5-2022

The Impact of Dietary Protein Supplementation as Part of a Time Restricted Feeding Eating Pattern on Sleep, Mood, and Body Composition in Adults with Overweight or Obesity

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The Impact of Dietary Protein Supplementation as Part of a Time Restricted Feeding Eating Pattern on Sleep, Mood, and Body Composition in Adults with Overweight or Obesity

A thesis submitted in partial fulfilment
of the requirements for the degree of
Master of Science in Food Science

by
Rebecca L. Bowie
University of Arkansas
Bachelor of Science in Food Science, 2020

May 2022
University of Arkansas

This thesis is approved for recommendation to the Graduate Council.

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ABSTRACT

Obesity in the United States continues to increase, and effective, long-term weight loss strategies are limited. Time restricted feeding (TRF) is a dietary intervention that has potential to serve as an effective long-term weight loss strategy, however more data is needed to establish the effectiveness of a TRF eating pattern on obesity and its related health outcomes. To our knowledge, manipulation of macronutrient composition during TRF intervention and the effect of TRF on sleep and mood have not been studied. Therefore, the objective of this study was to determine the effect of protein supplementation during TRF on mood, sleep, and metabolic health in overweight and obese adults. Overweight and obese men and women (36.6±7.3 years; BMI: 32.8±6.5) participated in this randomized, controlled 12-week TRF dietary intervention (8 hour eating window with a 16 hour fast). Participants were allocated to one of two groups: 1) control, TRF (n=7) 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. Anthropometrics, sleep (via Pittsburgh Sleep Quality Index Global Sleeping Score; PSQI GSS), total mood disturbances (TMD; via Profile of Mood States (POMS) including six affect states of depression, fatigue, anger, tension, confusion, and vigor subscales), appetite (using visual analog scales), and weighed 3-day dietary intake records were assessed at 0, 4, 8, and 12 weeks. Sleep was also measured at 0 and 12 weeks via wrist Actigraphy. Body composition was measured via DXA at 0 and 12 weeks. Data was analyzed using two-way ANOVA to assess the relationship between interventions and within intervention at each time point. Overall, both interventions improved total body fat percentage (p <0.05) and fat mass to fat-free mass ratio (p < 0.05) over 12 weeks. There was a significant difference in mood states of tension and confusion between intervention groups (p <0.05) and the protein group total mood disturbance was significantly different (p

<0.05) between week 1 and 12. However, there was no change in mood within the control group over the 12 week intervention period. Sleep, self-reported via PSQI, did not change between week 1 and week 12 within or between each of the dietary interventions. This pilot study suggests that protein supplementation during TRF has the potential to improve body composition by improving fat mass to fat free mass ratio in overweight or obese individuals. However, additional research is needed with a larger sample size to determine the long-term effect of protein supplementation in combination with TRF on mood, sleep, and body composition.

ACKNOWLEDGEMENTS

I would like to thank Dr. Jamie Baum for her guidance and mentorship towards this degree, as it has allowed me to grow both personally and professionally throughout my time here. I would also like to thank Mary Komp, Ada-Miette Thomas, Sydney Boudrey, Hannah Taylor, Brooke Martin, and Grace Martin. Without their collaborative efforts and dedication, this project would not have been possible. Lastly, I would like to thank Dr. Elisabet Børsheim, Dr. Erin Howie-Hickey, and Dr. Xinya Liang for serving on my thesis committee and their continued support and understanding throughout my degree.

DEDICATION

This thesis is dedicated to my father, Christopher Malone Bowie, and to the numerous family and friends that continue to support me. Thank you for your unyielding encouragement in all of my endeavors.

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CHAPTER 1: LITERATURE REVIEW

Prevalence and Impact of Obesity in the United States

Roughly half of adults in the United States adults were obese between 2017 and 2018, and another 31.1% were classified as overweight (CDC, 2018). By 2030 nearly 1 in 2 adults will have obesity and nearly 1 in 4 adults are projected to have severe obesity (BMI > 40) in the United States. (Ward et al, 2019). Obesity is associated with an increased risk for metabolic diseases such as cardiovascular disease, diabetes, and hypertension. Obesity also increases the risk of complications such as joint replacements, pancreatitis, several types of cancer, depression, and insomnia (Patterson, 2004). Popular treatments for obesity have focused on pharmacological, surgical, and lifestyle interventions.

Lifestyle interventions, including changes in diet and physical activity, have been the first-line therapy in efforts to combat obesity and its associated chronic diseases (Jebeile, 2019). However, these interventions have had limited success and follow-up of long-term weight loss studies have indicated that most patients return to their baseline weights within 3-5 years following the end of treatment (Brownell, 1987). This suggests that an improved intervention strategy for weight loss is needed (Chaix, 2014). Long-term success in maintaining weight loss continues to be a challenge. A meta-analysis of long-term weight loss maintenance of twenty-nine studies suggests that the average individual maintains a weight loss of greater than 3 kg and a reduced body weight of at least 3% of initial body weight five years after completing a structured weight loss study (Anderson, 2001). However, these numbers need to be improved to increase weight loss, management, and maintenance to reduce the rates of obesity in the United States (Lean, 2006).

Overconsumption of calories can lead to weight gain over time (Lluch et al., 2000; Pothos et al., 2009; Sung et al., 2009; van Strien et al., 2012). A contributing factor to overeating is the inability to adequately estimate portion sizes (Davis et al, 2007). Inability to consume proper portion sizes can result in excessive eating and negatively impact weight loss attempts (Davis et al, 2007). The average American self-reports eating over 12 hours throughout the day (Kant, 2014). Overeating results in increased weight gain and is a leading cause of obesity (Hall, 2017). Reducing the occurrence of overeating has the potential to increase weight loss. A specific type of intermittent fasting called time restricted feeding (TRF) has the potential to reduce occurrences of excessive eating by providing a rigid time frame that ranges from 8-10 hours and fasting for 14-16 hours per day (Jamshed et al, 2019).

TRF provides a potential solution as a weight loss strategy for individuals with obesity (Chaix, 2014; Melkani, 2017; Hatori et al., 2012). TRF refers to ad libitum energy intake within controlled time frames (Rothschild et al, 2014). Based on a review from 2021, 13 clinical trials have been conducted using TRF, with eating windows ranging from 4 to 10 hours (Varady et al, 2021). TRF has been shown to significantly improve cardiovascular disease risk markers such as triglycerides, total cholesterol, and LDL cholesterol, as well as decrease fat mass and fasting plasma glucose concentrations in overweight and obese adult participants who follow TRF eating patterns for at least 10 weeks (Che et al, 2021; Antoni et al, 2018; Sutton, 2018; Ravussin, 2019). It is assumed that eating earlier in the day may be superior to eating later, specifically in relation to the diurnal variations in glucose tolerance (Jarrett et al.1972; Van Cauter et al.1997), however there are few studies directly comparing early- versus late-TRF (Hutchison et al.2019; Lynch et al.2021). Although there is a lack of data in human participants, there have been dozens of studies using animal model to determine the effectiveness of TRF as a dietary intervention.

Through findings from animal studies, TRF is also thought to prevent the adverse effects of high-fat diet-induced metabolic diseases without altering caloric intake or nutrient composition (Hatori et al., 2012). These findings have encouraged further research related to the impacts of TRF on metabolic health and the ability provide a more achievable intervention for all lifestyles and dietary preferences. Additionally, while there have been promising findings from dietary interventions using TRF (Gill & Panda 2015; Gabel et al.2018; Cienfuegos et al.2020; Chow et al.2020; Wilkinson et al.2020), the role of specific macronutrient intake during TRF has not been investigated. TRF is still a relatively new eating pattern and data in humans is limited. Whether TRF will yield increased benefits when paired with changes in macronutrient composition, such as increased dietary protein intake is unclear and requires further investigation.

Diets higher in protein and lower in carbohydrate have been shown to improve body composition by preserving skeletal muscle mass and targeting fat mass loss during negative energy balance, while increasing satiety (Westerterp-Plantenga, 2012; Astrup et al, 2015). Subjective measurements of satiety after meals or foods also showed that protein is more satiating than fat or carbohydrate (Latner, 1999; Porrini et al, 1997; Rolls et al, 1988) and that it delays the return of hunger (Marmonier et al, 2000). The Daily Reference Intake Macronutrient Report defines an acceptable range of protein intake for adults to be between 10–35% of energy intake, indicating that there is potential for safe usage of increased protein as a dietary intervention. The potential benefits of protein when used in conjunction with TRF is unknown. The objective of this thesis chapter is to summarize the current scientific TRF literature and discuss the potential role of dietary protein intake during TRF for the prevention and management of obesity and related metabolic diseases.

Current Status of Knowledge

Time restricted feeding and intermittent fasting (IF) are often used interchangeably, however there are differences in the eating patterns between TRF and IF. Intermittent fasting is an umbrella term that refers to several different interventions that focus on feeding restrictions (Templeman et al, 2020). The first beneficial findings of unspecified IF suggesting IF improved apparent lifespan in littermate rats were published in 1946 (Carlson & Hoelzel, 1946). Use of IF as a weight loss strategy has increased in popularity over the past decade and has been shown to result in weight loss and improved metabolic health markers, comparable to standard daily calorie restriction (Barnosky et al, 2014). A 24-week trial using IF found up to a 7% reduction of body weight in obese women accompanied by improved LDL cholesterol levels (Harvie et al, 2011). Though these results show improvements in metabolic health, IF encompasses a broad spectrum of dietary interventions and the investigation into the application of specific IF approaches is limited (Templeman et al, 2020).

Ultimately, IF has been shown to yield similar results to continuous calorie restriction for both weight loss and improvement of cardiovascular risk factors, such as blood pressure and triglyceride concentrations (Sundfør et al, 2018; Harris et al, 2018; Harvie et al, 2011). In addition, in comparison to continuous calorie restriction, feelings of hunger are more pronounced during IF (Sundfør et al, 2018). While IF may not have significant weight loss reduction on its own, there is evidence that when IF is combined with exercise, results in significant improvements in body weight, body composition, and lipid indicators of heart disease than either exercise or IF produce individually (Bhutani et al, 2013). Therefore, there is potential that the effects of IF could be improved when combined with other weight loss strategies. A limitation to IF is subjects adhering to fasting for a duration of at least 24 hours in most interventions. A

recent meta-analysis of IF versus continuous eating restriction indicated that the adherence rate to IF was inconclusive (Enriquez et al, 2020). Long term adherence is the most important factor the success of a diet and the overall health benefits (Middleton et al, 2013). Due to the severity of several IF interventions, TRF has emerged as an alternative to traditional IF and may encourage increased compliance (Templeman et al, 2020). In a clinical trial conducted by Wilkinson et al. (Wilkinson et al, 2020), 63% of the participants were engaged in TRF dietary intervention 16 months after the end of the 12-week intervention. TRF refers to when eating is limited to a certain number of hours per day. For example, an 8-hour eating period could be from 10:00 am - 6:00 pm or from 12:00 - 8:00 pm. This same schedule would be repeated every day for a predetermined duration of days. In contrast, IF regimens generally require subjects to fast for 1–3 days consecutive per week. Due to the simplicity and shorter fasting periods, TRF could yield increased benefits in comparison to IF.

