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Effects of Time Restricted Feeding and Whey Protein Isolate Supplementation on Dietary Intake, Mood, and Sleep in a 12-week Randomized Controlled Trial

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Abstract

Background: Obesity is a quickly growing epidemic that is affecting adults in the United States leading to many chronic diseases and reduced well-being. Time Restricted Feeding (TRF) is type of dietary intervention that is gaining popularity among adults for weight loss. However, current data is lacking regarding the effectiveness of TRF on facets of well-being such as sleep and mood. Moreover, to our knowledge protein supplementation in conjunction with TRF has yet to be studied. The **objective** of this study was to determine the effect of time restricted feeding supplemented with whey protein isolate on food intake, sleep, and mood, in overweight or obese adults.

Methods: Nineteen participants were randomly assigned to the control group or experimental group: 1) control, TRF (n=10) and 2) TRF with whey protein supplementation (25 g/d; n=9). Protein supplements were consumed at the breaking of the fasting period each day and both groups followed a TRF dietary intervention (8 hour eating window with a 16 hour fast). Participants followed the assigned dietary intervention for 12 weeks. Subjects came to the Center for Human Nutrition for sample collection and measurements to be taken. Anthropometrics, including height and weight, were measured along with the Pittsburg Sleep Quality Index (PSQI) questionnaire, Profile of Mood States (POMS) questionnaire, and dietary record results every 4 weeks (baseline, week 4, week 8, and week 12) at the Center for Human Nutrition. Additionally, actigraphy measured objective sleep quality at week 1 and week 12.

Results: Overall, there were no significant differences between the control and protein group regarding sleep and mood parameters. The PSQI results indicated no difference in sleep between groups. After controlling for tension-anxiety scores for baseline, there was a significant decrease in the protein group compared to the control at week 12 ($p<0.01$). Lastly, total food consumption

was similar between groups with no significance except the amount of protein consumed each day, which was higher in the protein group ($p < 0.01$).

Conclusion: The results suggested whey protein isolate supplementation with TRF may improve outcomes of mood with no effect on sleep. Therefore, results from this study identify a need for further research to investigate the benefits from TRF and protein supplementation on sleep and mood.

Introduction

Obesity and Chronic Disease in the United States

Obesity is a quickly growing epidemic that is strongly associated with mortality and morbidity (1). Currently, more than two thirds of adults in the United States are overweight or obese, and all states and territories have an adult obesity rate of over 20% (2). Moreover, Arkansas ranks among the top four obese states in the nation with an average adult obesity rate of 36.4% surpassing the national average of 21% (2). In adults, obesity is defined as a body mass index of greater than 30 kg/m^2 (3). Obesity is statistically connected to many chronic diseases including types of cancer, disability, osteoarthritis, stroke, diabetes, cardiovascular disease, and high blood pressure (1, 3). When combined, these impacts make up for over 2 billion dollars spent every year on obesity related health care costs (4).

Not only does obesity increase risk of chronic disease, but it also has major impacts on well-being including sleep and mood (5, 6). According to the Center for Disease Control, well-being is an encompassing concept to describe one's overall life satisfaction of many aspects including sleep and mood (7). Depression has been found as a predictive factor of developing obesity (5, 6). While the literature is conflicting, decreased sleep has been linked with obesity due to causing increased caloric intake (8).

With effects of overweight and obesity being connected to many chronic diseases, including impacts on sleep and mood, many individuals are turning to varying methods of dietary intervention to lose weight including Time Restricted Feeding (TRF). Dietary changes, like macronutrient adjustments specifically targeted at increased protein consumption, have also become popular forms of weight reduction strategies. Our study combined these two dietary approaches testing the effects of a TRF intervention with increased protein consumption.

Currently, the research is lacking regarding the effects of TRF with increased protein on sleep and mood. Therefore, the purpose of this literature review and study was to investigate one form of reduced calorie intake combating obesity: time restrictive feeding and its effects on health and well-being. For the purpose of this study, TRF is defined as 8 hour eating period followed by a 16 hour fasting period.

Review of Literature

Well-Being in the United States

Well-being is an important aspect of life encompasing both physical and mental health. According to the Centers for Disease Control and Prevention (7), well-being generally includes various facets of life satisfaction and feelings ranging from depression to joy. A positive well-being includes the presence of positive emotions, satisfaction of life, and fulfillment of positive functioning. Many indicators are used to measure well-being (9, 10). However, this review will focus on measures of sleep and mood.

Sleep

More than a third of adults in the United States report not getting the recommended seven to nine hours of sleep per night to promote well-being. Research performed by the American Academy of Sleep Medicine and Sleep Research shows that sleeping less than seven hours per night regularly is associated with other adverse health outcomes such as weight gain, diabetes, insulin resistance, hypertension, heart disease, stroke, poor mood, and ultimately increased risk of death (11-13). A cross-cultural cohort comparison conducted in 1997-1999 between the United States (n = 3,027) and the United Kingdom (n = 6,472) found correlations between recommended sleep and changes in mood and body composition (14). Individuals who slept less than the desired amount were more likely to have increased body weight and higher central adiposity. Individuals who slept more than the desired amount (>8 hours) were associated with lower levels of physical activity. Moreover, a crossover randomized controlled trial conducted by Covassin et. al (15) on participants who were healthy and nonobese found that sleep restriction (<4 hours) led to increased energy intake in the absence of changes in energy expenditure. Additionally, a systematic review conducted by Patel et al. (8) found 11 of 19 evaluated cross-

sectional studies had an association between poor sleep and weight gain, suggested mechanisms were increased levels of ghrelin and reduced levels of leptin. In a meta-analysis evaluating randomized controlled trials that limited sleep, there was not a strong relationship between sleep restriction and body weight (16). Therefore, the current literature suggests some relationship between sleep and overall health outcomes, but further research needs to be conducted in order to evaluate the outcomes of sleep duration and quality.

Mood

Mood is very complex; it is reliant on many different factors including lifestyle, genetics (17), body composition (18), and the interactions between those factors (19). Statistics show that one in every six adults will suffer from depression in their lifetime (20). In addition, obesity and mood disorders are frequently associated. There are two main mood disorder types: bipolar disorders and major depressive disorders (18). Individuals who have major depressive disorder have 50% higher risk of developing obesity (21), and individuals who have bipolar disorders have an increased risk for increased visceral adiposity and waist circumference (18). In support, a meta-analysis of longitudinal studies found that when obesity or depression was present, there was an increase in the risk for the other (6). One biological mechanism suggests that inflammation caused by obesity can increase cortisol levels which can lead to neuron damage in venerable limbic regions of the brain associated with depression (22, 23). In addition, genome-wide association studies that evaluate loci of the brain found an association between BMI, appetite, and mood sections of the brain (23). Therefore, evidence suggests that obesity and mood may have a genetic link. Furthermore, dietary interventions have been shown to improve mood, although the mechanisms are unclear (24).

The bidirectional relationship of sleep and mood

Evidence suggests that the associations between mood and sleep are bidirectional. That is, poor sleep duration and quality may adversely affect emotional well-being via mood, and conversely, certain emotions such as anxiety and depression may lead to dysregulated sleep (11, 23, 25-27). A meta-analysis investigating the effects of sleep on depression found that individuals with short and long sleep durations are at a higher risk for developing depression (19). One explanation for this is inadequate levels of sleep increase daytime tiredness which is a predictor of depression (19). Another explanation is that obesity and poor diet may be influential factors exacerbating the decline in both sleep and mood in the U.S population (28). Although changes in diet may ameliorate poor sleep and mood via shifts in body composition, evidence is conflicting and mechanisms have yet to be fully investigated.

