

OMEGA-3 FATTY ACID SUPPLEMENTATION AND SLEEP

BY

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Abstract

Previous research has found omega-3 fatty acid supplementation advantageous in reducing depressive symptoms. One of the central diagnostic symptoms of depression is sleep disturbance. Accordingly, this study was designed to examine the effects of omega-3 fatty acid on self-reported insomnia. It was hypothesized that participants assigned to supplement their diet with omega-3 fatty acid would have improvements in sleep efficiency, sleep onset latency, sleep fragmentation and actual sleep time. Supplementary hypotheses examined were that omega-3 fatty acid might improve cognitive ability and sub-syndromal depressive symptoms. Participants were 27 undergraduate students reporting a variety of sleep difficulties as assessed by self report of insomnia symptoms. Participants were randomly assigned either to a treatment condition, with a daily supplement of 1500 mg omega-3 fatty acid and 30 IU of vitamin E, or a control group that received 30 IU daily of vitamin E. Sleep was monitored over a 28 day period, the last 21 days of which participants were instructed to take the assigned supplements. Participants were measured on objective (Actigraph) and subjective self-report measures of sleep for seven days prior to the intervention, and during the last seven days of the intervention. They also completed a self-report screen for depressive symptoms and several cognitive tasks immediately prior to and following the intervention. A 2x2 mixed factorial analysis of variance was performed on all outcome variables. The treatment group did not improve significantly more than the control group on any measures of sleep, or mood. One cognitive measure of processing speed did significantly improve for the treatment condition. These findings are discussed in light of study limitations and the existing literature, and recommendations made for additional research focused on the possibility of sleep improvements with omega-3 supplementation in a clinically depressed population.

Omega-3 Fatty Acid Supplementation and Sleep

Omega-3 fatty acid supplementation has recently gained considerable media attention for its potential efficacy in addressing a broad array of adverse health conditions. In fact, there exists published empirical evidence that it may be a useful intervention for serious medical conditions ranging from atherosclerosis (von Schacky, Angerer, et al, 1999) rheumatoid arthritis (Geusens, Wouters, Nijs, et al, 1994; Kjeldsen-Kragh, Lund, et al, 1992) hypertriglyceridemia (Wohl, Tien, et al, 2005) high blood pressure (Sacks, Hebert, et al, 1994) and skin conditions like eczema and psoriasis (Calder & Miles, 2000). Accordingly, the American Heart Association (AHA) has recently published a comprehensive list of medical uses for omega-3 fatty acids, and a rating system for their use based on empirical evidence of their likeliness to improve health. The AHA has also supported the use of omega-3 fatty acids for prevention of heart disease at a dosage of 1-3 grams. (Albert, Kyungwon, & Wang, 2004).

Omega-3 fatty acids are synthesized by plants, but they are acquired by humans through the consumption of animals which have eaten plants – especially grasses and algae – that contain omega-3s. There are three distinct molecular varieties of omega-3 fatty acids: alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). ALA has been shown to be essential for

heart and cardiovascular health, while DHA, a key constituent of the neural membrane, has been found to be important for brain growth and development (Parker, Gibson, et. al, 2006; Peet, 2003), and EPA has been shown to improve the overall efficiency of neural function (Logan, 2003). Dietary omega-3 deficiency has even been identified as a key vulnerability factor regarding the global burden of disease, including unipolar depression and bipolar disorder (Hibbeln et al, 2006).

Relevant epidemiological studies suggest that omega-3 fatty acids used to be much more prevalent in the human diet than they are today (Parker, Gibson, et. al, 2006; Peet, 2003). The contemporary deficiency appears to reflect the fact that meat products consumed today are no longer derived from naturally grazing animals – i.e., animals that consume the plants that contain omega-3 fatty acids. It is important to note in this context that humans cannot directly take in and digest the long-chain molecular versions of omega-3 fatty acid (EPA and DHA) in plant form. In order for humans to most efficiently consume EPA and DHA, they must ingest animals that have consumed these molecules in plant form. Animal meats and animal products are thus the richest potential sources of human-usable EPA and DHA (Parker, Gibson, et. al, 2006; Peet, 2003).

Examination of the list of potential uses for omega-3 fatty acids reveals a common factor across several of the diseases previously mentioned: they are all characterized by *inflammation*. This fact has led to an investigation of the mechanisms – including a robust anti-inflammatory effect (Tiemeier et al, 2003;

Edwards et al, 1998; Peet et al, 1998) – by which omega-3 fatty acid intake may improve such conditions.

Interestingly, major depressive disorder is also characterized by elevated inflammation (Glassman & Miller, 2007; Leonard, 2007; Sluzewska, Rybakowski, et al, 1996; Kenis & Maes, 2002), and individuals experiencing depression have significantly lower levels of omega-3 fatty acids present in adipose tissue and circulating blood plasma (Tiemeier et al., 2003; Maes et al., 1996; Edwards et al., 1998; Peet et al., 1998). Supplementation of omega-3 fatty acids in depressed individuals also results in a statistically significant improvement in depressive symptoms (Nemets et al, 2002; Peet & Horrobin, 2002), one of which is disordered sleep. Sleep difficulties, including sleep disruption, altered sleep architecture, and overall non-restful sleep, are central symptoms of major depressive disorder, and up to 90 percent of depressed individuals also experience adverse changes in sleep (Thase, 1999).

It has recently been estimated that 58 percent of the American adult population experiences at least occasional difficulties with sleep (Gelula, 2002). Accordingly, considerable research effort has been directed at the development of effective interventions for insomnia and other sleep disorders. Inasmuch as omega-3 supplementation is capable of reducing depressive symptomatology – including disordered sleep – it is possible that such supplementation may have potential as an intervention for sleep disturbance, even among non-depressed individuals. This

hypothesis has not yet been explicitly investigated, but there is evidence for regarding it as plausible.