Time restricted feeding is a type of IF that does not restrict calories and requires a specific eating period each day. The mechanism through which TRF may improve body weight regulation is that of an extended fasting duration. Similar to IF, extending the daily fasting duration likely promotes the mobilization of free fatty acids, increases fat oxidation, and increases the production of ketones which results in potential weight loss (Hatori et al, 2012). Following the fed state, the body enters the post-absorptive state which spans 8-12 hours after the last meal consumed. The post-absorptive state has been hypothesized to more readily burn fat due to reduced insulin levels (WH Freeman & Co., 2002).

The potential for TRF to increase weight loss has led to its increased popularity over the last several years. TRF limits the food consumption period which, in theory, should lead to fewer calories consumed, resulting in weight loss. While TRF does result in reduced calorie intake for

some (Rothschild 2014), other studies have shown participants do not eat fewer calories during TRF compared to their pre-intervention diet (Nematy, 2012). Dietary intervention studies in this area also depend on self-reported food diaries from participants, which can leave room for human error such as under-reporting daily food intake. However, the ability to eat *ad libitum* makes this intervention appealing for current societal behaviors.

Health Benefits of Time Restricted Feeding

TRF and Weight Loss

Recently, TRF has been shown to protect against excessive body weight gain without effecting caloric intake irrespective of dietary intake, time schedule, or initial body weight (Adafer et al, 2020). A meta-analysis of TRF studies through January 2019 found participants following TRF dietary patterns achieved greater weight loss compared to participants that did not follow TRF (Pellegrini et al, 2020).

A systematic review of TRF effects in human interventions found the overall adherence rate to TRF was 80%, with a 20% unintentional reduction in caloric intake, making this a promising approach for long-term weight loss and maintenance. Most of the studies in this review ranged in intervention lengths from 8-12 weeks. On average, TRF induced weight loss of 3%. A randomized, placebo-controlled trial involving resistance trained females showed a fat mass reduction of 4%-7% without any caloric restriction (Tinsley et al, 2019). Interestingly, TRF produced beneficial metabolic effects such as improvement to glucose tolerance, insulin resistance, and triglyceride levels, independently of weight loss (Adafer, 2020). Based on the findings described above, TRF appears to be a well-tolerated dietary pattern that is easy to follow, resulting in many beneficial health effects, suggesting that TRF could be sustainable

long-term dietary intervention. Despite the recent growth in popularity for TRF regimens, there is limited peer-reviewed, scientific literature for randomized, clinical trials using TRF. Animal studies have shown promising evidence supporting the success of TRF as a nonpharmacological strategy against obesity and associated diseases. A randomized rodent trial assigned isogenic, meaning the same or closely similar genotype mice to either a diet of standard composition or one with high-fat content under two food-access paradigms: ad lib or time-restricted access for more than 100 days. TRF improved metabolic and physiologic rhythms, and protected the mice from the adverse effects of a HFD. The time-restricted high-fat-fed mice showed significantly increased thermogenesis and improved rhythms in nutrient utilization, leading to reduced adiposity and liver steatosis, normal glucose tolerance, reduced serum cholesterol, increased bile acid production, and improved motor function. (Chaix, 2012). Hatori et al also suggested that TRF improved metabolic and physiologic rhythms and protected the mice from the adverse effects of a high-fat diet (Hatori et al, 2012).

TRF and Body Composition

Body composition describes the amount of fat, bone, water, and muscle in the body. Evidence suggests that maintaining a healthy body composition increases longevity and reduces the risk of heart disease, cancer, and diabetes (Chow et al, 2020). An 8-week randomized clinical trial using a single blind design of a TRF group versus a standard diet group in combination with resistance training. The TRF participants demonstrated a decrease in fat mass for the compared to the standard diet group and fat-free mass was maintained for both groups (Moro, et al. 2016). Another clinical trial of an 8-hour feeding window for TRF intervention study found, when compared to the non-TRF treatment reduced fat mass by 4.0%, lean mass by 3.0%, and visceral

fat by 11.1% over a span of 12 weeks (Chow, et al., 2020). Additionally, a randomized controlled trial found evidence of improved body composition during TRF over a four- week study period of elite cyclists. TRF reduced body weight (- 2%; $p = 0.04$) and fat mass percentage (- 1.1%; $p = 0.01$) with no change in fat-free mass. These findings suggested that a TRF program with an 8-h feeding window elicits weight loss, improves body composition in elite cyclists (Moro et al, 2020).

Bone mineral density has not been thoroughly investigated within TRF. A 12-week study of overweight adults suggested that the treatment time interaction for bone mineral content (BMC) was significant, such that BMC increased in the TRF group and decreased in the control group and does not adversely impact bone turnover (Lobene et al, 2021).

Time restrictive feeding has been minimally studied for its impact on skeletal muscle mass. Utilizing a randomized cross design, a study investigated skeletal muscle and serum metabolic and transcriptomic profiles from 11 men with overweight or obesity after TRF (8 hours) and extended feeding (15 hours). This study found that TRF increased the amplitude of oscillating muscle transcripts in comparison to extended feeding. However, muscle and serum metabolites did not increase, which provides evidence to suggest that TRF modulates the diurnal rhythm of lipid and amino acid metabolism (Lundell et al, 2020). Fat-free mass has been assessed in 11 randomized clinical trials which have had varying results. The impact of TRF on FFM is unclear with only one showing an increase during TRF (Tinsley et al, 2019). However, Tinsley et al. focused their study on the combination of resistance training and TRF which could account for differing body composition results than studies that did not use resistance training.

The limited findings analyzing body composition changes during TRF suggest a potential increased benefit for overall body composition when utilizing TRF. More research of how TRF impacts fat, bone and muscle needs to be conducted to develop more conclusive results.

TRF and Metabolic Disease

Metabolic disease is any disease or disorder that disrupts the process of converting food to energy on the cellular level (Enns, 2019). Metabolic diseases can lead to premature health complications such as heart disease, stroke, and type 2 diabetes (Enns, 2019). Diseases like obesity, arising from nutrient imbalance or excess, are often accompanied by disruptions of multiple pathways in different organ systems (Chaix et al, 2014). The benefits from TRF on metabolic disease support the use of TRF as a dietary intervention. Several studies have indicated that the beneficial effects observed on improvement of metabolic health markers is stimulated by weight loss and fat loss (Antoni et al, 2018; Smith et al, 2017; Gabel et al, 2018; Gabel et al 2019; Kesztyüs et al, 2019; Wilkinson et al, 2020; McAllister et al, 2020). Adiponectin has been identified as playing a key role in metabolic effects such as the loss of fat mass (Zhou et al, 2009), promotion of glycemic and lipid metabolism (Cnop et al, 2003; Higashiura et al, 2004), and a reduction in blood pressure (Baden et al, 2013). Adioponectin is also known to actively regulate the action of the circadian system (Hashinaga et al, 2012; Poggiogalle et al, 2018). Varady et al (2021) summarized that TRF has shown to improve cardiometabolic health by lowering blood pressure, insulin resistance, and oxidative stress.

A randomized animal trial using C57BL/6 male mice tested diverse nutritional challenges. The mice were randomized into either a normal chow diet, high sucrose diet, high fat diet showed that TRF attenuated metabolic diseases arising from a variety of obesogenic diets, and that benefits were proportional to the fasting duration when compared to the same diet

composition ad libitum. Additionally, protective effects were maintained even when TRF was temporarily interrupted by *ad libitum* access to food during weekends, a regimen particularly relevant to human lifestyle (Chaix, 2014).

Previous animal models have suggested that time-restricted high-fat-fed mice showed significantly increased thermogenesis and improved rhythms in nutrient utilization, leading to reduced adiposity and liver steatosis, normal glucose tolerance, reduced serum cholesterol, increased bile acid production, and improved motor function (Hatori et al, 2012; Sherman et al. 2012; Chaix et al. 2019). It has also been shown that TRF can reverse the progression of type 2 diabetes in mice with pre-existing diabetes (Chaix et al. 2014), reduce the rate of age-related cardiac deterioration in Drosophila (Gill et al. 2015) and contribute to improvements in markers of cardiovascular disease risk in rodents (Melkani & Panda 2017).

TRF and Sleep

Adequate sleep can lead to improved mood, boosted immune system, prevention of weight gain and other beneficial effects (Luyster et al, 2012). Sleep pattern impact on weight loss is a growing research area. Sleep restriction may adversely affect changes in body composition and "catch-up" sleep may not completely reverse it (Wang et al, 2018). Loss of only 1 hour per night five nights a week led to less proportion of fat mass loss in individuals undergoing hypocaloric weight loss, despite similar weight loss (Wang et al, 2018).

The circadian system represents all the physiological processes involved in a 24-h cycle, such as the sleep/wake cycle, blood pressure, heart rate, hormone secretion, cognitive performance, and mood regulation (Longo et al 2016). In 2019, adults with obesity (n = 23) followed an 8-hour TRF eating pattern for 12 weeks. Results from this study revealed Pittsburgh

Sleep Quality Index (PSQI) total score was below 5 at week 1 (4.7 ± 0.5) and week 12 (4.8 ± 0.7), indicating good sleep quality throughout the trial. Subjective measures of wake time, bedtime, and sleep duration remained unchanged. Findings from secondary analysis indicated that TRF does not alter sleep quality or duration in subjects with obesity (Gabel, 2019). A study indicated that TRF produced a significant and durable improvement of sleep quality and a 12-week single-arm trial evaluating TRF on 19 adults recorded an increase in sleep duration and efficiency in 84% of the participants (Gill et al, 2015). Improvement of sleep quality is known to be directly linked to the enhancement of the circadian system (Potter et al, 2016; Wright et al, 2015). These effects suggest that nutrition affects health not only through quantity or quality of the intakes, but also via the timing of food consumption according to the circadian clock (Adafer et al, 2020). The circadian system is regulated by a number of environmental stimuli such as food intake, light exposure and physical activity (Basolo et al, 2021). Growing evidence shows that the disruption of the circadian system is the cause of many metabolic pathologies like obesity (Arble et al, 2010; Longo et al, 2016). Greater understanding of TRF implications on the circadian system are needed to understand the mechanisms involved.

TRF and Mood

Mood can have overall impacts on productivity, desire to exercise, altered desire to eat and other physiological factors. There is little known regarding the impact of mood from TRF. The relevance of mood is demonstrated by describing some of the impacts on memory, task performance, helping behavior, socialization, self-concept, and health that have been observed (Hull, 1990). One notable mood regulating behaviors is food intake. The interaction between mood, emotional state, and feeding behaviors is complex and it is hypothesized that individuals

regulate their emotions and mood by changing both food choices and quantities (Singh, 2014). It is also apparent that mood can affect the self-rewarding mechanisms of food consumption (Morris & Reilly, 1987). Dopamine, which directly activates reward and pleasure centers, affects both mood and food intake (Cantello et al., 1989; Diehl and Gershon, 1992; Fochtmann and Fink, 1992; Black et al., 2002; Cawley et al., 2013), further supporting the link between psychology and eating behaviors (Singh, 2014).

