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The Effect of Protein and Omega-3 Fatty Acid Supplementation on Regulation of Sleep and Mood in Postmenopausal Women

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The Effect of Protein and Omega-3 Fatty Acid Supplementation on Regulation of Sleep and Mood in Postmenopausal Women

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Introduction and Background

The older population of the United States is in a period of rapid growth [1]. The population of those in the United States aged 65 or older will double to about 72 million in the next 25 year [1]. This population is at increased risk for obesity, chronic diseases, decreased quality of life, and premature death. Therefore, guidelines for healthy aging are desperately needed. In 2015 alone, 67.7% of adults in the United States aged 65 or older reported having two or more chronic conditions [2]. Treatment for those in the older population afflicted by multiple chronic conditions alone accounts for 66% of the United States' total health care budget [1]. In 2018, for the third year in a row, Arkansas was one of nine states with an adult obesity rate at or above 35 percent [3]. The obesity prevalence in Arkansans 65 years of age and older was reported to be 30.1% for 2018 and is projected to continue with an increasingly upward trajectory. In the United States, Arkansas was identified as having the 4th highest-ranked state with regard to overweight and obesity percentiles for adults. Arkansas was also identified as the 4th highest-ranked state for number of adults diagnosed with type 2 diabetes mellitus (T2DM). Additionally, in Arkansas alone 23.4% of adults aged 65 years of age or older were currently diagnosed with T2DM in 2015, with an expected increase in this percentage [2]. Poor sleep has been identified as one potential cause for this dramatic decline in health [4].

Sleep disorders are highly prevalent in older adults with 50% or more older adults being currently diagnosed with a clinical sleeping disorder [5]. A lack of sleep schedule regularity in addition to poor sleep quality puts older adults at significantly higher risk for cognitive and functional decline [5]. Sleep loss in older adults has been specifically linked to an increased risk of T2DM and obesity [4]. While aging is linked to a general

decline in total sleep time and sleep efficiency, sleep efficiency has been shown to significantly decrease past age 60 [6].

Adequate nutrition has the potential to combat dysregulated sleep and mood. Preference for fat-based dietary patterns have also been linked to poor sleep quality [7]. Those with poor quality sleep have been shown to typically select their diet from a smaller range of food groups, with low protein intake being cited as a common factor [7]. Omega-3 fatty acid (O3FA) supplementation has been shown to prevent cognitive impairments, in both the long- and short-term, that arise from chronic sleep deprivation [8]. Additionally, a shift in dietary intake away from O3FAs toward saturated fats in Western countries, such as in the United States, has been linked to the rise in depression and other neurological disorders [9]. In conjunction with this linkage, O3FAs have demonstrated potential use in an antidepressant role [9].

Nutrition is a key factor in the prevention of muscle loss and body fat gain often observed with age [10,11]. Diets high in O3FAs have been linked to healthy aging [10]. Increasing consumption of O3FAs has shown decreased risk of cardiovascular disease and potential sleep quality improvement through a decrease in overall body fat. In addition, high protein diets have also been linked to healthy aging through decreasing body fat and increasing muscle mass [11]. Protein needs increase as individuals age, and the impairments on physical function from a lack of protein is most obvious in older adults [11]. The synergistic effects of protein and O3FA supplementation on sleep quality, mood, and body composition are currently unknown.

For this study, measures to evaluate healthy aging will include weight, Body Mass Index (BMI), waist circumference measurements, and waist-to-hip ratios. BMI and waist

circumference measurements are both recognized as important measures of obesity and weight-related health risks [12]. Health risks increase as BMI increases, but within the obesity category (BMI ≥ 30) the National Institutes of Health (NIH) recognizes a graded scale at each obese category based upon waist circumference [12]. A higher waist circumference is associated with more health risks within a given BMI category. Specifically, a waist circumference >102cm for men or >88cm for women is considered a high waist circumference by the NIH [12]. Just as poor sleep is related to obesity and other chronic illnesses, obesity appears to also contribute to shorter sleep duration in a vicious cycle. Studies in adults have shown a significant decline in hours of sleep as BMI rises [13].

The effects of aging are experienced greatly in a specific demographic—post menopausal women [14,15,16,17]. Postmenopausal women have been found to present larger waist circumferences and larger waist-to-hip ratios than premenopausal women in studies [14]. Gain of abdominal fat during menopause for postmenopausal women has also been shown to display statistical significance even after adjustment for age and total body fat mass when compared to premenopausal women [15]. At the core of complaints given among those who are postmenopausal is sleep disturbance [16]. Mood disturbance among postmenopausal women is also exacerbated beyond that found with aging alone. Women are twice as likely as men to be afflicted by depressive symptoms and disorders, and this risk is heightened upon reaching the transition toward becoming postmenopausal [17].

The **objective** of this project was to determine whether increasing protein and O3FAs in the diet of aging postmenopausal women for 16 weeks would improve sleep

and overall well-being. We **hypothesized** that increased consumption of both protein and O3FAs would promote healthier aging in postmenopausal women through improved quality of sleep, reduced body weight, improved body composition, and improved mood. Signs of healthier aging would be shown through improved health measures—sleep quality, weight, BMI, waist-to-hip ratios, and overall mood. The demographic selected for this study was chosen as to negate the hormonal influence as well as to provide data on a population with little available research.

Materials and Methods

Subject Recruitment and Participation

Prior to subject recruitment, the study was submitted to the University of Arkansas' Institutional Review Board for approval and was registered on clinicaltrials.gov, clinical trial number: NCT0303041. Subjects were recruited on a voluntary basis through advertisement in the University of Arkansas Newswire, social media, and local businesses. In order to participate in the study, subjects were required to be postmenopausal women with a last reported menstrual cycle of 12 months or more prior, be absent of hormone replacement therapy (HRT), have no known food allergies, regularly eat breakfast, consume fatty fish 4 times or less per month, not taking medications that impact appetite, blood coagulation, metabolism, or blood lipids, not consuming protein or O3FA related supplements, and have an initial Pittsburgh Sleep Quality Index (PSQI) of 5 or greater, as this score indicated dysregulated sleep. All subjects completed a brief screening and filled out a consent form prior to enrolling in the study. Participants were recruited on a rolling basis and randomly assigned a treatment group. A total of 34 women, ages 32 to 82, completed the 16-week study.