How omega-3 fatty acids may influence sleep

The Hypothalamic-Pituitary-Adrenal Axis and Inflammation

As noted above, omega-3 fatty acids are anti-inflammatory, and this anti-inflammatory effect has a regulatory effect on the hypothalamic-pituitary-adrenal (HPA) axis. The HPA axis is an important part of the stress response system, and also plays a critical role in sleep. Because chronic inflammation in the body over-activates the HPA axis, it can in turn damage sleep quality and quantity.

By introducing omega-3 fatty acids into the body, inflammation can be decreased (Calder, 2002; Simopoulos, 2002; Motivala, Sarfatti, et al., 2005) and various studies have shown that omega-3 fatty acids inhibit chronic inflammatory responses (Calder, 2002). When inflammation is controlled, pro-inflammatory cytokines are no longer circulating in high quantities. Such cytokines, measurable markers of inflammation, also activate the stress response in the hypothalamic-pituitary-adrenal (HPA) axis, an event that can promote sleep disruption. Poor sleep has been linked to increases in circulating cytokines, providing evidence for a cycle of chronic inflammation and sleep difficulties.

There are multiple lines of evidence which suggest that increased inflammation, especially chronic inflammation, is associated with sleeping difficulties (Mullington, Hinze-Selch, et al, 2001; Song, et al, 1998). Also, inflammation can alter sleep architecture and sleep disturbances can, in turn, impair

immune functions (Song, et al, 1998). This promotes a vicious cycle of poor sleep contributing to dysregulated immune function, which again leads to poor sleep. It is hypothesized that omega-3 fatty acids may be able to break this cycle by decreasing inflammation, and thus inhibiting the runaway immune-inflammation response and its deleterious effect on sleep.

A prominent theory of sleep disorders involves dysregulation of the body's stress response system, particularly the hypothalamic-pituitary-adrenal axis (HPA axis) and its components. One of the important functions of the HPA axis is to maintain alertness and to regulate sleep (Buckley & Schatzberg, 2005). When the HPA axis is dysregulated it can cause disruption of sleep, and hyper-activity of the HPA axis can cause problems with various stages of sleep (Buckley & Schatzberg, 2005).

Elaboration on the effect of inflammation on the HPA axis is necessary to fully understand how omega-3 fatty acids may influence sleep. The HPA axis is structurally composed of the hypothalamus, the pituitary gland, and the adrenal gland. The hypothalamus signals the pituitary gland by way of a neurohormone, corticotrophin-releasing hormone (CRH), while the pituitary gland sends signals to the adrenal gland via a different neurohormone, adrenocorticotropin (ACTH). When an individual is exposed to a stressor, the hypothalamus produces CRH, which then triggers the release of ACTH, which is released into the bloodstream and travels to various systems throughout the body. The target location of importance in this study is the adrenal gland, which triggers the release of cortisol.

Corticotrophin releasing hormone (CRH), regulated by the hypothalamus, causes the HPA axis to become more active. Not surprisingly, an increase in circulating CRH increases wakefulness (Buckley, and Schatzberg, 2005) and in a study of healthy adult males without sleep disorders, CRH decreased slow wave sleep while increasing light sleep and wakefulness (Holsboer, von Bardeleben, and Steiger, 1988). CRH can be produced in response to stress, as well (Buckley, and Schatzberg, 2005). This begins a cycle of changes that result in chronic sleep problems. As excessive CRH hyper-activates the HPA axis, sleep is disturbed. The body becomes less capable of regulating the stress response in the future, which in turn lends itself to the cycle being replicated, with the HPA axis operating at a consistent level of hyperactivity.

ACTH activity is also of particular interest, as activation of the adrenal gland by ACTH triggers the release of glucocorticoid stress hormones (Lambert, K. and Kinsley, C.H., 2005). The glucocorticoid most relevant to the HPA axis function and sleep is cortisol. In a healthy HPA axis response, cortisol is the neurohormone critical to the deactivation of the HPA axis stress response. When cortisol does not effectively deactivate the HPA axis, hyper-activation occurs. Hyper-activation of the HPA axis is connected with depression, sleep difficulties, and anxiety (Lambert, K., and Kinsley, C.H., 2005). The HPA axis can also be hyper-activated by prolonged stress, and as mentioned, chronic inflammation in the body, which produces cytokines that continue to activate the release of CRH in the hypothalamus. When the body's inflammation response is appropriately regulated, it is hypothesized that

hyper-activation of the HPA axis can be decreased. Omega-3 fatty acids have strong anti-inflammatory effects in the body, and thus may reduce activation of the HPA axis.

Adequate and homeostatic levels of cortisol are imperative for brain and body functioning because it regulates glucose metabolism, blood pressure, insulin release, immune responses, and inflammatory functions (Brown, 2004). A chronic excess of circulating cortisol has myriad potential for negative effects in the body, such as impaired cognitive performance, decreased thyroid function, hyperglycemia, decreases in bone density and muscle tissue, high blood pressure, lowered immunity, increased abdominal fat, and increased heart attack and stroke susceptibility (Brown, 2004).

Cortisol is also influential in the sleep cycle. Cortisol levels fluctuate across the various stages of sleep and wakefulness. Most research examining the influence of cortisol on sleep has focused on its contribution to sleep disruption. Waking at night has been associated with spikes in the release of cortisol (Follenius, Brandenberger, et al, 1992; Spath-Schwalbe, Gofferje, et al, 1991). Research has also confirmed that chronic insomnia, even in the absence of depression, is associated with elevated cortisol levels (Rodenbeck, Hajak, 2001; Rodenbeck, Huether, et. al, 2002; Vgontzas, Tsigos, et al, 1998; Vgontzas, Bixler, et al, 2001). Research has also suggested that elevated cortisol levels may just be related to increased CRH activity as well (Buckley & Schatzberg, 2005). Cortisol levels from the evening before sleep also correlate with the number of nocturnal awakenings in both individuals with and

without insomnia (Rodenbeck, Hajak, 2001; Rodenbeck, Huether, et. al, 2002).

