The Effect of Whey Protein Supplementation at Breakfast on Tryptophan Levels, Food Intake, and Mood in Postmenopausal Women in a 16-Week Randomized Controlled Trial

Danielle L. Lamont
University of Arkansas, Fayetteville, dlwarren20@gmail.com

Jamie I. Baum
University of Arkansas, Fayetteville, baum@uark.edu

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Cover Page Footnote

Danielle Lamont is a 2021 honors program graduate with a major in Human Nutrition and Dietetics, and a minor in Human Development and Family Sciences. Jamie Baum, the faculty mentor, is an Associate professor in the Department of Food Science.

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Meet the Student-Author

Danielle Lamont

I graduated from Prairie Grove High School and am now a graduate of the University of Arkansas with a major in Human Nutrition and Dietetics, and a minor in Human Development and Family Sciences. I have been the recipient of several scholarships, including the Honors College Research Grant, the Honors College Academy Scholarship, the Arkansas Academic Challenge Scholarship, the University of Arkansas Alumni License Plate Scholarship, and the Albert and Mary Gartside Scholarship. As a graduate, I plan to earn experience in geriatric nutrition as a dietetic technician before obtaining a Master's in Human Nutrition and registration as a dietitian.

I would like to thank Dr. Jamie I. Baum, my Honors mentor and principal investigator for SHAPE. Without Dr. Baum, I would not have been able to have such a broad experience or have this experience at all. I would also like to thank Dr. Aubree Hawley, committee member, for sharing her knowledge and skill. I would also like to thank Dr. Kelly Webber, committee member, for her advice and expertise in the composition of this thesis. Thanks also to former graduate students Sam Walker and Angela Tacinelli for their advice, input, and leadership.

Danielle completing data entry work for her project from home.

Research at a Glance

- The Census Bureau estimates that by 2030, 25% of the United States population will be women ages 45 and up.

- Tryptophan levels, mood, and food intake were observed in postmenopausal women taking a whey protein supplement.

- There were no significant changes within the intervention group of 7 women. More research is needed.

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Danielle L. Lamont* and Jamie I. Baum[†]

Abstract

Whey protein isolate supplementation has been recognized as having potential for regulating appetite, thereby potentially improving mood and food intake. The objectives of this project were to 1) analyze the effects of high-quality whey protein intake on overall diet and 2) identify and examine a correlation between tryptophan levels and mood regulation. This research was conducted using a randomized experimental design. A total of 13 postmenopausal women (12+ months after last reported menstrual cycle) were recruited and allocated to one of two dietary intervention (DI) groups: 1) control (maintain current lifestyle; CON; n = 6), and 2) whey protein isolate (WPI; 25 g; n = 7). Protein was consumed prior to 10:00 AM daily. Both interventions were followed daily for 16 weeks. All laboratory visits required participants to arrive fasted with complete 3-day dietary logs. Participants completed the Pittsburgh Sleep Quality Index (PSQI) and Profile of Moods Questionnaire. Height, weight, and waist-to-hip ratio were measured. A blood draw was administered to assess sleep and metabolic blood markers. A one-way repeated measures analysis of variance (ANOVA) was used to assess the differences in body mass index (BMI) and Profile of Mood States (POMS). One-way ANOVA was used to calculate the POMS Total Mood Disturbance scores. Clinical biomarker differences were determined through repeated-measures ANOVA (statistically significant: $P < 0.05$). Prism GraphPad Software v. 9.0 (La Jolla, California) was used for all analyses. Results were inconclusive. We found no correlation between daily whey protein isolate supplementation and tryptophan levels, overall diet, or mood regulation.

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[†] Jamie Baum, the faculty mentor, is an Associate professor in the Department of Food Science.