Intervention

A total of 40 participants were recruited to partake in this 16-week randomized, single-blind, controlled study. Six participants dropped out of the study at various stages for different reasons—study visits were time-consuming to attend in the morning or they did not want to consume all necessary supplements. Thirty-four participants were randomly assigned to a control group or one of four treatment groups: 1) control—no supplements given, 2) 25 grams of whey protein, 3) 4.3 grams of omega-3 fatty acid supplements, 4) 25 grams of whey protein and 4.3 grams of soybean oil placebo supplements, or 5) 25 grams of whey protein and 4.3 grams of O3FAs supplements. Protein was consumed prior to 10:00 am, and the O3FA or placebo supplements were consumed in a 2.15 gram dose before 10:00 am as well as with the evening meal. Waist-to-hip ratio measurements, height, weight, Pittsburgh Sleep Quality Index (PSQI) questionnaire results, and Profile of Mood States questionnaire results were recorded at the baseline, week 4, week 8, week 12, and week 16 visits. All five laboratory visits required fasting the night before (10-12h), as the blood samples were drawn by a certified phlebotomist to assess sleep and metabolic blood markers. In addition, sleep was assessed at baseline, 8 weeks, and 16 weeks.

Educational Materials

Participants were given a booklet that corresponded to their randomly assigned groups' dietary intervention. All booklets included a standard study day schedule and checklist, as well as instructions for the sleep ActiGraph sleep monitors and sleep diary. Each booklet included a breakfast recipe section that was to be modified to incorporate the protein powder for participant groups assigned to a whey protein containing group.

Anthropometrics

Height, weight, and waist circumference, and hip circumference were measured at the baseline, week 4, week 8, week 12, and week 16 visits. Body height was measured to the nearest 0.01 cm using a stadiometer (Detecto, St. Louis, MO) while participants were barefoot, in the free-standing position. Body weight of participants was measured in the fasting state without shoes to the nearest 0.01 kg using calibrated balance scales (Detecto, St. Louis, MO). Body mass index (BMI) was calculated as weight (kg) divided by height (m) squared. Waist measurements were taken using the belly button as the standard position of the waist for each participant using a soft tape measure, and rounding to the nearest 0.1 cm. Hip measurements were taken at the widest point below the waist using the aforementioned soft tap measure, and rounding to the nearest 0.1 cm. Waist-to-hip ratios were recorded by dividing the waist measurement (cm) by the hip measurement (cm).

Sleep Assessment

Sleep quality was determined via wrist actigraphy. Wrist actigraphy utilizing the Cole-Kripke algorithm has been validated with high sensitivity, moderate specificity and high accuracy when compared to polysomnography, the current gold standard [18]. Each participant was required to wear an ActiGraph monitor on the non-dominant wrist for 7 days during the month of the intervention. Wrist actigraphy was utilized the 7 days prior to the baseline, week 8, and week 16 visits. 60Hz was utilized as the sample rate, as this rate has been verified for sleep quality analysis. In addition to wearing the ActiGraph monitor, participants also filled out a 7-day sleep diary to accompany the data collection

via wrist actigraphy. Sleep times were verified and corrected by comparing the sleep diaries against the raw data downloaded.

The Pittsburgh Sleep Quality Index (PSQI) questionnaire was utilized to measure subjective sleep quality. The PSQI questionnaire has high consistency and validity [19]. The PSQI questionnaire has also been specifically validated for use in older women of multiple ethnic groups [20]. The PSQI questionnaire was administered during all five visits—baseline, week 4, week 8, week 12, and week 16.

Mood

Mood was measured through administration of the commonly used Profile of Mood States (POMS) questionnaire [21]. The POMS questionnaire has been specifically validated and shown internal consistency in measuring mood in postmenopausal women [21]. The POMS questionnaire was administered at baseline, week 4, week 8, week 12, and week 16. Participants defined their mood on a 5-point Likert scale. The POMS questionnaire provided a score for Total Mood Disturbance (TMD), Anger-Hostility (Anger), Confusion-Bewilderment (Confusion), Depression-Dejection (Depression), Fatigue-Inertia (Fatigue), Tension-Anxiety (Tension), and Vigor-Activity (Vigor). For the Vigor measure a higher score, or a numerical increase in value after score comparison over time signifies an improvement in mood. For Total Mood Disturbance (TMD), Anger, Confusion, Depression, Fatigue, and Tension a lower score, or a numerical decrease in value upon comparing scores over time signified an improvement in mood. TMD was calculated using the following equation: TMD = (Tension-Anxiety) + (Depression-Dejection) + (Anger-Hostility) + (Fatigue-Inertia) + (Confusion-Bewilderment) – (Vigor-Activity).

Statistical Analysis

One-way repeated measures analysis of variance (ANOVA) was used to assess the differences in BMI, waist-to-hip ratio, Global PSQI Scores, wrist actigraphy, and POMS scores between the five treatments over the course of the 16-week intervention. One-way ANOVA was used to look at the total change from baseline compared to the end of the 16-week intervention for Global PSQI scores and POMS TMD scores. Clinical biomarker differences were determined using repeated-measures ANOVA. A *p-value* < 0.05 indicates statistical significance. Prism GraphPad Software Version 8.0 (La Jolla, CA) was used for all analyses.

Results

Participant Characteristics

A total of 34 participants completed the study. **Table 1** shows the baseline characteristics of the participants in the study including: age, height, weight, body mass index (BMI), waist-to-hip ratio, global PSQI score, and total mood disturbance POMS score. All baseline characteristics are reported in **Table 1** as the mean ± standard deviation.

Table 1. Participant Characteristics

Baseline Characteristics	Control (n = 6)	WPI (n = 4)	O3FAs (n = 10)	WPI + Placebo (n = 6)	WPI + O3FAs (n = 8)
Age (Years)	62.50 ± 8.92	62.75 ± 11.60	58.00 ± 12.10	60.33 ± 2.42	61.38 ± 8.45
Anthropometrics					
Height (m)	1.62 ± 0.08	1.64 ± 0.04	1.62 ± 0.05	1.65 ± 0.11	1.64 ± 0.08
Weight (kg)	74.30 ± 19.71	78.31 ± 12.65	76.44 ± 20.58	72.06 ± 20.62	73.53 ± 23.78
BMI (kg/m^2)	28.09 ± 6.06	29.02 ± 3.33	29.40 ± 8.52	26.42 ± 6.33	27.21 ± 8.68
Waist-to-Hip Ratio	0.85 ± 0.04	0.89 ± 0.02	0.88 ± 0.06	0.84 ± 0.04	0.80 ± 0.06
Sleep					
PSQI: Global Score	8.33 ± 3.01	6.25 ± 2.06	7.70 ± 2.87	7.67 ± 3.14	9.38 ± 2.50
Mood					
POMS: TMD	-1.83 ± 19.40	0.25 ± 23.04	0.40 ± 20.72	-2.33 ± 8.29	11.00 ± 33.30

Anthropometrics: *BMI, Waist Circumference, and Waist-to-Hip Ratio Measurements*

The calculated BMI, waist circumference, and waist-to-hip ratio measurements taken at each visit were compiled. The average of each per group was determined for each of the listed measures, and the results were standardized as change from baseline.

The line graph in **Figure 1A** shows the average BMI for each of the five treatment groups over the course of the study after controlling for baseline. A one-way repeated measures ANOVA did not result in any statistical significance. The bar graph in **Figure 1B** shows the average change in BMI from baseline at week 16 for each of the five treatment groups. A one-way ANOVA did not demonstrate any significance.