There is sufficient literature to determine that non-homeostatic levels of circulating cortisol contribute to sleep difficulties. A large influence on non-homeostatic levels of cortisol is directly related to the dysregulation of the HPA axis.

Chronic stressors contribute to the hyper-activation of the HPA axis. One mechanism in this regard is inflammation. Prolonged hyper-activation of the HPA axis leads to non-homeostatic levels of the glucocorticoid cortisol. The over-abundance of cortisol, in turn, contributes to repeated activation of the HPA axis; thus, a cycle is created in which the HPA axis cannot be properly regulated. Through research in the area of depression, it has been documented that markers of inflammation (cytokines) increase with exposure to chronic stressors as well as chronic sleep problems. These cytokines also activate the HPA axis. Thus, it is hypothesized that a vicious cycle may ensue, in which stress induces both HPA axis activation and excessive inflammation, which in turn contribute to the onset of sleep problems, which exacerbates stress, and so on.

In conclusion, HPA axis hyper-activity can lead to sleep fragmentation, shortened time spent in restorative slow wave sleep, and less time engaged in sleep. At the same time, sleep disturbances can make HPA axis dysfunction intensify. It is hypothesized that HPA axis hyper-activity is mediated by persistent release of CRH, which in turn signals non-homeostatic release of cortisol (Rodenbeck, Hajak, 2001; Rodenbeck, Huether, et. al, 2002), both of which have deleterious effects on the negative feedback loop that “turns off” CRH production in the HPA axis. It is further

hypothesized that increased CRH production may be due to high levels of pro-inflammatory cytokines in the bloodstream (Buckley & Schatzberg, 2005). Omega-3 fatty acids are likely to manage inflammation in the body so as to prevent a runaway cycle of inflammation that can damage sleep and thereby perpetuate further inflammation and thus further sleep difficulties.

In the case of sleep disorders and depression, as well as other disorders, a decrease in HPA axis hyper-activity results in decreased symptomatology. It is hypothesized that since omega-3 fatty acid has been shown in some studies to be efficacious in treating depression through possibly the same regulatory mechanism, that omega-3 fatty acid may also alleviate sleep difficulties.

Possible Benefits of Omega-3 Fatty Acids

Omega-3 fatty acid has been shown to have an effect on the HPA axis via the same mechanism through which they control inflammation. The pro-inflammatory cytokines activate the HPA axis by activating the release of CRH (Buckley & Schatzberg, 2005). Omega-3 fatty acids decrease the level of pro-inflammatory cytokines circulating in the body by binding to sites in the central nervous system that would otherwise be bound with Omega-6 fatty acids. Omega-6 fatty acids generally promote inflammation by serving as precursors for the production of pro-inflammatory hormones (eicosanoids). However, the typical American diet contains more omega-6 fatty acids, which leaves omega-3 fatty acids more available for the body's use. By making more omega-3 fatty acid available, the body is able to use

omega-3 fatty acids before resorting to omega-6 fatty acids. Omega-3 fatty acids are more efficient and have less negative inflammatory effects (Taperio, Ba, et al, 2002).

The hypothesized mechanisms by which omega-3 fatty acids may mediate the amelioration of sleep disturbances are not directly investigated in this study. Before such mechanisms can be investigated, it is useful merely to demonstrate that omega-3 fatty acid supplementation is an intervention that has a beneficial effect on sleep. Although there are some existing published studies that have reported beneficial sleep changes associated with omega-3 supplementation, these studies have mainly focused on changes in affect, with sleep examined only tangentially as a part of a battery assessing depressive or manic symptoms or learning and behavior problems (Stevens, Zentall, et al, 1996; Sorgi, Hallowell, et al, 2007).

Nevertheless, there is reasonable evidence to support the hypothesis that omega-3 fatty acid may help ameliorate sleep abnormalities. The primary aim of the present study was to examine this hypothesis, utilizing a supplemental omega-3 dose (1000mg of EPA and 500mg DHA daily) that has been supported as efficacious in addressing depressive symptoms, including sleep abnormalities (Kiecolt-Glaxer, Belury, et al, 2007; Peet & Horrobin, 2002). Specifically, supplement-related changes in nightly sleep quantity and quality will be assessed by means of continuous Actiwatch/Actigraph monitoring, in tandem with daily sleep diaries, among a sample of 27 undergraduates selected on the basis of self-reported sleep disturbance.

There is also substantial evidence to indicate that improved sleep can enhance cognitive performance (Randazzo, Muehlbach, et al., 1998; Ohayon, Vecchierini, 2005; Borak, Cieslicki, et al., 1996). Moreover, the effects of omega-3 fatty acid supplementation on cognitive function have also been examined with promising results (Kalmijn, van Boxtel, et al., 2004; Kalmijn, Launer, et al., 1997; Richardson, and Puri, 2002; Stevens, Zentall, 1995). Accordingly, the present study also examined the auxiliary hypothesis that cognitive function – specifically, attention, working memory, attentional flexibility, visual conceptual/visiomotor tracking, immediate memory, processing speed, and recall ability– may improve as a result of omega-3 fatty acid supplementation.

Method

Sample

Participants were 27 individuals with self-reported symptoms of sleep disturbance (52% female) as evaluated by an online survey of introduction to psychology students at a large Midwestern university. The mean age of participants was 19.9 years (SD = 2.81). The majority were Caucasian (78 %). Their average body mass index (BMI) was 23.5 (SD=2.7), calculated using the standard body mass index formula. See Table 1 for characteristics of treatment and control group participants. All subjects completed a medical history questionnaire prior to entrance into the study to ensure the absence of physical conditions (e.g., a blood clotting disorder) for which omega-3 fatty acid or vitamin E would be contraindicated. Pregnant women were also excluded from participation.