Introduction

The older adult population (≥65 years) in the United States is projected to double between 2017 and 2060 (Bureau, 2017). Two out of three older adults suffer multiple chronic diseases (The State of Aging and Health in America 2013, 2013). Additionally, in 2006, the Behavioral Risk Factor Surveillance System (BRFSS) indicated that in Arkansas, 8.6% to 12.4% of adults 50 years or older experienced depression (Directors, 2008). Depression can prevent successful treatment of chronic disease (Directors, 2008) and negatively impact overall diet through inducing unhealthy cravings (Chaput, 2014).

Projections estimate that by 2030, 25% of the United States population will be women ages 45 and up (Bureau, 2017). Postmenopausal women experience shifts in body composition, including an average 44% increase of visceral fat mass during menopause (Kozakowski et al., 2017; Panotopoulos et al., 1997), which may affect the way nutrients, such as tryptophan, are utilized. This population also experiences disrupted sleep cycles and fluctuating mood, partially due to hormone levels (Panotopoulos et al., 1997).

It is recommended to consume an evenly distributed, moderate amount of high-quality protein at each meal (Arentson-Lantz et al., 2015), but Western eating patterns are disproportionate, with breakfast providing a mere 16% of daily protein intake (Mishra et al., 2018). Furthermore, protein-containing foods consumed at breakfast are often of low protein quality (McCrory, 2014), resulting in inadequate consumption of essential amino acids (EEA) at the breakfast meal. This may contribute to low tryptophan levels and increased mood disturbances (Hoglund et al., 2019). Studies suggest that these factors may be reduced by whey protein supplementation (Wirunsawanya et al., 2018).

Whey protein isolate (WPI, 85% to 90% protein) (Flaim et al., 2017) has been associated with increased satiety (Hall et al., 2003), indicating that WPI may be useful in regulating appetite. The Recommended Dietary Allowance (RDA) of tryptophan for adults 19 years and older is 0.005 g/kg daily (Institute of Medicine, 2005). The RDA for this participant pool was approximately 0.3, 0.4, and 0.5 g/d for participants weighing 55.3, 73.4, and 91.5 kg, respectively. The Instantized BiPRO supplement used in this study contained approximately 0.8 g of tryptophan per 25 g daily serving. Therefore, this supplement provided tryptophan in amounts greater than the RDA for these participants.

Overall diet is influenced by sleep efficiency (Chaput, 2014). Tryptophan is the precursor to melatonin, which is involved in regulating the sleep-wake cycle (Peuhkuri et al., 2012). Insufficient sleep increases consumption of energy-rich foods to reduce psychological distress or relieve negative mood (Chaput, 2014). An increase in food intake, particularly high sodium- and carbohydrate-containing foods, contributes to the development of chronic disease (Zhou et al., 2016).

Tryptophan, also a precursor to the neurotransmitter serotonin, influences mood regulation, emotional processing, and alertness (Mohajeri et al., 2015). In a 19-day randomized-controlled trial, a test drink containing tryptophan was administered twice per day; researchers found improvements in emotional processing and reduced sensitivity to negative stimuli (Mohajeri et al., 2015). The results suggested that high levels of tryptophan supplementation improve mood and mitigate depressive episodes (Mohajeri et al., 2015).

The objectives of this project were to 1) analyze the effects of high-quality whey protein intake on overall diet and 2) identify and examine a correlation between tryptophan levels and mood regulation. We hypothesized that 25-g of whey protein isolate supplementation daily for 16 weeks would increase tryptophan levels, improve overall diet, and improve mood regulation in postmenopausal women.

Materials and Methods

Prior to subject recruitment, this study was approved by the University of Arkansas' Institutional Review Board and was registered on clinicaltrials.gov, clinical trial number: NCT0303041. Participants were recruited from July 2018 through April 2020.