The line graph in **Figure 2A** displays the average waist-to-hip ratio for each of the five treatment groups for each visit after controlling for baseline. A one-way repeated measures ANOVA demonstrated a significant difference ($p < 0.05$) between treatment groups. The bar graph in **Figure 2B** shows the average change in BMI from baseline at week 16 for each of the five treatment groups. A one-way ANOVA did not demonstrate any significance, although the general trend for all treatment groups, and not the control group, to decrease in waist-to-hip ratios from baseline to week 16. The **Figure 2C** displays the average waist circumference measurement for each of the five treatment groups after controlling for baseline. A one-way repeated measures ANOVA demonstrated a significant difference ($p < 0.05$) between treatment groups. All groups, except for the control group, tended toward a smaller waist circumference at the end of the 16-week intervention.

Figure 1.

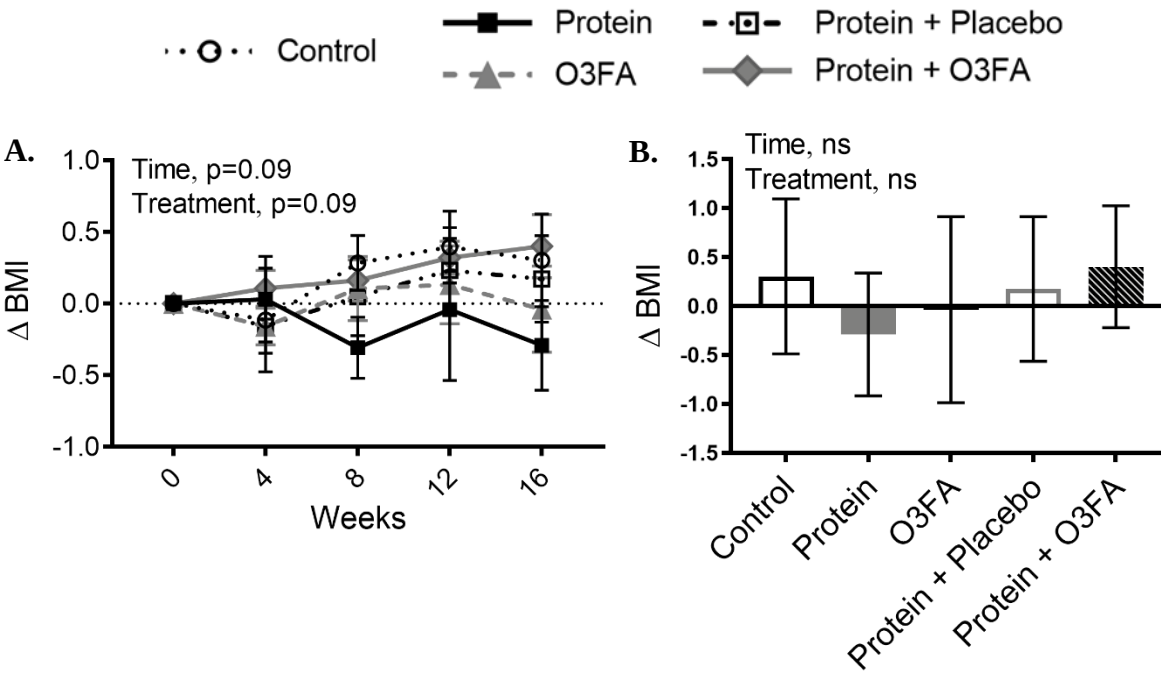

Figure 1. (**A**) The average BMI for each of the five treatment groups calculated for all five visits after controlling for baseline. No significance was found. (**B**) The average change in BMI from baseline at week 16 for each of the five treatment groups. No significance was found. Body mass index, BMI; Omega-3 fatty acids, O3FA.

Figure 2.