Overeating and obesity are often associated with depression and anxiety in humans which has also been reported in animal models (Novick et al., 2005; Simon and Von Korff, 2006; Kloiber et al., 2007; Singh et al., 2007, 2009; Akubuiro et al., 2013; Patterson and Abizaid, 2013; Sharma and Fulton, 2013). Chronic consumption of calorie-rich foods ultimately leads to obesity which in turn promotes vulnerability to depression and anxiety (Novick et al., 2005; Simon et al., 2006; Kloiber et al., 2007; Sharma and Fulton, 2013). Conversely, there are findings showing that prolonged high-fat feeding leads to negative emotional states, increased stress sensitivity, and altered basal corticosterone levels (Sharma et al., 2012). Due to this link between mood and eating behavior, understanding the implications TRF will have on overall mood is important for a successful intervention.

TRF and Energy Expenditure

Energy expenditure refers to the amount of energy an individual uses in the form of calories and can be estimated by measuring macronutrient, oxygen consumption, heat production or carbon dioxide production. The most common way to measure energy expenditure involves the measurement of oxygen consumption and carbon dioxide production via indirect calorimetry (Hills et al, 2014). Total energy expenditure is comprised of multiple components including

physical activity energy expenditure, resting energy expenditure, and the thermic effect of food (Hill et al, 2014).

Limited research has been completed analyzing energy expenditure in combination with TRF. Comparison of a TRF group to a consistent meal-time group resulted in no significant difference between groups for respiratory quotient, resting metabolic rate, or total energy expenditure. However, observation was made that both groups had a significant decrease in total energy expenditure (Lowe et al, 2020). Additionally, Moro et al measured resting energy expenditure and oxygen uptake (VO2) were measured via a standard open-circuit calorimetry (max Encore 29 System, Vmax, Viasys Healthcare, Inc., Yorba Linda, CA, USA) on a breath-by-breath modality and estimated by the modified Weir equation during a study of TRF in elite cyclists. Resting energy expenditure decreased in both groups (main time effect $p = 0.054$; main diet effect $p = 0.060$) and to an apparently greater extent after the TRE diet ($\sim -10\%$) compared to ND diet ($\sim -2\%$) (Moro et al, 2020). More research surrounding energy expenditure and TRF must be conducted to further understanding of the relationship.

Dietary Protein

Dietary protein is the most satiating of the macronutrients during energy restriction and energy balance conditions (Halton, 2004). There is convincing evidence that a higher protein intake increases thermogenesis and satiety compared to diets of lower protein content (Halton, 2004). The weight of evidence also suggests that high protein meals lead to a reduced subsequent energy intake (Halton, 2004; Eisenstein et al, 2002). There has also been evidence that the differential appetitive responses following increased protein intake at breakfast compared to other meal times suggest that the satiating property of dietary protein is influenced by the timing

of protein consumption (Leidy et al, 2008). Additionally, evidence suggests that high protein diets support appetite control, differential improvements in body composition, and longer-term weight loss maintenance (Leidy et al, 2015; Wycherley et al, 2012). A meta-analysis showed that high protein diets mitigate the loss of lean body mass during weight loss (Wycherley et al, 2012), which is pivotal for maintaining resting energy expenditure (Ravussin et al, 1986). Since protein has the highest thermic effect of feeding out of the macronutrients, a meal that is containing higher protein should promote a negative energy balance (Raben et al, 2003; Johnston et al 2004; Westerterp et al, 1999).

A prior study using a high protein diet protocol for 12 weeks incorporated energy restriction on a high protein diet for six days and fasting for one day. The average loss of body weight reached 10% but when assigned to weight loss maintenance on either a higher carbohydrate diet or a higher protein diet, only the protein group was able to maintain weight loss (Zuo et al, 2016). An additional dietary protein study from 2017 looked into the effect of animal protein and plant protein in combination with physical activity. Animal protein intake had higher percentage of skeletal muscle mass regardless of activity, whereas plant protein only had beneficial effects on active subjects for percent skeletal muscle mass (Bradlee, 2017). These findings suggest that dietary protein does have beneficial effects when consumed without caloric restriction. The effect of a standard protein diet (SPD) was compared to a high protein diet (HPD) in a study from 2017. The findings showed that there was a significant difference in mean weight loss (SPD -5.8% vs. HPD -9.5%) after adjusting for baseline BMI. Also, both groups demonstrated significant decreases in waist circumference, glucose, insulin, triglycerides, and VLDL cholesterol, but there were no differences between the groups (Campos et al, 2017).

Epidemiological data in humans also link the quality and quantity of dietary proteins to long-term health (Soultoukis & Partridge, 2016). Dietary protein may also influence sleep by supplying the body with amino acid tryptophan, which is a potential sleep-promoting nutrient (Peuhkuri et al, 2012). A cross-sectional study has reported that the intake of protein is positively correlated with sleep duration, quality and pattern (Zhou et al, 2016). The impact of dietary protein on mood focuses on the level of serotonin, specifically, the brain concentration of tryptophan (Wurtman et al, 2003). Although there is no compelling evidence indicating that high-protein diets negatively impact the mentality and induce or exacerbate mental disorders, these diets have been linked to worsening of mood, increasing fatigue, dizziness, irritability, headaches, confusion, and sleep problems from small-scaled clinical observations (Lloyd et al., 1994; Wells and Read, 1996; Butki et al., 2003).

Dietary Protein and TRF

The potential combination of dietary protein with TRF is a minimally researched concept. One research area that has analyzed high protein TRF is sleep studies. A higher protein diet may provide a higher concentration of circulating tryptophan. Higher tryptophan concentrations should promote flux across the blood–brain barrier to induce serotonin and melatonin synthesis—both are thought to improve sleep (Chaput 2014; Peuhkuri et al, 2012; Halson, 2014; Bravo et al, 2013). A sleep study from 2020 suggests that eating the recommended or higher value of dietary protein is associated with improved perceived sleep quality and daytime sleepiness among adults with overweight or obesity (Hudson et al, 2020).

The findings that support the increased intake or supplementation of dietary protein suggest that there is potential that, in combination with TRF, the overall health benefits from

each will be observed in the participant. Theoretically, during the TRF eating window, increased protein will allow for the benefits from protein to be obtained while simultaneously gaining the benefits from TRF. This could result in a more efficient TRF and yield more overall benefits for the participants. More research is needed related to the manipulation of specific nutrients within TRF to fully analyze the potential advantages or disadvantages associated. Research analyzing overarching health implications and benefits when consuming increased dietary protein during a TRF intervention is essential to finding the optimal weight loss strategy.

Gaps in the Literature

In conclusion, the prevalence of adult obesity is a primary health concern. The occurrence of obesity increases risk for developing metabolic diseases and decreasing overall well-being and therefore requires attention. Finding potential methods to aid in reducing the prevalence of adulthood obesity is necessary. One potential method is utilizing a TRF eating pattern. Increasing dietary protein within the diet has been found to promote weight loss, regulate markers of metabolic health, increase satiety, increase energy expenditure, and decrease energy intake. Current literature focuses on the potential impact of TRF within adults. However, further research is still needed to understand the potential impact of dietary protein supplementation in combination with TRF in adults with overweight of obesity.

Therefore, the overall objective of this thesis was to determine the effect of dietary protein supplementation during a TRF eating pattern as a method to combat adulthood obesity.

The objectives of each study aim were:

Aim 1: Determine the effect of TRF and protein supplementation on mood and sleep in adults with overweight or obesity.

Aim 2: Determine the effect of TRF and protein supplementation on overall body composition in adults with overweight or obesity.

Aim 3: Determine the effect of TRF and protein supplementation to reduce risk factors for metabolic disease in adults with overweight or obesity.

We hypothesized:

Aim 1: TRF in combination with protein supplementation will improve overall mood and sleep in adults with overweight or obesity.

Aim 2: TRF in combination with protein supplementation will improve overall body composition by improving the ratio of fat mass (FM) to fat-free mass (FFM) in adults with overweight or obesity.

Aim 3: TRF in combination with protein supplementation will increase metabolic health by improving markers of metabolic health in adults with overweight or obesity.

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CHAPTER 2

Abstract

Objective: The objective of this study was to determine if increasing protein intake during a time restricted feeding eating pattern for 12 weeks influences mood and sleep, overall body composition by increasing the ratio of FM to FFM, or metabolic health by improving fasting glucose and lipid levels.

Methods: Overweight and obese men and women (36.6±7.3 years; BMI: 32.8±6.5) participated in this randomized, controlled 12-week dietary intervention. Participants were allocated to one of two groups: 1) control, TRF (n=7) 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. Anthropometrics, appetite (via visual analog scales), sleep (via Pittsburgh Sleep Quality Index Global Sleeping Score; PSQI GSS), and mood including total mood disturbances (TMD; via Profile of Mood States (POMS) including six affect states of depression, fatigue, anger, tension, confusion, and vigor subscales) were assessed at 0, 4, 8, and 12 weeks. Sleep was also measured at 0 and 12 weeks via wrist Actigraphy. Body composition was measured via DXA at 0 and 12 weeks. Blood samples were taken at weeks 0, 4, 8 and 12, however analyses has not been conducted at this time. Data was analyzed using two-way ANOVA to assess the relationship.

Results: Overall, both interventions improved total body fat percentage ($p<0.05$) over 12 weeks. Appetite had no change with the exception of both interventions having a significant reduction in desire to snack ($p < 0.05$). There was a significant improvment in tension and confusion mood scores($p<0.05$) and total mood disturbance was significantly improved ($p<0.05$) between week 1 and 12 within the protein intervention. However, there was no change in mood

with control group over the span of 12 weeks. Sleep, self-reported via PSQI, did not change from week 1 and week 12 within or between each of the interventions.

Conclusions: This pilot study suggests that protein supplementation during TRF has the potential to improve body composition in overweight or obese individuals. However, additional research is needed to determine the long-term effect of protein supplementation in combination with TRF on mood, sleep, and body composition. Analysis of blood samples as well as caloric intake needs to be assessed and could be utilized in future research.

Introduction

Overweight and obesity continue to be a major public health concern. It is predicted that by 2030 nearly 1 in 2 adults will have obesity and nearly 1 in 4 adults are projected to have severe obesity in the United States (Ward et al, 2019). Additionally, by 2025 globally, over 2.5 billion adults will be overweight, approximately one billion will be classified as obese (WHO, 2020; World Obesity Federation, 2020). Weight loss and weight management strategies include surgery, physical activity regimens, pharmacological interventions, and dietary changes (Jebeile, 2019). One weight loss intervention gaining popularity is intermittent fasting (IF) (Paoli et al, 2019; Longo & Panda, 2016; Brandhorst & Longo, 2019). IF can be defined as time periods of eating with time periods of fasting. There are three main categories of IF (Figure 1): 1) alternate day fasting (ADF), 2) the 5:2 diet, and 3) time restricted feeding (TRF). ADF generally refers to a feast day alternated with a fast day (Hoddy et al, 2014). The 5:2 diet, on the other hand, is a modified version of ADF that involves 5 feast days and 2 fast days per week (Varady et al, 2021). TRF, in contrast, differs from ADF and the 5:2 diet in that it requires individuals to fast for a period of time every day. TRF involves confining the eating window to a specified number

of hours per day, and fasting with zero-calorie beverages for the remaining hours of the day (Varady et al, 2021). Throughout all variations of intermittent fasting, *ad libitum* eating with no restrictions on quantity or type of food is observed during feasting times (Figure 2).