Recruitment was ended earlier than anticipated due to the COVID-19 pandemic. Participants were recruited voluntarily through advertisements in the University of Arkansas' daily news email, social media, word-of-mouth, and flyers. Eligibility required that participants were postmenopausal women with a last reported menstrual cycle 12 months or more in the past; were not taking hormone replacement therapy (HRT); had no known food allergies; regularly ate breakfast (>5 times per week); were not taking medications that impact appetite, blood coagulation, metabolism, or blood lipids; were not regularly consuming protein supplements; were not consuming more than 0.8 g/kg of protein per day (assessed via food frequency questionnaire); and have an initial Pittsburgh Sleep Quality Index (PSQI) of 5 or greater, as this score indicated dysregulated sleep. All subjects completed a phone screening and signed an informed consent form prior to enrolling. Participants were recruited on a rolling basis and were randomly assigned a treatment group. A total of 13 women, ages 46 to 72, completed the 16-week study.

This research was conducted using a randomized experimental design. A total of 13 postmenopausal women (12+ months after last reported menstrual cycle) were recruited and allocated to one of two dietary intervention (DI) groups. The demographic of postmenopausal women was chosen to negate hormonal influence and to provide

data on a population with limited available research. Upon acceptance into the study, participants were assigned randomly to 1 of 2 dietary intervention (DI) groups. The DI groups were as follows: 1) control (maintain current lifestyle; CON; n = 6), and 2) whey protein isolate (WPI; 25 g; n = 7). Protein was consumed prior to 10:00 AM daily. Both interventions were followed daily for 16 weeks.

The WPI supplement, Instantized BiPRO (Davisco Foods International, Le Sueur, Minnesota), was given in 28 single-serving bags at the baseline, 4-, 8-, and 12-week laboratory visits. Participants were instructed to return empty supplement packages to the researchers upon the next visit to ensure compliance. All 5 laboratory visits required participants to arrive fasted with complete 3-day dietary logs.

Participants received a booklet corresponding to their randomly assigned intervention. All booklets contained a standard study day schedule and checklist, and breakfast recipes that were modified to include protein powder for those participants assigned whey protein.

Body composition was measured via DEXA at baseline and week 16. Height, weight, and waist-to-hip ratio were measured at baseline, 4-, 8-, 12-, and 16-weeks. Height was measured to the nearest 0.01 cm using a stadiometer (Detecto, St. Louis, Missouri) with participants barefoot in the free-standing position. Weight was measured to the nearest 0.01 kg using calibrated balance scales (Detecto, St. Louis, Missouri), with participants in the fasting state without shoes. Body mass index (BMI) was calculated as weight (kg) divided by height (m) squared. Waist measurements were taken at the level of the umbilicus using a soft tape measure and were rounded to the nearest 0.1 cm. Hip measurements were taken at the widest point below the waist using the soft tape measure and were rounded to the nearest 0.1 cm. Waist-to-hip ratios were recorded by dividing the waist measurement (cm) by the hip measurement (cm).

At baseline, 4-, 8-, 12-, and 16-weeks, participants completed 3-day food records. Food data was recorded for two weekdays and one weekend day; for example, Sunday, Monday, and Tuesday, food data would be recorded prior to a Wednesday test day. Participants were trained to accurately record amounts of food using provided food scales (Greater Goods, LLC, St. Louis, Missouri) and beverages. Participants were asked to record brand names and food preparation methods. A researcher reviewed the 3-day food records with the participants on each study day to confirm details. The Nutrition Data System for Research (Nutrition Coordinating Center, University of Minnesota, Minneapolis, Minnesota) analysis software was used to analyze the energy, micronutrient, and macronutrient composition of the 3-day food records.

Sleep was assessed at baseline, 8-, and 16-weeks, using the PSQI. The PSQI is validated as an instrument of subjective measure of sleep quality (Mollayeva et al., 2016).

Mood was assessed at baseline, 8 weeks, and 16 weeks, via the Profile of Mood States (POMS) questionnaire. The POMS questionnaire has been validated in postmenopausal women (Wyrwich and Yu, 2011). The questionnaire consists of 65 questions containing a one-word adjective of mood to measure and identify six affective states. Those states are tension, depression, anger, vigor, fatigue, and confusion. Participants define their mood on a 5-point Likert scale. Response options are 0 = not at all; 1 = a little; 2 = moderately; 3 = quite a lot; 4 = extremely. Researchers were available to answer questions regarding the meaning of a word.