Dietary Approaches to Treat Obesity

The fundamental cause of obesity is a surplus of energy between calories consumed and expended. Evidence has shown that a weight loss as little as 5-10% in the span of 6 months reduces risk factors of comorbidities and can be seen by a change in biochemical markers (29). According to Freire (29), there are three main ways in which individuals can promote weight loss. The first method focuses on the alteration of macronutrient distribution such as low fat or high protein. The second method is focused on the restriction of certain foods such as vegan, paleo, or gluten-free dietary practices. Lastly, there are diets that are focused on manipulating the timing of when food is consumed such as fasting. Throughout this review we will focus on the combination of macronutrient distribution and time manipulation via high protein diets or dietary protein supplementation and time restricted feeding.

Macronutrient Intake and Weight Loss

While there are many methods that are used to encourage weight loss, most are associated with increased hunger and reduced fullness (30). Therefore, there has been specific research on what macronutrient distribution is most beneficial for weight loss. Research suggests that high-protein diets may promote weight loss by influencing energy balance and improved body composition in obese adults (31). For instance, increased protein consumption can be linked to higher levels of satiety and energy expenditure. In a randomized control trial conducted by Smeets et al. (32), healthy participants who consumed a high protein lunch, characterized by 20-30% protein, were fuller longer than participants who consumed a lower protein lunch at 10-15% protein. In addition, a randomized control trial investigating the effects of a high protein diet with a total diet replacement supplement on normal weight adults found a higher energy expenditure, fat oxidation, and negative fat balance (33). Furthermore, a 12 month randomized control trial conducted on subjects who were overweight on a high protein diet (25% protein) compared to a medium protein diet (12% protein), caused a greater weight loss by 3.5 kg (34). Beyond increased total energy expenditure and promoting satiety, high protein diets are also associated with an increased thermic effect of feeding which is a contributing factor in weight loss (35). Moreover, protein quantity and quality are suggested to influence the effectiveness of dietary protein as a treatment strategy for obesity, as reviewed below.

Protein Quantity

Recently the 2020-2025 Dietary Guidelines for Americans was released, keeping the Dietary Reference Intake (DRI) for recommended amounts of protein at 0.8g/kg a day (36). On average, adult Americans get the required number of grams of protein per day, with men consuming more than women (37). However, current research suggests reservations to the current Recommended

Dietary Allowance (RDA) that is set. The RDA was established by finding the minimum amount of protein needed to eliminate lean muscle mass loss determined by nitrogen balance. The amount of these studies that have been conducted is small, and none have addressed whether protein consumption above the RDA may be beneficial aside from maintaining nitrogen balance (38). Not only that, but most of the studies conducted have taken place on college-aged men (38, 39). In addition to the RDA, the Food and Nutrition Board has set forth Acceptable Macronutrient Distribution Ranges (ADMR). This range recommends that 10-35% of total calories come from dietary protein (37). Based on this data, experts recommend protein consumption should be closer to 1.0 or 1.5 g/kg, near the upper end of the AMDR, to decrease the risk of obesity and promote well-being (38, 39).

Protein Quality

While most Americans receive the proper amount of protein based on the RDA, the quality of the protein is also an important factor to consider. Protein quality is dependent on its digestibility and indispensable amino acid score (40). For example, a high quality protein such as cheese, milk, eggs or fish, contain all nine of the essential amino acids in proportion to the body's requirements, while a low quality protein such as vegetables, grains, or nuts, have a limiting essential amino acid (40). Overall, animal proteins such as beef and milk protein stimulate muscle protein synthesis more effectively than plant-based proteins such as soy (38). Therefore, a higher consumption of lean protein foods has been associated with greater gains in lean body mass (38). In addition to being higher quality, animal proteins such as lean meats require a lower consumption of kilocalories to meet protein goals in comparison to protein from plant-based products such as legumes. Therefore, energy restriction in conjunction with dietary

protein goals are easier to achieve. Further research is needed to determine the effect of protein quality on well-being.

One example of a high-quality protein widely used in weight loss interventions is dairy, specifically whey. Whey is a general term that denotes the liquid that is leftover after cheese manufacturing. Whey protein isolate (WPI) is whey in its most concentrated form, above 90% protein (41). This protein is considered high-quality because of its high protein content and branched chain amino acid composition (41). In addition, it is highly bioavailable and has components of glycolmacropeptide and alpha-lactoglobulin which are associated with satiety (42). Furthermore, a few randomized controlled trials have linked WPI supplementation to improved well-being outcomes. For example, 9.5 grams of WPI supplementation has been found to improve subjective sleep quality (via PSQI) and decrease cortisol levels (43). However, further research is needed to establish a relationship between WPI and well-being.

The Role of Dietary Protein in Weight Loss and Maintenance

A high protein diet while losing weight can support weight loss efforts. In a 12 month randomized control trial conducted on subjects who were overweight on a high protein diet (25% protein) compared to a medium protein diet (12% protein), high protein diet caused a greater weight loss by 3.5 kg (34). Furthermore, an increased protein diet can also help maintain weight loss. In a clinical trial investigating the effects of an increased protein diet on participants who were overweight and obese following a 4 week period of low energy intake (2.1 MJ/day) found a higher protein diet (18% compared to 15%) resulted in 50% lower body weight regain (44). Additionally, a trial investigating the effects of protein supplementation on older adults with obesity during intentional weight loss found a significant difference in appendicular muscle mass

over the 13 week study with a preservation of muscle mass (45). Moreover, increased protein consumption is linked to decreased desire to eat as evident by a RCT conducted by Gwin et al. (46) which examined a high protein energy restricted diet and found a decrease in satiety throughout the day. With all the combined literature, it is evident that a high protein diet is an effective method of weight loss and retention.

The Role of Dietary Protein in Well-Being

Sleep

While protein supplementation is commonly associated with diets with a positive effect on weight loss and retention, its direct effect on sleep and mood remain unclear. In a meta-analysis conducted by Wirth et al.(47) investigating the effects of higher protein consumption on subjective sleep in twelve intervention studies, there was not a clear relationship between increased protein consumption and sleep. Another meta-analysis was carried out to examine the effect of sleep restriction on body weight found no association (16). Parallel to these findings, a randomized controlled crossover trial specifically examining the effects of implementation of 9.5g of dietary protein supplementation on healthy adults led to improved subjective sleep quality during some days of the study while other days there was no significant difference (43). Overall changes in the PSQI were not different between placebo and whey protein supplementation groups. However, one time point of data during the study produced significantly lower global PSQI score. In addition, there was a trend of lowered sleep latency in the supplement group (43). In another RCT, a high protein diet was found to improve the PSQI global sleeping score in overweight and obese adults (48). A study investigating normal sleepers and insomniacs, insomniacs were found to have an average protein intake of 6 grams lower than

normal sleepers (49). Furthermore, increased protein consumption has been found to increase sleeping metabolic rate, resulting in higher caloric expenditure (50, 51). Overall, increased protein consumption and the effect it has on sleep is a newly emerging area of research.

