The Association between Dietary Omega-3 Fatty Acid Intake and Sleep Quality among Healthy Adults

A Thesis submitted in partial fulfillment of the requirements for the degree of Master of Science, Nutrition at George Mason University

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Summer Semester 2015
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DEDICATION

I dedicate this thesis to my family. Thank you for your love and support.

ACKNOWLEDGEMENTS

I would like to acknowledge my thesis committee members, Dr. Sina Gallo, Dr. Lilian de Jonge, Dr. Amber Courville, and Dr. Margaret Slavin for their guidance throughout this process. I would also like to thank the NIH for partnering with the GMU Department of Nutrition and Food Studies making this research happen. Finally, I would like to acknowledge Dr. Monica Skarulis for inviting me to be an associate investigator on the Obesity Phenotype protocol, Shanna Bernstein and Dr. Ninet Sinaii for their guidance throughout the analysis and making this project fun and interesting.

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LIST OF ABBREVIATIONS

AA	Arachidonic acid
ADD	Attention Deficit Disorder
AI	Adequate Intake
ALA	α-linolenic acid
AMDR	Acceptable Macronutrient Distribution Range
BMI	Body Mass Index
BRFSS	Behavioral Risk Factors Survey System
CDC	Center for Disease Control
CSHQ	Child Sleep Habits Questionnaire
CLOCK	Circadian Locomotor Output Cycles Kaput
DHA	Docosahexaenoic acid
EPA	Eicosapentaenoic acid
ESS	Epworth Sleepiness Scale
FAO	Food and Agriculture Organization of the United Nations
FDA	Food and Drug Administration
FFQ	Food Frequency Questionnaire
HEI	Healthy Eating Index
IL-1	interleukin-1
IL-2	interleukin-2
IL-6	interleukin-6
ISSFAL	International Society for the Study of Fatty Acids and Lipids
LA	Linoleic acid
MSLT	Multiple Sleep Latency Test
NDSR	Nutrition Data System for Research
NHANES	National Health and Nutrition Examination Survey
NIEHS	National Institute of Environmental Health Sciences
NIH	National Institutes of Health
NREM	Non-Rapid Eye Movement
PSQI	Pittsburgh Sleep Quality Index
PUFA	Polyunsaturated fatty acid
RD	Registered dietitian
REM	Rapid Eye Movement
SAS	Statistical Analysis Software
SFA	Saturated fatty acid

SPSS	Statistical Package for Social Sciences
TFEQ	Three-Factor Eating Questionnaire
U.S.	United States
WALI	Weight and Lifestyle Inventory
WHO	World Health Organization

ABSTRACT

THE ASSOCIATION BETWEEN DIETARY OMEGA-3 FATTY ACID INTAKE AND SLEEP QUALITY AMONG HEALTHY ADULTS

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Previous research has suggested possible associations between dietary fat intake, obesity and sleep. In a mHypoE-37 neuron cell culture model, saturated fat was found to disrupt regulation of the Circadian Locomotor Output Cycles Kaput (CLOCK) gene (implicated in circadian rhythms) but the addition of docosahexaenoic acid (DHA) attenuated this disruption. DHA supplementation in children has yielded positive sleep outcomes, but there is a paucity of such data in adults. Therefore, the aim of this thesis was to determine the relationship between total dietary fat, omega-3 fatty acids, and DHA intake with sleep quality among healthy adults. Data were from an observational study, aimed to phenotype healthy adults, conducted at the National Institutes of Health (NIH) Clinical Center (Bethesda, MD). Adults (n=226) completed 7 day food records to determine dietary intake of total fat and long chain fatty acids. The Pittsburgh Sleep Quality Index (PSQI) assessed overall sleep quality as well as seven subcomponents: (1) subjective sleep quality, (2) sleep latency, (3) sleep duration, (4) habitual sleep efficiency, (5) sleep disturbances, (6) use of sleeping medication, and (7) daytime dysfunction.

Medication, demographics and anthropometric measurements were obtained from medical records. Univariate regression analyses explored predictors of total PSQI score and its subcomponents. Medication use, Body Mass Index (BMI) and sex were consistently related to sleep quality. Adjusting for these covariates, percent energy from fat, omega-3 (g/1000 g) intake, and DHA (g/1000 g) intake were not significant predictors of overall sleep quality. However, when examining PSQI subcomponent scores in adjusted analyses, omega-3 intake was a statistically significant predictor of sleep latency (Adj. R^2=0.050, β=-0.340, p=0.042). While total omega-3 intake was not associated with overall sleep quality, this thesis suggests the potential role for omega-3 in shortening sleep latency. As short sleep is associated with chronic illness and weight gain, nutritional interventions aimed at increasing sleep duration may lead to improvements in overall health. Thus, further investigation examining the association between omega-3 fatty acid and sleep quality is warranted.

CHAPTER 1

Literature Review

The Importance of Sleep

Sleep's association with disease has been a debated topic for decades. Some scientists believe lack of sleep is a risk factor to poor health while others concede sleep as a confounder or risk marker for disease. Whether poor sleep causes disease or not, an association between poor sleep and poor health has been established.

Research suggests that individuals who consistently get a poor night's sleep are at greater risk of diabetes and obesity.[1,2] Individuals who slept less had increased glucose intolerance and leptin-ghrelin (hunger regulating hormones) ratios,[1] leading to an increased appetite. However, some have concluded the short sleep durations are only weakly associated with weight gain.[3,4] While cross-sectional studies showed short sleep associated with BMI,[4] some prospective and longitudinal studies did not.[5] Thus, one cannot rule out reverse causality (e.g. BMI causing poor sleep).

Individuals with consistent short sleep duration trend toward greater rates of depression, low socio-economic status, chronic illness, obesity, and poor health-related quality of life.[6–10] While short sleep duration has shown significant associations with poor health, the negative effects of longer-than-average sleep duration is still being debated by health professionals.[11] For men, long sleep was associated with decreased

physical activity levels and lower health-related quality of life.[12] Whether long sleep is a consequence of chronic morbidities or a risk marker of detecting other health related issues still remains a question, and research has not yet proven a negative health outcome as a causal consequence to poor sleep.

Decreased sleep duration over time can predict cardiac outcomes and can serve as a marker for some cancer risks, while increased sleep over time may be a predictor of non-cardiovascular mortality. One study observed impaired glucose intolerance and a 70% increase in leptin to ghrelin ratios after sleep restriciton.[1] One potential mechanism for this metabolic consequence suggests a decrease in hypothalamic activity following a decrease in sleep duration.[13,14] Whether causal or a confounder, sleep's association with poor health is not ignored, and scientists continue to research methods of improving sleep as a way to deter illness for individuals of all sizes and even treat obesity.

Sleep and Obesity Connection

Ensuring the human body has adequate rest is important to all genders, age groups and health levels, but it is especially important for people with obesity.[15] More than a third of the U.S. adult population is now classified as obese, and with obesity comes a multitude of health problems including diabetes, metabolic syndrome, nutrient deficiencies, anxiety, and sleep disturbances.[16] Research has revealed an association between poor sleep quality and obesity[17] with a few possible explanations: increased activity of the sympathetic nervous system slowing metabolism,[18] imbalanced ghrelin and cortisol ratios causing increased appetite,[2] and a decreased inhibition of hypothalamic

activity.[13,14] However, some research has also suggested that weight gain is caused by stress induced by mechanical stimuli as opposed to chronic sleep loss.[19]

A variety of stimuli could lead to negative neuro-endocrinological impacts, stemming from stressors in the socioeconomic, socio-cultural, and physical environments. For example, a stressor from one's environment may lead to a decrease in sleep which in turn alters the body's ghrelin and cortisol ratio, increases appetite, and increases caloric intake which leads to obesity. Conversely, those same environmental stressors could lead to obesity which in turn could cause sleep apnea, poor sleep quality and an increase in the circulation of inflammatory cytokines (a common marker for individuals diagnosed with sleep apnea).[17]

Scientists continue to study the impact of cytokines on sleep and have observed an imbalanced regulation of interleukin IL-1, IL-2, and IL-6 during disrupted sleep cycles. While they have concluded a possible mechanism lies with short sleep durations instead of solely circadian rhythm, this research also suggests that cytokines produced during sleep disturbances may lead to an increased production of prostaglandins,[20] something that omega-3 fatty acids are known to combat.

Although not all individuals who experience sleep disturbances have obesity, those who do have frequent insufficient sleep are more likely to be obese (odds ratio of 1.5).[21,22] This begs the question why this association exists and if improving sleep could be a treatment option.

Sleep cycles

There are two different types of sleep cycles: Non-Rapid Eye Movement (NREM) and Rapid Eye Movement (REM). The NREM has three different phases. NREM stage 1 is light sleep where one is easily awakened. NREM stage 2 is a slighter deeper sleep with slower brain waves. An individual is half-asleep during this stage. NREM stage 3 is a deep restorative sleep and the most important stage in order to get enough rest and feel energized the next morning. Individuals spend about one-fifth of their sleep duration in this phase. Finally, the REM stage is where dreaming occurs, the body is temporarily paralyzed, and the brain is stimulated to learn and make memories. About one-fifth of an individual's sleep duration is spent in this stage. NREM sleep stage 3 is important to health, and when acutely or chronically disturbed, there are associations with negative health outcomes.[15]

During REM sleep and wake cycles, the sympathetic nervous system (the system associated with the fight or flight response during stress) is in a heightened state.[18] During the NREM sleep cycle, epinephrine and norepinephrine (the hormones associated with the sympathetic nervous system) decrease in circulation. In a depressed sympathetic nervous system, leptin is no longer inhibited and the hunger response is low. In contrast, when the sympathetic nervous system is activated; there is an increase in fatty acids in the blood due to direct innervation of the adipose tissue/lipolysis, and the hunger response is high. Therefore one potential explanation for this relationship between sleep and obesity may due to the increased sympathetic nervous system's activity during the different sleep cycles however, these mechanisms are still not completely understood.[18]

What is a good night's sleep?

Health professionals suggest that a good night's rest is paramount to daytime effectiveness, and it consists of 6-8 hours of uninterrupted sleep (8 hours being ideal), but it actually depends on age.[23] An expert panel from the National Sleep Foundation published the sleep duration recommendations for healthy individuals by age group in January 2015 as shown in **Table 1.1**.

However, according to the Centers for Disease Control (CDC), Americans' sleep quality needs improvement.[24] In 2009, randomly selected participants responded to a sleep questionnaire over the telephone via the Behavioral Risk Factor Surveillance System (BRFSS).[24] Of the 74,571 respondents spanning 12 states, 35.3% reported having less than 7 hours sleep on average during a 24 hour period, 48% reported snoring, 37.9% reported unintentionally falling asleep during the day at least 1 day during the past 30 days, and 4.7% reported nodding off or falling asleep while driving in the previous 30 days **(Table 1.2)**.[24]

A similar CDC Morbidity and Mortality report published in 2009 revealed Americans' perceived insufficient sleep status. This 2008 BRFSS randomly called 403,981 individuals from all 50 states, D.C., and U.S. territories. The survey asked, "During the past 30 days, for about how many days have you felt you did not get enough rest or sleep," then the response was stratified into one of four groups: 0 days, 1-13 days, 14-29 days, and 30 days. A total of 30.7% reported zero days of insufficient rest/sleep, 41.3% 1-13 days, 16.8% 14-19 days, and 11.1% 30 days. Additionally, males differed

from females; 12.4% of females reported 30 days of insufficient rest compared to 9.9% of males. As age increased, the likelihood of reporting zero days of insufficient sleep increased, 13.8% of participants aged 25-34 years reported 30 days of insufficient rest while those age ≥ 65 years were less likely at 7.4%. Consequently, there was a decreased rate of perceived insufficient rest with aging.[25]

Compared to other countries, the United States (U.S.) falls short of the recommended sleep duration. The National Sleep Foundation's 2013 poll on adults aged 25-55 years concluded that the U.S. and Japan get an average of 30 to 40 minutes less sleep per weeknight compared to Germany, Mexico, the United Kingdom, and Canada.[26]

Individuals with obesity have reported less sleep per 24 hour period than those of normal weight status. Vorona et al. assessed 1,001 individuals' sleep status via questionnaire which gathered information on demographics; the presence, frequency, and duration of naps; bed time, wake time, and total estimated sleep time per 24 hours; general medical problems; diagnosed sleep disorders; and caffeine, tobacco, alcohol use. The results concluded that individuals with obesity slept significantly less ($p=0.04$) than those of normal weight however, this was not the same for overweight individuals, as there was no significant difference in sleep times between participants who were normal and overweight ($p=0.31$).[27]

Similar to the CDC BRFSS 2009 results previously discussed, the CDC also conducted a BRFSS Health Related Quality of Life survey in 18 states in 2002. All 79,625 respondents answered how many days within the past 30 they had gotten an insufficient amount of rest or sleep. Responses were then categorized into <14

("sufficient") or ≥ 14 ("insufficient"). Of the individuals with obesity (BMI≥30), 23.9% reported frequent insufficient sleep. Those with insufficient sleep were almost 1.5 times more likely to be obese (Adj. OR 1.4, 95% CI: 1.3-1.5).[22] Others have reported similar results; individuals who report less than 7 hours in bed are almost 3 times more likely to be obese (OR 2.93, 95% CI: 1.06, 8.09).[21]

With these statistics, the Institute of Medicine Committee on Sleep Medicine and Research recommends an interdisciplinary approach to the treatment of sleep disturbances, requiring an integration of health care efforts.[28] However, research in this area is hindered by the lack of accurate sleep assessments.