Many of the popular weight loss interventions have had limited success and long-term follow-up of weight loss studies indicates that most patients return to their baseline weights within 3-5 years after the end of treatment (Brownell, 1987). Time restricted feeding may be a potential long-term weight management strategy. Several indicators of overall health have been analyzed to gauge the success of TRF including body composition, cardiometabolic health markers, sleep, mood, and energy expenditure. However, the effect TRF in humans has not been well-studied,13 human clinical trials have been conducted using TRF eating patterns, with eating windows ranging from 4 to 10 hours (Varady et al, 2021). In addition, the TRF studies in published literature have not investigated the impact of macronutrient composition impact on obesity-related health outcomes.

The average American self-reports eating over 12 hours a day (Kant, 2014). Overeating has been shown to result in increased weight gain and is a leading cause of obesity (Hall, 2017). TRF reduces the potential for overeating by providing a rigid time frame that is 8-10 hours and fasting for at least 14 hours per day (Jamshed et al, 2019).

Additionally, protein has been shown to be the most satiating of all the macronutrients (Holt et al, 1995) and could therefore lead to reduced subsequent energy intake (Skov et al, 1999; Weigle et al, 2005; Halton, 2004; Eisenstein et al, 2002). Higher-protein diets have been suggested cardiometabolic risk factor improvements (Westerterp-Plantenga et al, 2012; Dong et al, 2013). Higher-protein *ad libitum* diets have led to unintentional weight loss caused from reductions in daily energy intake, which may have occurred as a result of increased satiety (Skov

et al, 1999; Weigle et al, 2005). There is suggestion that protein source plays a role in the overall success of high protein diets. In some protein focused research (Veldhorst et al, 2009; Hall et al, 2003), but not all (Bowen et al, 2006; Alfenas et al, 2010), studies the consumption of whey protein elicited a greater reduction in postprandial hunger and a greater increase in postprandial satiety than consumption of casein and/or soy.

The potential combination of dietary protein with TRF is a minimally researched concept. There is suggestion that dietary protein supplementation, when in combination with TRF, could positively impact the overall health benefits observed in ad libitum TRF programs. Theoretically, during the TRF eating window, increased protein will allow for the benefits from protein to be obtained while simultaneously gaining the benefits from TRF. This could result in a more efficient TRF and yield more overall benefits for the participants. More research needs to be done on the alteration of specific nutrients within TRF in order to fully analyze the potential advantages or disadvantages associated. Research analyzing overarching health implications when consuming increased dietary protein during a TRF intervention is essential to finding an optimal weight loss strategy.

The objective of this thesis was to determine the effects of TRF, in combination with protein supplementation, on body composition, cardiometabolic health markers, sleep, and mood. We hypothesize that the combination of protein supplementation with TRF will improve body composition by improving the ratio of fat mass to fat-free mass, cardiometabolic health markers, sleep, and mood in adults with overweight and obesity.

Methods

Participants. Male and female participants between the ages of 25 and 50 years were recruited to participate in this randomized, controlled study (n=16; 12 females and 4 males; age 36.6 + 7.3). Individuals who had food allergies, dietary restrictions (e.g., vegan, vegetarian), recently dieted (within the last three months), were taking medication that may impact study outcomes, had a body mass index (BMI) less than 25, or had any other diet-related conditions that would prevent them from consuming their assigned dietary intervention, were excluded from the study. In addition, individuals who used illicit drugs or consumed more than four alcoholic beverage per week, and/or were pregnant or breastfeeding were excluded from the study. The age range of 25 to 50 was chosen to encourage compliance and to potentially exclude changes to metabolism that occur with aging that could impact outcomes of this study.

Participants were recruited on a rolling basis and randomly assigned to one of two dietary intervention groups. A total of forty-one individuals were screened and twenty-one participants enrolled in the study (Figure 2). Twenty individuals screened did not meet the study criteria. Sixteen participants completed the study and were used for data analysis. Ethical approval for the study was obtained from the Office of Research Compliance Institutional Review Board of the University of Arkansas (Fayetteville, AR). This study was registered at clinicaltrials.gov (NCT04949451). Written consent was obtained from all participants prior to beginning the study.

Study Design. This was a randomized, controlled dietary intervention. Participants followed their assigned dietary intervention for 12-weeks. A time restricted feeding (TRF) model was used for this study, in which participants ate during a defined eight-hour period during the day and then fasted for the remaining 16 hours (Ravussin et al, 2019). The dietary interventions were as follows: 1) control, TRF without dietary modification (participants were advised to

follow their current dietary pattern ; (n=7; F: 5, M: 2; age: 37.1 + 7.8; BMI: 30.5 + 3.7); or 2) TRF plus whey protein supplementation (Lifeplus, Batesville, AR) at 25 grams (n=9; F: 7, M: 5; age: 36.2 + 7.3; BMI: 37.1 + 7.2), at the start of the daily eating cycle. All protein supplements were provided to the participants.

Participants came to the Center for Human Nutrition at the University of Arkansas every four weeks (baseline, 4-, 8-, and 12-weeks) for sample collection and metabolic measurements. Participants were provided with detailed educational guides, met monthly with study personnel, and could request meetings with study personnel in between study visits to ensure compliance. Participants were instructed to fast overnight and limit their physical activity prior to each study day. Upon arrival, measurements (height, weight, hip-to-waist ratio, blood pressure, hand grip strength, glucose, triglycerides, cholesterol, mood, self-reported sleep) were collected. Participants were instructed to record 3-day food logs and wear sleep monitors outside of the study visits during baseline week and week eleven.

Clinical Assessments

Clinical assessments were conducted at baseline, 4-, 8-, and 12-weeks.

Anthropometrics. Body height was measured to the nearest 0.1 cm using a stadiometer (Detecto, St. Louis, MO) with subjects barefoot, in the free-standing position. Body weight was measured in the fasting state with subjects without shoes to the nearest 0.05 kg using calibrated balance scales (Detecto, St. Louis, MO). Waist-to-hip ratio was also measured. When measuring waist-to-hip ratio, participants were asked to stand with arms at the sides, feet positioned close together and weight evenly distributed across the feet. Hip circumference was measured around the widest portion of the buttocks and waist circumference was measured at the top of the iliac

crest using a standard measuring tape, which was held snugly and at a level parallel to the floor. Body composition, including FM and FFM, was assessed by dual energy X-ray absorptiometry (DEXA; Lunar Prodigy, GE Healthcare).

Appetite and Palatability Measurements. On day 1, appetite and palatability was assessed using a traditional 100-mm visual analog scale (VAS) with opposing anchors (e.g., "extremely hungry" or "not hungry at all") at baseline. Questions consisted of: "how hungry do you feel at this moment", "how full do you feel at this moment", "how strong is your desire to eat at this moment" and "how much food do you think you can eat at this moment". Appearance ("how much do you like or dislike appearance of the breakfast foods") and palatability ("how much do you like or dislike the smell and taste of the breakfast foods") was assessed during fasted visits using a traditional 100-mm VAS with opposing anchors "dislike extremely" or "like extremely". Participants were asked to place an "X" on the 100-mm VAS in the place that pertains to their perceived appetite feelings at each time point (Flint et al, 2000).

Fingerstick. Cardiometabolic health marker samples were measured using the fingerstick method via the Alere Cholestech LDX Analyzer (Abbott; Chicago, IL). One blood sample (40 μL) per time point was collected in a Cholestech LDX Lipid Profile•GLU Cassette (Abbott Laboratories; Chicago, IL) which measures total cholesterol (TC), a measure of the total amount of cholesterol in the blood, high density lipoprotein (HDL) cholesterol, and triglycerides (TRG) as well as glucose (GLU), the TC/HDL ratio, non-HDL cholesterol, and estimates the level of LDL cholesterol.

Dietary Assessment. Prior to beginning the study, all participants were asked to complete a three-day (two weekdays and one weekend day) weighed food record to assess baseline dietary intake. Scales, measuring cups, and teaspoons were provided to each participant

to help with accuracy of reporting food intake. Participants met with study personnel to review the food records and have any questions answered before beginning the intervention. Participants were also required to maintain one three-day weighed food record during each month of the study (a total of four, three-day food records). The records were returned in person on a date assigned by study personnel. To ensure dietary compliance, participants were contacted (via email, text message or phone call) with a reminder to complete the three-day weighed food records. In addition, study personnel used a food frequency questionnaire to validate the food intake reported in the self-reported food records. Food records were analyzed for nutrient composition using Nutrition Data System for Research nutrient analysis software (University of Minnesota - http://www.ncc.umn.edu/ncc-news/ncc-news-fall-2014/).

Activity Monitors. Each participant was required to wear an activity monitor one week prior to beginning the study and at week 12. The activity monitor was only to be removed during activities where the monitor will be submerged in water (e.g., bathing, swimming). The ActiGraph activity monitor used Bluetooth Smart wGT3X-BT features utilized validated 3-axis accelerometer and digital filtering technology and included integrated wear time and ambient light sensors. The activity monitor was worn on the wrist, similar to a watch, to capture and record continuous, high resolution physical activity and sleep/wake information.

Strength Testing. Hand-grip strength was assessed using a standard hand-grip dynamometer (Takei Scientific Instruments, Niigata-City, Japan). Participants were properly fitted to the dynamometer so that their middle finger is at a 90-degree angle, then instructed to squeeze maximally for three seconds. Three trials were completed on each hand with a 60 second rest period between trials.

Mood. Mood was assessed using the Profile of Mood States (POMS) questionnaire. POMS is a factor analytically derived inventory that has been developed to measure six identifiable mood or affective states. The six mood states include tension-anxiety, depression-dejection, anger-hostility, vigor-activity, fatigue-inertia, and confusion-bewilderment. The POMS is a 65-question 5-point rating scale. POMS is appropriate for persons of a 7th grade educational level. The EITS manual for the profile of mood states was utilized as a guide for administration, scoring and interpretation of POMS. The administration time took approximately five to ten minutes. Participants defined their mood on a five-point Likert scale. Response options were: not at all; a little; moderately; quite a lot; extremely. To obtain a score for each mood factor, the sum of the responses is obtained for the adjectives defining the factor. A Total Mood Disturbance Score (TMD) may be obtained by summing the scores (while subtracting Vigor) on the six primary mood factors.

Pittsburgh Sleep Quality Index. Sleep was assessed using the Pittsburgh Sleep Quality Index (PSQI) self-rated questionnaire. The questionnaire has 19 self-reported questions that account for 7 different sleep components. The administration time took approximately five to ten minutes. Then participants were given a score for seven components: subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleep medication, and daytime dysfunction. These components were combined resulting in one global PSQI score. A global PSQI score less than or equal to 5 is associated with good sleep quality, while a score greater than 5 is associated with poor sleep quality.

Statistical Analysis. Data were analyzed as a complete randomized block design. Diet served as the experimental unit. Two-way ANOVA was used to compare differences between baseline and week 12 between diet groups. Results are reported as means ± standard deviation.

All analyses were conducted using GraphPad Prism Software, version 8.3.1. Probability values (P values) less than or equal to alpha = 0.05 were considered significant. Sex and age were not controlled for during analysis due to the small sample size.