Mood

Not only does increased protein consumption have effects on sleep quality, but it also impacts mood. A supplementation of WPI is related to a lowering of salivary cortisol, suggesting that WPI could reduce stress (43). Moreover, the results of the National Health and Nutrition Examination Survey (NHANES), concluded that the intake of total protein and milk products were negatively associated with depressive symptoms (52). While protein supplementation suggests an increase in sleep quality and mood, the existing literature is conflicting, and the mechanism of action has yet to be established. However, tryptophan has been suggested as a way to improve well-being

The Role of Tryptophan in Well-Being

WPI is high in the essential amino acid tryptophan. In a single serving of WPI supplementation, there is 862 mg of tryptophan. The recommended consumption of tryptophan is between 250-425 mg/day, while the average daily intake is 900-1000 mg. Therefore, the average person is getting sufficient tryptophan to optimally support muscle health and body composition (53). Tryptophan plays a role in protein synthesis along with the regulation of numerous physiological mechanisms (53) including acting as a precursor for peripherally and centrally produced serotonin and melatonin (54). This means that adequate tryptophan intake could be associated with higher sleep duration and lower levels of depression (55). In fact, in the past 20 years many studies have examined the effects of tryptophan consumption on sleep, and most

evidence suggest an increase in at least one sleep outcome (56). In a randomized control trial conducted by Markus et al., the intake of WPI increased TRP-LNAA (tryptophan levels), indicating there was more to be taken up by the brain, and a projected increase in central serotonin and increased mood (57).

Time Restricted Feeding as a Weight Loss Strategy

To combat the major rise in obesity and improve outcomes of well-being, many forms of dietary intervention and behavioral changes have been created (58, 59). All of these methods aim to reduce caloric intake in some form, with the most popular being continuous energy restriction (CER). However, CER adherence usually declines within 1-4 months, and individuals usually regain the weight they lost within one year. Therefore, alternate approaches to weight loss have been created (60). One proposed method to reduce caloric intake and improve well-being is through intermittent fasting (61, 62). Intermittent fasting is a broad term that encompasses a variety of eating methods that use extended periods of fasting between meals of normal intake in order to reduce calorie intake (63). The term Time Restrictive Feeding (TRF) characterizes a subcategory of intermittent fasting in which an eating pattern is restricted to 8 hours or less per day (60). Evidence suggests TRF may be an effective strategy to achieve a caloric deficit and improve metabolic health in obese adults (60). For example in a 12 week study, conducted by Wilkinson et al., on participants with metabolic syndrome, TRF was found to decrease systolic and diastolic blood pressure, total cholesterol, LDL-C, non HDL, and high fasting glucose levels (62). Similarly, a non-randomized controlled clinical trial conducted on women who were obese found the TRF group exibited a decrease in weight, BMI, fat mass, and percentage body fat (64). Therefore, data suggests that TRF could have a positive impact on sleep and mood. However, the

mechanism by which TRF may influence sleep and well-being has yet to be discovered. Other studies find no effect of TRF and weight loss. Therefore, coupling TRF with caloric restriction and other dietary interventions is needed.

High-Protein Diet with Time Restricted Feeding

TRF is a dietary pattern that may promote calorie restriction. To our knowledge, long-term manipulation of macronutrients while following a TRF regimen has not been extensively studied. It is therefore unclear if TRF in conjunction with WPI supplementation improves sleep and mood. However, one study has investigated the short-term impact of intermittent fasting together with protein consumption (65). For this study, 40 participants who were obese consumed a high quality high protein low calorie diet (HPLC) (30% protein) with one 24 hour fast per week for 10 weeks. This was followed by a 52 week follow up of either a high protein diet (n=10) with a one day fast per week or a heart healthy diet (n=14). Over the 10 week period of the HPLC diet, there was a decrease in body weight (10%), BMI, triglycerides, and blood pressure. Overall, the outcomes of this study indicate the important role that dietary protein and intermittent fasting may play in weight loss and retention. Furthermore, it warrants further research investigating the effect of intermittent fasting and high protein on additional outcomes including those related to well-being such as sleep and mood.

Conclusion

Nutrition is a key factor in the prevention of muscle loss, body fat gain, and sustained well-being during weight loss. The addition of whey protein isolate (WPI) is commonly used as a key supplement in weight loss intervention; however, little is known about the effectiveness of

WPI combined with TRF on weight loss and outcomes of well-being. Therefore, the **objective** of this study is to determine the effect of time restricted feeding supplemented with whey protein isolate on food intake, sleep, and mood, in overweight or obese adults. We **hypothesize** that time restricted feeding with supplementation of whey protein isolate will improve sleep quality and mood compared to time restricted feeding alone in overweight or obese adults.

Materials and Methods

Subject Recruitment:

Subjects were recruited between June 2021 and August 2022 through the university digital newspaper, advertisements on the Center for Human Nutrition website, Food Science Department website, social media (e.g., Facebook, Twitter, and Instagram), and by word of mouth. There were three phases of recruitment. Phase 1 was advertisement of the study as listed above. Phase 2 was conducted through a phone screening. Candidates were phone interviewed to meet the following requirements: must not have taken protein supplements or any other supplements that may interfere with metabolism, no food allergies, non-smoking, consumed alcohol less than four times per week, non-breastfeeding, not used illicit drugs or have dieted in the past three months. Participants who met all requirements qualified to participate in the study. 91 adults underwent an initial phone screening and 27 eligible adults enrolled into the study. 12 participants were allocated to control and 15 to treatment. The control group had 3 dropouts and the treatment group had 5 dropouts. There were varying reasons for dropout including personal reasons, discontinuing of intervention, and breaking protocols. A total of 19 participants completed the study, 10 were in the intervention group and 9 were in the control group. All participants were overweight or obese (BMI $\geq$ 25 kg/m^2). Participants signed a consent form following a complete explanation. The protocol was submitted and approved by the University of Arkansas Institutional Review Board before subjects were recruited. This trial was registered at clinicaltrials.gov as NCT04949451.

Intervention:

The 12 week study was conducted as a randomized control trial with one control group and one dietary intervention group. Participants were randomly assigned to the control group or

experimental group: 1) control, TRF (n=10) and 2) TRF with powdered whey protein supplementation (20 g/d; n=9) (refer to **Table 1** for participant characteristics and **Figure 1** for nutrient composition). Protein supplements were allocated in powder form to individuals satchets and were consumed at the breaking of the fasting period each day and both groups followed a TRF dietary intervention (8 hour eating window with a 16 hour fast). Participants followed the assigned dietary intervention for 12 weeks. Subjects came to the Center for Human Nutrition for sample collection and measurements to be taken. Anthropometrics including height and weight were measured. Participants were asked to complete two questionnaires, the Pittsburg Sleep Quality Index and Profile of Mood States questionnaire. Results were measured every 4 weeks (baseline, week 4, week 8, and week 12) at the Center for Human Nutrition. Objective sleep quality was measured via accelerometer at baseline and week 12. Participants were compensated 250 dollars for completion of the study (see **Figure 2** for study day timeline).

Education Materials:

Participants were provided with a booklet that corresponded with their dietary intervention. All booklets provided a standard study schedule as well as a study day checklist. The booklet also provided a guide for TRF and example schedules that the participants could follow. Details for the ActiGraph sleep monitor, and sleep diaries instructions for filling out food records were also included. Booklets given to participants in TRF and WPI supplementation group included a section with easy and quick recipes for protein supplementation consumption.