Sleep Assessment Methods

Depending on the goals of the primary care provider or the researcher, sleep can be assessed in three primary ways: physiological indicators, observation and self-report. A popular method of quantitative physiological sleep assessment is polysomnography. This test monitors sleep quality and disturbances through measuring air flow, blood oxygen level, body position, brain waves, breathing effort and rate, muscle activity, eye movement, and heart rate. The test is conducted by strapping electrodes to the chin, scalp, and outer edge of the eyelids as well as monitors attached to the chest to record heart rate and breathing. These instruments also measure sleep latency, how long it takes to fall into REM sleep and the number of times breathing stops.[29] However, these tests are costly and require more time and resources compared to other methods. Like polysomnography, actigraphy is another method of quantitative physiological sleep assessment. However, unlike polysomnography, actigraphy is less cumbersome and

requires only a wrist monitor to be worn on the non-dominant hand. The monitor tracks the wearer's movements and determines sleep-wake states, and some have concluded an actigraph measuring all three axes (like the Mini Motionlogger) is the most accurate measure of sleep-wake cycles.[30]

Behavioral observations of sleep cycles can also be assessed through real time observation of the patient's sleep or video recordings. Although physiological assessments such as polysomnography are the most quantitative of the sleep assessment options, sleep questionnaires and observations may capture the less tangible sleep information such as family history, medical history, medication use, the sleeping environment, and psychological confounders.[31]

Examples of self-reported sleep assessments include sleep diaries and questionnaires, including the Pittsburgh Sleep Quality Index (PSQI). The PSQI was initially published as a sleep assessment tool in 1989 and has been used as a self-reported tool since its publication. The PSQI was validated in the U.S. over an 18 month period among three distinct groups of men and women: good sleepers as the control (n=52), poor sleepers with major depressive disorders housed in a psychiatric institute (n=34), and poor sleepers referred by a physician to the Sleep Evaluation Center (n=62). All participants were evaluated by medical history, physical examination and routine polysomnography.[32]

Two methods were used to test validity. The first examined the degree which the PSQI detected differences among the distinct groups that were previously assessed by a combination of clinical interviews, structured interviews, and polysomnographic data.

The second method compared each group's PSQI scores with the polysomnographic data for REM %, delta %, sleep latency, sleep efficiency, and sleep duration. Before sensitivity and specificity were calculated, the two groups of poor sleepers were combined into one and compared to the good sleeper control group. The results concluded a PSQI score less than 5 yielded an 89.6% sensitivity and 86.5% specificity distinguishing between the two groups.[32]

Individuals who suffer from sleep disruptions also are known to be excessively sleepy throughout the day accompanied by daytime dysfunction. The Epworth Sleepiness Scale (ESS) is an 8 item questionnaire which measures overall daytime sleepiness. It is scored on a scale of 0 to 24, with a high score correlating to increased daytime sleepiness. A score of 16 or more is categorized as excessive daytime sleepiness. Originally published for use in 1991, the ESS was validated for use by testing 180 participants – 30 controls with normal sleep habits and 150 participants with various diagnosed sleepiness disorders. A total of 138 patients of the 150 sleepy participants completed an overnight polysomnography, and 12 completed the Multiple Sleep Latency Test (MSLT). The MSLT is known to be an accurate measure of daytime sleepiness on the day the test is conducted. ESS scores were compared with polysomnography ($r=-0.379$, $n=138$, $p<0.001$) and MSLT results ($r=-1.514$, $n=27$, $p<0.01$). Additionally, there was a significant difference of ESS scores between the control and sleepy groups ($p<0.0001$).[33]

Due to the strengths and limitations of each type of sleep assessment method, primary care physicians and researchers tend to use a combination of assessment methods to achieve the most accurate sleep diagnosis.[17,31]

The Association between Diet and Sleep

A good night's sleep is not only important for feeling well-rested but for overall health. Those experiencing sleep restriction are more likely to develop chronic illnesses such as hypertension, diabetes, obesity, and cancer along with higher risks of depression, mortality, and reduced quality of life and daytime productivity.[24] We see a majority of nutrition and sleep research focusing on macro- and micronutrient dietary intake alone, but there is a growing body of evidence displaying an association between sleep and BMI, energy intake, diet quality, and morning tiredness.[34–37]

While patients were previously encouraged to eat a balanced diet, exercise regularly, and avoid caffeine before bedtime in order to maximize potential for a good night's rest, scientists have found correlations between diet and sleep quality/duration.[38] A negative correlation has been established between BMI and sleep duration[35] and between energy intake and sleep duration.[34–36]

Short sleep duration is associated with a decreased ability to control food intake.[34] A total of 267 adults completed a Three-Factor Eating Questionnaire (TFEQ), which measures the intent to control food intake, the overconsumption of food in response to cognitive or emotional cues, and food intake in response to feeling hunger. Three-day food records and a sleep questionnaire which asked, "On average, how many hours do you sleep per day?" were also completed. Sleep responses were divided into 3 groups

(≤6 hours, 7-8 hours and ≥9 hours). Short sleep duration was significantly associated with high disinhibition eating behaviors and increased odds of gaining weight over the 6 year period ($p<0.05$, OR 4.49, 95% CI: 3.06-6.06).[34]

In order to elucidate the relationship between obesity and short sleep duration, the role of habitual diet was explored.[39] After examining 459 women from the Women's Health Initiative, the researchers concluded that short sleep duration was negatively correlated with dietary fat intake when sleep was measured via actigraph and controlled for the following confounders: age, income, education, total dietary grams, BMI, and physical activity. Sleep was also measured subjectively via a daily sleep diary but yielded insignificant results. Dietary data was measured by Food Frequency Questionnaire (FFQ) over a 3 month period, and a summary of the results may be found on **Table 1.3**.[39]

In contrast, Yamaguchi et al. found no significant associations between dietary fat and sleep results using FFQ and subjective sleep measures (n=1,368 Japanese adults).[40] Lindseth et al. also found no significant difference in actigraph sleep measures between participants consuming a high fat diet and the control group (n=44). Although, significant associations were found between high protein and high carbohydrate groups.[41]

Grandner's et al. 2013 examined major dietary nutrients and sleep duration. Based on the 2007-2008 National Health and Nutrition Examination Survey (NHANES),[42] individuals who reported 7-8 hours of sleep were associated with the greatest food variety, measured by the number of foods ($p<0.001$) consumed. Those who reported <5 hours were associated with decreased cholesterol intake however, this was

not statistically significant ($p<0.10$). Individuals who reported >9 hours were associated with decreased saturated fatty acid (SFA) ($p<0.05$), monounsaturated fatty acid ($p<0.05$), and cholesterol intake ($p<0.01$) however, these results were insignificant after adjustment.[42] Upon careful analysis of the NHANES data assessment, one can conclude that diet can contribute to determining sleep patterns however, the data lacked specific fatty acid analysis, and sleep and diet were not well quantified.[42] The NHANES measured diet based on 24-hour recall and one sleep question, "How much sleep do you usually get at night on weekdays or workdays?" Grandner et al. concluded these associations require further research to determine causality due to appetite dysregulation, sleep duration, or whether nutrients have physiological effects on sleep regulation.[42] Based on this work,[39,41] diet diversity has a positive association with normal sleep duration (7-8 hours), but the results of dietary fat's relationship to sleep quality are inconsistent.

Haghighatdoost et al. also examined diet diversity, BMI and sleep, but among a different sample - female Iranians. This study conducted in 2012 concluded that participants with low Healthy Eating Index (HEI) and Diet Diversity scores had poor sleep patterns.[35] Female Iranians age 18-28 years self-reported their sleep duration and were separated into one of three groups: <6 hours sleep, 6-8 hours sleep, and >8 hours sleep. Those participants reporting <6 hours sleep a night had significantly higher BMI ($p=0.0001$) and caloric intake ($p=0.01$) as well as low HEI ($p=0.002$) and diet diversity scores ($p=0.001$).[35]

Individuals from U.S./Puerto Rico had similar results. Over 27,000 women from the National Institute of Environmental Health Sciences (NIEHS) Sister Study completed a FFQ and a sleep questionnaire, where they asked "about how much sleep do you get per night on average?" HEI scores were calculated from the FFQ data and analyzed via general linear regression with the sleep data (hours) split among 7 time groups. They concluded that the tendency to eat during unconventional eating hours was associated with shorter sleep durations of <5 hours, increased snacking as well as an increased intake of fat and sweets.[43] Both of these studies only examined females, therefore more research is needed to compare these results among both sexes. Yet, there remains a scientific consensus that individuals with poor sleep quality indicators have greater odds of being obese.[21,22]

Even though people with active lifestyles tend to have lower BMI,[44,45] when one's sleep is disturbed, the feeling of morning tiredness can lead to a lack of motivation to perform any physical activity. As seen in a 2012 study, adolescents who reported getting less than eight hours of sleep per night also reported a subjective feeling of morning tiredness. Although, this significantly reduced participation in leisure time physical activity in males (OR 0.64, 95% CI: 0.45-0.93), these results were not significant among females (OR 1.01, 95% CI: 0.75-1.36).[37] Differences in sleep between sexes was examined further by Goel (2005) where they concluded that females overall slept better than males and had a shorter sleep latency ($p=0.009$, $d=1.11$).[46] Although this study used quantitative sleep measures by actigraph, it consisted of a small sample size (n=31, 16

male, 15 female, age 18-30 years). Additionally, some have concluded female sleep latency can increase during the luteal phase of the menstrual cycle.[47]

Even though scientists have concluded an association exists between BMI, energy intake, morning tiredness, diet quality, and sleep there remains a lack of established causality of these conditions. Whether poor sleep causes obesity or obesity causes poor sleep is unclear. While cross-sectional research established an association, it is important to note the bidirectional nature of these relationships.[18] While some research has examined sleep's relationship to BMI, energy intake, overall diet, and some of the macronutrients (including fat), few have expanded this research into the realm of a detailed nutrient panel in humans. However, discoveries in cell cultures[48] and secondary observations in human studies[49,50] have revealed the positive impact omega-3 fatty acid has on sleep measures.

Omega-3 Fatty Acid Status among Americans

Polyunsaturated fats are a special class of lipids containing one or more double bonds in their structure and known for their multitude of health benefits. Omega-3 and omega-6 fatty acids are types of long chain polyunsaturated fatty acids (PUFA), which are essential and need to be obtained through exogenous sources. Deficiencies of these fatty acids may lead to neurological, cardiovascular, cerebrovascular, autoimmune, metabolic diseases as well as cancer.[51] Eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) are two types of omega-3 fatty acids known for their health promoting effects. In particular, DHA has benefits for brain development during pregnancy and infancy.[50] Both DHA and arachidonic acid (AA, 20:4n-6) are

highly concentrated in the cell membranes of the brain and retina and accumulate rapidly during the fetus's rapid brain development.[52]

Omega-3 fatty acids are derived primarily from fish while omega-6 fatty acids are derived from mainly vegetable oils. It is partially for this reason that the Food and Drug Administration (FDA) encourages a balanced diet,[53] consisting of 2 servings of fatty fish every week with an adequate intake (AI) of 0.6 to 1.2% of total energy intake.[54] If trying to reduce cardiovascular disease risk, studies have shown taking 500 mg per day of EPA and DHA can be beneficial.[55] Considering the average American's dietary EPA and DHA intake only amounts to about 150 mg daily,[54] this proves challenging for most Americans/individuals, so some have resorted to supplementation. The International Society for the Study of Fatty Acids and Lipids (ISSFAL) recommends a linoleic acid (LA) adequate intake of 2% of total energy, α-linolenic acid (ALA) healthy intake of 0.7% total energy, and combined EPA and DHA of 500 mg per day (minimum) for cardiovascular health.[56]. The World Health Organization (WHO) also has similar recommendations as shown in **Table 1.4**.[57]

Eicosanoids are the key mediators and regulators of inflammation. A large proportion of inflammatory cell structure consists of omega-6 fatty acids with a lower proportion of other types of 20-carbon PUFAs like omega-3 EPA. Because of this large proportion of omega-6 in inflammatory cell lipid profiles, AA is identified as the primary eicosanoid synthesis substrate and is a common target for anti-inflammatory treatments. Prostaglandins, thromboxanes, and leukotrienes are three types of eiconsanoids, and eicosanoid synthesis from AA or EPA can be a determining factor for inflammatory

markers **(Figure 1.1)**.[58] Fatty acids compete for enzyme desaturase, with enzymes metabolizing fatty acids in the following order of preference: omega-3 > omega-6 > omega-9.[59] Alpha-linolenic acid (18:3n-3) and linoleic acid (18:2n-6) are essential fatty acids derived only from the diet, and Americans consume far more omega-6 fatty acids than omega-3. In fact some studies estimate Americans consume 20 times more omega-6 fatty acids than omega-3.[59]

When humans ingest a higher ratio of omega-3 to omega-6 fatty acids, the omega-3 fatty acids replace the omega-6 in cell membranes of the body, especially platelets, erythrocytes, neutrophils, monocytes, and liver cells. This in turn has cascading effects decreasing the production of harmful prostaglandins.[59] ALA, EPA, and DHA all contribute as an anti-inflammatory however, ALA tends to be less effective than both EPA and DHA as an inflammatory which are already 20 and 22 carbons, respectively (**Figure 1.1**).

EPA and DHA from exogenous sources have been attributed to decreasing the production of harmful prostaglandins by assisting the release of AA from the cell membrane phosolipid pool; however, the molecular mechanism behind this fatty acid release is not completely understood. If scientists knew how to incite the release of AA from the phosolipid pool, the treatment options for inflammatory disorders would be boundless.[60]

Omega-3 Fatty Acids and Sleep

Dietary fat intake is shown to affect daytime sleepiness in mice.[61] Mice were separated into either a high fat (more beef fat) or low fat (no beef fat) group and observed

over an eight week period. At the conclusion of the study, the high-fat diet mice slept more than low-fat diet mice during the nighttime, when they would normally be active (p=0.001).[61]

Greco, et al. (2014) discussed the possible protective effect omega-3 DHA's presence has on the circadian rhythm, countering the negative sleep effects experienced from high SFA consumption.[48] The human body's circadian rhythm is run by genes, the surrounding environment's cues, and lifestyle choices.[62] CLOCK genes are key components to the generation of circadian rhythms. A change to any one of the *Bmal1*, *Per2*, and *Rev-erba* CLOCK genes can lead to sleep disturbances [48] or increase the risk for metabolic syndrome.[63] For example, the *mPer2* gene is specifically associated with appetite control.[64] A 2014 cell culture study added palmitate and DHA to neuronal cultures and observed how DHA helped protect the *Bmal1* CLOCK gene from negative circadian rhythm-altering affects caused by palmitate.[48]

Similarly, using a mouse model, Barnea et al. (2009) observed overall fat intake and its effect on the CLOCK gene.[65] Six C57BL mice age 2-3 weeks were split into two groups and observed over a seven week period. One group was fed a low fat diet (no palm oil) while the second group was fed a high fat diet (with palm oil). Upon examination of the hepatocytes derived from the mouse livers, it was concluded that the mice on the high fat diet had a three hour phase delay in the *mPer1* CLOCK gene. This meant that during the daytime when the mice would normally be awake and functioning, the mice fed the high fat diet did not awaken until 3 hours later than the mice who were fed the low-fat mice.[65] With both the Barnea and Greco studies observing associations

between fat and sleep on both the in vitro and animal models, it begs to question whether a similar association exists between fat and sleep quality in humans.