Results

Participant Characteristics. Participant characteristics are presented in Table 1. There was no significant difference in age, height, weight, BMI, BMI percentile, fat mass, lean mass, or fat free mass between diet groups at baseline. There were 4 participants that dropped out of the study. One of the dropouts reported not following the intervention protocol and was therefore removed from the study. One participant dropped out due to personal scheduling conflicts with the fasted blood draw appointment times. One participant dropped out for personal reasons. One participant reported having an adverse reaction to the whey supplementation and requested to withdraw from the study.

Anthropometrics. Body composition, including fat mass, lean mass and FM to FFM ratio are presented in Table 2. There was a significant effect of time ($p < 0.05$) on FM to FFM ratio within both the control and protein interventions. Change in FM-to-FFM ratio from baseline to week 12 is shown in Figure 3. There was a significant effect of time ($p < 0.05$) on total body fat for both the control intervention and the protein intervention. The change in percent total fat over time is shown in Figure 4. Weight and waist-to-hip ratio are represented in Table 3, there was not a significant change within or between interventions for weight or waist-to-hip ratio. Hand grip strength, presented in Table 4, had a significant improvement ($p < 0.05$) on the right hand in the control intervention for week 4. Grit is also represented in Table 4. There was a

significant effect of time ($p < 0.05$) on grit within the TRF intervention at week 8. There was not a significant effect on grit within TRF + protein or between interventions.

Appetite and Palatability. Appetite results are presented in Table 5. There is a significant decline over time ($p < 0.05$) for desire for a snack in both the control and protein interventions. There were no effect of time or diet on appetite within or between interventions.

Fingerstick. Cardiometabolic markers are presented in Table 6. There were no significant differences in glucose, cholesterol, HDL, or triglycerides within or between interventions over the 12-week dietary intervention.

Mood. The Profile of Mood States Questionnaire results are presented in Table 7. There was a positive significant effect of time ($p < 0.05$) on tension scores for the protein intervention for weeks 8 and 12 (Figure 5). There was a positive significant effect of time ($p < 0.05$) on confusion scores for the protein intervention (Figure 6). Additionally, there was a positive significant difference of time ($p < 0.05$) on the total mood disturbance score for the protein intervention (Figure 7). There was no effect of time or dietary intervention on depression, anger, fatigue, or vigor within or between interventions.

Sleep: Activity Monitors and Pittsburg Sleep Quality Index. Sleep was quantified using the Actigraph Activity Monitors (Table 8). There was a significant decline over time x dietary intervention ($p < 0.05$) on total sleep time within the TRF + protein intervention, the total sleep time decreased (Figure 8). There was a significant incline between dietary intervention ($p < 0.05$) on average awakening length for the control and protein interventions, the average awakening length increased (Figure 9). There was no significant effect of time or diet on sleep latency, efficiency, wake after sleep onset, or number of awakenings within or between interventions. Results of self-reported sleep are presented in Table 9. There were not any

significant differences for subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleep medication, daytime dysfunction, or global sleep scores within or between interventions.

Discussion

To our knowledge, this is the first study to examine the role of protein supplementation for 12 weeks in a TRF eating pattern in adults with overweight or obesity on body composition, appetite, cardiometabolic markers, mood, and sleep. Our results indicate that protein supplementation (25 g/day) led to a significant improvement in total FM percentage and FM-to-FFM ratio for both interventions. Reduction of self-reported desire to snack was also observed for both interventions over the duration of the study. Also, there was improvement in the mood states tension and confusion as well as an improved total mood disturbance score for the TRF + protein intervention over time. Collectively, this data suggests that a TRF eating pattern may improve mood and body composition and TRF + protein supplementation may offer additional benefits.

The beneficial effects of dietary protein support the improvement of body composition (Donaldson et al, 2018; Kim et al, 2016) and promotion of weight loss (Beavers et al, 2015; Halton, 2004) in adults. However, there is limited research on the effects of dietary protein within TRF eating pattern. Previous TRF studies focus on adults without changes in macronutrient composition (Varady et al, 2021); whereas this study focuses on overweight or obese adults and increased protein consumption via supplementation at the end of the fasting period. In this study, the protein intervention provided 25g of whey protein daily. Diets higher in

protein and lower in carbohydrate have been shown to improve body composition by preserving skeletal muscle mass and targeting fat mass loss during negative energy balance, increasing satiety, and increasing postprandial energy metabolism (Westerterp-Plantenga, 2012; Astrup et al, 2015). The reliability of TRF to increase fat utilization sufficiently has been questioned. A study by Klein et al. examined the time course of lipid metabolism following various fasting periods demonstrated that more than 50% of the total increase in fat oxidation would occur during the time interval between 12 and 24 hours of fasting (Klein et al, 1993). This finding suggests that the typical fasting window required by TRF is adequate in facilitating fat oxidation. In the current study, increased protein consumption for 12 weeks improved FM-to-FFM ratio. High protein intake helps preserve lean body and muscle mass during weight loss (Cava et al., 2017). Retaining lean muscle translates into increased body strength, increased basal metabolic rate and increased bone strength (Stiegler & Cunliffe, 2006). The retention of lean muscle during weight loss may be related to the leucine's ability to stimulate muscle synthesis (Layman, 2003). Additionally, given that FFM represents a key determinant of the magnitude of resting metabolic rate, it follows that a decrease in lean tissue could hinder the progress of weight loss (Stiegler & Cunliffe, 2006). Therefore, with respect to long-term effectiveness of weight-loss programs, the loss of FM while maintaining FFM and resting metabolic rate is desirable (Stiegler & Cunliffe, 2006). The high leucine content (50–75% more than other common food proteins) of whey proteins (Layman, 2003) coupled with fast absorption (Boirie, 1997) make whey protein ideal as a protein supplement during weight loss.

This study found significant improvement to body composition demonstrating improved FM-to-FFM ratio within and between both interventions. The randomized clinical trials utilizing TRF that have been conducted include 11 studies that presented data on FFM, three reported a

reduction (Cienfuegos et al, 2020; Chow et al, 2020; Lowe et al, 2020), seven shown no change (Moro et al, 2016; Moro et al, 2020; Brady, 2021; Domaszewski et al, 2020; Gabel et al, 2018; McAllister et al, 2020; Stote et al, 2007), and one found an increase (Tinsley et al, 2019) in FFM following TRF that ranged from 1-12 weeks. In the study that showed an increase in FFM, TRF was administered in conjunction with resistance training in normal-weight individuals (Tinsley et al, 2019). Our study aligned with the majority reporting no change in FFM. Although minimal changes were seen in body composition within the interventions of this study, the results found for body composition are similar to other TRF randomized clinical trials. Similar to the findings of our study, 10 of the 15 studies that measured body composition showed a significant reduction in percent body fat or FM as a result of TRF. Additionally, the changes to body composition we observed may be further improved with a longer intervention duration since diet-induced weight loss can require 4-6 months to see measurable changes via DXA (Strychar, 2006). Additionally, this study showed non-significant improvement to lean mass within TRF + protein and non-significant improvement in FFM for the TRF + protein intervention. Lean mass is the weight of everything in the body minus the non-essential fat, whereas FFM is the lean mass minus the essential fat (Smith, 2004). Previous studies have utilized hand grip strength to access fitness and performance indicators during TRF. In our study there was no change for hand grip strength with the exception of the control group having increased hand grip strength in the right hand. Previous studies have found that TRF had either no effect on these performance indices (Moro et al, 2016; Anton et al, 2019; Lowe et al, 2020) or these indices improved similarly between TRF and control (Stratton et al, 2020; Tinsley et al, 2017; Tinsley et al, 2019).

To our knowledge, there are no published studies measuring appetite changes in response to TRF. Our study found no change for most parameters of appetite, however, there was a

decrease in desire to snack over the 12-week intervention for both the TRF and TRF + protein interventions.

Previous TRF studies demonstrate significant improvements in cardiovascular disease risk factors such as triglycerides, total cholesterol and LDL cholesterol, as well as fasting glucose concentrations in overweight and obese adult participants after at least 10 weeks (Che et al, 2021; Antoni et al, 2018; Sutton, 2018; Ravussin, 2019). The duration of previous studies has varied, with most being less than 12 weeks with eating windows ranging from 4 to 10 hours (Varaday et al, 2021). Of the 16 studies that have measured fasting glucose levels, five showed a decrease (Moro et al, 2016; Antoni et al, 2018; Cienfuegos et al, 2020; Chow et al, 2020; Hutchison et al, 2019), 10 found no change (Moro et al, 2020; Brady et al, 2021; Gabel et al, 2019; Lowe et al, 2020; McAllister et al, 2020; Schroder et al, 2021; Stote et al, 2007; Sutton et al, 2018; Tinsley et al, 2019; Wilkinson et al, 2020), and one observed an increase (Carlson et al, 2007) in this measure following TRF. These findings are similar to our study findings showing no change to fasting glucose. Additionally, of the 16 studies that have measured fasting blood triglyceride levels, four showed a reduction (Moro et al, 2016; Chow et al, 2020; Gabel et al, 2018; Stote et al, 2007), 11 found no change (Moro et al, 2020; Antoni et al, 2018; Brady et al, 2021; Cienfuegos et al, 2020; Hutchison et al, 2019; Kesztyüs et al 2019; Lowe et al, 2020; McAllister et al, 2020; Schroder et al, 2021; Tinsley et al, 2019; Wilkinson et al, 2020), and one reported an increase (Sutton et al, 2018) in this measure following TRF. Results from this study align with the majority of published research, no change of fasting blood triglyceride levels. There are 12 studies that measured fasting blood cholesterol levels, one reported a decrease (Wilkinson et al, 2020), nine found no change (Moro et al, 2016; Moro et al, 2020; Antoni et al, 2018; Gabel et al, 2018; Kesztyüs et al 2019; Lowe et al, 2020; Schroder et al, 2021; Sutton et al,

2018; Tinsely et al, 2019), and two showed an increase (McAllister et al, 2020; Stote et al, 2007) in this measure following TRF. The findings from these studies align with our study with cardiometabolic markers having a no change throughout the duration of the study. The measurement of insulin could indicate additional information regarding change to cardiometabolic markers.

There has been limited research of the impact TRF has on mood. Of the studies that have been conducted, one study assessed the health-related quality of life and fatiguability using a questionnaire. Anton et al. 2019 utilized a 4-week intervention of 8 hour TRF. Through this they showed that participants who followed TRF experienced a moderate but non-significant improvement to health-related quality of life in overweight older adults (Anton et al, 2019). These findings are similar to our results showing a significant improvement to the TRF + protein intervention for confusion, tension, and total mood disturbance scores.