Anthropometrics:

Each visit (baseline, 4, 8, and 12 weeks), anthropometric measurements were taken. The lab team used a stadiometer (Detecto, St. Louis, MO) to measure body height to the nearest 0.1 cm while subjects were barefoot, in the free-standing position. Body weight was measured to the

nearest 0.05 kg using calibrated balance scales (Detecto, St. Louis, MO). Waist to hip ratios were also measured. Participants were asked to stand with arms to their sides and feet positioned close together and weight evenly distributed across their feet. Waist circumference was measured at the top of the iliac crest and hip circumference was measured at the widest portion of the buttocks.

Sleep Assessment:

Objective sleep quality and duration were assessed via an ActiGraph triaxial wrist accelerometer, a validated method of sleep assessment. This method uses the actigraphy algorithm (Cole-Kripke) that has been validated with high accuracy when compared to the current gold standard of polysomnography (66). Participants wore the ActiGraphs for one week before the start of the study (baseline) and one week prior to their final visit (week 12). The sample rate was 60Hz as this has been verified for sleep quality analysis (67). A 7-day average was be calculated for each sleep outcome (sleep efficiency, sleep latency, wake after sleep onset, number of awakenings, and length of awakenings). While wearing the ActiGraph, participants kept a sleep diary to confirm sleep schedule and awakenings.

Subjective sleep quality was self-assessed using the Pittsburg Sleep Quality Index (PSQI) questionnaire. This questionnaire is the most widely used and accepted for subjective sleep quality, and has a moderate convergent validity with depression and quality of life (68). The administration took five to ten minutes and was completed at each study visit (baseline, week 4, week 8, and week 12). A compiled global score of the seven scored sleep components (sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleep medication, and daytime dysfunction) distinguished good sleepers (≤ 5) from poor sleepers.

Profile of Moods States:

We administered the Profile of Mood States (POMS). This survey has been used since 1971 and exhibits construct and predictive validity of the 6 POMS subscales (69). This questionnaire consisted of 65 questions, taking around 10 minutes to complete. The test contained a one-word adjective of mood to measure and identify six affective states. The six identifiable mood/affective states were tension-anxiety, depression-dejection, anger-hostility, vigor-activity, fatigue-inertia, and confusion-bewilderment. Total Mood Disturbance (TMD) score is calculated by summing the scores across all six factors (weighting vigor negatively). The Profile of Mood States (POMS) questionnaire assessed fasting mood every lab visit (baseline, week 4, week 8, and week 12). Higher subscores for all affect states but the vigor domain represent poorer mood. The TMD score is the most clinically relevant and ranges from -32 (best possible score) to 200 (worst possible score). Mood was quantified with a 5-point Likert scale by participants.

Dietary Intake:

Each participant completed a 3-day (2 weekdays and 1 weekend day) food intake record every four weeks of the intervention. Scales were provided to ensure accuracy of the record. Participants met with study personnel to ensure expectations at the beginning of the study. Each lab visit (baseline, week 1, week 4 and week 12) subjects had their food records reviewed by study personnel for inconsistencies and lack of specifics. Food records were reviewed and input into the Nutrition Data System for Research (NDSR) software. Total energy intake, macronutrients, total dietary fiber, total saturated fatty acids, total trans-fatty acids, omega-3 fatty acids, and essential amino acids including tryptophan amounts were analyzed immediately following 3-day food record collection via the NDSR software providing a report and analysis of nutrient consumption. Averages were accumulated for for energy, macronutrients, saturated fatty

acids, trans fatty acids, omega-3 fatty acids, fiber, tryptophan, and niacin at baseline, week 4, week 8, and week 12.

Statistical Analysis:

Student t-tests were used to determine difference between the control and intervention group in participant characteristics including age, weight, PSQI GSS, POMS TMD, and sleep efficiency at baseline. Repeated-measures analysis of variance (ANOVA) were used to determine the differences in height, weight, food intake, Global PSQI scores, POMS scores, and wrist actigraphy over the course of the 12 week period. Two-way ANOVA was used to determine the difference between the beginning and end of the intervention. All tests were two sided with P-values ≤ 0.05 indicating significance. Analysis of data was conducted by using statistical software GraphPad Prism version 9.0.

Results

Participant Characteristics:

A total of 19 participants were included in the final data analysis (n=14 females) (n=5 males). The physical characteristics and demographics are presented in **Table 1**. The control group had a significantly lower BMI (p=0.04) and weight (p=0.03) when compared to the protein group, however there were no differences in height, global sleeping score, total mood disturbance or sleep latency between groups at baseline.

POMS Scores:

Results from POMS scores are presented in **Figure 4**. There was significant difference in treatment (p=0.01) for tension-anxiety scores, with the protein group having lowering scores over the duration of the study. After controlling tension-anxiety scores for baseline, there was a significant decrease in the protein group compared to the control at week 12 ($p<0.01$). Additionally, anger-hostility scores decreased over time in both groups after controlling for baseline. Furthermore, total mood disturbance (TMD) scores exhibited a significant decrease in both groups at week 12 compared to baseline with the control group having significantly lower TMD at week 12 (p=0.03). There were no other differences in results including depression-dejection, anger-hostility, fatigue-inertia, confusion-bewilderment, and total mood disturbance. However, we did identify a trend of reduced total mood disturbance score in the protein group (p=0.068).

PSQI Scores:

Figure 5 depicts PSQI global sleeping scores and sub scores. Significance was found in treatment of sleep latency (p=0.02), as the protein group's scores started below and stayed below the control. When sleep latency was controlled for baseline, it increased in the control group

compared to the protein group at week 4 and week 8 with no differences at week 12 ($p<0.01$). Furthermore, when global sleeping score was controlled for baseline, there was a significant decrease in the protein group compared to control at week 8 with no differences at week 4 or week 12 ($p<0.01$). No other statistical significance was found between global sleeping score, sleep duration, habitual sleep efficiency, sleep disturbances, and total daytime dysfunction.

Actigraphy:

Table 2 illustrates the results from sleep actigraphy at week 1 and week 12. There was difference in the protein group that had a significantly higher awakening length compared to the control group ($p<0.01$). There was no time effect nor treatment effect on total sleep time or total minutes in bed. However, there was a significant group by time interaction ($p<0.05$). No other results were statistically significant including sleep latency, sleep efficiency, and wake after sleep onset.

Dietary Intake:

Dietary intake is depicted in **Table 3**. The amount of protein consumed by the protein group was significantly higher than the control group ($p <0.01$), as well as a increasing trend for tryptophan (0.09). There was also a significant group effect for total energy consumed, however when total energy consumption and protein intake values are controlled for baseline there are no differences. All other dietary intake remained similar throughout the study.

Discussion

The rising levels of overweight and obese individuals have increased the need for weight loss interventions to combat the widespread effects of obesity (2). These effects include negative impacts on well-being such as poor sleep and mood. Therefore, various strategies have been implemented to assist individuals in losing weight including TRF and protein supplementation. However, to the best of our knowledge, this is the first RCT to examine the concomitant effects of TRF and protein supplementation on outcomes of well-being.

Current literature suggests that the implementation of protein in a diet increases mood by improving functions of neurotransmitters (70). Furthermore, no literature exists examining the effects of protein consumption on specific mood components in conjunction with TRF. However, a case control study conducted with older Italians identified a decrease in signs of mental health distress in those who exhibited TRF patterns (71). Contradictory to those findings, results published by Anton et al. (72) who investigated older adults for a 4 week period of TRF did not find statistical significance in well-being via Health Related Quality of Life Scales. Parallel to those results, we found that there was only a significant difference in treatment for tension-anxiety sub scores of the POMS, with no other major differences in preliminary results. However, after controlling the data for baseline, we did find a significant difference in TMD and anger-hostility which decreased across the study in both groups. Consequently, the results could have been significant with a larger treatment group. Therefore, further research needs to be conducted to fully evaluate TRF while consuming protein supplementation on mood.