Human Studies

An observational cohort study published in 2010 followed 810 children age 5-12 years all diagnosed with Attention Deficit Disorder (ADD) over a twelve week period.[50] PUFA have been known to play a role in preventing and treating certain mental health disorders like ADD, but previous trials involving DHA supplementation alone have had mixed results, suggesting that EPA may also play an important role in ADD treatment.[50] Students were recruited in school and each given one ESPRICO® supplement containing 400 mg omega-3 EPA, 40 mg omega-3 DHA, omega-6, magnesium, and zinc daily. Sleep patterns were assessed by asking the students' parents if their children had trouble falling asleep (yes or no) during each checkup. At the end of the twelve week period (and as a secondary observation), there was a decrease in "trouble falling asleep" from 79.5% to 45.4%.[50]

Montgomery et al. (2014) examined omega-3 supplementation in children in a randomized controlled trial.[49] Children (n=392) age 7-9 with below average literacy rates were separated into either placebo (corn/soybean oil) or DHA supplement groups and were compared about 16 weeks. Sleep patterns were measured using the Child Sleep Habits Questionnaire (CSHQ) and accelerometer. Blood samples were taken throughout the trial. The supplement group of children showed increased blood fatty acid concentrations and the following sleep effects as compared to the placebo group: 58 more minutes asleep (p=0.029) as measured by actigraph, 7 fewer wake episodes (p=0.013)

actigraph, 44 fewer minutes awake (p=0.068), and an overall 8% increase in sleep efficiency (t:2.000, p=0.052). However the CSHQ results were not as statistically significant.[49]

Conclusion

Sleep is important to health, but whether it has been established as a confounder or risk marker for disease has yet to be determined. Epidemiological evidence demonstrates an association exists between poor sleep quality and poor health, especially for chronic diseases like obesity, diabetes as well as depression, daytime dysfunction and poor quality of life. The human diet's association with sleep quality has also been established, but further research needs to be conducted in order to gain full understanding of each nutrient's impact on sleep quality and health. Some studies have observed omega-3 fatty acid's positive influence on sleep quality in vitro models, and omega-3 interventions in children have also shown correlations between omega-3 consumption and sleep quality as a secondary observation.

Table 1.1 National Sleep Foundation Sleep Recommendations throughout the lifecycle.[23]

Age	**Recommended Sleep Duration (hours)**
0-3 months	14-17
4-11 months	12-15
1-2 years	11-14
3-5 years	10-13
6-13 years	9-11
14-17 years	8-10
18-25 years	7-9
26-64 years	7-9
≥65 years	7-8

Table 1.2 Adults Reporting Selected Sleep Behaviors in 12 States by Characteristics, Behavioral Risk Factor Surveillance System, U.S., 2009[24]

	Sleeping on average <7 hrs in 24-hr period (n=74,571)	Snoring (n=68,462)	Unintentionally fell asleep during day at least once in the past month (n=74,063)	Nodded off or fell asleep while driving in the past month (n=71,578)
Total	35.5%	48%	37.9%	4.7%
Age (years)				
18 to 24	30.9%	25.6%	43.7%	4.5%
25 to 34	39.4%	39.6%	36.1%	7.2%
35 to 44	39.3%	51.0%	34.0%	5.7%
45 to 54	39.0%	59.3%	35.3%	3.9%
55 to 64	34.2%	62.4%	36.5%	3.1%
≥ 65	24.5%	50.5%	44.6%	2.0%
Sex				
Male	35.5%	56.5%	38.4%	5.8%
Female	35.2%	39.6%	37.3%	3.5%

Table 1.3. Partial correlations between dietary nutrient variables and objective sleep duration.[39]

Dietary nutrient	**R**	**p-value**
Fat	-0.15	0.0004
PUFA*	-0.168	0.0012
Total energy	-0.162	0.0019
% calories from fat	-0.143	0.0060

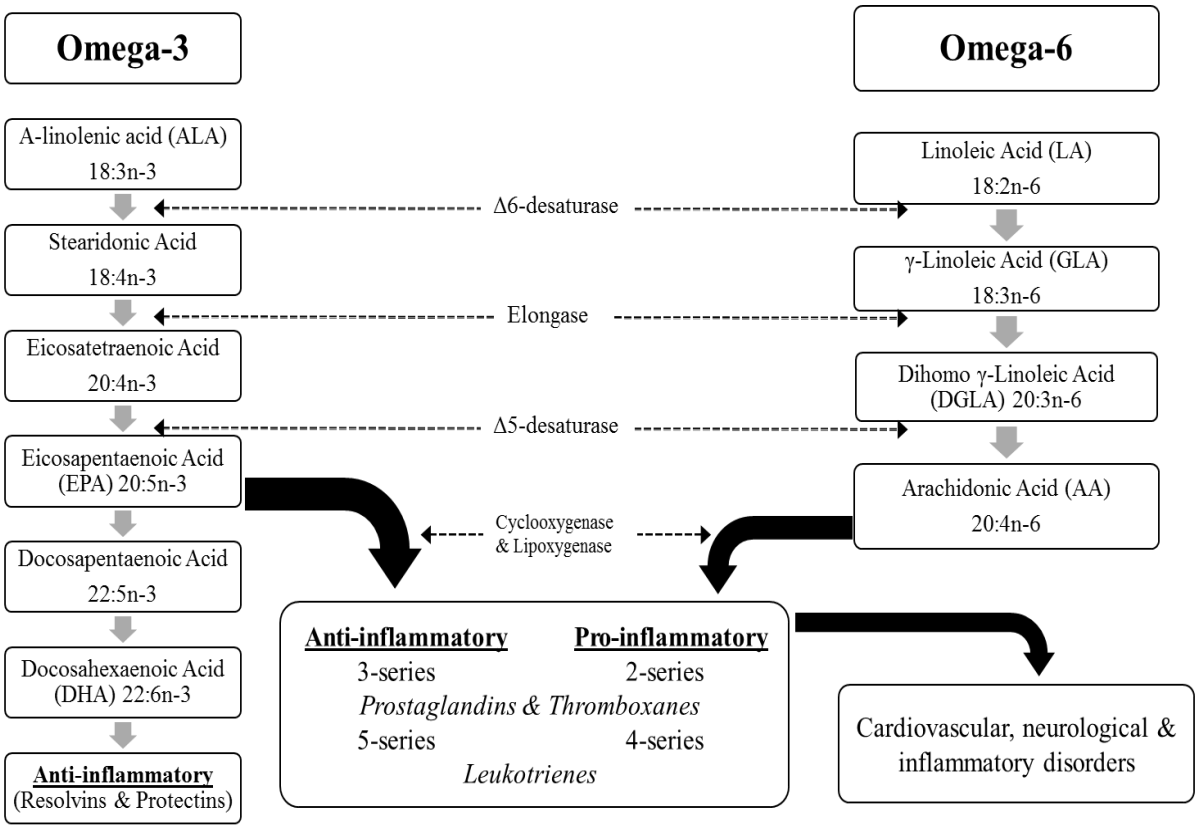
*PUFA (polyunsaturated fatty acid)

Table 1.4. Food and Agriculture Organization of the United Nations (FAO) and the WHO PUFA recommendations, expressed in % of energy intake. [57]

	Intake to prevent deficiency	**Healthy dietary intake**
PUFA	2.5-3.5%	6-11%
LA	2-3%	2.5-9%
ALA	0.5-0.6%	
EPA + DHA		2% (upper level)

PUFA (polyunsaturated fatty acid), LA (linoleic acid), ALA (α-linolenic acid), EPA (eicosapentaenoic acid), DHA (docosahexaenoic acid).

Figure 1.1



Metabolism of omega-3 and omega-6 essential fatty acids[58]

CHAPTER 2

Rationale, Objectives, and Hypotheses

Rationale

With this quickly growing body of research on the topic of dietary fat intake and sleep, there is still a scarcity/limited studies which examined dietary fat intake and sleep in adults. The 2014 in vitro study added palmitate and DHA to neurons and observed that DHA helped protect the *Bmal1* CLOCK gene from the negative circadian rhythm altering affects caused by the SFA palmitate.[48] Similarly, using a mouse model, Barnea et al. (2013) observed how high overall fat intake was associated with a three (3) hour phase delay in the *mPer1* CLOCK gene.[65] DHA supplements in humans have been examined – however, both studies were in children,[49,50] and one of which looked at sleep patterns as a secondary effect.[50] One third of the American population has obesity, and with the established association between poor sleep quality, obesity and poor health, it is important to expand this research to include adults.

In vitro and child studies found a significant association between sleep and supplemental DHA, but more research needs to be accomplished before we can draw the same conclusions for healthy adults, whether supplemental or dietary. When SFA is accompanied with DHA, it can attenuate the disruption of the CLOCK gene and circadian rhythm in cell cultures.[48] It is also known that high overall fat intake was associated with

a 3 hour phase delay in the mPer1 CLOCK gene in animal models,[65] poor diet is associated with poor sleep quality in adults,[35,42,43] and DHA supplementation improved sleep in children.[49,50] However, the mechanisms behind these associations are still not completely understood, some of the previous literature lacked robust sleep assessment data, and there is a paucity of omega-3/sleep research in adults.

For example, the 2009 NHANES analysis was based upon only one question, "how much sleep do you get on average per night."[36] Previous research lacked quantitative assessment of sleep duration and quality.[38] This shows significant gaps in data but also the potential for future research. In order to improve scientific rigor, sleep assessment should be measured with validated sleep methods such as full length questionnaires (e.g. PSQI, ESS etc.) or actigraphy.

Finally, more analysis is needed to correlate specific dietary nutrients with sleep quality and duration. When studying sleep in adults, previous literature had focused on overall macronutrient intake,[65] weight gain,[34] diet patterns[15,24,36] as opposed to specific dietary fatty acids. The few studies which have examined specific fatty acids' relationship to sleep either yielded inconclusive results[38] or were supplemental interventions on children[49,50] with sleep observed as a secondary research objective.

Objectives

Due to the progress in the field of omega-3 research and the significant gaps in literature concerning this topic, the aim of this study was to determine whether an association exists between dietary omega-3 fatty acid and sleep quality among an

ethnically diverse group of healthy adults. One primary and two secondary objectives have been established to address these gaps in the research literature.

Primary Objective

Objective 1.1: To assess the association between dietary omega-3 fatty acid, DHA, and overall fat intake and sleep quality (measured by the PSQI) in healthy adults.

Hypothesis 1.1: The null hypothesis states that no association exists between sleep quality (measured by global PSQI score), and overall total dietary fat, omega-3, and DHA intake. The alternative hypothesis states that overall dietary fat, omega-3 and DHA intake are significant predictors of sleep quality as measured by the global PSQI score.

Secondary Objectives

Objective 2.1: To examine the PSQI subcomponents' relationship with total dietary fat, omega-3, and DHA intake.

Hypothesis 2.1: The null hypotheses states no association exists between the PSQI subcomponent scores (subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleeping medication, and daytime dysfunction) and total dietary fat, omega-3 and DHA intake. The alternative hypothesis states that total dietary fat, omega-3 and DHA intake are significant predictors of PSQI subcomponent scores.

Objective 2: To examine the association between total dietary fat, DHA, and omega-3 fatty acid intake and daytime sleepiness (measured by the ESS).

Hypothesis 2.2: The null hypothesis states no association exists between ESS score and total dietary fat, omega-3 and DHA intake. The alternative hypotheses states total dietary fat, omega-3 and DHA intake are significant predictors of daytime sleepiness (measured by ESS score).

CHAPTER 3

Manuscript

ABSTRACT

Background

Previous research has suggested possible associations between dietary fat intake, obesity and sleep. In a mHypoE-37 neuron cell culture model, saturated fat was found to disrupt regulation of the CLOCK gene (implicated in circadian rhythms), but the addition of DHA attenuated this disruption. There is a paucity of such data in humans.

Objective

The aim of this study was to determine the relationship between total dietary fat, omega-3 fatty acids, and DHA intake with sleep quality among healthy adults.

Methods

Data were from an observational study, aimed to phenotype healthy adults, conducted at the NIH Clinical Center (Bethesda, MD). Adults (n=226) completed 7 day food records to determine dietary intake of total fat and long chain fatty acids. The PSQI assessed overall sleep quality as well as seven subcomponents: (1) subjective sleep quality, (2) sleep latency, (3) sleep duration, (4) habitual sleep efficiency, (5) sleep disturbances, (6) use of sleeping medication, and (7) daytime dysfunction. Medication, demographics and anthropometric measurements were obtained from medical records. Multiple regression

analyses explored predictors of total PSQI score and its subcomponents.

Results

Medication use, BMI and sex were consistently related to sleep quality. Adjusting for these covariates, percent energy from fat, omega-3 (g/1000 g) intake, and DHA (g/1000 g) intake were not significant predictors of overall sleep quality. However, when examining PSQI subcomponent scores in adjusted analyses, omega-3 intake was a statistically significant predictor of sleep latency (Adj. R^2=0.050, β=-0.340, p=0.042).