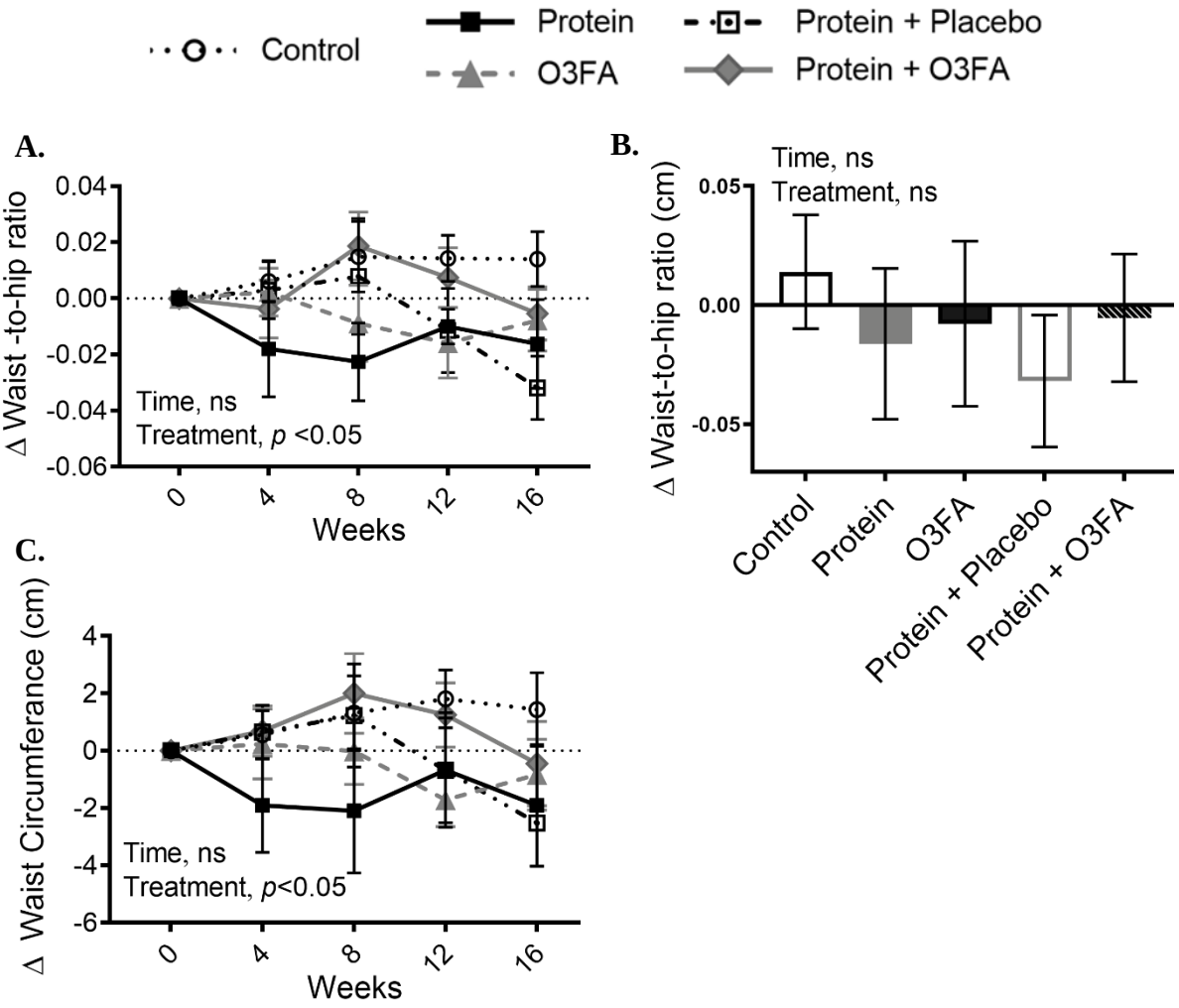

Figure 2. (**A**) The average waist-to-hip ratio for each of the five treatment groups calculated for all five visits after controlling for baseline. Significance was found between treatment groups ($p < 0.05$). (**B**) The average change in waist-to-hip ratio from baseline at week 16 for each of the five treatment groups. No significance was found. (**C**) The average waist circumference measurement for each of the five treatment groups calculated for all five visits after controlling for baseline. Significance was found between treatment groups ($p < 0.05$). Omega-3 fatty acids, O3FA.

Sleep Quality

Sleep quality was assessed by utilizing both The Pittsburgh Sleep Quality Index questionnaire (PSQI), as well as wrist actigraphy, during the course of the study.

Sleep: The *Pittsburgh Sleep Quality Index (PSQI)*

The PSQI Global Scores taken at the beginning of each participants' visit were calculated and recorded with the scores of the other participants in their respective treatment groups. The average PSQI Global Scores for each of the five treatment groups was calculated for each visit. The line graph in **Figure 3A** shows the average PSQI Global Score for each of the five treatments groups for each visit after controlling for baseline A one-way repeated measures ANOVA demonstrated a strong significant effect ($p < 0.0001$) over time. The bar graph in **Figure 3B** shows the average change in PSQI score from baseline to week 16 for each of the five treatment groups. A one-way ANOVA demonstrated significant differences ($p < 0.01$) between treatments groups, specifically the O3FA and the Protein + O3FA groups.

Figure 3.

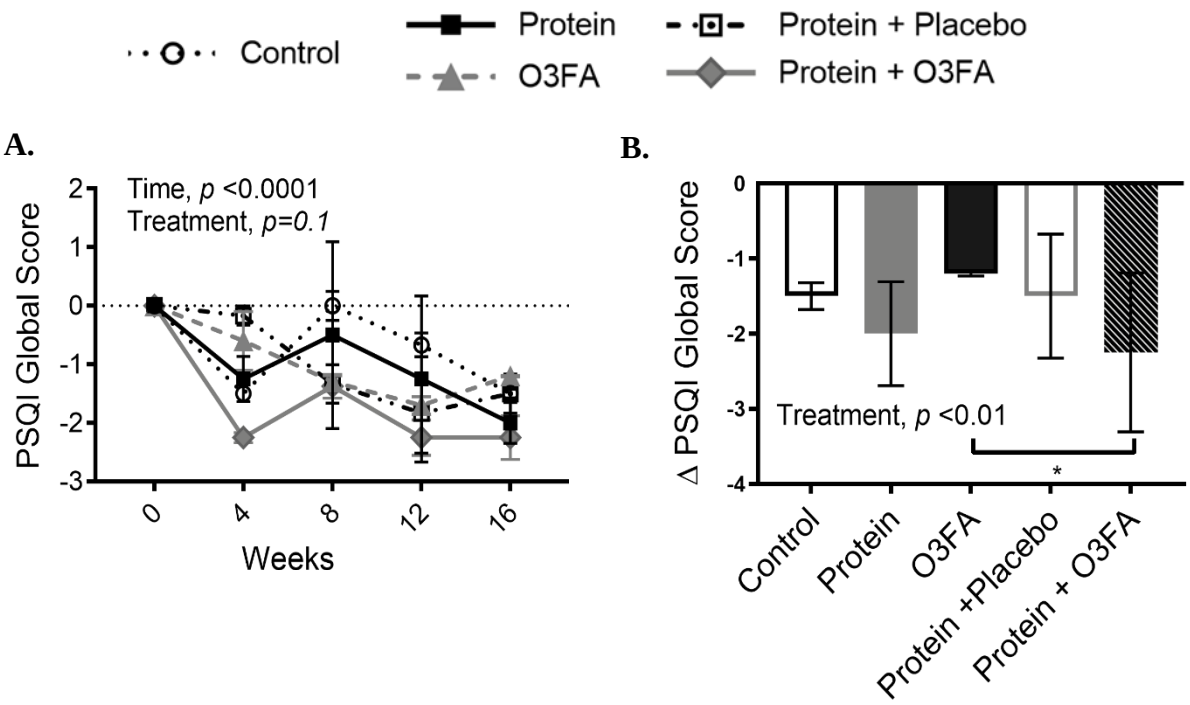

Figure 3. (**A**) The Global PSQI Score average for each of the five treatment groups calculated for all five visits after controlling for baseline. Significance was found for time ($p < 0.0001$). (**B**) The average change in Global PSQI score from baseline at week 16 for each of the five treatment groups. Significance was found for treatment, between the O3FA and the Protein + O3FA groups ($p < 0.01$). Omega-3 fatty acids, O3FA; Pittsburgh sleep quality index, PSQI.

Sleep: *Wrist Actigraphy*

The ActiGraph monitors were collected from each participant at the beginning of the baseline, week 8, and week 16 visits along with the corresponding sleep diary. Wrist actigraphy data was downloaded and analyzed using ActiLife v6.13.4 software (Pensacola, FL). Sleep times were verified and corrected by comparing the sleep diaries against the raw data downloaded. The sleep data was then analyzed to find total sleep time, total minutes in bed, sleep efficiency, number of awakenings, and length of awakenings.

The line graph in **Figure 4A** displays the average Sleep Efficiency (total sleep time divided by time in bed) as a percentage, for each treatment group after controlling for baseline sleep efficiency. A one-way repeated measures ANOVA did not demonstrate significance. The line graph in **Figure 4B** displays the average Total Sleep Time in minutes for each treatment group after controlling for baseline. A one-way repeated measures ANOVA did not demonstrate significance. The line graph in **Figure 4C** displays the average Total Time in Bed in minutes for each treatment group after controlling for baseline. A one-way repeated measures ANOVA did not demonstrate significance. **Figure 5A** shows a line graph depicting the average Number of Awakenings for each group after controlling for baseline. A one-way ANOVA demonstrated significance for time ($p < 0.05$), as well as between treatments ($p < 0.05$). **Figure 5B** shows a line graph depicting the average Wake after Sleep Onset in minutes for each group after controlling for baseline. A one-way ANOVA demonstrated significance for time ($p < 0.05$), as well as between treatments ($p < 0.05$).

