drinks) did you have?* Enter the number of caffeinated drinks you consumed yesterday.

13b. *What time was your last caffeinated drink?* If you had a caffeinated drink, enter the time of day in hours and minutes of your last drink. If you did not have a caffeinated drink, write "N/A" (not applicable).

14. *Did you take any over-the-counter or prescription medication(s) to help you sleep?* If so, list the medication(s), dose, and time taken: List the medication name, how much you took EACH different medication you took to help you sleep. Include medication available over the counter, prescription medications, and herbals. If every night is the same, write "same" after the first day.

Appendix C: Ingredient List of Daily Self Defense (Revised EMPowerplus formula)

Ingredient	1 capsule		3 capsules		6 capsules	
Vitamin A	666.7	IU	2000.0	IU	4000.0	IU
Vitamin C	50.0	mg	150.0	mg	300.0	mg
Vitamin D	500.0	IU	1500.0	IU	3000.0	IU
Vitamin E	43.7	IU	131.0	IU	261.9	IU
Vitamin K_1	15.0	mcg	45.0	mcg	90.0	mcg
Vitamin K_2	5.0	mcg	15.0	mcg	30.0	mcg
Thiamin	10.0	mg	30.0	mg	60.0	mg
Riboflavin	2.7	mg	8.0	mg	16.0	mg
Niacin	8.7	mg	26.0	mg	52.0	mg
Vitamin B_6	10.0	mg	30.0	mg	60.0	mg
Folate	66.7	mcg	200.0	mcg	400.0	mcg
Vitamin B_{12}	66.7	mcg	200.0	mcg	400.0	mcg
Biotin	63.0	mcg	189.0	mcg	378.0	mcg
Pantothenic acid	5.0	mg	15.0	mg	30.0	mg
Calcium	81.0	mg	242.9	mg	485.8	mg
Iron	3.0	mg	9.0	mg	18.0	mg
Phosphorus	51.5	mg	154.6	mg	309.1	mg
Iodine	40.0	mcg	120.0	mcg	240.0	mcg
Magnesium	36.8	mg	110.4	mg	220.8	mg
Zinc	2.9	mg	8.8	mg	17.7	mg
Selenium	12.5	mcg	37.5	mcg	75.0	mcg
Copper	0.4	mg	1.3	mg	2.6	mg

Manganese	0.6	mg	1.8	mg	3.5	mg
Chromium	38.3	mcg	114.9	mcg	229.8	mcg
Molybdenum	8.8	mcg	26.4	mcg	52.8	mcg
Potassium	14.7	mg	44.2	mg	88.3	mg
Choline bitartrate	28.7	mg	86.1	mg	172.2	mg
Shilajit	12.5	mg	37.5	mg	75.0	mg
Spirulina	11.0	mg	33.0	mg	66.0	mg
Larch arabinogalactan	11.0	mg	33.0	mg	66.0	mg
Inositol	9.6	mg	28.7	mg	57.4	mg
Rhodiola rosea root extract	6.7	mg	20.0	mg	40.0	mg
Astragalus root extract	6.0	mg	18.0	mg	36.0	mg
Royal jelly 3X	4.0	mg	12.0	mg	24.0	mg
Grape seed extract	2.4	mg	7.2	mg	14.4	mg
Ginkgo biloba leaf extract	1.9	mg	5.8	mg	11.5	mg
Germanium sesquioxide	1.3	mg	3.8	mg	7.6	mg
Boron	147.2	mcg	441.6	mcg	883.2	mcg
Vanadium	73.2	mcg	219.7	mcg	439.4	mcg
Lithium orotate	61.4	mcg	184.1	mcg	368.2	mcg
Nickel	1.8	mcg	5.5	mcg	11.0	mcg
Cellulose	55.2	mg	165.6	mg	331.2	mg
Glycine	45.0	mg	135.0	mg	270.0	mg
Citric acid	26.8	mg	80.4	mg	160.9	mg
Magnesium stearate	24.0	mg	72.0	mg	144.0	mg
Silicon dioxide	20.0	mg	60.0	mg	120.0	mg