Conclusion

While total omega-3 intake was not associated with overall sleep quality, this study suggests a potential role for omega-3 in shortening sleep latency. As short sleep is associated with chronic illness and weight gain, nutritional interventions aimed at increasing sleep duration may lead to improvements in overall health. Thus, further investigation is warranted.

BACKGROUND

Poor sleep quality has been associated with obesity and other accompanying illnesses like diabetes, metabolic syndrome, nutrient deficiencies, anxiety, and sleep disturbances.[16,24] Sleep is important for human health, and previous research has revealed an association between poor sleep quality and higher BMI[4] in addition to dietary factors including more snacking, higher fat diets, and unhealthy eating.[43]

Dietary intervention is a way to combat poor sleep quality and improve overall health.[15] Evidence from in vitro[48] and human[49,50] models showed positive effects of

omega-3 fatty acids on sleep. Particularly, DHA protected the CLOCK gene from circadian altering effects of SFA.[48] DHA supplementation trials conducted in children have shown improved sleep but did not examine dietary intake and lacked robust sleep assessment data.[49,50] Therefore, there is currently a gap in literature examining the association between dietary fat and sleep in adults.

This study aims to fill this gap in current literature by examining the association between dietary omega-3 fatty acid, DHA, and dietary fat intake and sleep quality among healthy adults and as a secondary objective, measure its association with daytime sleepiness.

METHODS

Participants

This was a secondary analysis of participants enrolled in the clinical *Study of the Phenotype of Overweight and Obese Adults* (protocol number 07-DK-0077, clinicaltrials.gov identifier NCT00428987) at the National Institutes of Health located in Bethesda, MD. This cross-sectional, observational study began January 2007 with a projected end date of 2030. The study was approved by the National Institute of Diabetes, Digestive and Kidney Diseases Institutional Review Board and the present secondary analysis was approved by the George Mason University Institutional Review Board. Inclusion criteria for the main study included both male and female adult participants with a BMI ≥18.5 upon initial approval of participation. General exclusion criteria included significant physical limitations, current, unstable medical conditions, or

psychiatric conditions which would preclude the participant from completing required study assessments. Further exclusion criteria used for the purposes of the secondary analysis included chronic, un-controlled disease or illness. All demographics were self-reported, and both race and ethnicity were defined by the categories of the 2000 U.S. Census.[66] Only data from participants' baseline study visit were included in this present analysis.

Medications and Supplement Use

Medication and supplement usage was taken from both medical records, assessed by a health professional upon initial visit, and the Weight and Lifestyle Inventory (WALI) **(Appendix A.12)** as documented via the WALI self-administered questionnaire. If a medication or supplement was listed for a subject in either record, it was included in this analysis. Each medication listed was labeled and coded for sleep side effects including excessive tiredness/drowsiness, insomnia/trouble sleeping, both drowsiness and insomnia, prescribed for sleep, or no sleep side effects. Sleep side effects were determined by cross referencing each medication on Medline, or if not listed in Medline, WebMD, or the drug's website. Medication side effects were then categorized as "yes" for sleep side effects or "no" for no sleep side effects. Omega-3 supplement use was categorized "yes" or "no" based on lists taken from both the medical records and the self-reported WALI. Supplement dosages were not available for this sample.

Anthropometrics

Height in centimeters was measured using a wall stadiometer, and weight was measured in kilograms by electronic scale in the morning after patients abstained from

food and water for >8 hours. Both were measured in triplicate and the average of the three measures was used to calculate BMI (kg/m^2). BMI <25 was categorized as normal, 25 ≤ BMI < 30 overweight, and BMI ≥ 30 obese. Data on sleep apnea diagnosis was not collected as part of this study, as neck circumference is highly associated with incidence of sleep apnea, neck circumference was used in analyses as a proxy for sleep apnea.[67]

Sleep Assessment

Sleep quality data was taken from the PSQI questionnaires (**Appendix A.7**) completed by all participants during their baseline visit. All incomplete questionnaires were excluded. The PSQI was self-administered and participants completed their PSQI via either hard copy or on a computer by answering ten multi-part multiple-choice questions. Component and global PSQI scores were calculated in accordance with published PSQI protocols.[32] Global PSQI scores measure overall sleep quality and range from 0 to 21 with higher scores equating to poorer sleep quality. Scores ≤5 were associated with good sleep quality, and scores >5 were associated with poor sleep quality. Subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleeping medication, and daytime dysfunction were assessed by PSQI components 1 through 7 respectively.

Daytime sleepiness data was taken from the ESS questionnaires **(Appendix A.8)**. Similarly, all incomplete questionnaires were excluded. The ESS is a self-administered test, and all participants completed their ESS via computer by answering 8 questions on a scale of 0 to 3. ESS composite scores were calculated in accordance with published,

approved ESS instruction standards.[33] Total ESS scores range from 0 to 24 with higher ESS scores associated with high levels of daytime sleepiness.

Dietary Assessment

The participants' dietary data was taken from 7-day food records. Each subject was given instructions explaining how to record their dietary intake and told to not alter their typical diet during the assessment period. Records included 7 consecutive days of dietary intake data including all foods, drinks and condiments consumed during the assessment period. All participants recorded a description of the food consumed along with type of preparation and total amount/serving size. Upon completion, each subject met with a registered dietician (RD) or nutrition technician and reviewed their records with 3 dimensional food models to ensure accuracy. Dietary data was then coded into the Nutrition Data System for Research (NDSR) where all macro- and micronutrient data was analyzed. Dietary fat percentage was calculated dividing total dietary fat (kilocalories) by total energy intake (percent of kilocalories). Omega-3 density was calculated dividing omega-3 (g) by total food intake (g) times 1000 g. DHA density was calculated dividing DHA (g) by total food intake (g) times 1000 g. Prior research has suggested adjusting nutrients for total energy intake[69] or body weight and physical activity (in epidemiological studies).[70] Finally, Hu et al. also concluded that units expressed as calories or grams do not affect analyses when adjusting for total energy intake.[71] Nutrients may be analyzed as absolute amounts or in relation to total intake,[68] therefore densities were used in order to control for overall food intake in grams. Participants with incomplete 7-day food records were excluded from analysis.

Statistical Analysis

Descriptive data including race, ethnicity, sex, BMI categories, medication use, and omega-3 supplement usage were assessed via frequency distribution tables and reported by sample percent distribution. The distributions of all of the continuous variables were fully assessed using tests for normality (Shapiro-Wilk test) as well as visual inspection. Log-transformed data were also similarly assessed to determine if there was any improvement in the distributions. For the regression modeling, use of data on the original scale did not violate assumptions or lead to a different conclusions hence, results from regression analyses are reported using data on their original scale due to ease of interpretation. Continuous variables including BMI, age, and neck circumference are reported as means and standard deviations. Non-normally distributed data including dietary and sleep variables are reported as medians and ranges. Simple linear regression models were used to assess the potential confounding effects of possible confounder variables (medications with sleep side-effects, omega-3 supplement use, caffeine intake, alcohol use, BMI, age, sex, ethnicity, race, and neck circumference) on sleep outcomes. As the simple regression models showed correlations within the results, separate correlation analyses were not used to test for confounders. Nonparametric testing was considered but not used due to parametric tests yielding the best fit models. Assumptions of all statistical tests were tested and evaluated for regression models including independence of observations, normality and constant variance of random error terms/residuals, as well as diagnostics for influential observations and collinearity. Univariate linear regression models established medication use and sex as significant

predictive confounders for sleep quality. BMI was also a significant confounder for sleep quality, established by previous literature.[1,6–8,10] Therefore this study's statistical analyses controlled for these three confounding variables in all regression models. The effect of the primary independent variable omega-3 density on (1) global PSQI, (2) PSQI component 1 subjective sleep quality, (3) PSQI component 2 sleep latency, (4) PSQI component 3 sleep duration, (5) PSQI component 4 habitual sleep efficiency, (6) PSQI component 5 sleep disturbances, (7) PSQI component 6 use of sleep medication, (8) PSQI component 7 daytime dysfunction, and (9) total ESS score for daytime sleepiness were tested in different multiple linear regression models while controlling for medication use, BMI and sex. The effects of DHA density and dietary fat percentage on sleep measures were also tested using multiple linear regression models. A p-value <0.05 was considered significant. Statistical Analysis Software (SAS) v. 9.2 (SAS Institute, Inc, Cary, NC) was used to test for confounders and the range of statistical assumptions. Statistical Package for Social Sciences (SPSS) v. 21.0 (IBM Corporation, Armonk, NY) was used to calculate descriptive statistics and conduct regression analyses.

RESULTS

Data was reviewed in December 2014; a total of 410 baseline visits of which 336 were considered healthy and 226 had complete diet and sleep data, thus yielding a final sample size of 226 participants for the purposes of this analysis **(Figure 3.1)**. The average BMI of the population was categorized as obese at 33.3±10.0 kg/m^2. The average age was 40 years, and 55% of the sample was white, Caucasian. Almost two-

thirds of the sample was female (66%). Half of the sample took medications which could impact sleep quality, but only 12% of the sample reported taking an omega-3 supplement **(Table 3.1)**. Mean omega-3 and omega-6 dietary intake were within the AMDR recommendations.[54] The dietary fat breakdown revealed the participants' cholesterol, monounsaturated fatty acid, polyunsaturated fatty acid, omega-6, omega-3, EPA, and DHA would be comparable to the American population. Their SFA intake was a little higher than the recommended 10% of dietary intake at 11% **(Table 3.2)**. Overall, 47% of the participants had good sleep quality as defined by a PSQI ≤5 and 95% had a low level of daytime sleepiness according to the ESS ≥16. **(Table 3.3 - 3.4)**. There were no significant effects of dietary omega-3 density or dietary fat percentage on global PSQI score **(Table 3.5)** (**Objective 1.1**). There was a trend (p=0.086) for DHA density on global PSQI score when controlling for medications, BMI, and sex (β=-5.347, SE=3.098) **(Table 3.5)**. No significant effects of diet were observed on any of the PSQI subcomponents (**Objective 2.1**) except sleep latency (PSQI component #2). Dietary omega-3 fatty acid intake was a significant predictor of sleep latency, (β=-0.340, SE=0.166, p=0.042). Thus, every 1 unit increase in dietary omega-3 fatty acid consumption predicted a 0.340 decrease in sleep latency score after adjusting for medications, BMI, and sex. Additionally, a trend (p=0.093) in DHA's relationship to sleep latency was reported (β=-1.408, SE=0.834) **(Table 3.6)**. When sexes were examined separately in a sub-group analysis, dietary omega-3 intake was no longer a significant predictor of sleep latency and differed by sex. In females, a trend (p=0.069) was observed in omega-3 intake as a predictor of sleep latency (β=-0.368 ±SE 0.201)

(Table 3.7) however, for the male participants this was not significant (p=0.458) **(Table 3.8)**.

Daytime dysfunction, as assessed by ESS score, was a secondary objective study however (**Objective 2.2**), due to the limited range in the scores (95% of the sample categorized as low levels of daytime sleepiness) further regression analysis was not included. Additional regression outcomes for all PSQI components can be found in **Appendices A.1. through A.6**.

DISCUSSION

This was the first study to explore dietary fatty acid intake with sleep quality in adults, and this study provided novel data suggesting that dietary omega-3 intake was a significant predictor of sleep latency. Although, the primary objective's results found that total fat intake, omega-3 intake and DHA intake were not significant predictors for sleep quality as measured by the global PSQI, there was an effect of omega-3 intake on sleep latency. Thus, these results offer promise for a role of dietary fat, particularly omega-3 intake in sleep research.

Omega-3 intake was a significant predictor of sleep latency in our study population, thus participants consuming higher intakes of dietary omega-3 took less time to fall asleep. A previous study by Montgomery et al. conducted with children concluded a significant improvement in sleep duration post omega-3 supplementation however, sleep latency was not significantly different.[49] Our results appear inconsistent with Montgomery et al. yet, sleep was assessed using actigraph which some have shown to be

the most accurate method of sleep assessment.[30] The CSHQ results proved insignificant. Additionally, the Montgomery study only examined children which would likely have a different sleep pattern compared to adults.[72]

Full mechanisms of omega-3 fatty acids are still not completely understood which leaves some question as to how omega-3 has a significant negative association with sleep latency in healthy humans. Individuals with disrupted sleep cycles have imbalanced regulation of IL-1, IL-1, and IL-6 and an overall increase in cytokine production and inflammatory markers.[20] EPA and DHA are attributed to decreasing the production of harmful prostaglandins and inflammation,[60] therefore further research is warranted in order to examine the chemical mechanisms possibly involved in prostaglandin reduction during the sleep cycle.