Figure 4.

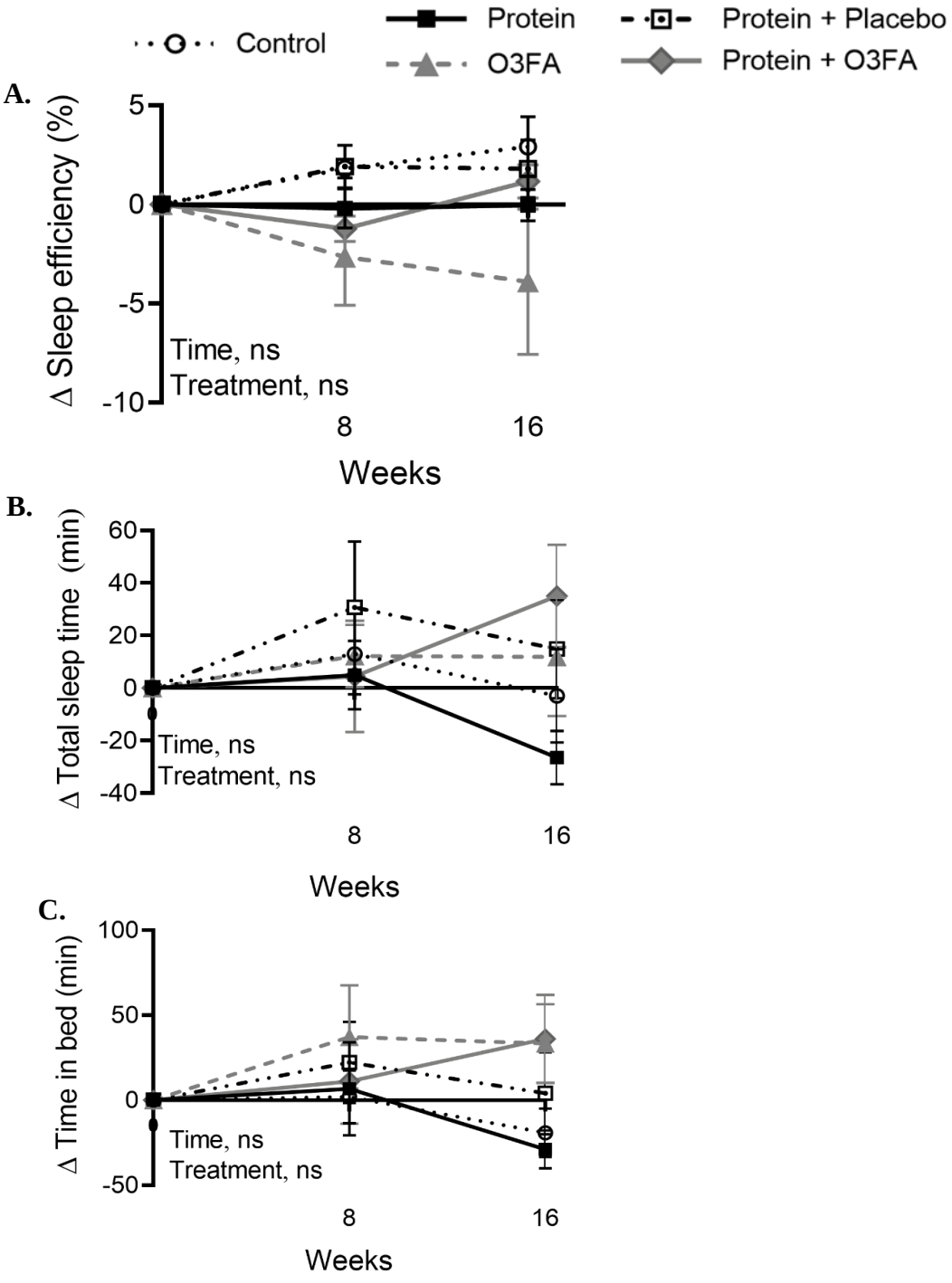

Figure 4. (**A**) The average Sleep Efficiency for each of the five treatment groups calculated for all five visits after controlling for baseline. No significance was found. (**B**) The average Total Sleep Time for each of the five treatment groups calculated for all five visits after controlling for baseline. No significance was found. (**C**) The average Time in bed for each of the five treatment groups calculated for all five visits after controlling for baseline. No significance was found. Omega-3 fatty acids, O3FA.

Figure 5.