In addition to mood, little to no literature exists on TRF with the effect of protein supplementation on sleep, moreover increased protein consumption is not consistently linked with better sleep outcomes (47). Some studies indicate that increased protein consumption can

lead to better sleep quality. For example, Sutanto et al. (73) found an association between dietary tryptophan levels and sleep quality. Conversely, in a study conducted by Kim et al. (74) there were no significant differences in structure of sleep in a 4 week TRF intervention in metabolically healthy adults among a Korean version of the PSQI. Similarly, in our study the results from the PSQI did not yield significance. The only significant results identified were in the sleep latency category, with significantly lower sleep latency in the control group. After controlling for baseline, we identified that there was an increase in the control group compared to the protein group at week 4 and week 8 with no significant differences at week 12. Trends were also identified in GSS, and after controlling these results for baseline, there was a significant improvement in the protein group compared to the control group at week 8 with no differences at week 4 or week 12. Therefore, subjective sleep quality was not improved by the intervention in this study.

In addition to our findings of subjective sleep quality, objective sleep quality measured by wrist accelerometers found some differences between the protein and control group. Over the duration of the study, there were identified differences in total sleep time and total minutes in bed with the protein group spending significantly less time in bed at week 12 versus week 1 compared to the control group. Moreover, as total sleep time and total minutes in bed decreased in the protein group, the sleep efficiency remained stable. Although not statistically significant ($p=0.30$) the control group had a mean decrease of 9.9% compared to a 0.6% decrease sleep efficiency in the protein group which may have led to increased sleep time in the control group. However, due to the lack of significance a link cannot be established and further research is needed (75, 76).

Lastly, dietary intake between groups was the same except for protein consumption. Within the control group, the average protein consumption was 15.5% where the protein group consumed 20% of calories from protein. Previous studies investigating a high protein diet consumed between 25-35% of protein therefore the protein supplement consumption may have not been adequate to see results (32, 34). There were no significant differences in other dietary components between groups eliminating aspects of diet as confounding variables.

Although these results did not support our hypothesis that TRF while consuming protein would improve markers of sleep and mood this study presented many strengths. This research is the forefront of examining a popular form of intermittent fasting while altering macronutrient consumption adding to its relevance to the field. This study allowed participants to eat ad libitum so the effect of caloric restriction in TRF was not a confounding factor. Additionally, both subjective and objective sleep were measures strengthening the results. Currently, few studies exist examining the effects of TRF with supplementation. Therefore, this study develops a baseline for future research in the field of TRF. Specifically, more research on the impacts of TRF and protein consumption on sleep and mood would be beneficial.

There were also some limitations that could have impacted the results. First, our sample size was low because it was hard to recruit metabolically healthy participants, especially post COVID-19. For future studies, it could be beneficial to eliminate some of the inclusion criteria to recruit more individuals. Group differences between BMI and weight at the beginning of the study was another restriction. Also protein intake was not significant between groups as expected. Furthermore, participants may have had similar eating schedules to the TRF pattern before the study or consumed similar number of calories during the TRF period, making the

results less notable. There may also be a need to increase the percentage of of calories consumed from protein to 25%-35%.

Conclusion

As the problems of obesity and its impacts on sleep and mood shape our current society, it has become necessary to turn to dietary interventions as means to improve overall well-being. While some aspects of the study demonstrated significance and with others being insignificant, it is necessary to pursue future research in this field to improve the lives of those who face these problems. Protein consumption and TRF may have the potential to impact markers of well-being.

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Tables

Table 1. Participant Characteristics at Baseline

	Control		**Protein**		**P- Values**
Sex	Female (n=7)	Male (n=2)	Female (n=7)	Male (n=3)	
Age	39.14±7.69	32.5±3.54	38. 86±5.93	25.7±2.31	0.45
BMI	29.13±3.04	27.10±1.84	35.0±8.42	34.2±8.29	0.04
Body Weight (lbs.)	175.43±3.04	181±8.49	226.75±65.72	238±51.29	0.03
Height	64.71±1.60	68.00±0.00	65.5±3.23	70.07±5.11	0.30
Ethnicity					
African American	1	0	0	0	
Caucasian	6	1	6	2	
Hispanic	0	0	0	1	
Asian	0	1	1	0	
PSQI					
Global Sleeping Score	7.00±2.78		5.80±2.30		0.33
POMS					
Total Mood Disturbance	30.00±24.64		16.80±19.84		0.21
Actigraphy					
Sleep Latency	2.52±1.87		1.63±1.14		0.24

Table 1. Participant Characteristics

[1] All baseline and 12-week values are means ± SD. P-value <0.05 indicates significance.

Actigraphy

Table 2. Effects of the 12-week dietary supplementation intervention on objective sleep duration and quality in the dietary intervention and control group[1].

	0	12 wk	Group	Time	Group X time
7-day ActiGraph					
Sleep latency, min			0.53	0.48	0.27
CON	2.52±1.87	2.65±3.31			
PRO	1.66±1.14	2.26±2.70			
Sleep efficiency, %			0.64	0.41	0.84
CON	91.77±2.28	81.82 ± 30.47			
PRO	90.80±3.53	90.19±5.35			
TST, min			0.59	0.22	0.01
CON	350.41±68.53	390.13±64.18			
PRO	411.67±100.63	355.52±105.74			
Awakenings, #			0.12	0.38	0.12
CON	11.42±4.00	12.19±2.57			
PRO	12.03±5.65	9.99±4.02			
Average Awakening Length			<0.01	0.06	0.62
CON	2.18±0.45	2.58±0.67			
PRO	3.32±0.68	3.97±1.30			
WASO			0.12	0.85	0.31
CON	24.94±10.31	29.35±9.55			
PRO	39.81±18.52	36.81±23.56			
Total Minutes in Bed			0.56	0.72	0.02
CON	377.87±74.98	422.12±68.05			
PRO	453.14±109.08	394.58±120.21			

[1] All baseline and 12-week values are means ± SD. Control n=9; whey protein isolate, PRO, n=10; WASO denotes time from sleeping to first period of wakefulness**;** "Sleep latency" denotes time from "lights out" to "fell asleep"; "Sleep efficiency" (%) denotes the proportion of time spent asleep of time in bed (100% × sleep duration/the time between bed time and get up time). TST, total sleep time; WASO, wake after sleep onset. P-value <0.05 indicates significance.