Previous literature had established differences in sleep quality and sleep latency between males and females, but these outcomes depended on the age of the sample.[46,47] Young women trended toward having shorter sleep latency and better sleep quality than men[47] however, post-menopausal women and women in the luteal phase of the menstrual cycle had longer sleep latency and poor sleep quality.[46] In fact in this sample, sex was a stronger predictor of sleep latency (β=-0.375 ±SE 0.137, p=0.007) than omega-3 density (β=-0.340 ±SE 0.166, p=0.042). Since this study did not account for the female participants' menstrual cycles or menopausal status, this should be further examined in future research. When the sexes were analyzed separately, the females ate more omega-3 (1.25 omega-3 g/1000 g) compared to males (0.90 omega-3 g/1000 g) on average however, when compared, this was not statistically significant (p=0.278). This difference

in omega-3 consumption between the sexes is consistent with the American population; in fact, according to 2009-2010 NHANES data for ages ≥20 years, women consistently ate more ALA, LA, EPA, and DHA per 1000 kcal than men **(Table 3.9).**[73] These results beg to question how hormonal differences and differences in dietary intake between the sexes may play a role in lipid metabolism and the sleep quality. Previous research has also established that women have higher erythrocyte DHA levels, most likely due to females having more enzyme desaturase activity, incited by estrogen.[74] If women consume more omega-3 fatty acids per 1000 g and have increased enzyme desaturase activity, this may help explain why omega-3 was a stronger predictor of sleep latency for the female compared to the male participants in our sample. Dietary fat's association with sleep quality has been examined with varied results; some report negative sleep effects[42] and some report no association.[41,40] Therefore it was not unexpected to see a lack of association between total dietary fat intake and sleep quality in this population. When examining total dietary fat, the data does not take into account stratifying the more harmful fats like saturated fat from the polyunsaturated fats. As a high amount of saturated fat proved to correlate with negative sleep effects in vitro in prior literature,[48] it is important to distinguish between the types of fat when examining sleep quality. Dietary omega-3 density was not a significant predictor for overall sleep quality. Although these results were somewhat unexpected, the positive skewness of the sample may explain why these results were insignificant. Omega-3 intake ranged from 0.427 g to 5.279 g across the population with a median intake of 1.846 g and an average intake of 1.977 g. The positive skewness may have negatively impacted the results. Additionally,

previous research focused on DHA when examining the effects on sleep quality, finding that in vitro, DHA had a protective effect on the CLOCK gene's circadian cycle when subjected to SFA.[48] Greco et al. (2014) analyzed the impact of 25 µM DHA on 25 µM palmitate in vitro.[48] Possible future studies could compare these sleep quality results to SFA and DHA ratios in humans.

The lack of a significant association between DHA intake and global PSQI could be explained by a lack of variability in intake among the population **(Figure 3.3).** The average intake of DHA was 0.125 g among this sample, which is actually more than the average American intake as reported by the 2009-2010 NHANES (0.06 g).[73] Separated by sex, this sample's female group reported an average intake of 0.121 g DHA, and males reported an average of 0.163 g DHA. The 2009-2010 NHANES females reported 0.06 g DHA and males reported 0.08 g DHA on average.[73] The DHA dietary intake distribution of our sample was positively skewed. In fact, 9.7% consumed ≥0.159 g DHA, so with this skewness, it is difficult to ascertain DHA's effect on sleep quality and sleep latency.

Sex, medication use, and BMI were significant confounding predictors for overall sleep quality in multiple analyses. In fact, in many models BMI was the strongest predictor of sleep quality. This complimented previous literature's conclusion; there is a need to examine the relationship between obesity and sleep.[4,35] Prior research suggests that both caffeine[75,76] and alcohol[77] intake are significant predictors of sleep quality. In this study, both were tested but were not found to be significant predictors of sleep quality. This was most likely due to highly skewed data. A total of 17.7% of the sample did not consume any alcohol in their diet, and 48.2% consumed less than 90 mg of

caffeine. An additional multiple regression analysis was conducted examining whether BMI and sex were significant predictors of percent fat, omega-3 density (g/1000 g), and DHA density (g/1000 g) in the sample. BMI was a significant predictor of percent dietary fat intake (β=0.171, SE±.045, p=0.000), and sex was a significant predictor of omega-3 density (g/1000 g) (β=-0.125, SE±0.055, p=0.024). However, no other results were significant.

Prior research suggests that timing of food intake, specifically meals close to bedtime can negatively impact sleep latency ($r^2 = 0.45$; $r = 0.67$, $p < 0.001$) (n = 15) and sleep efficiency ($r^2 = 0.26$; $r = -0.51$, $p = 0.007$) in women (n=15) but not in men.[76] However, the timing of food consumption was not taken into account for this analysis.

In addition, there was no reliable measure of sleep apnea, so neck circumference was included as a proxy as prior research suggests a significant correlation between the two; a neck circumference ≥38 cm had a 58% sensitivity and a 79% specificity in predicting sleep apnea.[67] Neck circumference was not a significant confounder, and this could be explained by its high correlation with BMI, which was already established as a confounding variable. Since there was multi-collinearity between BMI and neck circumference, neck circumference was not included in the final regression model.

Although data has been published examining the effects of age and race/ethnicity on sleep quality,[78,79] due to its possible impact on other health determinants, it was included in this analysis although not found to be significant predictors. Although previous research suggests that variations in specific sleep patterns were observed in different races and ethnicities, they concluded these patterns were most likely due to

differences in socio-economic status.[79] Socio-economic status data was not available for this analysis, and this may explain why race and ethnicity did not prove to be significant predictors. This study's sample consisted of healthy volunteers from the D.C. area, therefore it is also possible that this study's sample did not have much variation among socio-economic status. This may be why there was also not a difference between races. One may consider socio-economic status as a variable in future sleep research.

The quality and quantity of data available for this secondary analysis were significant strengths to this research. Although, no gold standard in dietary assessment exists, seven day food records currently provide the best estimate of usual dietary intake as compared to other methods of dietary assessment, and the rigorous process of reviewing these records for completeness contributed additional strength to this dataset. Finally, the sample size was large considering the amount of complete seven day food records, PSQI and ESS questionnaires. Although dietary supplement data was ascertained in medical records and WALI questionnaires, dosage amounts were not included or assessed to the level commensurate with NHANES data.[80] This analysis was unable to take these supplement doses into account in the regression models; however, only 12% reported taking an omega-3 supplement, so this most likely would not have impacted the results. In a previous study published in 2010, Grandner et al. found a negative correlation between dietary fat intake and sleep quality when sleep was measured via actigraph. However, the sleep data they collected using daily sleep diaries yielded insignificant results.[39] This study only measured sleep by self-reported questionnaires. Therefore, in order to collect the most accurate sleep measures, future

research could consider a combination of multiple sleep assessment methods. The NIH NIDDK *Study of the Phenotype of Overweight and Obese Adults* has blood lipid profiles and unicorder data available for their participants. Future research examining diet and sleep for this sample should consider including unicorder data as an additional method for sleep assessment as well as lipid profile data to examine the relationship between dietary fat intake vs. absorption.

CONCLUSION

This study helped confirm a relationship between diet and sleep quality and filled a previous gap in literature by examining adult data. Previous literature had established a relationship between sleep quality, BMI, and diet, but its relationship regarding dietary fat and omega-3 was not fully understood. Some research has found an association between high fat diets and sleep disturbances while others found fat to be of no predictive significance. While in vitro models have suggested DHA's protective effects on the CLOCK gene while exposed to saturated fat, no such data had been examined in adults. These novel results establish omega-3 dietary density as a significant predictor of sleep latency in healthy adults and opens the research possibilities in the realm of dietary treatments for sleep disturbances. As poor sleep has been associated with obesity and other chronic inflammatory diseases, these results could prove invaluable as we continue to learn more about the possibilities of treating obesity.

Table 3.1 Population descriptive statistics (n=226). Continuous variables reported as Mean ± SD and categorical as % of sample.

Population Characteristic	
BMI (kg/m^2)	33.3 ± 10.0
Age (years)	40.6 ± 12.8
Neck circumference (cm)	37.3 ± 4.6
Race	
White	55.3%
Black	33.6%
Asian, multi-race, other	11.1%
Ethnicity	
Not Latino or Hispanic	85%
Latino or Hispanic	15%
Sex	
Male	34.1%
Female	65.9%
BMI categories[1]	
Normal (<25 kg/m^2)	22.1%
Overweight (25 – 29.9 kg/m^2)	21.2%
Obese (≥30 kg/m^2)	56.6%
Medication use[2]	
Yes	50%
No	50%

Omega-3 supplement use[3]	
Yes	11.9%
No	80.1%

[1]In accordance with CDC guidelines

[2]Yes = participant used medications that could cause sleep disturbance side-effects (common or rare) according to Medline, WebMD, or the drug's website, under normal usage conditions.

[3]Yes = taken in addition to normal dietary consumption.

Table 3.2 Dietary component description (n=226). Presented as Median [Range].

Characteristic	**Median [Range]**	**Recommendation**[54]
Total energy (kcal)	2090 [623, 4317]	
Fat (g) / (%)	80.5 [11.4, 234.6] / 33.5 [10.2, 65.6]	20-35% of total energy
Cholesterol (g)	289.2 [1.293, 2486.1]	
SFA (g) / (%)	25.6 [1.821, 80.9] / 10.7 [2.5, 22.1]	
MUFA (g) / (%)	30.1 [4.6, 109.2] / 12.3 [3.7, 30.5]	
PUFA (g) / (%)	17.5 [3.6, 50.6] / 7.2 [1.9, 14.8]	
PUFA : SFA ratio	0.758 [0.3005, 2.6013]	
LA (g) / %	15.201 [2.680, 46.424] / 6.546 [1.65, 14.06]	5-10% of total energy
ALA (g) / %	1.6 [0.532, 3.403] / 0.695 [0.14, 1.97]	0.6-1.2% of total energy
ω-6 (g)	15.5 [2.9, 47.0]	
ω-3 (g)	1.846 [0.427, 5.279]	
ω-6 : ω-3 ratio	8.8 [2.2, 19.6]	
ω-3 (g per 1000 g)	0.935 [0.207, 2.917]	
EPA (g per 1000 g)	0.017 [0.0002, 0.2322]	
DHA (g per 1000 g)	0.0412 [0.0, 0.4399]	
Carbohydrate (g) / (%)	249.9 [65.2, 567.6] / 47.7 [7.0, 76.1]	45-65 % of total energy
Protein (g) / (%)	83.9 [23.6, 214.3] / 16.6 [10.4, 38.1]	10-35% of total energy
Alcohol (g) / (%)	0.171 [0.0, 70.1] / 0.052 [0.0, 21.9]	
Caffeine (mg)	98.6 [0.0, 1360.8]	

SFA (saturated fatty acid), MUFA (monounsaturated fatty acid), PUFA (polyunsaturated fatty acid), LA (linoleic acid), ALA (α-linolenic acid).

Table 3.3 Sleep quality as assessed by the PSQI **(n=226)**. Data reported as % of sample and Median [Range].

Component	**0 (%)**	**1 (%)**	**2 (%)**	**3 (%)**	**Median [Range]**
Global PSQI / % good / % poor*					5 [0, 18] / 47.3% / 52.7%
(1) PSQI subjective sleep quality	22.1	53.1	19.9	4.9	1 [0, 3]
(2) PSQI sleep latency	31.0	38.5	18.1	12.4	1 [0, 3]
(3) PSQI sleep duration	54.0	27.0	11.5	7.5	0 [0, 3]
(4) PSQI habitual sleep efficiency	70.4	13.7	7.1	8.8	0 [0, 3]
(5) PSQI sleep disturbances	6.6	66.8	25.7	0.9	1 [0, 3]
(6) PSQI Use of sleep medication	78.8	8.0	4.0	9.3	0 [0, 3]
(7) PSQI daytime dysfunction	40.3	42.9	15.0	1.8	1 [0 ,3]

*Global PSQI <5 = good sleep quality. Global PSQI ≥ 5 = poor sleep quality[32]

Table 3.4 Daytime sleepiness assessed by the ESS (n=218).

Component	**Median [Range]**
Total ESS score	7.0 [0, 24]
Low level daytime sleepiness (ESS<16)	95.4%
High level daytime sleepiness (ESS≥16)	4.6%

* ESS ≥ 16 = excessive daytime sleepiness. ESS<16 = low level of daytime sleepiness[33]

Table 3.5 Multiple linear regression models for sleep quality (Global PSQI). Data presented as β [SE] p-value.

Dependent variable: Sleep Quality (Global PSQI)			
	Model 1	Model 2	Model 3
Intercept	β=3.199 [1.026], p=0.002	β=2.743 [0.905], p=0.003	β=3.400 [1.351], p=0.013
ω-3 (g/1000 g)	β=-1.002 [.618], p=0.100		
DHA (g/1000 g)		β=-5.347 [3.098], p=0.086	
Fat (% energy)			β=-0.039 [0.036], p=0.284
Medication (ref=no)	β=1.836 [0.487], p=0.000	β=1.875 [0.488], p=0.000	β=1.818 [0.488], p=0.000
BMI, kg/m^2	β=0.096 [0.024], p=0.000	β=0.088 [0.024], p=0.000	β=0.099 [0.025], p=0.000
Sex (ref=female)	β=-1.239 [0.513], p=0.016	β=-1.071 [0.507], p=0.036	β=-1.156 [0.510], p=0.024
	R^2 = 1.65, p=0.000	R^2 = 1.66, p=0.000	R^2 = 1.59, p=0.000

Table 3.6 Multiple linear regression models for sleep latency (PSQI Component #2). Data presented as β [SE], p-value.

Sleep Latency (PSQI Component #2)			
	Model 1	Model 2	Model 3
Intercept	β=1.164 [.275], p=0.000	β=0.981 [0.244], p=0.000	β=1.078 [0.364], p=0.003
ω-3 (g/1000 g)	**β=-0.340 [0.166], p=0.042**		
DHA (g/1000 g)		β=-1.408 [0.834], p=0.093	
Fat (% energy)			β=-0.008 [0.010], p=0.438
Medication (ref=no)	β=0.207 [0.131], p=0.114	β=0.216 [0.131], p=0.102	β=0.200 [0.132], p=0.130
BMI, kg/m^2	β=0.009 [0.007], p=0.152	β=0.007 [0.007], p=0.282	β=0.009 [0.007], p=0.164
Sex (ref=female)	β=-0.375 [0.137], p=0.007	β=-0.322 [0.136], p=0.019	β=-0.341 [0.137], p=0.014
	R^2 = 0.067, p=0.004	R^2 = 0.061, p=0.007	R^2 = 0.052, p=0.019

Table 3.7 Multiple linear regression model for sleep latency (PSQI Component #2) for subgroup females. Data presented as β [SE], p-value.

Sleep Latency – (PSQI Component #2)	
Intercept	β=0.871 [0.350], p=0.014
ω-3 (g/1000 g)	β=-0.368 [0.201], p=0.069
Medication	β=0.327 [0.168], p=0.053
BMI	β=0.017 [0.008], p=0.042
	R^2 = 0.072, p=0.012

Table 3.8 Multiple linear regression model for sleep latency (PSQI Component #2) for subgroup males. Data presented as β [SE], p-value.