To our knowledge, sleep has not been analyzed in many TRF focused studies. Our study indicated no change to sleep for the TRF group and a negatively significant change to total sleep time and length of awakenings for the TRF + protein intervention based on Actigraph activity monitor reported measurements. While many studies suggest that protein intake improves sleep, there have been some findings that protein consumed right before bed could result in sleep disturbances due to digestion slowing during sleep (Salas, 2022) and could provide a potential explanation of the results in this study. A randomized clinical trial of 8-hour TRF for 12 weeks used PSQI to measure self-reported sleep from obese adults (Gabel et al, 2019). Similarly to findings within our study, there was no change to PSQI scores over time indicating that TRF does not impact sleep quality or duration in obese adults. To our knowledge, Actigraph activity monitors have not been used to measure sleep within a TRF eating pattern. However, a study

using a repeated-measures, counterbalanced, crossover study design was used to administer treatment diets to 44 adult participants. Participants served as their own control and consumed high-protein, high-fat, high-carbohydrate, and control diets. This study found that high-protein diets were associated with fewer wake episodes and that specific macronutrient composition may have a significant influence on sleep (Lindseth et al, 2013). Our study reported no change for wake episodes within the TRF + protein intervention, however, protein source could potentially play a role in sleep impact measured via activity monitors (Binks et al, 2020).

Study Limitations: This study recruited a small sample size, therefore, an increased sample size is needed to determine if protein supplementation in combination with time restricted feeding could potentially serve as a method for improving mood, sleep and body composition within overweight or obese adults. The aim of recruitment was to balance sexes between intervention groups however both intervention groups were primarily female. There were also imbalances related to age, ethnicity and baseline weight. An additional limitation of this study is that beyond the protein supplementation provided, no other dietary components were controlled. This may have contributed to the limited effects observed between or within diet intervention groups. Despite participants being provided with tools to improve reporting accuracy (i.e. measuring cups, measuring spoons, food scales, detailed instruction) the study relied on self-reported 24-hour dietary intake records, and therefore could have led to inaccuracies (Dhurandhar et al, 2015). Future research should focus on increasing the sample size of the study to gain a more representative understanding of the effects of TRF and protein supplementation. Additionally, alteration of other macronutrients in combination with TRF is a potentially interesting research gap.

In conclusion, consumption of protein supplementation in addition to TRF participation did not show an overall improvement to cardiometabolic markers or sleep, however there was an improvement of total mood disturbance compared to the TRF intervention. Future research should focus on increased sample size and altering macronutrients other than protein. Alteration of other dietary macronutrients could help provide a deeper understanding of the role TRF eating patterns play as a weight management strategy. The improvement to body composition is possible with TRF eating patterns through improvement to the fat mass to fat-free mass ratio. Collectively, additional research is needed to understand the effects of protein supplementation within a TRF eating pattern.

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FIGURES

Figure 1: Intermittent fasting categories (Created with BioRender.com)