Study Design

Procedures

Participants were selected from an introductory psychology class. Students from this class were required to participate in research as a requirement of course completion. Participants completed an initial screen for insomnia online (see appendix for screening questions) were invited to participate in the study via email. In order to qualify for participation, participants had to report that their sleep was not restful, that they experienced feeling tired during the day to the point where it made

it difficult to complete tasks at work, school, or at home. They also had to endorse experiencing one of the following three sleep disturbances at least 5 or more nights of the week: having difficulty falling asleep, having difficulty staying asleep, or waking early in the morning and not being able to fall back to sleep. Prior to the study, all subjects were informed in detail about the investigation, the measures being collected, and the requirements they would need to meet, and gave their informed written consent. The study protocol was approved by the relevant institutional review board.

Contact with participants occurred in a psychological laboratory on campus; this included completion of the consent process and all further screening procedures. Eligibility was confirmed after participants completed a medical history form to rule out possible contraindications for omega-3 fatty acid or vitamin E supplementation. After eligibility was determined participants completed a survey, the Pittsburg Sleep Quality Index (PSQI), to assess sleeping habits, the Beck Depression Inventory-II (BDI-II), and four cognitive tasks (Letter-Number Sequencing, the Rey Auditory and Verbal Learning Test [RAVLT], Trails A and B, and the Stroop test). Finally, they were be given a device called an Actigraph/Actiwatch, which was worn on their non-dominant hand for the next 7 days. They were also instructed to fill out two sleep logs; (a) a sleep diary (National Sleep Foundation, 2005), and (b) a measure examining sleep and emotions (Buysse, Ancoli-Israel, et al., 2006). At the end of the designated 7 days, participants returned to the lab to give back the watch in exchange for their assigned supplements (either vitamin E, or omega-3 fatty acid and vitamin

E). Participants were randomly assigned to a control or treatment condition prior to their arrival for the first session. The control condition was assigned to take a 30IU pill of vitamin E each day, while the treatment condition received 30 IU of vitamin E and approximately 1500mg of omega-3 fatty acid daily (in the form of 5 pills). In summary, the control condition took 1 pill a day (30IU vitamin E) and the treatment condition took a total of 6 pills (5 pills omega-3 fatty acid and 1 pill vitamin E). Participants were instructed to take the pills for the remainder of their time in the study (21 days) and encouraged to take them just prior to bedtime. On the last 7 days of the ensuing 21-day period, the participant wore the Actiwatch/Actigraph again. Upon the completion of the study, participants returned to the laboratory to return the watch, turn in 28 days' worth of sleep diaries, and again complete the same cognitive tests administered before the intervention; the Beck Depression Inventory, and Pittsburgh Sleep Quality Index.

Omega-3 fatty acid and vitamin E administration

To control for the different supplements participants received based on their assigned group, all supplements were given in unmarked containers. The pills themselves look similar, inasmuch as both are gel caps of the same color and shape. Size is the only immediately apparent difference between the treatment and control supplements, with the omega-3 fatty acid supplement pills being approximately 3 times larger than the vitamin E supplement. Participants were asked to indicate each day on their sleep logs if they took their assigned supplements.

Sleep Measures

Actigraph

The primary objective method of measuring sleep behavior was the data collected using actigraphy. The actigraph is a piece of technology that looks much like a normal wristwatch, which is used to measure body movement. There are accelerometers within the watch, which measure and collect data regarding movement of the user. (Littner, M. et al, 2003). Data is gathered about the wearer's activity level throughout the day. Data gathered by the watch is then downloaded on to a computer and analyzed to give estimates regarding various indicators of sleep elements (Littner, M. et. al, 2003). The theory for examining the data is based on the principal that there is less movement during sleep than when an individual is awake. (Littner, M. et. al, 2003). By looking at this information it is possible to determine sleep onset, wakenings, the quality of sleep, and duration of the sleep period. Regarding psychometric properties, it has been shown to be an efficient, economical, and adequate measure of sleep (Littner, M. et al, 2003). The actigraph device was worn on the non-dominant hand throughout the specified duration of the study. Data collected by the actigraph regarding body movement is a good indicator of sleep quantity, quality, and architecture (Littner, M. et al, 2003). Factors measured by the actigraph technology include sleep onset latency, actual sleep time, the number of wake bouts during sleep, the number of minutes moving during sleep, and a

calculation of sleep efficiency. Accordingly, data collected from the actigraph is an objective way of monitoring relevant sleep processes.

In this study overall sleep efficiency was measured by the “Sleep Efficiency” score produced by the actigraph algorithm. Sleep onset was measured by the “Sleep Latency” score, actual sleep time was measured by the “Actual Sleep Time” score, also produced by an actigraph algorithm, and an objective estimate of awakening during sleep periods was determined by the “Number of Wake Bouts” score and supplemented with the “Number of Minutes Moving” score, also produced by the actigraph algorithm.

Pittsburgh Sleep Quality Index

The Pittsburgh Sleep Quality Index (PSQI) was administered to each participant at study entry and termination to assess sleep during the preceding month. It assesses subjective quality of sleep as well as subjective report of typical sleeping patterns, including typical bedtime, what time the participant gets up in the morning, an estimate of how long it takes them to fall asleep, as well as possible reasons for waking during sleep. A total PSQI score is generated from the responses and has been found to be a reliable and valid measure of subjective sleep disturbance (Backhaus, Junghanns, et al, 2002). A total PSQI score of 5 or greater is indicative of significant sleep disturbance (Buysse, Reynolds, et al, 2000).