Sleep Latency – PSQI Component #2)	
Intercept	β=1.228 [0.395], p=0.003
ω-3 (g/1000 g)	β=-0.218 [0.292], p=0.458
Medication	β=0.035 [0.204], p=0.865
BMI	β=-0.005 [0.010], p=0.635
	R^2 = 0.012, p=0.821

Table 3.9. NHANES 2009-2010, average polyunsaturated fatty acid intake comparison by sex (presented as total g and g/ 1000 kcal).[73]

	Male	**Female**
ALA (18:3n-3)	1.77 g 0.705 g/1000 kcal	1.38 g 0.776 g/1000 kcal
LA (18:2n-6)	17.84 g 7.102 g/1000 kcal	13.33 g 7.497 g/1000 kcal
EPA (20:5n-3)	0.04 g 0.016 g/1000 kcal	0.03 g 0.017 g/1000 kcal
DHA (22:6n-3)	0.08 g 0.032 g/1000 kcal	0.06 g 0.034 g/1000 kcal

ALA (α-linolenic acid), LA (linoleic acid), EPA (eicosapentaenoic acid), DHA (docosahexaenoic acid)

Figure Legend

Figure 3.1 Consort Diagram

Figure 3.2 Dietary Omega-3 Intake (g) Distribution

Figure 3.3 Dietary DHA Density (g/1000 g) Distribution

Figure 3.1

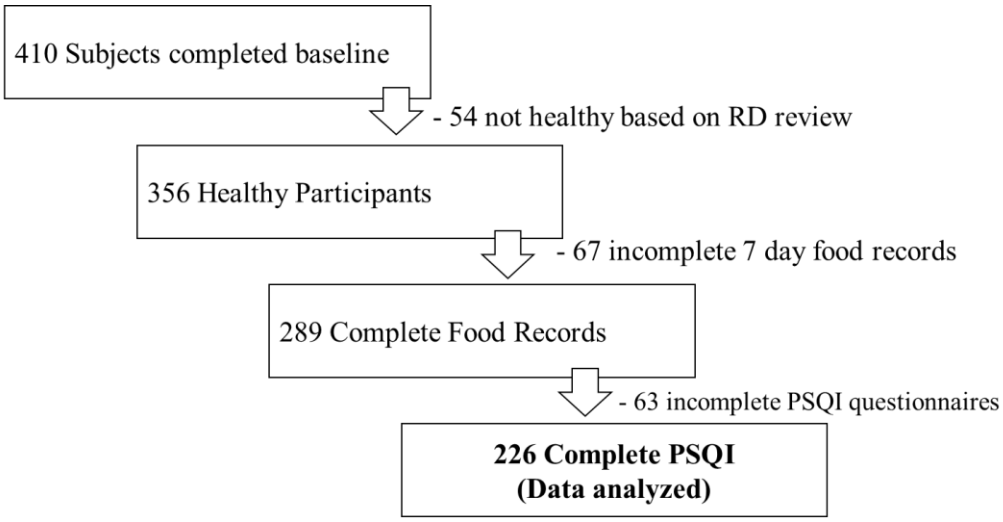

410 Subjects completed baseline
- 54 not healthy based on RD review
356 Healthy Participants
- 67 incomplete 7 day food records
289 Complete Food Records
- 63 incomplete PSQI questionnaires
226 Complete PSQI
(Data analyzed)

Figure 3.2

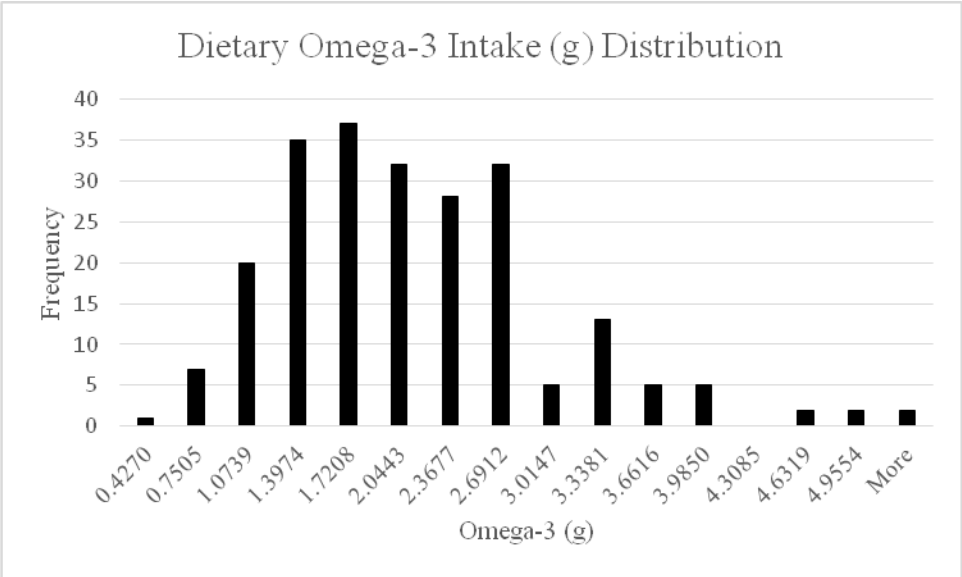
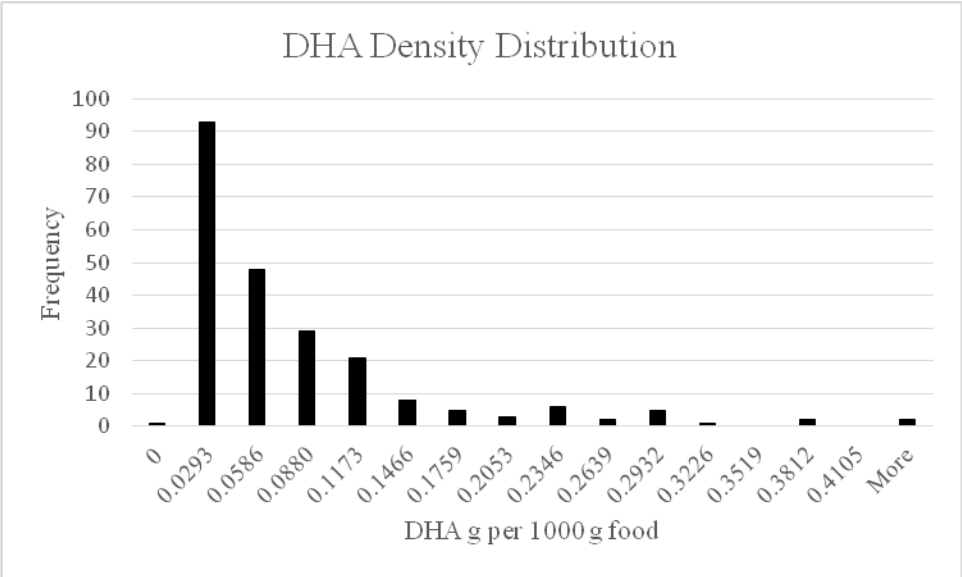

Dietary Omega-3 Intake (g) Distribution
Frequency
40
35
30
25
20
15
10
5
0
0.4270
0.7505
1.0739
1.3974
1.7208
2.0443
2.3677
2.6912
3.0147
3.3381
3.6616
3.9850
4.3085
4.6319
4.9554
More
Omega-3 (g)

Figure 3.3


DHA Density Distribution
Frequency
100
90
80
70
60
50
40
30
20
10
0
0
0.0293
0.0586
0.0880
0.1173
0.1466
0.1759
0.2053
0.2346
0.2639
0.2932
0.3226
0.3519
0.3812
0.4105
More
DHA g per 1000 g food

CHAPTER 4

Summary

Over one third of Americans have obesity, and obesity is accompanied by chronic illness and inflammatory disorders such as diabetes, metabolic syndrome, nutrient deficiencies, anxiety, and sleep disturbances.[16] Obesity is often treated through dietary intervention, physical activity and sleep intervention,[15] but the mechanisms explaining the associations among sleep, diet and chronic illness are still not completely understood. This study's results confirmed an association between diet and sleep quality, was the first to explore dietary fatty acid intake with sleep quality, and overall provided novel data suggesting that dietary omega-3 intake was a significant predictor of sleep latency.

These results could prove invaluable to the future of obesity research. Literature has previously established that long sleep latency is associated with daytime sleepiness, daytime dysfunction and low physical activity levels. If dietary omega-3 is a significant predictor of sleep latency, this marks the initial step into the future of obesity research and treatment options. The interconnection between dietary omega-3, sleep latency, daytime dysfunction and physical activity offers the possibility of multiple positive health outcomes stemming from one treatment approach – dietary omega-3 intake.

This study's findings add to the realm of diet/sleep literature because it is the first study of its kind, examining fatty acids' relationship to sleep quality. Although omega-3

proved to be a significant predictor of sleep latency among the sample population, once a sub-analysis was completed examining males and females separately, omega-3 was no longer significant. Interestingly, in females, a trend (p=0.069) was observed in omega-3 intake as a predictor of sleep latency however, for the male participants this was not significant. Sex-specific differences in sleep has been examined in previous literature, as females have trended toward longer sleep latency times[79] or shorter depending on age, menstrual cycle/menopause onset[46] however, this begs to question the hormonal and metabolic reactions causing these differences. In relation to this study specifically, further research is needed to establish why dietary omega-3 fatty acids are associated with shorter sleep latency among women and the physiological and metabolic mechanisms associated with this reaction.

One limiting factor of this study included the lack of physiological sleep measures. Grandner et al. had previously established a correlation between fat intake and physiological sleep measures, but this correlation did not apply to the self-reported sleep measures of the same sample. This suggests that physiological measures may be a more accurate reflection of the data. Additionally, approximately two thirds of this study's sample was female. Since sex has proven a significant predictor of sleep quality, more accurate results could come from an evenly distributed sample between male and female.

Going forward, this study offers promise for future clinical trials examining the relationship between dietary omega-3 and sleep quality. A future trial could examine two groups (equal number of healthy males and females in each group with similar BMI) – one control group and one intervention group. The two groups would be equicaloric

except for differing levels of total dietary omega-3. The control group would have the average American consumption of omega-3 and the intervention group's diet would have 2-3-fold higher amount of omega-3 in the diet. Sleep outcomes would be measured via a physiological assessment method such as. Additionally, since one of the postulated mechanisms behind obesity's relationship to poor sleep quality has been explained by an imbalanced ghrelin and cortisol ratio,[1] a secondary objective could examine ghrelin and cortisol levels throughout the study's duration to determine if omega-3 improves that hormonal balance and helps satiety throughout the daytime.

Another option for a future trial could include testing omega-3 intake in a poor sleep population while controlling omega-3 intake or supplementation. This could help elucidate the underlying mechanism regarding the effect of omega-3 on sleep.

BMI, sleep quality, energy intake, diet diversity, omega-3, DHA, medication use, sex, sleep apnea, neck circumference, sleep latency, daytime dysfunction, physical activity, healthy eating, inflammation, eicosanoids, eating times and chronotype are all connected. The key to future obesity treatment lies in the mechanisms behind these connections, and omega-3 could be a positive step toward improving sleep latency among the obese population and eventually improving health.

APPENDICES

A.1. Multiple linear regression models for subjective sleep quality (PSQI Component #1). Data presented as β [SE], p-value.

Subjective Sleep Quality (PSQI Component #1)			
Intercept	β=0.611 [0.214], p=0.005	β=0.552 [0.189], p=0.004	β=0.483 [0.282], p=0.088
ω-3 (g/1000 g)	β=-0.164 [0.129], p=0.206		
DHA (g/1000 g)		β=-1.021 [0.646], p=0.115	
Fat (% energy)			β=-0.001 [0.008], p=0.933
Medication (ref=no)	β=0.247 [0.102], p=0.016	β=0.255 [0.102], p=0.013	β=0.242 [0.102], p=0.018
BMI, kg/m^2	β=0.017 [0.005], p=0.001	β=0.016 [0.005], p=0.003	β=0.016 [0.005], p=0.002
Sex (ref=female)	β=-0.179 [0.107], p=0.097	β=-0.150 [0.106], p=0.157	β=-0.159 [0.107], p=0.137
	R^2 = 0.097, p=0.000	R^2 = 0.101, p=0.000	R^2 = 0.091, p=0.000

A.2. Multiple linear regression models for sleep duration (PSQI Component #3). Data presented as β [SE], p-value.

Sleep Duration (PSQI Component #3)			
Intercept	β=0.013 [0.264], p=0.960	β=0.078 [0.232], p=0.736	β=0.466 [0.344], p=0.177
ω-3 (g/1000 g)	β=0.032 [0.159], p=0.842		
DHA (g/1000 g)		β=-0.436 [0.796], p=0.585	
Fat (% energy)			β=-0.015 [0.009], p=0.108
Medication (ref=no)	β=0.254 [0.125], p=0.043	β=0.260 [0.125], p=0.039	β=0.258 [0.124], p=0.039
BMI, kg/m^2	β=0.016 [0.006], p=0.011	β=0.016 [0.006], p=0.013	β=0.019 [0.006], p=0.004
Sex (ref=female)	β=0.062 [0.132], p=0.638	β=0.061 [0.130], p=0.637	β=0.041 [0.130], p=0.752
	R^2 = 0.055, p=0.013	R^2 = 0.056, p=0.012	R^2 = 0.066, p=0.004

A.3. Multiple linear regression models for habitual sleep efficiency (PSQI Component #4). Data presented as β [SE], p-value.