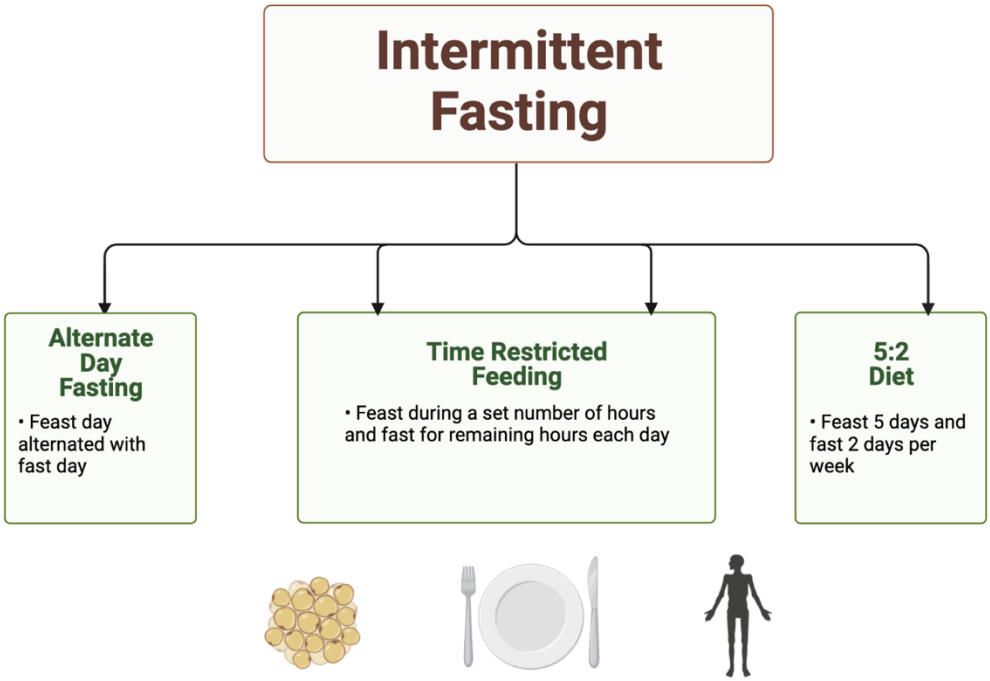


Figure 2: Participant Recruitment Process

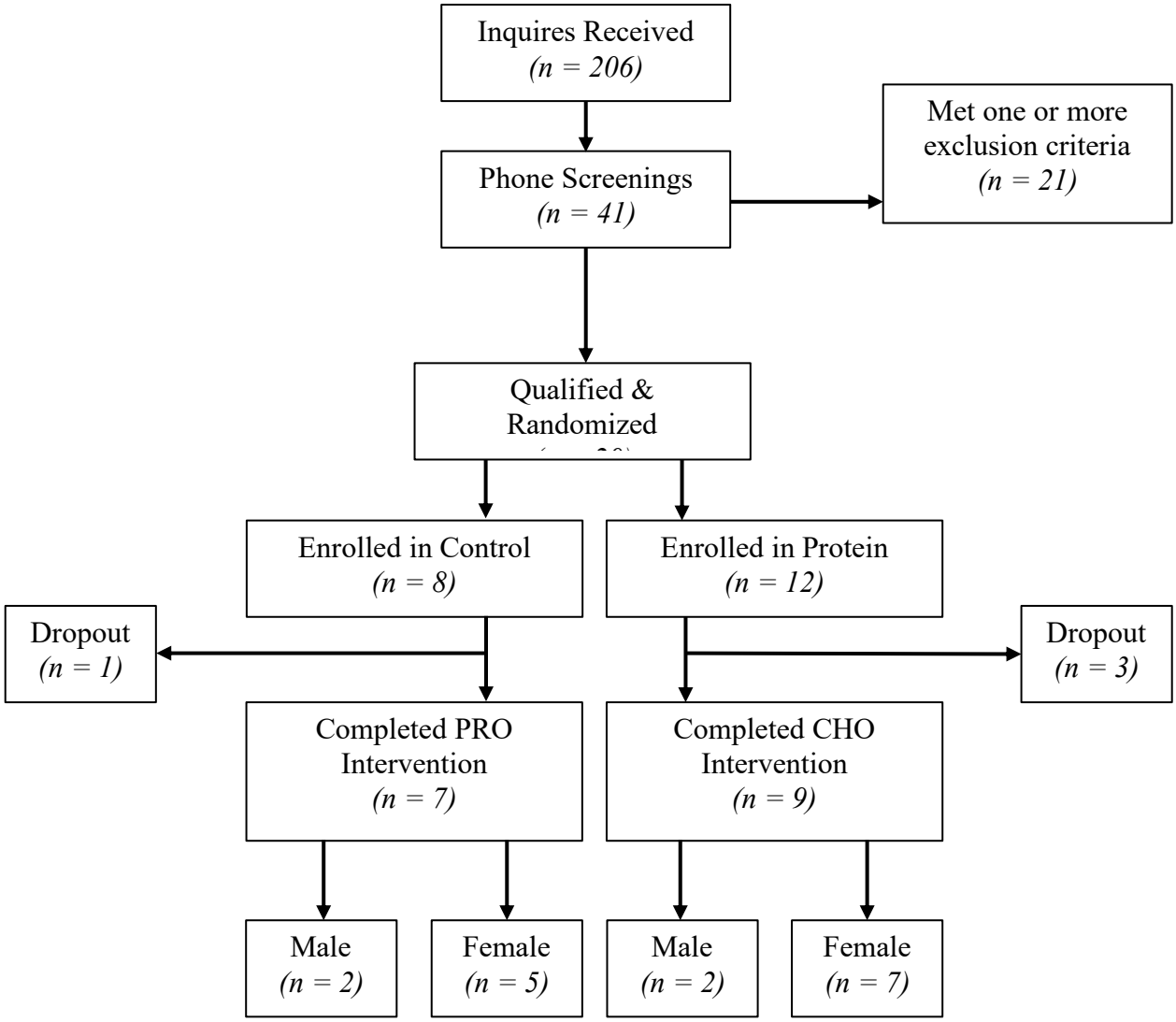


Figure 3: Change in FM to FFM Ratio from Baseline to Week 12

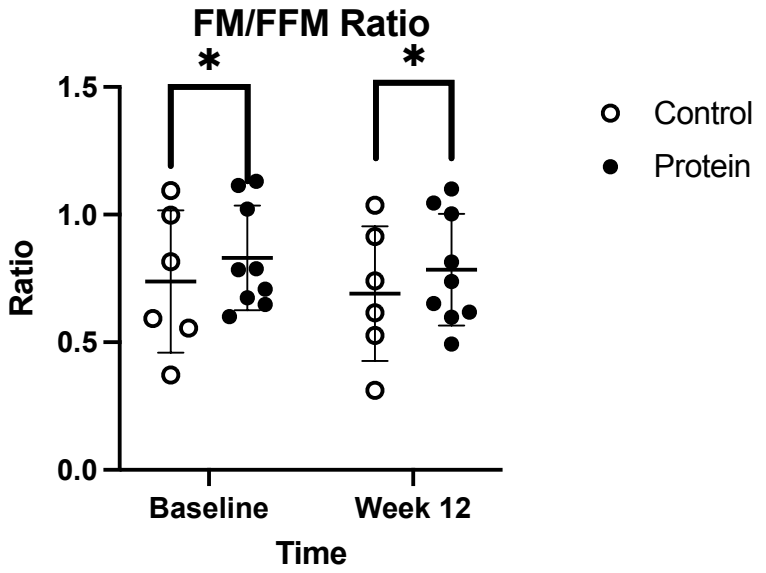


Figure 4: Change in % Total Fat from Baseline to Week 12

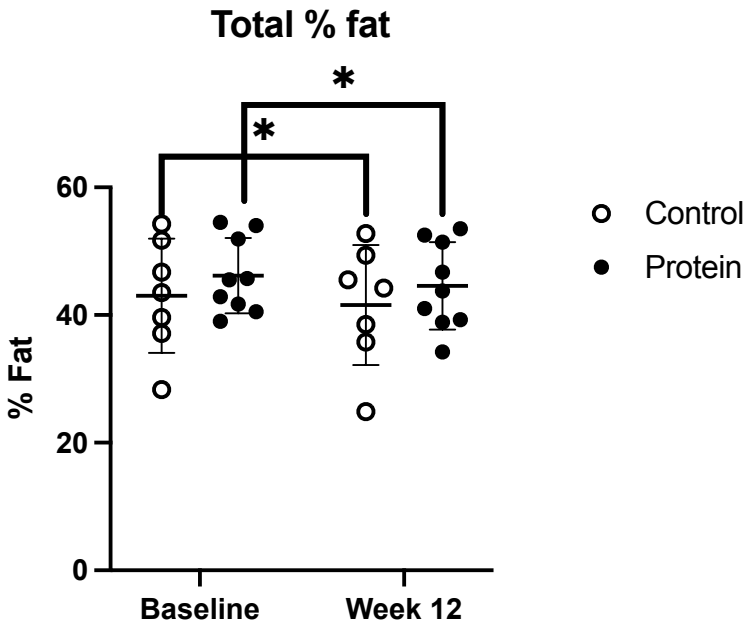


Figure 5: Mood POMS Tension Scores

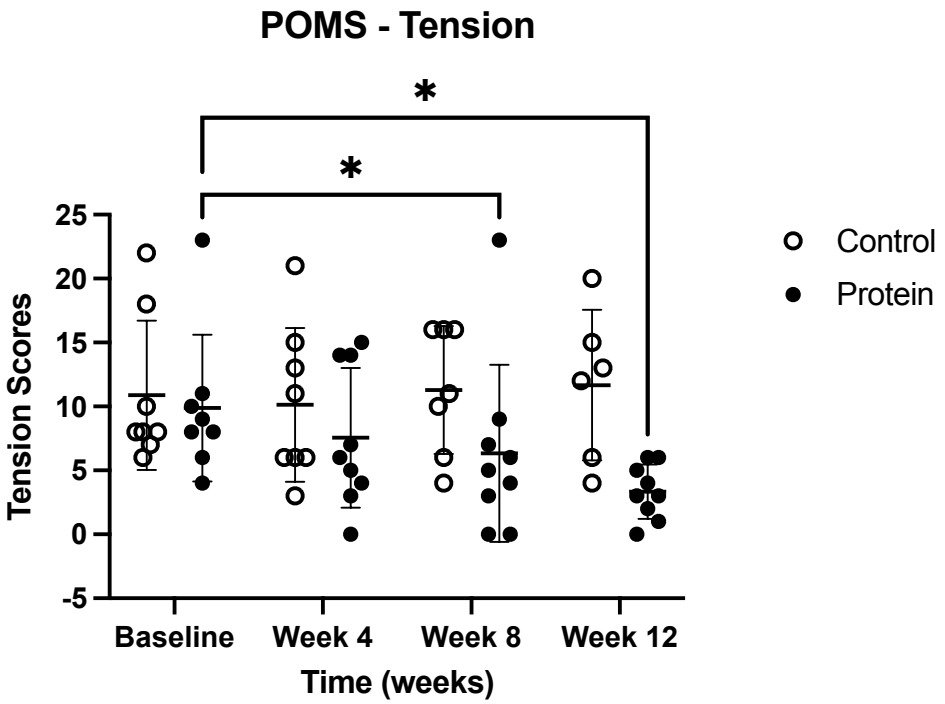


Figure 6: Mood POMS Confusion Scores

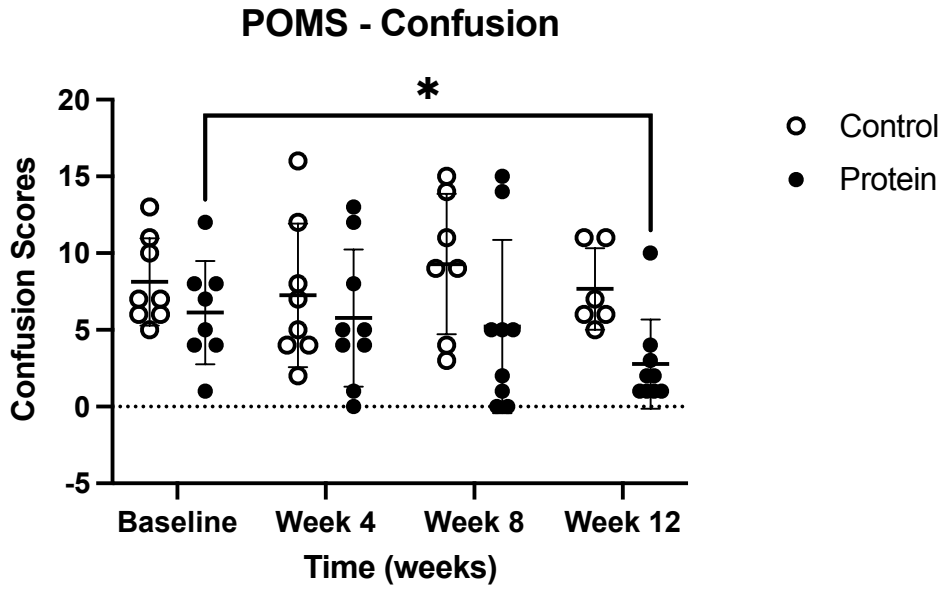


Figure 7: Mood POMS TMD Scores

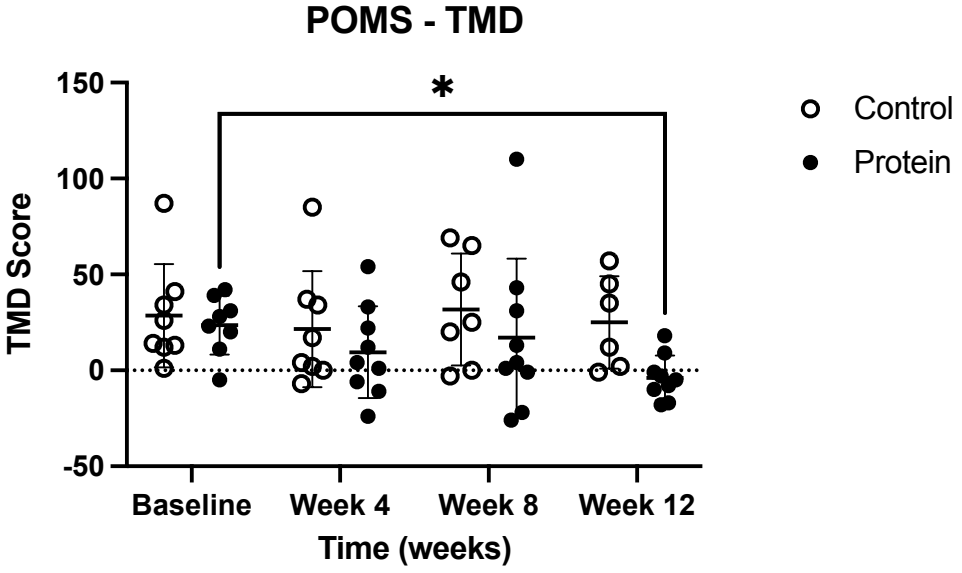


Figure 8: Sleep via Actigraph Activity Monitors – Total Sleep Time

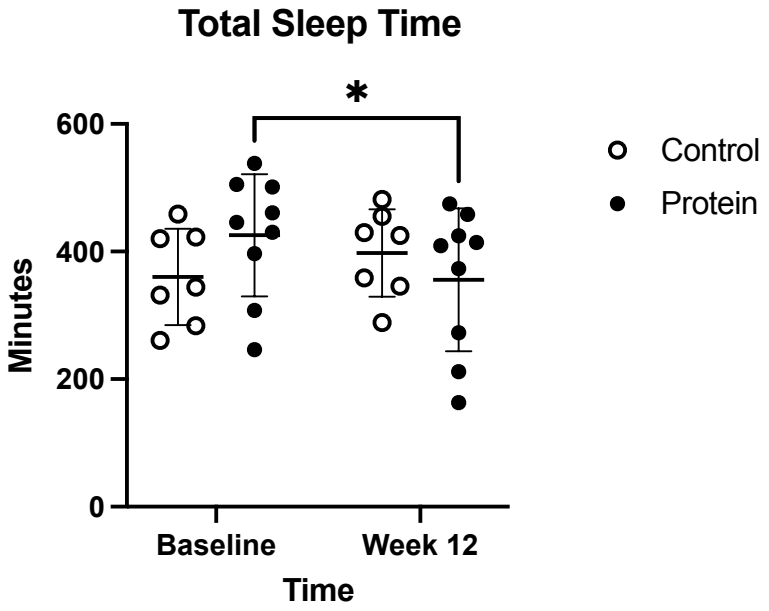
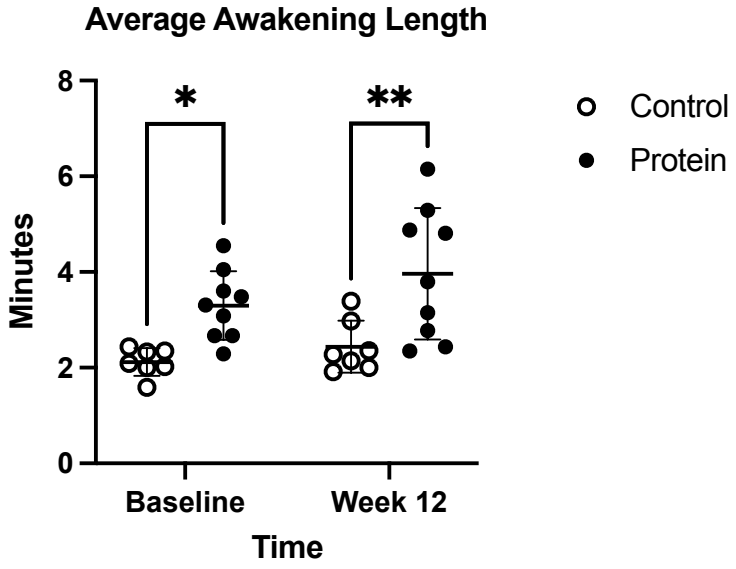


Figure 9: Sleep via Actigraph Activity Monitors – Average Awakening Length



TABLES

Table 1**:** Participant characteristics

	Control (n=7)		Protein (n=9)	
Characteristic	Male (n=2)	Female (n=5)	Male (n=2)	Female (n=7)
Age, years	32.5 ± 3.5	39.0 ± 8.6	27 ± 0.0	38.9 ± 5.9
Ethnicity				
Caucasian	1	4	1	5
African American	-	1	-	-
Hispanic	-	-	1	1
Asian	1	-	-	1

Values are presented as means ± SD.