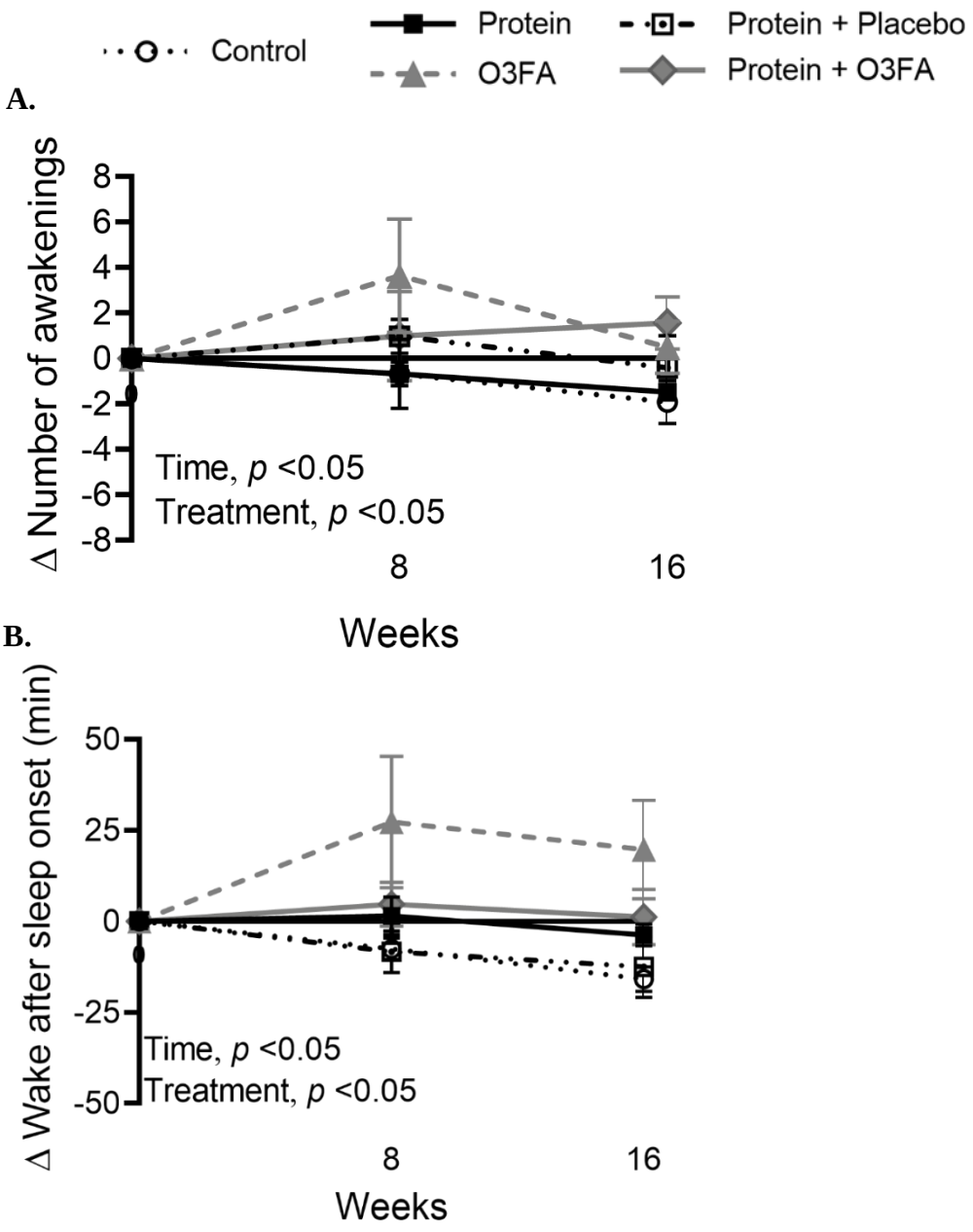

Figure 5. (**A**) The average Number of Awakenings for each of the five treatment groups calculated for all five visits after controlling for baseline. Significance was found for both time ($p < 0.05$), as well as between treatment groups ($p < 0.05$). (**B**) The average Number of Awakenings for each of the five treatment groups calculated for all five visits after controlling for baseline. Significance was found for both time ($p < 0.05$), as well as between treatment groups ($p < 0.05$). Omega-3 fatty acids, O3FA.

Mood: *Profile of Mood States Questionnaire (POMS)*

The POMS questionnaires filled out at the beginning of each participants' visit were recorded and compiled to produce a score for each of the following: Total Mood Disturbance (TMD), Anger, Confusion, Depression, Fatigue, Tension and Vigor. These scores were recorded with those of the other participants in their respective treatment groups. The average POMS score for each of the five treatment groups was calculated per mood distinction category for each visit.

The line graph **Figure 6A** shows the average TMD score for each of the five treatments groups for each visit after controlling for baseline. A one-way repeated measures ANOVA did not demonstrate significance. The bar graph in **Figure 6B** shows the average change in TMD score from baseline at week 16 for each of the five treatment groups. A one-way ANOVA demonstrated no significance.

Figure 7A displays the average Anger score for each of the five treatments groups for each visit after controlling for baseline. A one-way repeated measures ANOVA did not demonstrate significance. **Figure 7B** displays the average Confusion score for each of the five treatments groups for each visit after controlling for baseline. A one-way repeated measures ANOVA demonstrated significance ($p < 0.05$) between treatment groups. **Figure 7C** displays the average Depression score for each of the five treatments groups for each visit after controlling for baseline. A one-way repeated measures ANOVA did not demonstrate significance. **Figure 7D** displays the average Fatigue score for each of the five treatments groups for each visit after controlling for baseline. A one-way repeated measures ANOVA did not demonstrate significance **Figure 7E** displays the average Tension score for each of the five treatments groups for

each visit after controlling for baseline. A one-way repeated measures ANOVA demonstrated significance ($p < 0.05$) between groups. **Figure 7F** displays the average Vigor score for each of the five treatments groups for each visit after controlling for baseline. A one-way repeated measures ANOVA demonstrated significance ($p < 0.05$) between treatment groups.

Figure 6.

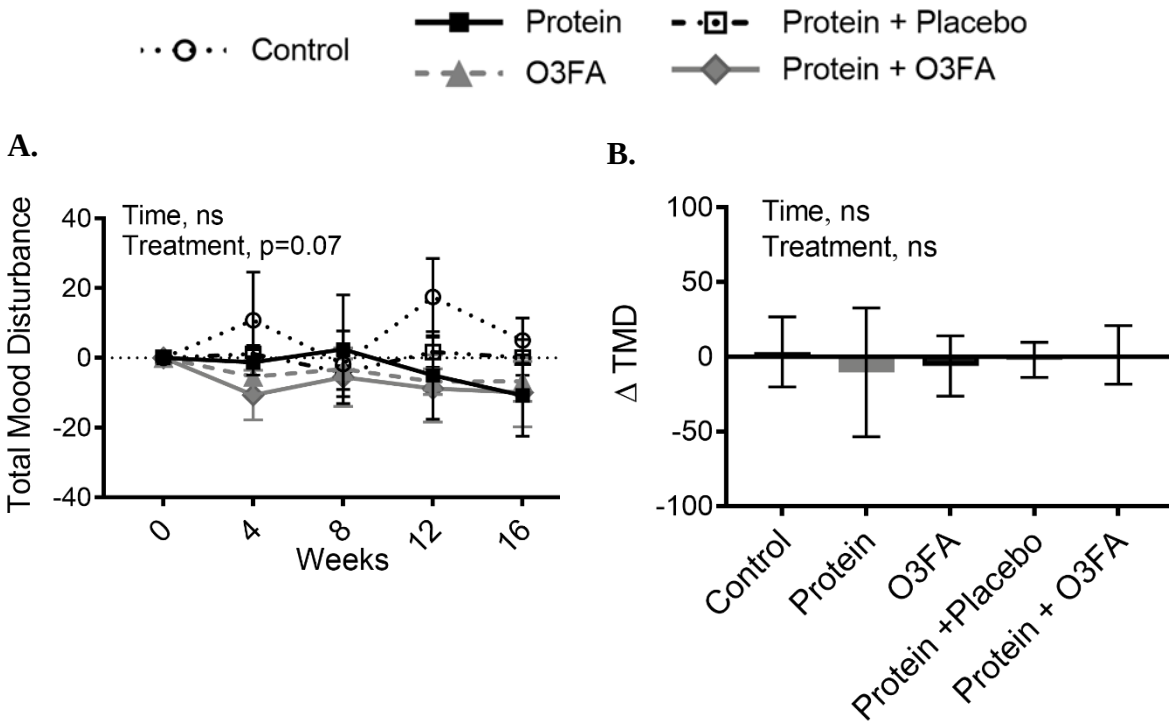

Figure 6. (**A**) The average TMD score for each of the five treatments groups for each visit after controlling for baseline. No significance was found. (**B**) The average change in TMD score from baseline at week 16 for each of the five treatment groups. No significance was found. Omega-3 fatty acids, O3FA.

Figure 7.