Habitual Sleep Efficiency (PSQI Component #4)			
Intercept	β=-0.100 [0.265], p=0.707	β=-0.184 [0.234], p=0.432	β=0.235 [0.346], p=0.497
ω-3 (g/1000 g)	β=-0.104 [0.160], p=0.514		
DHA (g/1000 g)		β=-0.099 [0.801], p=0.902	
Fat (% energy)			β=-0.015 [0.009], p=0.107
Medication (ref=no)	β=0.184 [0.126], p=0.146	β=0.182 [0.126], p=0.150	β=0.184 [0.125], p=0.142
BMI, kg/m^2	β=0.022 [0.006], p=0.001	β=0.022 [0.006], p=0.001	β=0.024 [0.006], p=0.000
Sex (ref=female)	β=-0.256 [0.132], p=0.054	β=-0.242 [.131], p=0.066	β=-0.260 [0.131], p=0.048
	R^2 = 0.087, p=0.000	R^2 = 0.085, p=0.001	R^2 = 0.096, p=0.000

A.4. Multiple linear regression models for sleep disturbances (PSQI Component #5). Data presented as β [SE], p-value.

Sleep Disturbances (PSQI Component #5)			
Intercept	β=.954 [.153], p=0.000	β=.901 [.135], p=0.000	β=.834 [.202], p=0.000
ω-3 (g/1000 g)	β=-.112 [.092], p=.229		
DHA (g/1000 g)		β=-.546 [.464], p=.240	
Fat (% energy)			β=.001 [.005], p=.891
Medication (ref=no)	β=.257 [.073], p=.001	β=.261 [.073], p=.000	β=.254 [.073], p=.001
BMI, kg/m^2	β=.008 [.004], p=.021	β=.008 [.004], p=.037	β=.008 [.004], p=.036
Sex (ref=female)	β=-.140 [.077], p=.069	β=-.122 [.076], p=.109	β=-.125 [.076], p=.102
	R^2 = .105, p=.000	R^2 = .105, p=.000	R^2 = .099, p=.000

A.5. Multiple linear regression models for use of sleep medication (PSQI Component #6). Data presented as β [SE], p-value.

Use of Sleep Medication (PSQI Component #6)			
Intercept	β=0.062 [0.259], p=0.811	β=-0.042 [0.229], p=0.853	β=0.103 [0.341], p=0.762
ω-3 (g/1000 g)	β=-0.192 [0.156], p=0.221		
DHA (g/1000 g)		β=-0.780 [0.783], p=0.320	
Fat (% energy)			β=-0.007 [0.009], p=0.417
Medication (ref=no)	β=0.445 [.123], p=0.000	β=0.450 [0.123], p=0.000	β=0.442 [0.123], p=0.000
BMI, kg/m^2	β=0.012 [0.006], p=0.059	β=0.010 [0.006], p=0.093	β=0.012 [0.006], p=0.054
Sex (ref=female)	β=-0.142 [0.129], p=0.272	β=-0.113 [0.128], p=0.381	β=-0.127 [0.129], p=0.324
	R^2 = 0.090, p=0.000	R^2 = 0.088, p=0.000	R^2 = 0.087, p=0.000

A.6. Multiple linear regression models for daytime dysfunction (PSQI Component #7). Data presented as β [SE], p-value.

Daytime Dysfunction (PSQI Component #7)			
Intercept	β=0.495 [0.211], p=0.020	β=0.458 [0.185], p=0.014	β=0.201 [0.277], p=0.469
ω-3 (g/1000 g)	β=-0.143 [0.127], p=0.263		
DHA (g/1000 g)		β=-1.057 [0.635], p=0.097	
Fat (% energy)			β=0.006 [0.007], p=0.432
Medication (ref=no)	β=0.243 [0.100], p=0.016	β=0.252 [0.100], p=0.012	β=0.237 [0.100], p=0.019
BMI, kg/m^2	β=0.011 [0.005], p=0.025	β=0.010 [0.005], p=0.047	β=0.010 [0.005], p=0.058
Sex (ref=female)	β=-0.209 [0.105], p=0.049	β=-0.183 [0.104], p=0.080	β=-0.184 [0.105], p=0.079
	R^2 = 0.076, p=0.001	R^2 = 0.082, p=0.001	R^2 = 0.074, p=0.002

A.7. Pittsburgh Sleep Quality Index Questionnaire

Protocol: 07-DK-0077 Physical and Behavioral Traits of Overweight and Obese Adults

Form Name: Pittsburgh Sleep Quality (Sleep Questionnaire 3)

Form Description:

The following questions relate to your sleep habits during the past month only.
Your answers should indicate the most accurate reply for the majority of days and nights in the past month. Please answer all questions.

1. When have you usually gone to bed (Enter time as hh:mm)

AM or PM ○ AM ○ PM

2. How long (in minutes) has it taken to fall asleep each night ○ 0-15 minutes ○ 16-30 minutes ○ 31-60 minutes ○ 61-75 minutes ○ 76-90 minutes ○ 91-120 minutes ○ greater than 120

3. When have you usually gotten up in the morning (Enter time as hh:mm)

AM or PM ○ AM ○ PM

4. How many hours of actual sleep did you get that night (This may be different than the number of hours you spent in bed)

Interval ○ Baseline ○ Annual Visit 1 ○ Annual Visit 2 ○ Annual Visit 3 ○ Annual Visit 4 ○ Annual Visit 5 ○ Annual Visit 6

5. During the past month, how often have you had trouble sleeping because you...

(a) Cannot get to sleep within 30 minutes ○ Not during the past month ○ Less than once a week ○ Once or twice a week ○ Three or more times a week

(b) Wake up in the middle of the night or early morning ○ Not during the past month ○ Less than once a week ○ Once or twice a week ○ Three or more times a week

(c) Have to get up to use the bathroom ○ Not during the past month ○ Less than once a week ○ Once or twice a week ○ Three or more times a week

(d) Cannot breathe comfortably ○ Not during the past month ○ Less than once a week ○ Once or twice a week ○ Three or more times a week

(e) Cough or snore loudly ○ Not during the past month ○ Less than once a week ○ Once or twice a week ○ Three or more times a week

(f) Feel too cold ○ Not during the past month ○ Less than once a week ○ Once or twice a week ○ Three or more times a week

(g) Feel too hot ○ Not during the past month ○ Less than once a week ○ Once or twice a week ○ Three or more times a week

(h) Had bad dreams ○ Not during the past month ○ Less than once a week ○ Once or twice a week ○ Three or more times a week

(i) Have pain ○ Not during the past month ○ Less than once a week ○ Once or twice a week ○ Three or more times a week

(j) Other reason(s), please describe

How often during the past month have you had trouble sleeping because of this?	Not during the past month Less than once a week Once or twice a week Three or more times a week
6. During the past month, how often have you taken medicine (Prescribed or "over the counter") to help you sleep?	Not during the past month Less than once a week Once or twice a week Three or more times a week
7. During the past month, how often have you had trouble staying awake while driving, eating meals, or engaging in social activity?	Not during the past month Less than once a week Once or twice a week Three or more times week
8. During the past month, how much of a problem has it been for you to keep up enough enthusiasm to get things done?	Not during the past month Less than once a week Once or twice a week Three or more times week
9. During the past month, how would you rate your sleep quality overall?	Very good Fairly good Fairly bad Very bad

A.8. Epworth Sleepiness Scale Questionnaire

Protocol: 07-DK-0077 Physical and Behavioral Traits of Overweight and Obese Adults

Form Name: Epworth Sleepiness Scale (Sleep Questionnaire 4)

Form Description:

How likely are you to dose off or fall asleep in the following situations, in contrast to just feeling tired? This refers to your usual way of life in recent times. Even if you have not done some of these recently, try to work out how they would have affected you.

Choose the most appropraite answer for each situation.

1. Sitting and reading
   - Would never dose
   - Slight chance of dozing
   - Moderate chance of dozing
   - High chance of dozing
2. Watching TV
   - Would never doze
   - Slight chance of dozing
   - Moderate chance of dozing
   - High chance of dozing
3. Sitting inactive in a public place
   - Would never doze
   - Slight chance of dozing
   - Moderate chance of dozing
   - High chance of doing
4. Being a passenger in a motor vehicle for an hour or more
   - Would never doze
   - Slight chance of dozing
   - Moderate chance of dozing
   - High chance of dozing
5. Lying down in the afternoon
   - Would never doze
   - Slight chance of dozing
   - Moderate chance of dozing
   - High chance of dozing
6. Sitting and talking to someone
   - Would never doze
   - Slight chance of dozing
   - Moderate chance of dozing
   - High chance of dozing
7. Sitting quietly after lunch (no alcohol)
   - Would never doze
   - Slight chance of dozing
   - Moderate chance of dozing
   - High chance of dozing
8. Stopped for a few minutes in traffic while driving
   - Would never doze
   - Slight chance of dozing
   - Moderate chance of dozing
   - High chance of dozing

Interval
- Baseline
- Annual Visit 1
- Annual Visit 2
- Annual Visit 3
- Annual Visit 4
- Annual Visit 5
- Annual Visit 6

A.9. Ethics Certificate

Office of Research Integrity and Assurance

Research Hall, 4400 University Drive, MS 6D5, Fairfax, Virginia 22030
Phone: 703-993-5445; Fax: 703-993-9590

DATE:	January 29, 2015
TO:	Lilian de Jonge, PhD
FROM:	George Mason University IRB
Project Title:	[691380-1] The Association Between Dietary Omega-3 Fatty Acid Intake and Sleep Quality among Healthy Adults
Reference:	
SUBMISSION TYPE:	New Project
ACTION:	DETERMINATION OF NOT HUMAN SUBJECT RESEARCH
DECISION DATE:	January 29, 2015

Thank you for your submission of New Project materials for this project. The Office of Research Integrity & Assurance (ORIA) has determined this project does not meet the definition of human subject research under the purview of the IRB according to federal regulations.

Please remember that if you modify this project to include human subjects research activities, you are required to submit revisions to the ORIA prior to initiation.

If you have any questions, please contact Karen Motsinger at 703-993-4208 or kmotsing@gmu.edu. Please include your project title and reference number in all correspondence with this committee.

This letter has been electronically signed in accordance with all applicable regulations, and a copy is retained within George Mason University IRB's records.

A.10. Seven day food record form

Phenotyping study	PLACE	TIME	FOODS AND BEVERAGES	AMOUNT	COMPLETE DESCRIPTION	REVIEWER'S COMMENTS
NAME:						
Day of Week:						
Date: / /						
Is this a typical day? __yes __no If no, give reason:						
Daytime phone: ( )						
Birthdate: / /						
Age:						
Caller business:						
Intake: T M L						
Info: R I U						
Substudy __ __						
Visit __ __ __						

RD/HT: _____

continue on reverse side

Side 2 (continued from the front side)

Pheotyping study	PLACE	TIME	FOODS AND BEVERAGES	AMOUNT	COMPLETE DESCRIPTION	REVIEWER'S COMMENTS
NAME:						
Date: / /						

Additional information, recipes, etc.

If you have any questions, call dietitians Diane Dellavalle at (301) 496-8248 or Amber Courville at (301) 594-8051.

A.11. Seven day food record instructions

Keeping Food Records for the Phenotyping Study

Welcome to the Phenotyping Study! As you begin the study, we need to assess your usual food and beverage intake. For this reason, we are asking you to write down everything you eat and drink for 7 typical days. You will need to keep additional food records periodically during the study.

When should the food records be kept? Write down all foods and beverages you consume for 7 consecutive days. We suggest you record:
_____ through _____ .

These days should represent a fairly typical week for you. Eat normally on these days. Don't change your food choices or methods of food preparation just because you are recording your intake. There is no right or wrong way to eat for this evaluation.

How and what should be recorded? Use a new form each day. It is best to record what you eat *immediately* after each meal and snack.

You should write down:

- the time you begin eating each meal and snack.
- all foods and beverages you consume, except for plain water. Don't forget to record candy, mints, etc.
- all condiments (such as ketchup, margarine, mayonnaise, salad dressing, sauces, gravy, sugar, etc). You *do not* need to record salt, pepper, herbs and spices.
- all medicines, vitamins, minerals, and/or other supplements taken.

For each food and beverage that you list, include an amount and a description. To guide you, refer to the sample food record and the hints for recording amounts and description information on the next few pages.

What do I do with the forms after they are filled out? Bring them to NIH the next time you come. A dietitian will meet with you to go over the food records in case any items need clarification.

If you have any questions or concerns, please do not hesitate to contact us.

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Hints for Completing the Amount Column

A. Measure foods *after* preparation and cooking is completed.

B. Measurements can be listed in 4 ways:

1. The number of items.

Examples:	saltines	6
	white bread	2 slices
	grapes	12
	ketchup	2 packets

2. In household measures, using standard level measuring cups and spoons.

Examples:	applesauce	1/3 cup
	popcorn	3 cups
	jelly	1 ½ TB [tablespoons]
	2% milk	1.5 CP [cup]

(Do not use non-standard measures like "handful" or "serving")

3. By weight or by volume, as listed on a package (or by using a kitchen scale if you have one).

Examples:	ginger ale	12 FL OZ [fluid ounces]
	yogurt	4.5 oz [ounces]
	Almond Joy	49 gm [grams]
	roast beef	2 ½ oz
	pretzels	1/3 of 6.5 oz bag

4. By dimensions, using a ruler.

Examples:	pancake	5" diameter
	meatball	1 ¼" diameter
	lasagna	3 ½" x 4" x 1½" cube
	pizza	1/8 of 14" diameter [or draw it with dimensions]

C. Remember not all food that is served is eaten, and at other times, you may go back for seconds. You may need to adjust portion sizes to reflect the amount you actually ate.

Hints for Completing the Description Column

A. Describe foods completely.

Examples:

sirloin steak, *fat partially trimmed*
80% lean ground beef (or 80/20 ground beef)
chicken drumstick, *skin removed before cooking*
reduced fat or *2%* milk
baked potato, *skin eaten*
carrot cake *with cream cheese frosting*

B. Include brand names whenever possible. Also include terms like *calcium-fortified, light*, and *reduced calorie* if listed on the label.