Dietary Intake

TABLE 3. Effects of a 12-week supplementation intervention on energy and macronutrient intake in dietary intervention and control groups[1]

	Weeks of Intervention						
Energy & Macronutrients	0	4	8	12 wk	Group	Time	Group X time
Energy, kcal/d					0.04	0.89	0.60
CON	1737.30±483.74	1543.00±375.77	1543.91±460.15	1619.78±691.72			
PRO	1954.87±638.94	1821.75±515.23	2015.96±495.15	1902.80±943.62			
Fat, g/d					0.23	0.78	0.99
CON	70.79±25.10	62.98±22.43	67.26±21.56	68.92±30.4			
PRO	80.82±68.14	68.14±30.27	78.73±27.84	76.70±43.86			
Carbohydrate, g/d					0.38	0.87	0.89
CON	205.76±63.21	177.57±46.02	175.78±60.49	185.50±87.31			
PRO	206.04±95.42	193.19±64.12	215.88±56.12	194.81±103.02			
Protein, g/d					<0.01	0.63	0.76
CON	66.76±19.77	63.05±12.34	65.07±19.64	64.84±43.03			
PRO	99.84±24.34	82.30±26.07	102.33±22.41	104.72±49.29			
Saturated Fatty Acids g/d					0.13	0.38	0.66
CON	22.21±8.64	20.43±7.82	21.39±8.44	24.80±10.68			
PRO	31.18±11.64	19.39±11.54	29.00±12.86	28.15±21.88			
Trans Fat g/d					0.70	0.08	0.66
CON	2.79±2.92	2.29±1.79	1.63±0.94	1.66±0.62			
PRO	3.25±1.91	1.57±1.15	2.14±1.11	2.01±1.08			
Omega 3 g/d					0.94	0.67	0.99
CON	2.08± 0.81	1.79±0.65	1.73±1.13	1.70±1.07			
PRO	2.18±2.08	1.64±0.86	1.80±0.93	1.77±0.98			
Fiber g/d					0.60	0.89	0.84
CON	16.92±7.22	14.14±5.23	17.03±7.38	14.89±7.46			
PRO	15.91±7.71	17.73±6.49	17.44±5.76	15.75±10.12			

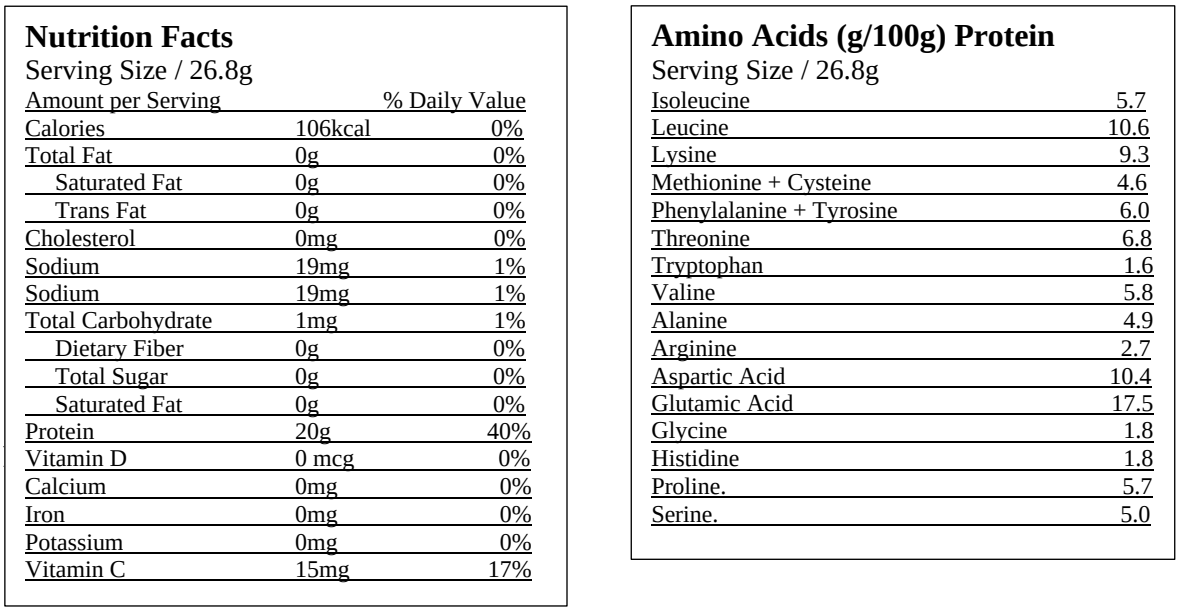
Tryp mg/d					0.09	0.12	0.18
CON	1.63±1.63	0.75±0.16	0.76±0.23	0.77±0.57			
PRO	1.31±0.42	1.09±0.34	1.34±0.31	1.35±0.66			
Niacin, g/d					0.37	0.78	0.91
CON	19.50± 7.23	17.87± 4.44	19.16± 8.33	19.08±11.41			
PRO	20.32± 6.96	17.96± 11.08	23.24±5.53	22.44±15.20			

[1] Values are mean ± SD. Control, no intervention CON, n=9; whey protein isolate, PRO, n=10;

[2] Treatment effect between groups were tested by one-way ANOVA with baseline measurements subtracted from 16-week values. P-value <0.05 denotes significant differences: * $P < 0.05$

Figures

Figure 1: Protein supplement nutrition breakdown of macronutrients, micronutrients, and individual amino acids.



Nutrition Facts

Serving Size / 26.8g

Amount per Serving		% Daily Value
Calories	106kcal	0%
Total Fat	0g	0%
Saturated Fat	0g	0%
Trans Fat	0g	0%
Cholesterol	0mg	0%
Sodium	19mg	1%
Sodium	19mg	1%
Total Carbohydrate	1mg	1%
Dietary Fiber	0g	0%
Total Sugar	0g	0%
Saturated Fat	0g	0%
Protein	20g	40%
Vitamin D	0 mcg	0%
Calcium	0mg	0%
Iron	0mg	0%
Potassium	0mg	0%
Vitamin C	15mg	17%

Amino Acids (g/100g) Protein

Serving Size / 26.8g

Isoleucine	5.7
Leucine	10.6
Lysine	9.3
Methionine + Cysteine	4.6
Phenylalanine + Tyrosine	6.0
Threonine	6.8
Tryptophan	1.6
Valine	5.8
Alanine	4.9
Arginine	2.7
Aspartic Acid	10.4
Glutamic Acid	17.5
Glycine	1.8
Histidine	1.8
Proline.	5.7
Serine.	5.0