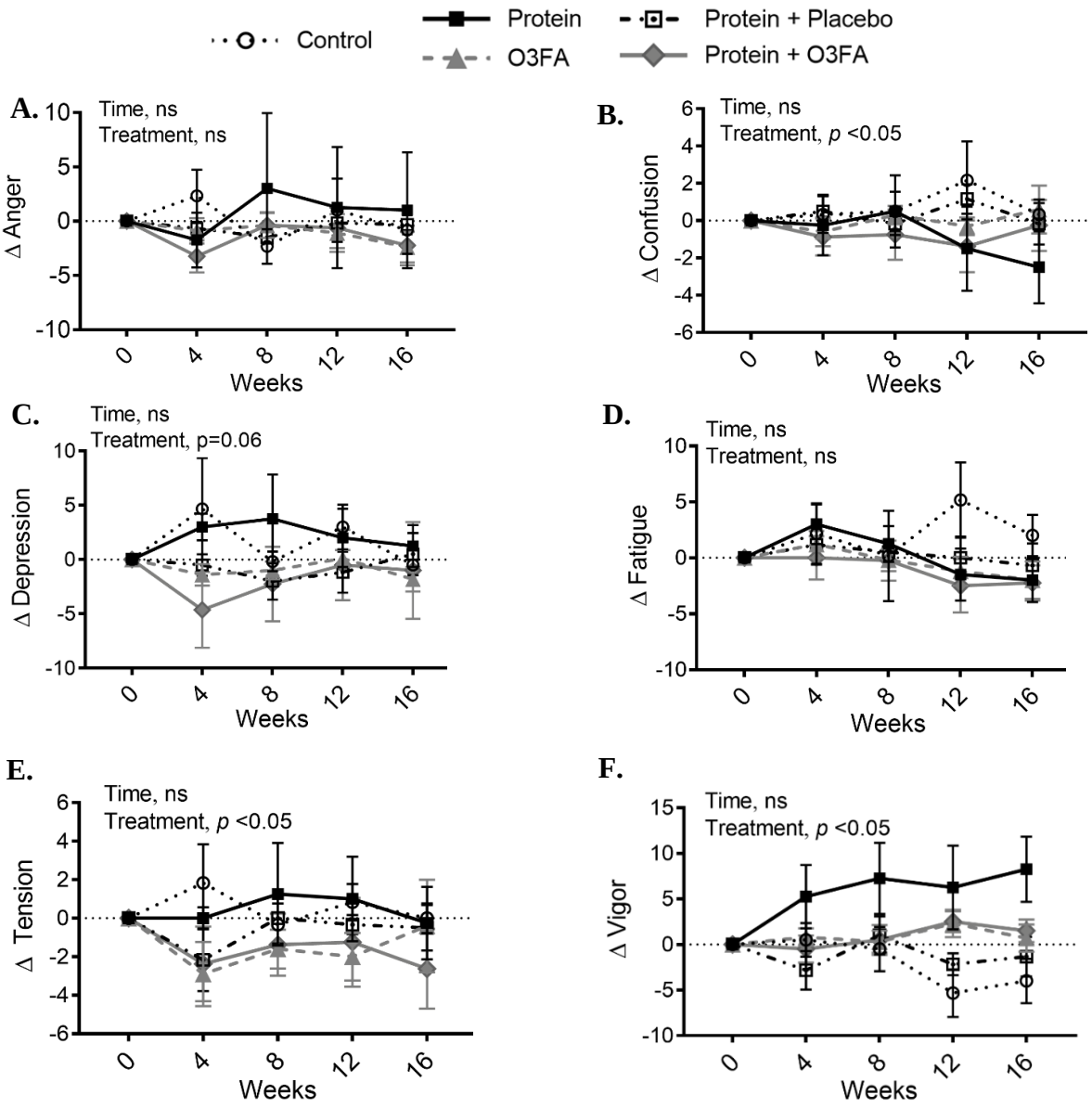

Figure 7. (**A**) The average Anger score for each of the five treatments groups for each visit after controlling for baseline. No significance was found. (**B**) The average Confusion score for each of the five treatments groups for each visit after controlling for baseline. Significance was found between treatment groups ($p < 0.05$). (**C**) The average Depression score for each of the five treatment groups after controlling for baseline. No significance was found. (**D**) The average Fatigue score for each of the five treatments groups for each visit after controlling for baseline. No significance was found. (**E**) The average Tension score for each of the five treatments groups for each visit after controlling for baseline. Significance was found between treatment groups ($p < 0.05$). (**F**) The average Vigor score for each of the five treatments groups for each visit after controlling for baseline. Significance was found between treatment groups ($p < 0.05$). Omega-3 fatty acids, O3FA.

Discussion

The rapid growth of the older population of the United States' and this population's increasing health risks including obesity, chronic diseases, decreased quality of life, and premature death are expected to slow down anytime soon [1]. Determining the health risks associated with particular obesity levels has been shown to best be measured through BMI with waist measurements distinguishing health risks for a given BMI group [4]. The risk of obesity as well as poor sleep increases with age [22]. Poor sleep in turn, may lead to more emphasized signs of aging, obesity, and other hardships. Nutrition continues to be a key factor in preventing both muscle loss and body fat gain. Nutrition further emerges, as a preventative strategy to decrease sleep and mood dysregulation that comes with aging. Studies have shown that healthy aging is correlated with high O3FA consumption [10], as well as high protein diets via decreased body fat [11]. Guidelines for the promotion of healthy aging are needed.

Through this particular study, sleep, mood, BMI, waist circumference, and waist-to-hip ratio measurements were all utilized in an attempt to determine the synergistic effect of protein and omega-3 fatty acid supplementation in a particular sector of the aging population—postmenopausal women. While the intervention lasted 16 weeks, improvements in any of the above measures were recognized as potential clues to nutrient supplementation's effect on healthy aging.

Current literature suggests that poor sleep quality, as assessed by a variety of measures, is linked to low protein intake [7]. The findings of this study are aligned with this idea, as a major trend for the Protein + O3FA group to decrease in PSQI Global Score more than any other group from baseline to week 16. The significantly lowered

Global PSQI score from baseline to week 16 for the Protein + O3FA group as compared to the just O3FA group also aligns with the literature stating that increased intake of dietary protein has shown the ability to improve sleep quality as measured by Global PSQI scores [23]. High-protein diets have been found to improve sleep quality through decreasing the occurrence of wake episodes during sleep [7]. ActiGraph measurements for sleep quality did not align with the current literature for improved sleep with increased intake of dietary protein. The supplementation of protein did not show consistently clear improvement for these measures of sleep quality, but this could have due to small group sizes and the short duration of the intervention.

Postmenopausal women's sleep disturbances have been linked to mood symptoms in current literature, particularly depression and anxiety [16]. Current literature has presented the possibility of O3FAs to decrease depression [9]. POMS Depression scores trends aligned with the literature. The O3FA and Protein + O3FA groups both demonstrated the greatest trends in a decreased depression score from baseline to week 16, showing an improved mood state with respect to the measurement of depression. In fact, the Protein + O3FA group both tended to trend toward a lower, improved mood score from baseline to week 16 for: TMD, Anger, Depression, Fatigue, and Tension.