Examples:

Tropicana calcium-fortified orange juice
Oreo reduced-fat cookies
Miller Lite draft beer
Hellmann's fat-free cholesterol-free mayonnaise
Dannon lowfat fruited yogurt
sliced peaches in *light* syrup

C. Include information about preparation and cooking methods.

Examples:

skinless chicken breast	*floured and pan-fried in corn oil*
canned corn	*tub margarine and sugar added*
Campbell's tomato soup	*made with water*
mac and cheese	*from box mix, made with whole milk and stick margarine*

D. For mixed dishes and recipe items, you only need to list major ingredients. You do not need to write down the entire recipe.

Examples:

potato salad	*made with potatoes, eggs, regular mayo*
chocolate chip cookies	*homemade with real butter, walnuts added*
meatloaf	*made with 85/15 ground beef, oatmeal, ketchup, egg*

E. For fast food items from major chains, you only need to name the item. No description is necessary, unless you "special order" an item. Also, note if all of it wasn't eaten.

Examples:

Biggie Fries (Wendy's)
Quarter Pounder with Cheese (McDonald's) - didn't eat pickles
Meat-lovers Pan Pizza (Pizza Hut)

F. Remember to record any additions made at the table, such as margarine, sugar, ketchup, mustard, sauces, mayonnaise. List them separately, and include amounts.

A.12. Weight and Lifestyle Inventory (WALI) sections K-N.

Protocol: 07-DK-0077 Physical and Behavioral Traits of Overweight and Obese Adults

Part VII

Please do not submit any part of the Lifestyle Questionnaires until you have completed all seven parts. Once you complete a part, select "Save: I'll Finish Later." When all parts are saved, review each part and add any additional information you may have missed. If you are finished reviewing all questionnaires select "Submit: I'm Finished."

Please select the name of your current visit. (if you are unsure, please ask):

Section K: Physical Activity

1. To what extent do you enjoy physical activity? (Select one)

○ Not at all ○ Slightly ○ Moderately ○ Greatly

2. Do you have any physical problems that limit your physical activity? (Select one) ○ Yes ○ No

If yes, please describe

3. Check the types of physical activity that you enjoy. Check only those that you have participated in during the last 6 months.

- ☐ a. Walking outside
- ☐ b. Walking (indoors, including treadmill)
- ☐ c. Jogging
- ☐ d. Running
- ☐ e. Biking outside
- ☐ f. Biking (Stationary)
- ☐ g. Aerobic class
- ☐ h. Tennis/racket sports
- ☐ i. Swimming
- ☐ j. Basketball
- ☐ k. Golf
- ☐ l. Dancing
- ☐ m. Strength trainning
- ☐ n. Other

If other, Please describe

4. For your most preferred activity, how many times have you participated in this activity in the past 6 months? (times)

5. How many hours of TV do you watch on an average weekday?

6. How many hours of TV do you watch on an average weekend day?

7. How many hours do you spend sitting while using a computer or handheld device on an average weekday?

8. How many hours do you spend sitting while using a computer or handheld device on an average weekend day?

9. Approximately how many city blocks or the equivalent do you regularly walk each day? (blocks: 12 blocks = 1 mile)

10. How many flights of stairs do you climb up each day? (flights a day: 1 flight = 10 steps)

11. Please describe your daily lifestyle activity (i.e., how active you are) by picking any number from 1 to 10 in which 1 = very sedentary and 10 = very active. Your number is:

Section L: Family and Living Arrangments

1. Currently, I am
- living alone
- living with a spouse/partner
- living with a significant other
- living with children
- living with parents/step parents

2. Please indicate the total number of persons living in your home.

3. If you are currently involved in an intimate relationship (significant other), please answer these questions. What is this person's attitude towards your efforts to lose weight?

○ strongly supports my efforts ○ supports my efforts ○ neutral ○ opposes my efforts ○ strongly opposes my efforts

Please describe briefly what this person does either to help or hinder your efforts to lose weight.

nih.gov/ctdb/form/form.do?action=view_form&id=1258 1/20/2

4. How satisfied are you with your overall relationship with this person?

very satisfied satisfied neutral dissatisfied very dissatisfied

5. Will other people support your efforts to lose weight? Yes No

If yes, how many people will?

Who are these people?

How many of these people are actively helpful to you?

6. How many people do you talk with about your weight when you are upset about it?

How many of these people are helpful to you?

7. Will other people oppose or undermine your efforts to lose weight? Yes No

If yes, how many will?

Who are these people?

Section M: Self-Perceptions

1. How satisfied are you with your current weight?

very satisfied moderately satisfied slightly satisfied neutral slightly dissatisfied moderately dissatisfied very dissatisfied

2. How satisfied are you with your current shape (i.e., figure or physique)?

very satisfied moderately satisfied slightly satisfied neutral slightly dissatisfied moderately dissatisfied very dissatisfied

3. How satisfied are you with your current overall appearance?

very satisfied moderately satisfied slightly satisfied neutral slightly dissatisfied moderately dissatisfied very dissatisfied

4. Pick the one sentence that best describes your overall feelings about yourself, "In general, I am..."

very happy with who I am | happy with who I am | ok with who I am but have some mixed feelings | unhappy with who I am | very unhappy with who I am

5. "As compared with most people, I think I have..."

very good self-esteem | good self-esteem | average self-esteem | poor self-esteem | very poor self-esteem

6. Pick the one sentence that best describes your feelings about the way you looked the last time you lost a lot of weight. "I was..."

very happy with the way I looked | happy with the way I looked | ok with the way I looked, but with some mixed feelings | unhappy with the way I looked | very unhappy with the way I looked

7. How much weight did you lose?

At what weight did you start to diet during this time?

Section N: Timing

1. Please indicate if you are currently experiencing any greater than usual stress in your life related to the following events. Complete each item by selecting the appropriate answer.

a. Work ○ Yes ○ No

b. Health ○ Yes ○ No

c. Relationship with spouse/significant other ○ Yes ○ No

d. Activities related to your children ○ Yes ○ No

e. Activities related to your parents ○ Yes ○ No

f. Legal/financial trouble ○ Yes ○ No

g. School ○ Yes ○ No

h. Moving ○ Yes ○ No

i. Other ○ Yes ○ No

Please explain in a sentence any items to which you responded yes:

2. Are you planning any major life changes (i.e., new job, moving, relationship, etc.) during the next 6 months? ○ Yes ○ No

If yes, please briefly describe below

3. How stressful has your life been during the last 6 months?

○ much less stressful than usual ○ less stressful than usual ○ average level of stress ○ more stressful than usual ○ much more stressful than usual

4. How stressful do you think that your life will be in the next 6 months excluding your efforts to lose weight?

○ much less stressful than usual ○ less stressful than usual ○ average level of stress ○ more stressful than usual ○ much more stressful than usual

5. How motivated are you to lose weight at this time? Pick a number between 1 and 10, in which 1= not motivated and 10 greatest motivation you have ever had. Your number is:

6. Why do you want to lose weight right now, as compared to 1 year ago? What has prompted you to lose weight now?

7. What is the single most important thing that you hope to achieve as a result of losing weight?

8. People who want to achieve long-term weight control need to spend at least 30 minutes a day, for a minimum of 6 months trying to change their eating, exercise, and thinking habits. Please select the sentence below that best describes you:

○ I definitely will not be able to devote 30 minutes daily to weight control
○ I'm not sure if I can find 30 minutes daily for weight control
○ I can definitely find 30 minutes daily for weight control
○ I can devote more than 30 minutes daily to weight control

9. Rate how confident you are that you will be able to significantly change your eating and exercise habits. Pick a number from 1 to 10 in which 1 = not at all confident and 10 = extremely confident. Your number is:

Section O: Medications and Supplements

List all medications you currently take (including vitamins, herbals/botanicals, and other supplements). Please indicate the dosage and frequency (number of times s day) of each medication.

	Medication	Dosage	Frequency	Reason for taking
1.				
2.				
3.				
4.				
5.				
6.				
7.				
8.				
9.				
10.				

Additional Information: Please use the space below to provide any additional information that you think is important to understanding you or your weight problem, as well as the goals you seek.

A.12. Medications coded for sleep side effects. (0=no sleep side effects, 1=drowsiness/tiredness, 2=insomnia, 3=both drowsiness and/or insomnia, 4=prescribed for sleep).

Medication Name	Side Effect Code	Medication Name	Side Effect Code
AcipHex	0	Baby ASA (baby aspirin)	0
Actos	0	Bayer aspirin	0
Adderall	3	Benadryl	1
Adderall ER	3	Benanepril	1
Advair	0	Benicar	0
Advil	0	Benzaclin	0
Albuterol	3	Bisoprolol-HCTZ	1
Albuterol inhaler	0	Bupropion	3
Aldomet	1	Caduet (amlodipine+atorvastatin)	0
Alesse	0	Calcium carbonate	0
Aleve	3	Catapres-TTS-2 patch	3
Allegra	0	Celebrex	1
Allegra D	2	Celexa	1
Allopurinol	0	Cetirizine HCL	1
Alprazolam	1	Chondroitin	1
Altace	1	Citalopram	1
Ambien	4	Citrucel	0
Ambien CR	4	CLA	0
Amlodipine	1	Claritin	2
Amlodipine mesylate	1	Claritin-D	2
Amlodipine/valsartan	1	Clonidine	1
APAP/codeine	1	Clucaphage	0
ASA	0	Colace	0
Aspirin	0	Colazal	1
Astelin nasal spray	1	Concerta	3
Atacand	0	Condritin/glucosamine	1
Atacand	0	CoQ10	3
Atenolol	1	Coreg	1
Ativan	1	Cosamin DS	1
Atorvastatin	1	Coumadin	1
Avapro	0	Cozaar	0
Azithromycin opthalmic solution	0	Cozaar HCTZ	0

Medication Name	Side Effect Code	Medication Name	Side Effect Code
Creatine	0	Generic Lotrelle	0
Crestor	2	Geodon	1
Curcumin	0	Ginseng	2
Cyclobenzaprine	1	Glucatrol	0
Cymbalta	1	Glucomannan	0
Depo-provera	3	Glucophage	0
Detrol LA	0	Glucosamine	1
Diclofenac	1	Glucosamine & chondroitin	1
Diclofenac sodium	1	Glucotrol	0
Dilantin	2	Glyburide	0
Diltiazem CD	1	HCTZ	0
Diovan	1	HCTZ-triamterene	1
Diovan - HCT	1	Hytrin	1
Doxycycline	1	Hyzaar	0
Effexor XR	1	Ibuprofen	0
Elavil	1	Imitrex	1
Enalapril	0	Imuran	0
Enteric-coated aspirin	0	Inderal	3
Esjic	1	Insulin	0
Estrace cream	2	Januvia	0
Estradiol	0	K-dur	0
Excedrin	2	Klonoprin	1
Excedrin migraine	2	Lamictal	1
Exforge	0	Lantus	0
Famotidine	0	Lasix	0
Fenofibrate	0	Levora	1
Ferrous sulfate	0	Levothryoxine	2
Fexofenadine	0	Lexapro	1
Flexeril	1	Lidex cream	0
Flomax	3	Lipitor	1
Flovent	1	Lipo6	2
Fluticasone oral inhalation	1	Lisinopril	1
Fluvastatin	3	Lisinopril/HCTZ	1
Fosamax	0	Lithium	1
Furosemide	0	Loestrin	1
Garcinia cambogia	0	Loestrin 24 Fe	1
Gen Mevacor	1	Loestrin Fe	1

Medication Name	Side Effect Code 1	Medication Name	Side Effect Code
Lo-ovral	1	Olopatadine opthalmic solution	0
Loratadine	2	Omeprazole	1
Losartan	0	Ortho Tri-Cyclen	1
Losartan-HCTZ	0	Ortho-cyclen	1
Lotrel	0	Paxil	1
Lovastatin	1	Pepcid	0
Lutera	1	Phenobarbital	1
Lyrica	1	Phentermine	2
Medroxyprogesterone	2	Piroxicam	1
Medtrol dose pack	2	Plaquenil	0
Melaleuca	0	Potassium	0
Melatonin	4	Pottasium chloride	0
Metamucil	0	Pravastatin	1
Metformin	0	Prevacid	1
Metformin ER	0	Preventil inhaler	0
Metformin HCL	0	Prevpac	1
Methimazole	1	Prilosec	1
Metoprolol	0	Prilosec OTC	1
Mevacor	1	Proair	0
Micardis	0	Progestin	0
Mirena	0	Propecia	0
Mobic	1	Prostrate SR	0
Mononessa	1	Protonix	1
Motrin	0	Prozac	1
MSM	3	Remicade	0
Mucinex	0	Rozerem	4
Mycophenolate mofetil	2	Seasonique	1
Nabumetone	2	Sertraline	1
Naproxen	3	Simvastatin	1
Nasonex	0	Singulair	1
Neurontin	1	Sirolimus	0
Nexium	1	Sleep MD	4
Nicotine patch	0	Solia	1
Nizoral Nazal spray	3	Soriatane	2
Norvasc	1	Sprionolactone/HCTZ	1
Novolog	0	Symbicort	0
Ocuvite	0	Synthroid	2

Medication Name	Side Effect Code	Medication Name	Side Effect Code
Tegretol	1	Ventolin	3
Telmisartan	0	Viagra	2
Tenormin	1	Verapamil	1
Teveten HTC	1	Vytorin	1
Tiazide	0	Vyvance	2
Timolol	1	Warfarin	1
Topamax	1	Wellbutrin	3
Toprol	0	Wellbutrin SR	3
Toprol XL	0	Wellbutrin XL	3
Tramadol	3	Whey protein	1
Trazadone	1	Xanax	1
Triaminic	1	Xopenex	0
Tricor	0	Yaz	1
Tri-sprintec	0	Zaditor eye drops	0
Tums	0	Zetia	1
T-up	0	Ziac	1
Tylenol	0	Zocor	1
Tylenol #4	1	Zoloft	1
Tylenol simply sleep	4	Zolpidem	4
Vagifem	2	Zopenex	0
Vagifem estradiol tablet	2	Zyrtec	1
Valerian	4	Zyrtec p.r.n.	1
Valtrex	0		

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