National Sleep Foundation Sleep Diary

Participants completed the National Sleep Foundation (NSF) Sleep Diary (Buysse, Ancoli-Israel, et al, 2006) for every day they were in the study. The NSF

sleep diary was used to acquire the participant's subjective experience of sleep for the duration of the study. It generates information regarding bedtime, awakenings, duration of sleep, medication taken, exercise, subjective restfulness of sleep, and alcohol and caffeine use. Sleep efficiency was calculated by dividing the estimated hours of sleep by the estimated time in bed. Sleep latency and number of awakenings were determined by the participant's estimates recorded in the sleep diary.

Cognitive Functioning

All cognitive tests were administered on the initial visit to the laboratory and the debriefing session on the last day of the experiment.

Letter-Number Sequencing

Letter-Number Sequencing, a subtest from the Wechsler Adult Intelligence Scale-III (WAIS-III) was administered to examine attention and working memory (Crowe, S.F., 2000; Wechsler, 1997). The administrator reads a list of letters and numbers to the participant. The participant then mentally reorganizes the list of letters and numbers and must recall the list back to the examiner with numbers repeated first in order followed by the letters, also in alphabetical order (see appendix). Participants start with a list of just two stimuli to reorganize and repeat and the list gets progressively harder pending the success of the participant on the subsequent administrations.

Trails A and B

Trails A and B was used to assess visual conceptual and visuomotor tracking as well as processing speed. Participants are timed on how quickly they can draw lines in between numbers, searching in numerical order. Trails B is more difficult, for it requires participants to trace both letters and numbers in order. For example, the participant will draw a line from “1 to A to 2 to B, and so on”. Trails A and B have been found to be valid (Gaudino, E.A., Geisler, M.W. & N. K. Squires, 1995) but there are practice effects that make reliability difficult to assess (Fals-Stewart, W, 1992).

Rey Auditory and Verbal Learning Test

The Rey Auditory and Verbal Learning Test (RAVLT) were used to assess immediate memory, effects of interference, and recall ability (Mungas, D, 1983). The first list is read and participants repeat back as many words as they can remember from the list. Then a second similar interference list is read and participants repeat back words they can remember. After the interference list is repeated back, the participant is asked to recall as many words from the first list that they can. Lastly, a checklist is provided with words from the first list, the interference list, and words on neither list. Participants are instructed to indicate which words were on the first list but not the second by placing a check mark next to the “first list” words. It has been found to be both reliable and valid (King, J.H., Gefeller, J.P. & Davis, H.P. 1998).

Stroop Test

The Stroop test was administered via a computer program. It is designed to assess directed attention and attentional flexibility. It is administered in three segments. The first segment participants are instructed to name the color word, which appears in black text, which appears on the screen. For example, “Blue”. Participants press the space bar on the computer keyboard to progress as quickly as they can through the computer generated list of color words. The second segment is administered the same way, except colored “X”s appear on the screen and participants are instructed to name the color of the “X”s as quickly as possible. The third and final segment is color words written in a different color than the word written. For example, the word on the screen is “Blue” but it is written in the color yellow. Participants are instructed to name the color the word is written in, and not the word written. This last test provides a measure of processing speed in the face of a distracter and of attentional function. It has been found to be both a reliable and valid measure of these constructs (Koch, K, 2003). Processing speed and interference were also calculated based on the results of the three Stroop test segments. Processing speed was generated by adding the scores obtained from the Stroop color naming task and the word naming task (where each score indicates the total number of colors or words named correctly in the allotted 60 seconds per trial). This technique has been determined to be an advanced and accurate way to examine processing speed (Denney, D. & Lynch, S., 2008). Interference was calculated by regressing the Stroop color-word naming score on the color naming score, and thus obtaining a residual score for each participant, and then adding the overall group

mean from the Stroop color-word naming test to the participants' residual score (Denney, D. & Lynch, S., 2008).

Mood

Beck Depression Inventory-II

A Beck Depression Inventory-II (BDI-II) (Beck, Steer, and Brown, 1996) was administered to subjects upon first evaluation in the laboratory and upon the last day of the study. It is a 21-item self-report measure of depressive symptomatology (see appendix). Participants indicate how much they agree with a statement regarding a depressive symptom. A high score on the BDI-II is correlated with endorsing more depressive symptomatology than a lower score. The Beck Depression Inventory-II has been found to be both a reliable and valid measurement of depressive symptoms (Longwell, 2005; Beck, Steer, et al, 1996).

Data Analyses

Primary study hypotheses regarding between-group differences on actigraphy-derived sleep measures as well as subjective sleep measures (PSQI, NSF Sleep Diary) and cognitive measures were tested by means of 2-by-2 multi-factorial analysis of variance. Sleep data was averaged over the 7 nights of subjective and objective measures of sleep to produce aggregated scores on all sleep measures, taking into account the variation of sleep processes over the 7 day period. Observed time by treatment interaction effects were further examined by means of one-way analyses of covariance (using pre-treatment score as the covariate and post-treatment

score as the dependent variable) and paired-samples t-tests within each treatment group. An alpha level of .05 was used to determine statistical significance in all analyses. Twenty-eight individuals participated in this study, however, one participant was simultaneously engaged in another sleep intervention and that participant's data was removed due to the nature of the secondary sleep intervention.

Results

The treatment and control groups were compared in terms of potential confounding variables; namely age, body mass index, gender, and minority status. There were no significant differences between the treatment and control groups on any of these demographic variables (see Table 1 and Table 2).

Treatment and control groups were also compared at baseline, using independent samples t-tests, on the study's outcome variables of interest: self-report measures of sleep disturbance (PSQI) and depressive symptomatology (BDI-II), sleep-diary and actigraphy measures of sleep efficiency, sleep onset latency, sleep wakening, and total sleep time, and four cognitive tasks (Letter-Number Sequencing, Rey Auditory and Verbal Learning Task, Stroop, and Trails A and B). The results of these tests can be found on Table 3, Table 4, and Table 5. The means and standard deviations for the adherence variable can be found in Table 2. No significant differences were observed on any of these baseline variables between groups.