While BMI values did not display much improvement, both waist circumference and waist-to-hip ratios displayed a trend for all treatment groups, and not the control group, to demonstrate a decline from baseline to week 16. This is supported by the literature, as O3FA and protein consumption are known, individually, to decrease body fat [10, 11]. Postmenopausal women are afflicted by a great gain in waist circumference and waist-to-hip ratio when compared to their premenopausal counterparts [14]. Current

literature has found that dietary supplementation may aid to combat the fat gains experienced by postmenopausal women [10, 11]. The statistical significance found between treatment groups for waist-to-hip ratios, waist circumference, and the general trend shown in for all groups with dietary supplementation of whey protein and/or O3FAs to decline in waist-to-hip ratios and waist circumference measurements, aligns with the current literature.

The effect of additional protein, O3FAs, and the combination of both protein and O3FA supplementation showed potential to counter the risks often associated with aging adult by promoting health aging. Protein and O3FAs appeared to positively impact the nutrition of aging adults and thus affect aging through improving various aspects of subjective sleep, mood, and anthropometric measurements.

Limitations of this study included a small sample size, anthropometric human error, sleep diary human error, and subject compliance. Recruitment for human studies, combined with inclusion and exclusion criteria, is never an easy task. As the intervention was over the course of 16-weeks and included morning study day visits, total of 34 participants fully completed the study. This sample size is not larger enough to be fully representative of the given population—aging United States' postmenopausal women. Despite standardizing points to measuring waist circumferences, hip widths, and utilizing the same stadiometer and balance scales for all visits and participants, anthropometric human error during the course of the intervention could have occurred. The sleep diaries given to participants included instructions, but human error in recording proper times could have altered ActiGraph results when verifying the ActiGraph monitors with potentially incorrect sleep diaries. Finally, as the consumption of supplements—whether

whey protein, O3FA, or soybean oil placebo—was left to the participant on their own time in between visits, subject compliance cannot be fully verified. Subjects checked boxes as reminders for supplement consumption, returned empty supplement packaging, and were verbally asked their compliance from visit-to-visit, but this does not ensure correct compliance if perhaps a supplement dosage was mistakenly missed.

Further study on the exact processes affected by protein and O3FAs in the aging adults' body both individually and combined are needed. The epidemics of obesity, chronic diseases, and overall decreased quality of life currently impact many aging adults. This supports deeper inquiry into the bodily impact of nutrient supplementation. Postmenopausal women, in particular, tend to experience significant fat gain, highly report sleep disturbance, and are prone to mood disturbances at a higher rate compared to their male counterparts [15, 16, 17]. This study considered a demographic with much need for healthy aging guidelines that is overall underrepresented in research. Protein and O3FAs appear to have the potential to assist in achieving healthier aging, and more research is called for to assist both the afflicted aging, and specifically postmenopausal, demographic.

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Appendices

Appendix 1: IRB Approval Letter (August 20th, 2018)

UNIVERSITY OF ARKANSAS

To:	Jamie I Baum FDSC N2216
From:	Douglas James Adams, Chair IRB Committee
Date:	08/21/2018
Action:	**Approval**
Action Date:	08/20/2018
Protocol #:	1708023785R002
Study Title:	The Effect of Protein and Omega-3 Fatty Acid Supplementation on Body Composition, Sleep, Cardiometabolic Health, and Strength in Postmenopausal Women
Expiration Date:	09/11/2019
Last Approval Date:	09/12/2018
Risk Level:	

The above-referenced protocol has been approved following Full Board Review by the IRB Committee that oversees research with human subjects.

If the research involves collaboration with another institution then the research cannot commence until the Committee receives written notification of approval from the collaborating institution's IRB.

It is the Principal Investigator's responsibility to obtain review and continued approval before the expiration date.

Protocols are approved for a maximum period of one year. You may not continue any research activity beyond the expiration date without Committee approval. Please submit continuation requests early enough to allow sufficient time for review. Failure to receive approval for continuation before the expiration date will result in the automatic suspension of the approval of this protocol. Information collected following suspension is unapproved research and cannot be reported or published as research data. If you do not wish continued approval, please notify the Committee of the study closure.

Adverse Events: Any serious or unexpected adverse event must be reported to the IRB Committee within 48 hours. All other adverse events should be reported within 10 working days.

Amendments: If you wish to change any aspect of this study, such as the procedures, the consent forms, study personnel, or number of participants, please submit an amendment to the IRB. All changes must be approved by the IRB Committee before they can be initiated.

You must maintain a research file for at least 3 years after completion of the study. This file should include all correspondence with the IRB Committee, original signed consent forms, and study data.

cc: Aubree L Worden, Investigator
Hexirui Wu, Key Personnel
Megan Elizabeth Rosa-Caldwell, Key Personnel
Angela M Tacinelli, Key Personnel
Samuel Preston Belt Walker, Key Personnel
Michelle Gray, Key Personnel
Jamie Lauren McDermott, Key Personnel
Caroline A. Baughn, Key Personnel
Justine Gaelle Jossic, Key Personnel

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Appendix 2: IRB Approval Letter (September 3rd, 2019)

UNIVERSITY OF ARKANSAS

To:	Jamie I Baum FDSC N2216
From:	Douglas James Adams, Chair IRB Committee
Date:	09/11/2019
Action:	**Expedited Approval**
Action Date:	09/03/2019
Protocol #:	1708023785A009
Study Title:	The Effect of Protein and Omega-3 Fatty Acid Supplementation on Body Composition, Sleep, Cardiometabolic Health, and Strength in Postmenopausal Women
Expiration Date:	09/02/2020
Last Approval Date:	09/03/2019

The above-referenced protocol has been approved following expedited review by the IRB Committee that oversees research with human subjects.

If the research involves collaboration with another institution then the research cannot commence until the Committee receives written notification of approval from the collaborating institution's IRB.

It is the Principal Investigator's responsibility to obtain review and continued approval before the expiration date.

Protocols are approved for a maximum period of one year. You may not continue any research activity beyond the expiration date without Committee approval. Please submit continuation requests early enough to allow sufficient time for review. Failure to receive approval for continuation before the expiration date will result in the automatic suspension of the approval of this protocol. Information collected following suspension is unapproved research and cannot be reported or published as research data. If you do not wish continued approval, please notify the Committee of the study closure.

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Amendments: If you wish to change any aspect of this study, such as the procedures, the consent forms, study personnel, or number of participants, please submit an amendment to the IRB. All changes must be approved by the IRB Committee before they can be initiated.

You must maintain a research file for at least 3 years after completion of the study. This file should include all correspondence with the IRB Committee, original signed consent forms, and study data.

cc: Aubree L Worden, Investigator
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