Figure 2: Test day schematic

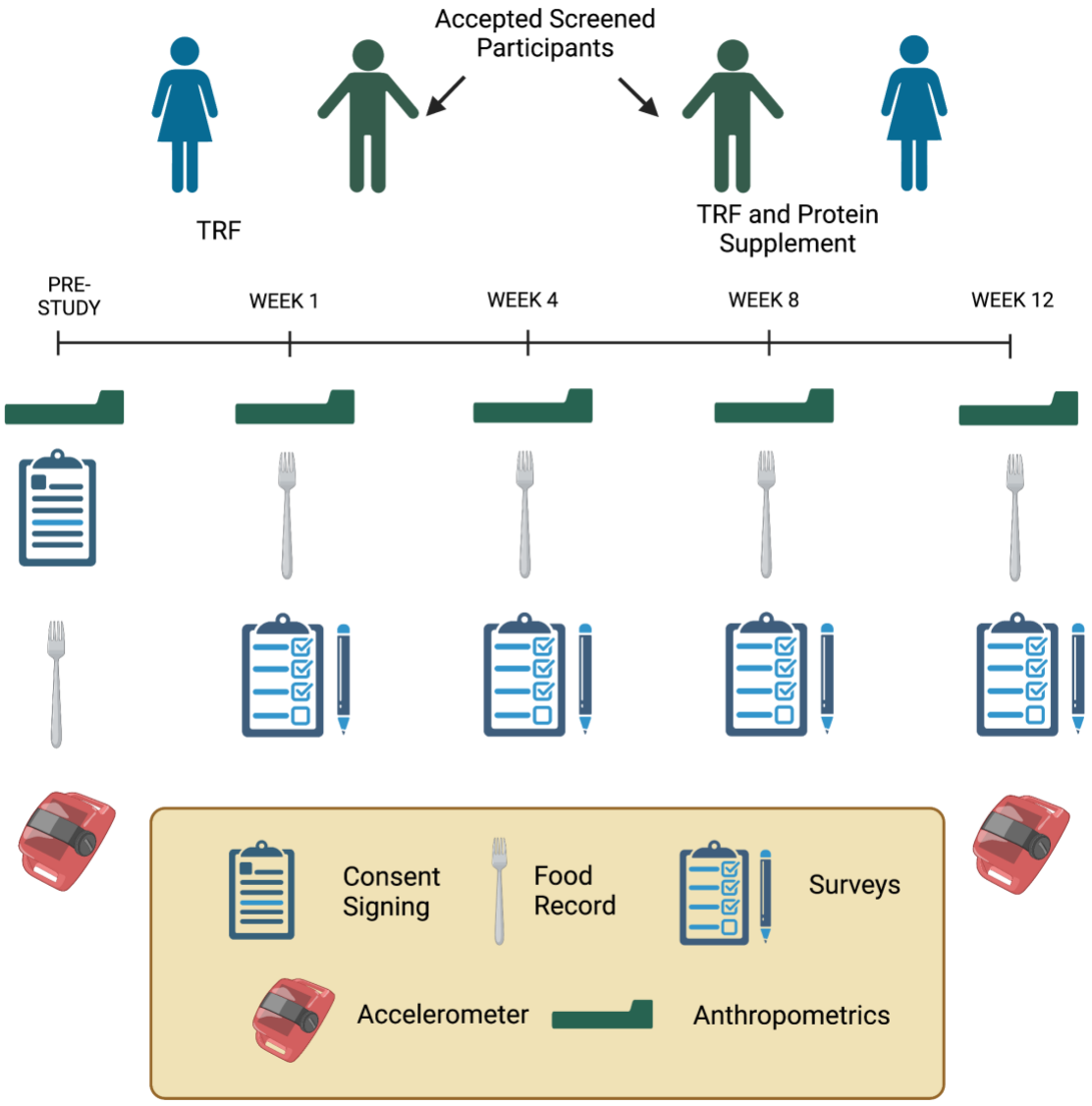


Figure 3: Recruitment process and analyzation of participants

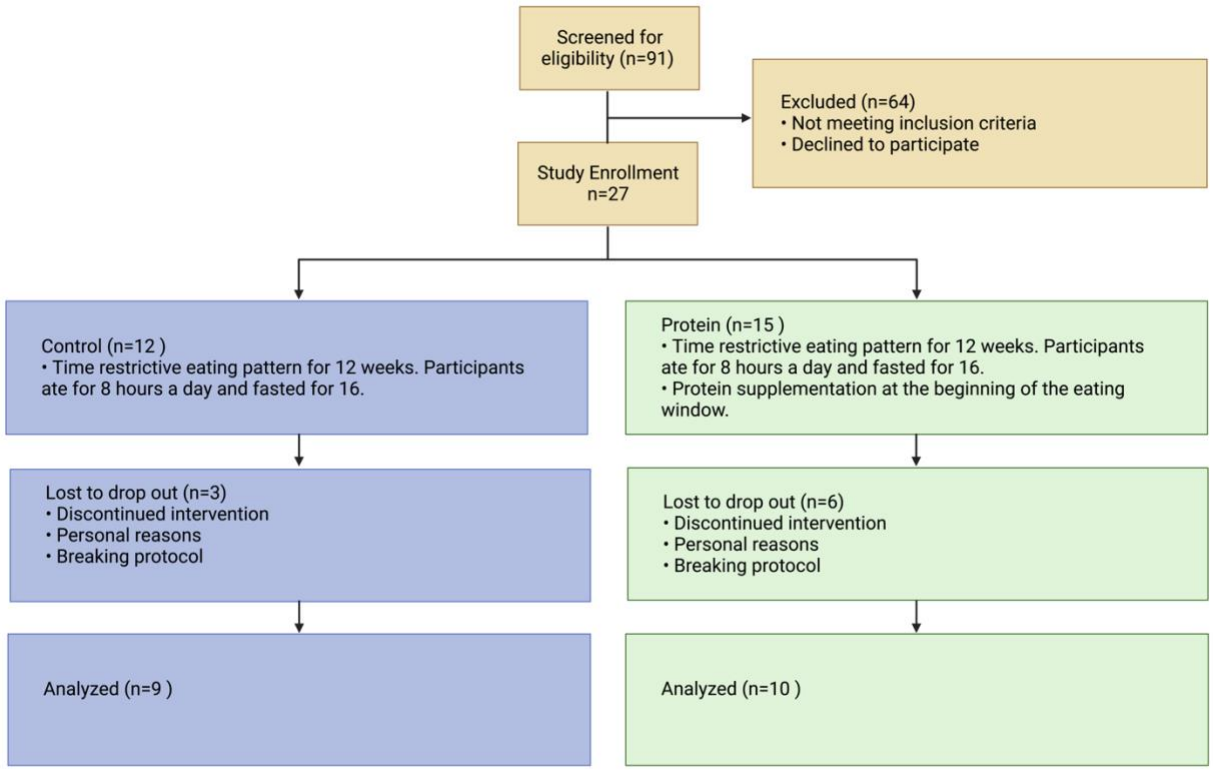


Figure 4. POMS Scores

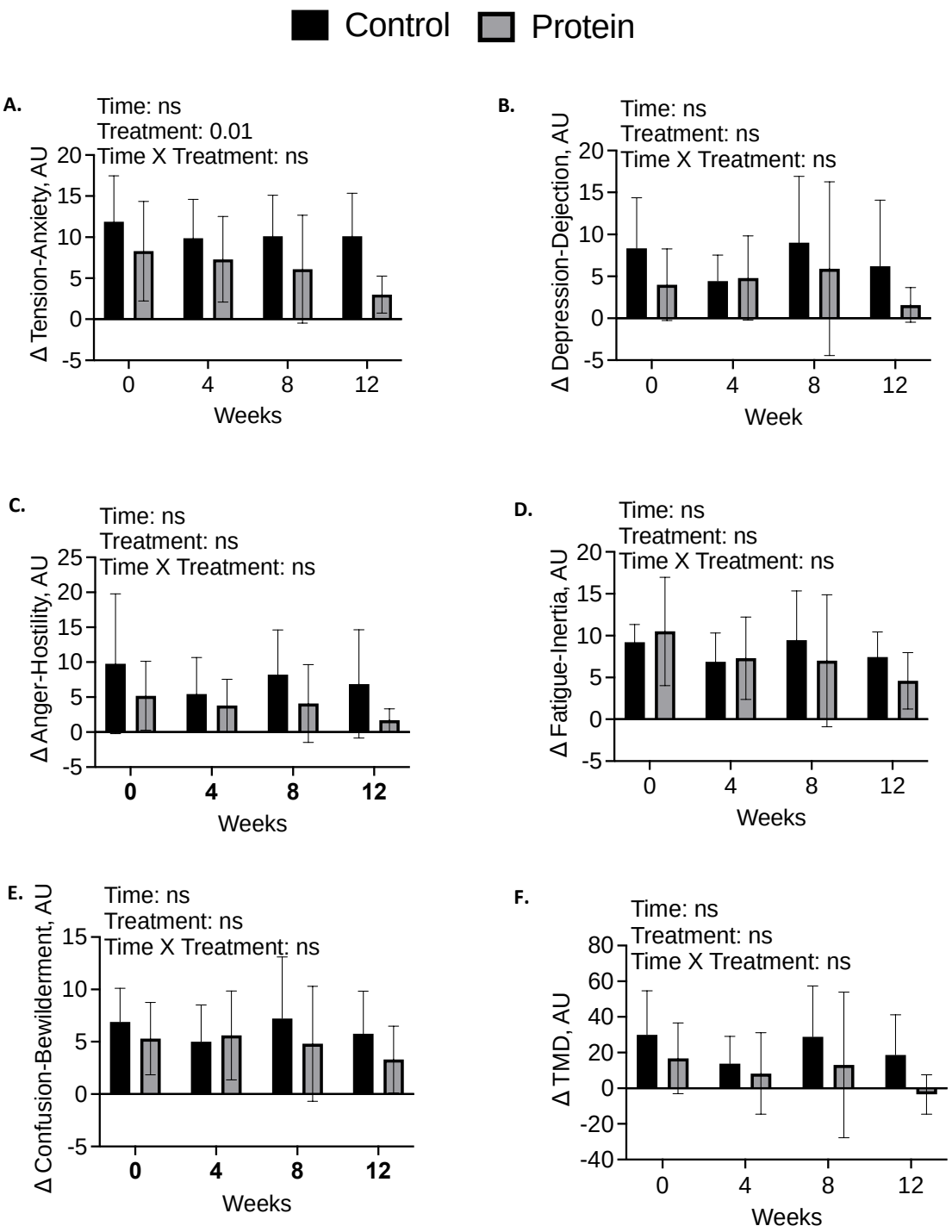


Figure 3 (A.) The mean tension-anxiety score of control and treatment groups. Significance was found for treatment (p=0.0124) **(B.)** The mean depression-dejection score of control and treatment groups. **(C.)** The mean of anger-hostility of control and treatment groups. **(D.)** The mean fatigue-inertia of control and treatment groups. **(E.)** The mean confusion-bewilderment of control and treatment groups. **(F.)** The mean total mood disturbance (TMD) of control and treatment groups.

Figure 5. PSQI Scores

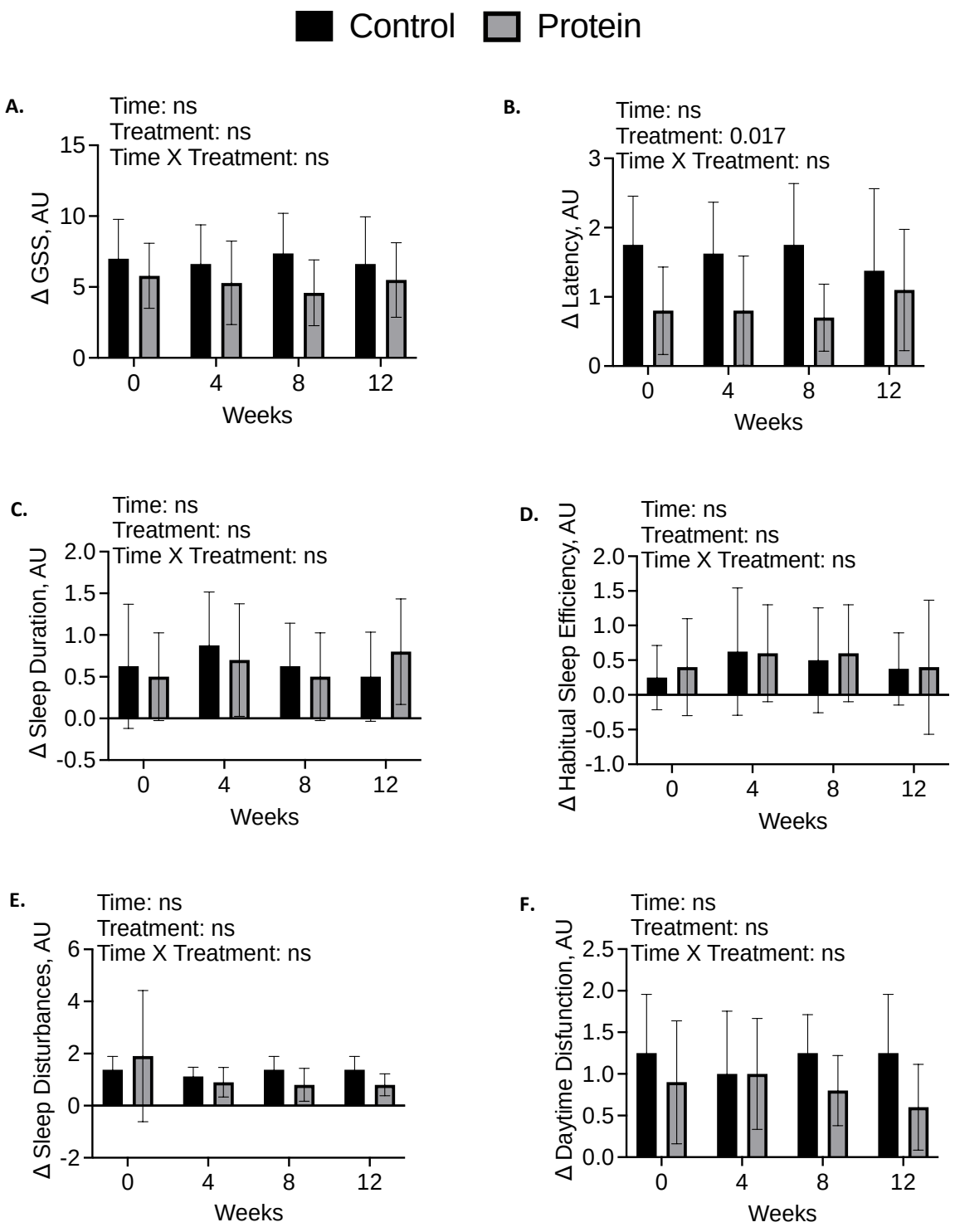


Figure 4 (A.) The mean PSQI Global Sleeping score of control and treatment groups. **(B.)** The mean sleep latency score of control and treatment groups. **(C.)** The mean sleep duration of control and treatment groups. **(D.)** The mean habitual sleep efficiency control and treatment groups. **(E.)** The mean sleep disturbances of control and treatment groups. **(F.)** The mean total daytime dysfunction of control and treatment groups.

Appendices

Appendix1: IRB Approval Letter 12/23/2019

UNIVERSITY OF ARKANSAS

To:	Jamie I Baum FDSC N2216
From:	Douglas James Adams, Chair IRB Committee
Date:	12/23/2019
Action:	**Approval**
Action Date:	12/23/2019
Protocol #:	1912236045
Study Title:	Time Restricted Feeding Intervention for Muscle and Metabolic Health (TRIMM)
Expiration Date:	12/10/2020
Last Approval Date:	
Risk Level:	No greater than minimal risk.

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: Jennifer Celene Veilleux, Key Personnel
Lindsey Susan Aloia, Key Personnel
Samuel Preston Belt Walker, Key Personnel
Aubree Leigh Hawley, Key Personnel
Angela M Tacinelli, Key Personnel