An adherence variable was also calculated based on the percentage of assigned pills that the participant reported having consumed. There was no

significant difference between groups regarding self-reported adherence to the treatment regimen. The means and standard deviations for the adherence variable can also be found in Table 2.

In order to test for treatment effects on the outcome variables of interest, a set of 2x2 mixed factorial analyses of variance was conducted, with pre- and post-treatment measures for each variable. These analyses allow for a test of the main effects of time (significant pre-post improvements) as well as a time-by-treatment interaction effect, which indicate between-group differences in the effect of treatment.

Among measures of sleep (objective and subjective) there were no significant time-by-treatment interaction effects, a finding which indicates no substantial difference in improvement over time between the two groups. However, significant main effects for time were observed on 3 self-report (sleep diary) measures of sleep: sleep latency (pre-treatment mean= 31.634, standard error= 3.279; post-treatment mean= 21.458, standard error= 2.593, $F(1,23)=10.811$, $p=.003$), estimated total sleep time (pre-treatment mean= 439.800, standard error= 12.761; post-treatment mean= 474.988, standard error= 13.783, $F(1,23)=8.817$, $p=.007$), and number of awakenings (pre-treatment mean= 2.038, standard error= .269; post-treatment mean= 1.086, standard error= .165, $F(1,23)=13.392$, $p=.001$). Likewise, there was no significant time-by-treatment interaction observed for the PSQI, but a significant main effect for time (pre-treatment mean= 10.309, standard error= .676; post-treatment mean= 7.512, standard error= .567, $F(1,24)=21.285$, $p<.001$). There were no significant

main effects for group on any study sleep measures, including the PSQI, nor was there significant main effect or interaction for the BDI-II score.

With respect to cognitive measures, one significant time-by-treatment interaction effect was observed; specifically, it occurred on the Stroop processing speed measure ($F(1,18)=5.134, p=.036$). As shown in Figure 1, the treatment group showed a substantial trend toward an increase in processing speed from pre- to post-treatment (pre-treatment mean= 157.123, s.d.= 10.650; post-treatment mean= 175.250, s.d.= 12.318, $t= -2.29, p=.056$). The control group also experienced a slight pre-post increase in processing speed, but it was not statistically significant (pre-treatment mean= 145.667, standard error= 8.696; post-treatment mean= 147.083, standard error= 10.058, $t= -.472, p=.646$). In order to better account for the potential influence of baseline Stroop performance on the post-treatment outcome measure, an analysis of covariance was conducted, with pre-treatment Stroop score used as a covariate. The treatment group still performed significantly better than the control group ($F(1, 20)= 4.52, p=.048, \text{partial } \eta^2=.210$) when covarying for baseline score. There was also a significant main effect of time on the Stroop processing speed measure (pre-treatment mean= 151.396, standard error= 6.875; post-treatment mean= 161.167, standard error= 7.951, $F(1,18)=7.023, p=.016$). This is suggestive of an increase in processing speed.

There was also a significant main effect of time on all 3 components of the RAVLT: immediate recall (pre-treatment mean= 8.588, standard error= .543; post-treatment mean= 9.964, standard error= .510, $F(1,24)=7.460, p=.012$), delayed recall

(pre-treatment mean= 5.705, standard error= .691; post-treatment mean= 7.481, standard error= .719, $F(1,23)=10.632$, $p=.003$), and recognition (pre-treatment mean= 9.282, standard error= .639; post-treatment mean= 10.370, standard error= .440, $F(1,24)=5.156$, $p=.032$). No other main effects or interaction effects were observed for any study cognitive measures.

Discussion

This investigation represents the first reported placebo-controlled trial of the effect of omega-3 fatty acid supplementation on sleep disturbance. Among a group of 27 college undergraduates with self-reported symptoms of sleep disturbance, no specific beneficial effects of omega-3 ingestion on sleep quality or quantity were observed. Specifically, there were no significant differences in outcome between the omega-3 and placebo (vitamin E) groups on any of the objective (Actigraph) or subjective (sleep diary and PSQI) sleep measures of sleep efficiency, sleep latency, awakenings, total sleep time, or depressive symptomatology.

There were, however, significant pre-post improvements in sleep observed in both groups, as indicated by four self-report measures. Self-reported measures of sleep latency decreased by approximately 67%, awakenings decreased by approximately 53%, and total sleep time increased by 7%. Also, the total score on the PSQI self-report sleep measure substantially improved in both treatment groups. These self-reported improvements appear consistent with placebo-like positive expectancy effects among participants in both treatment conditions- a hypothesis

supported by the fact that no significant improvements in sleep were observed on any of the study's objective, Actigraphy-based measures. Presumably, if significant improvements in sleep quality and quantity had actually occurred, this would have been evident on both objective and subjective study measures.

Among study cognitive measures, however, one significant between-group difference was observed. Specifically, on a component of the Stroop test that measures processing speed, participants receiving the omega-3 supplement were found to be significantly faster at post-treatment than they had been at baseline (pre-treatment). This finding is consistent with some previously published reports which have also found omega-3 supplementation to enhance various cognitive processes, including processing speed (Kalmijn, et. al., 2004; Kalmijn, 2000, Kalmijn and Launer, 1997). However, this particular study finding should be interpreted with caution, given that the Stroop task was only completed by 20 study participants, only 8 of whom were in the omega-3 condition. Moreover, there was no observed effect of omega-3 supplementation on two other study measures of processing speed (Trails A and B).

Research investigating the relationship between Alzheimer's disease and inflammation has found that inflammatory processes are involved in the pathogenesis of the disorder's characteristic cognitive decline (Kalmijn, et.al, 2004). Related research has found that omega-3 supplementation and fish consumption are associated with decreased cognitive decline in Alzheimer's patients (Kalmijn, et.al, 2004). This was predominantly displayed in measures of processing speed. The

supported hypothesis is that omega-3's affect speed of cognition through the inflammatory mechanism because they decrease the inflammation commonly associated with cognitive decline (Kalmijn, et.al., 2004).