Table 2: Body Composition via Dual Energy X-ray Absorptiometry Scanning

	Control		Protein	
	Baseline	Week 12	Baseline	Week 12
Lean Mass (kg)	46.3 ± 6.7	46.3 ± 5.2	54.7 ± 12.7	56.4 ± 14.8
Fat Mass (kg)	35.5 ± 10.3	34.2 ± 11.0	47.9 ± 15.5	46.8 ± 18.2
Fat Free Mass (kg)	49.4 ± 6.7	49.4 ± 5.4	57.9 ± 13.3	59.5 ± 22.3
Bone Mineral Content (kg)	3.1 ± 0.3	3.1 ± 0.4	3.0 ± 0.5	3.0 ± 0.6
Total Body Fat (%)	43.0 ± 8.9	41.6 ± 9.4 *	46.2 ± 5.9	44.6 ± 6.8 *
Fat Mass/Fat Free Mass Ratio	0.74 ± 0.3	0.69 ± 0.3 *	0.83 ± 0.2	0.79 ± 0.2 *

Values are presented as means ± SD. * indicates significant difference between baseline and week 12 time-points, $P < 0.05$

Table 3: Anthropometrics – Waist-to-Hip Ratio and Weight over 12 Weeks

	Control				Protein			
	Baseline	Week 4	Week 8	Week 12	Baseline	Week 4	Week 8	Week 12
Waist to Hip Ratio	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	1.0 ± 0.1	1.0 ± 0.0	0.9 ± 0.1	1.0 ± 0.1
Weight (kg)	85.4 ± 10.8	84.5 ± 10.7	84.1 ± 10.5	83.0 ± 12.2	103.5 ± 27.6	102.9 ± 28.3	106.0 ± 27.6	105.8 ± 28.1

Values are presented as means ± SD. * indicates significant difference between control and protein groups compared to baseline, $P < 0.05$

Table 4: Grit and Hand Grip Strength

	Control				Protein			
	Baseline	Week 4	Week 8	Week 12	Baseline	Week 4	Week 8	Week 12
Grit Score	3.4 ± 0.6	3.6 ± 0.5	3.8 ± 0.2 *	3.7 ± 0.3	3.6 ± 0.5	3.7 ± 0.5	4.0 ± 0.5	3.9 ± 0.5
Hand Grip Strength Left Hand	28.8 ± 3.4	30.9 ± 4.0	30.0 ± 4.2	30.9 ± 5.5	30.2 ± 11.6	31.7 ± 9.1	30.1 ± 9.0	30.6 ± 11.2
Hand Grip Strength Right Hand	29.2 ± 4.7	31.5 ± 4.0 *	32.4 ± 4.6	32.7 ± 5.1	31.3 ± 10.6	33.1 ± 10.7	30.4 ± 9.6	33.6 ± 10.5

Values are presented as means ± SD. * indicates significant difference between control and protein groups compared to baseline, $P < 0.05$

Table 5: Appetite Change from Baseline to 12 Weeks

	Control				Protein			
	Baseline	Week 4	Week 8	Week 12	Baseline	Week 4	Week 8	Week 12
Hunger	43.5 ± 28.6	36.1 ± 32.4	28.6 ± 27.8	16.2 ± 12.7	34.8 ± 35.3	26.3 ± 33.8	39.7 ± 39.5	19.3 ± 32.0
Fullness	25.0 ± 19.9	33.5 ± 32.3	39.3 ± 27.4	46.8 ± 27.5	50.2 ± 31.2	38.6 ± 29.2	34.6 ± 30.5	51.1 ± 31.7
Desire to Eat	47.4 ± 28.2	36.4 ± 35.0	35.4 ± 29.6	21.3 ± 19.5	36.9 ± 30.6	36.2 ± 32.7	41.6 ± 37.2	24.6 ± 32.4
Desire for salty	36.6 ± 32.7	20.9 ± 22.2	31.7 ± 26.4	19.8 ± 25.3	16.6 ± 26.0	30.0 ± 33.4	23.8 ± 31.8	17.4 ± 26.4
Desire for Sweet	46.5 ± 17.6	38.1 ± 33.6	39.1 ± 29.2	24.8 ± 21.8	28.7 ± 31.3	16.9 ± 22.3	26.1 ± 25.7	18.8 ± 19.5
Desire for snack	52.0 ± 34.7	32.5 ± 27.7	33.3 ± 30.2	25.2 ± 19.1 *	30.8 ± 31.0	17.7 ± 22.6	24.3 ± 22.4	11.8 ± 14.2 *

Values are presented as means ± SD. * Indicates significant difference between control and protein groups compared to baseline, $P < 0.05$

Table 6: Cardiometabolic markers via Cholestech fingerstick

	Control				Protein			
	Baseline	Week 4	Week 8	Week 12	Baseline	Week 4	Week 8	Week 12
Glucose, mg/dL	91.9 ± 6.2	91.5 ± 7.4	85.6 ± 11.3	83.4 ± 12.6	97.4 ± 10.2	95.1 ± 6.5	94.0 ± 4.4	90.6 ± 18.4
Cholesterol, mg/dL	168.4 ± 38.6	173.0 ± 24.0	181.7 ± 37.3	168.9 ± 37.7	196.9 ± 24.5	181.6 ± 16.4	182.9 ± 31.8	191.7 ± 27.5
HDL, mg/mL	44.4 ± 11.3	47.9 ± 11.3	48.3 ± 20.2	44.7 ± 17.2	44.3 ± 12.7	43.3 ± 8.9	41.4 ± 5.0	41.3 ± 8.9
Triglycerides, mg/dL	103.6 ± 59.3	96.1 ± 61.1	82.1 ± 24.0	97.9 ± 70.6	126.0 ± 49.8	125.6 ± 71.5	146.5 ± 105.3	142.3 ± 74.0

Values are presented as means ± SD. HDL, high density lipoprotein. * Indicates significant difference between control and protein groups at baseline and week 12 time-points, $P < 0.05$

Table 7: Mood via Profile of Mood States Questionnaire Results

	Control				Protein			
	Baseline	Week 4	Week 8	Week 12	Baseline	Week 4	Week 8	Week 12
Tension	10.9 ± 5.8	10.1 ± 6.0	11.3 ± 5.0	11.7 ± 5.9	9.9 ± 5.7	7.6 ± 5.5	6.3 ± 6.9 *	3.3 ± 2.1 *
Depression	7.6 ± 6.7	7.0 ± 8.7	9.7 ± 8.8	8.3 ± 9.1	5.0 ± 4.2	5.1 ± 5.2	6.4 ± 10.8	1.6 ± 2.2
Anger	9.0 ± 10.0	6.1 ± 5.6	7.7 ± 6.1	8.8 ± 9.0	6.4 ± 4.8	3.6 ± 3.9	4.2 ± 5.9	1.8 ± 1.7
Fatigue	8.3 ± 3.3	8.6 ± 4.8	9.4 ± 4.7	7.8 ± 3.2	12.3 ± 6.0	7.3 ± 5.2	7.8 ± 8.0	4.3 ± 3.5
Confusion	8.1 ± 2.9	7.3 ± 4.7	9.3 ± 4.6	7.7 ± 2.7	6.1 ± 3.4	5.8 ± 4.5	5.2 ± 5.7	2.8 ± 2.9 *
Vigor	15.4 ± 6.2	17.6 ± 4.6	15.7 ± 5.8	19.2 ± 3.1	16.0 ± 4.2	17.4 ± 4.9	13.0 ± 8.0	17.8 ± 6.0
TMD	28.5 ± 27.0	21.5 ± 30.3	31.7 ± 29.1	25.0 ± 24.1	23.6 ± 15.3	9.4 ± 23.9	17.0 ± 41.3	-3.9 ± 11.6 *

Values are presented as means ± SD. TMD, total mood disturbance. * Indicates significant difference between control and protein groups between time-points, $P < 0.05$

Table 8: Quantitative Sleep Measurements via Actigraph Activity Monitors

	Control		Protein	
	Baseline	Week 12	Baseline	Week 12
Latency (min)	2.2 ± 2.0	1.6 ± 2.2	1.6 ± 1.2	2.2 ± 2.9
Efficiency (%)	92.0 ± 1.9	91.5 ± 2.5	90.9 ± 3.7	90.3 ± 5.7
Total Sleep Time (min)	389.4 ± 82.3	397.9 ± 68.6	425.7 ± 95.8	355.7 ± 112.2 *
Wake After Sleep Onset	27.0 ± 10.5	31.0 ± 10.2	41.4 ± 19.0	36.5 ± 25.0
Number of Awakenings	12.5 ± 3.8	13.3 ± 5.3	12.5 ± 5.7	9.4 ± 5.2
Average Awakening Length (min)	2.1 ± 0.3	2.4 ± 0.5 *	3.3 ± 0.7	4.0 ± 1.4 *

Values are presented as means ± SD. * Indicates significant difference between control and protein groups at baseline and week 12 time-points, $P < 0.05$

Table 9: Qualitative Sleep Measurements via PSQI Questionnaire

	Control				Protein			
	Baseline	Week 4	Week 8	Week 12	Baseline	Week 4	Week 8	Week 12
Subjective Sleep Quality	1.1 ± 0.9	1.4 ± 0.5	1.6 ± 0.8	1.2 ± 0.4	1.0 ± 0.5	0.6 ± 0.5	0.8 ± 0.4	1.0 ± 0.5
Sleep Latency	2.1 ± 0.7	1.6 ± 0.7	1.7 ± 1.0	1.5 ± 1.1	0.8 ± 0.7	0.7 ± 0.7	0.7 ± 0.5	1.1 ± 0.8
Sleep Duration	0.9 ± 0.7	1.1 ± 0.6	0.7 ± 0.5	0.8 ± 0.8	0.6 ± 0.5	0.7 ± 0.7	0.6 ± 0.5	0.8 ± 0.7
Habitual Sleep Efficiency	0.4 ± 0.5	1.0 ± 1.2	0.9 ± 0.9	0.8 ± 1.2	0.4 ± 0.7	0.7 ± 0.7	0.7 ± 0.7	0.4 ± 1.0
Sleep Disturbances	1.4 ± 0.5	1.3 ± 0.5	1.4 ± 0.5	1.2 ± 0.4	1.1 ± 0.3	0.9 ± 0.6	0.8 ± 0.7	0.8 ± 0.4
Use of Sleep Medication	1.0 ± 1.3	0.5 ± 0.8	0.4 ± 1.1	0.5 ± 1.2	0.4 ± 1.0	0.8 ± 1.2	0.6 ± 1.0	0.6 ± 1.1
Daytime Dysfunction	1.3 ± 0.8	1.4 ± 0.9	1.3 ± 0.5	1.3 ± 0.5	1.0 ± 0.7	0.9 ± 0.6	0.8 ± 0.4	0.6 ± 0.5
Global Score	8.3 ± 2.9	8.3 ± 3.0	8.0 ± 2.7	7.3 ± 3.2	5.3 ± 1.9	5.1 ± 3.1	4.8 ± 2.4	5.1 ± 2.5

Values are presented as means ± SD. * Indicates significant difference between control and protein groups at baseline and week 12 time-points, $P < 0.05$

CONCLUSION

In conclusion, consumption of a dietary protein supplementation within a TRF eating pattern, compared to an ad libitum TRF eating pattern, for 12 weeks did not show an overall improvement to cardiometabolic health markers, sleep or appetite in overweight or obese adults ages 25-50 years old. However, there was improvement to total mood disturbance. Additionally, TRF dietary intervention still serves as a potential intervention method, however future research may require larger sample sizes and macronutrient manipulation. Further research is necessary to determine effective strategies to combat obesity.

APPENDIX

UNIVERSITY OF ARKANSAS

To:	Jamie I Baum FDSC N2216
From:	Douglas J Adams, Chair IRB Full Board
Date:	05/27/2021
Action:	**Approval**
Action Date:	05/27/2021
Protocol #:	1912236045A006
Study Title:	Time Restricted Feeding Intervention for Muscle and Metabolic Health (TRIM
Expiration Date:	12/10/2021
Last Approval Date:	05/27/2021
Risk Level:	

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

If the research involves collaboration with another institution then the research cannot commence until the Co 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 dat

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

Adverse Events: Any serious or unexpected adverse event must be reported to the IRB Committee within 48 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, stu personnel, or number of participants, please submit an amendment to the IRB. All changes must be approved 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.

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