A main effect of time was also observed with all 3 components of the RAVLT. Performance on immediate recall, delayed recall, and recognition of presented stimuli improved over time for both treatment conditions. It is possible that this improvement over time- with an absence of between-group-differences- is due to practice effects. Several participants remarked that they remembered words from the initial administration of the RAVLT on their post treatment assessment. No other cognitive effects of omega-3 supplementation were found on the other cognitive tests included in the study.

If the principal study findings- i.e., no specific benefit of omega-3 supplementation regarding sleep- are replicated by other investigators, this would provide valuable information of conceptual and practical clinical utility. At a theoretical level, it could indicate less support for the hypothesized role of inflammation and hyperactivation of the HPA axis in sleep disturbance. Given that dietary omega-3 fatty acids promote decreased inflammation, which should in turn lead to decreased HPA axis hyperactivity, these results, if replicated, would suggest the likelihood of other factors contributing to sleep disturbance beyond merely inflammation and HPA axis hyperactivity.

Clinically, these results, if replicated, also indicate that omega-3 fatty acid is not an effective treatment for at least some forms of sleep disturbance- specifically,

problems with sleep efficiency, sleep onset, and frequent awakening. It would remain to be shown whether or not such supplementation would be of benefit for sleep problems among depressed individuals- a population shown in previous investigations to experience an overall reduction in depressive symptomatology with omega-3 supplementation.

There may be several factors that contributed to the study's largely null results. First, the literature on which this study was based had focused on the treatment of depression and HPA-axis hyperactivity, while the study sample comprised a non-clinical group of non-depressed individuals. It is possible that those who suffer from depression, and commensurate HPA-axis hyperactivity, may find sleep benefits from omega-3 fatty acid supplementation more so than the participants in this study. According to the BDI-II scores obtained before and after the intervention, the great majority of participants scored in the low range, which indicates that they were not experiencing clinical levels of depressive symptomatology.

Also, this study only used one particular dosage combination of the two prominent long-chain of omega-3 fatty acids: 1000mg of EPA and 500 mg of DHA daily. It may be that higher dosages could prove to be more effective. Clinical studies investigating other disorders, such as depression and schizophrenia have found that a higher omega-3 dosage may actually be related to increased symptom reduction (Peet and Stokes, 2005). Also, it is possible that a different ration of EPA and DHA may be more beneficial for reduction in sleep disorder symptoms. This

investigation only used one particular ration of EPA/DHA (2:1) that has been found beneficial in those with depressive symptoms.

This study also neglected to look at blood plasma ratios of omega-6 to omega-3 fatty acids. It has been hypothesized that high omega-6 ratios common among those who consume the typical American diet, may require higher levels of omega-3 fatty acids to counter the deleterious effects of pro-inflammatory omega-6 fatty acids (Hibbeln, et al., 2006).

Although all but one study subject scored above a “5” on the baseline PSQI, indicating a high probability of at least some significant sleep difficulties, the sample was not formally screened for clinically significant experiences of insomnia. It may be that omega-3 fatty acid supplementation only works to reduce symptoms of insomnia in a more severely sleep-disordered population. It is possible, in other words, that the study’s null results are related to its truncated range of sleep difficulties, and that participants’ sleep disturbances were not clinically significant enough to permit measurement of intervention-related change.

It is also likely that some participants in this college sample experienced sleep difficulties due to lifestyle factors instead of chronic or prolonged biologically based insomnia. A qualitative review of participant sleep diaries revealed many sleep disruptions due to environmental irregularities, such as roommate noise and uncomfortable room temperature. These environmentally-based disruptions are different from the disturbances that would be expected in a population experiencing insomnia for biologically-based reasons. It may be, therefore, that omega-3 fatty acid

is helpful in addressing some sleep difficulties of an organic nature (like HPA axis dysregulation), as opposed to the sleep-related difficulties of an environmental nature that appear to have been commonplace among study participants. Also, insomnia is a heterogeneous disorder in which there are multiple reasons for poor quality and quantity of sleep. Some forms of insomnia are easily measured scientifically, while others are less measurable. Insomnia that takes on a more psycho-physiological pattern may be less observable by scientific measurement, yet participants still may report feeling tired or that their sleep isn't restful and may meet criteria for clinical insomnia. However, the scientific measurement of sleep quality and quantity may not adequately display their sleep disturbances or poor quality of sleep. It is possible that this sample of college-age students is experiencing a clinical level of insomnia that may be more psycho-physiological in nature and thus less easily measured.

There are also several prominent study limitations worth noting. First, this study employed a relatively small sample size, with only 27 total participants- and only 12 in the treatment condition. Thus, the study suffered from low statistical power to detect anything but large-magnitude effects; it is possible, therefore, that a larger sample size would have yielded different results. This study was also limited by the truncated age range of participants. It is possible that supplementation with a wider age range may reveal important information about effectiveness at different ages. Also, there was no external measure of participant adherence to the supplementation protocol, and the investigator could only rely on self-report of adherence. Therefore, it is not known if the participants actually complied with the

entire treatment protocol. Other measures of adherence, such as blood samples testing for levels of circulating omega-3 fatty acids, could substantially improve confidence in the measurement of adherence. Also, this investigation did not control for participant medication use or other substance use- e.g., stimulants, antidepressants, alcohol- that have an established potential to affect sleep quality and quantity. This study also did not directly measure the use of prescription sleep aids. As mentioned previously, participants in this study were not formally screened for diagnostic-level insomnia, nor were they categorized by the type of sleep disturbance they were experiencing, and it cannot be ruled out that they utilized sleep medication while participating in the study.

Directions for Future Research

As noted, future research is needed to examine the use of omega-3 fatty acid supplementation on insomnia and other sleep problems in a clinically depressed population. Also, examination of changes in sleep using a higher dosage or longer duration of omega-3 fatty acid supplementation may prove influential on sleep processes. Research should also be conducted to assess for an optimal ratio of omega-6 to omega-3 fatty acids that may reduce symptoms of insomnia.

It would also be beneficial to see future investigations that utilize a non-college sample in order to attempt to control for unique environmental influences on sleep that characterize college populations. Likewise, it would be wise to measure and control for prescription stimulant and sedative medication use in future studies.

Also, it is suggested that future research focus on using participants with a high likelihood of biological underpinnings for their sleep disturbance, as it is hypothesized that this population, as opposed to a non-clinical college population, would evidence a favorable response to an omega-3 intervention.

Also, given the significant finding with respect to improvement in cognitive processing speed, a replication study is warranted, as is future research regarding the benefits of increased dosage. Investigations using omega-3 supplementation for the purpose of examining cognitive processing speed should focus on not only dosage effects, but also type of omega-3, and duration of supplementation that is most likely to produce increases in cognitive performance. Strong research investigating the possible benefits of omega-3 supplementation on cognitive processing speed should include multiple methods of measuring processing speed, given that participants in this study performed significantly better on only one of the three study indicators of this construct.

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Table 1. Treatment and Control Groups: Sample Characteristics

| Measure | | Total Sample (N=27) | Treatment Group (N=12) | Control Group (N=15) |
|----------------|--------|------------------------|---------------------------|-------------------------|
| Gender (M/F) | M (SD) | 13/14 | 6/6 | 7/8 |
| Age | M (SD) | 19.89 (2.81) | 19.91 (2.68) | 19.87 (3.00) |
| Minority (Y/N) | M (SD) | 6/21 | 4/8 | 2/13 |
| BMI | M (SD) | 23.51 (2.71) | 22.62 (2.65) | 24.23 (2.63) |

Table 2. Independent Samples t-test for baseline values

| Baseline Variable | Treatment Group Mean (SD) | Control Group Mean (SD) | t (df) | p |
|-------------------|------------------------------|----------------------------|-------------|------|
| Age | 19.92 (2.68) | 19.87 (2.99) | .045 (25) | .964 |
| Gender | 6/6 | 7/8 | -.166 (25) | .870 |
| BMI | 22.62 (2.65) | 24.23 (2.63) | -1.577 (25) | .127 |
| Minority | 4/8 | 2/13 | 1.231 (25) | .230 |
| Adherence | 93.15 (14.78) | 96.51 (8.15) | -.743 (24) | .465 |

Table 3. Independent Samples t-tests for baseline values

| Baseline Variable | Treatment Group Mean (SD) | Control Group Mean (SD) | t (df) | p |
|-------------------------------------|---------------------------|-------------------------|-------------|------|
| Sleep Efficiency- Actigraph | 82.38 (5.05) | 79.27 (5.74) | 1.477 (25) | .152 |
| Sleep Efficiency- Sleep Diary | 89.47 (7.11) | 88.55 (8.48) | .285 (23) | .778 |
| Sleep Latency- Actigraph | 22.67 (14.52) | 29.85 (13.77) | -1.315 (25) | .200 |
| Sleep Latency- Sleep Diary | 30.97 (20.90) | 32.30 (11.95) | -.203 (23) | .841 |
| Number of Wake Bouts- Actigraph | 38.00 (9.60) | 38.06 (12.48) | -.014 (25) | .989 |
| Number of Wakenings- Sleep Diary | 2.03 (0.83) | 2.05 (1.55) | -.028 (23) | .978 |
| Number of Minutes Moving- Actigraph | 49.81 (32.71) | 45.84 (15.85) | .415 (25) | .682 |

Table 4. Independent Samples t-test for Baseline Values for Cognitive Tests

| Baseline Variable | Treatment Group Mean (SD) | Control Group Mean (SD) | t (df) | p |
|--------------------------|---------------------------|-------------------------|-------------|------|
| Trails A | 12.67 (2.99) | 11.60 (2.67) | .987 (25) | .337 |
| Trails B | 60.58 (14.18) | 52.47 (12.28) | 1.594 (25) | .123 |
| Stroop Words | 82.12 (19.87) | 77.62 (18.37) | .530 (19) | .602 |
| Stroop Color/Words | 157.13 (30.97) | 144.08 (28.89) | .979 (19) | .340 |
| Stroop Interference | 54.32 (6.32) | 58.66 (10.64) | -1.041 (19) | .311 |
| Letter-Number Sequencing | 5.67 (0.78) | 5.87 (1.06) | -.546 (25) | .590 |
| RAVLT List A | 8.67 (2.93) | 8.27 (2.58) | .377 (25) | .709 |
| RAVLT Recall | 5.67 (3.39) | 5.50 (3.41) | .124 (24) | .902 |
| RAVLT Recognition | 9.33 (3.23) | 9.20 (3.10) | .109 (25) | .914 |

Table 5. Independent Samples t-tests for Baseline Values for PSQI, BDI-II, and Sleep Time

| Baseline Variable | Treatment Group Mean (SD) | Control Group Mean (SD) | t (df) | p |
|-------------------------|---------------------------|-------------------------|-------------|------|
| PSQI Total | 10.33 (3.08) | 10.80 (3.86) | -.340 (25) | .736 |
| Sleep Time- Actigraph | 420.51 (35.23) | 396.30 (62.56) | 1.269 (25) | .217 |
| Sleep Time- Sleep Diary | 440.14 (50.08) | 439.46 (69.35) | .027 (23) | .979 |
| BDI-II | 11.73 (4.56) | 16.07 (5.24) | -2.175 (23) | .040 |

Figure 1.) Stroop Processing Speed