The sum of the responses for each adjective is calculated to score each affective state subscale. Higher subscale scores for all except vigor represent a poor mood. The Total Mood Disturbance score (TMD) is determined through the summation of the scores of the six factors (weighting vigor negatively). The TMD is calculated by the following equation:

$$TMD = (Tension - Anxiety) + (Depression - Dejection) + (Anger - Hostility) + (Fatigue - Inertia) + (Confusion - Bewilderment) - (Vigor - Activity)$$

A blood draw was administered by a certified phlebotomist to assess metabolic blood markers. Tryptophan (and all amino acids) was measured using the commercially available EZ Faast amino acid analysis kit via gas chromatography/mass spectrometry.

One-way repeated measures analysis of variance (ANOVA) was used to assess the differences in BMI and POMS. One-way ANOVA was used to calculate the POMS Total Mood Disturbance scores. Clinical biomarker differences were determined through repeated-measures ANOVA (statistically significant: $P < 0.05$). Prism GraphPad Software Version 9.0 (La Jolla, California) was used for all analyses. All measurements were reported as the mean ± standard deviation.

Results and Discussion

In this 16-week study, tryptophan levels, mood, and food intake were evaluated in order to determine the effect of whey protein supplementation on postmenopausal women. Though the study size was relatively small, any significant effects could lead to a deeper understanding of the impact of whey protein supplementation on the health of postmenopausal women.

There were no significant differences for plasma tryptophan concentrations with 16-weeks of protein supplementation (Fig. 1). There were also no significant mood regulation changes, as shown in Figs. 2 and 3. Current research indicates that high-quality protein supplementation can reduce mood disturbances and stress-related mental
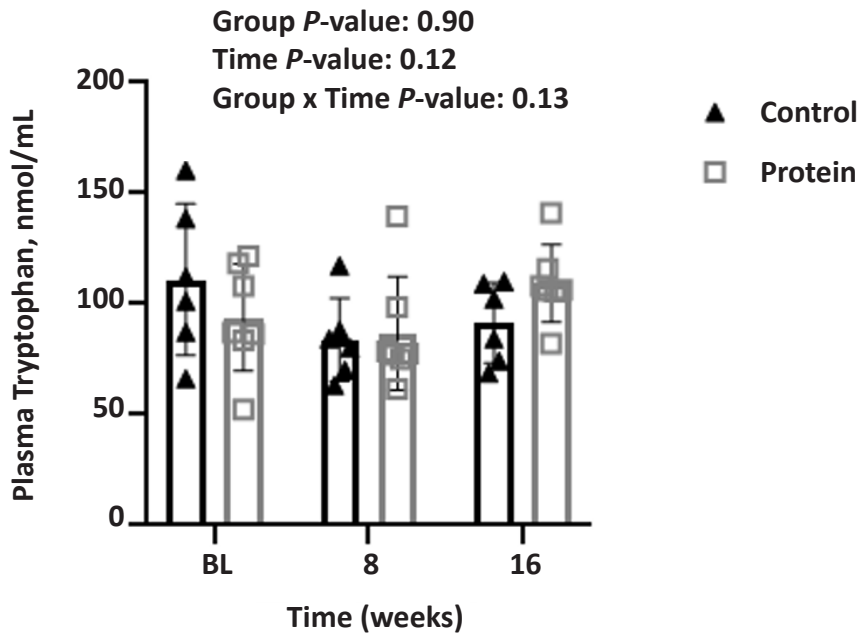
Fig. 1. Plasma tryptophan levels over the course of the intervention. Control, indicated by black triangles, represents the no intervention and free living group, n = 6. Protein, indicated by the gray squares, represents the whey protein isolate intervention group, n = 7. Plasma tryptophan levels for the control group lowered after baseline (BL) and increased after week 8. Plasma tryptophan levels for the protein group lowered slightly after baseline and increased after week 8. Significant differences: * $P < 0.05$. Results not significant.
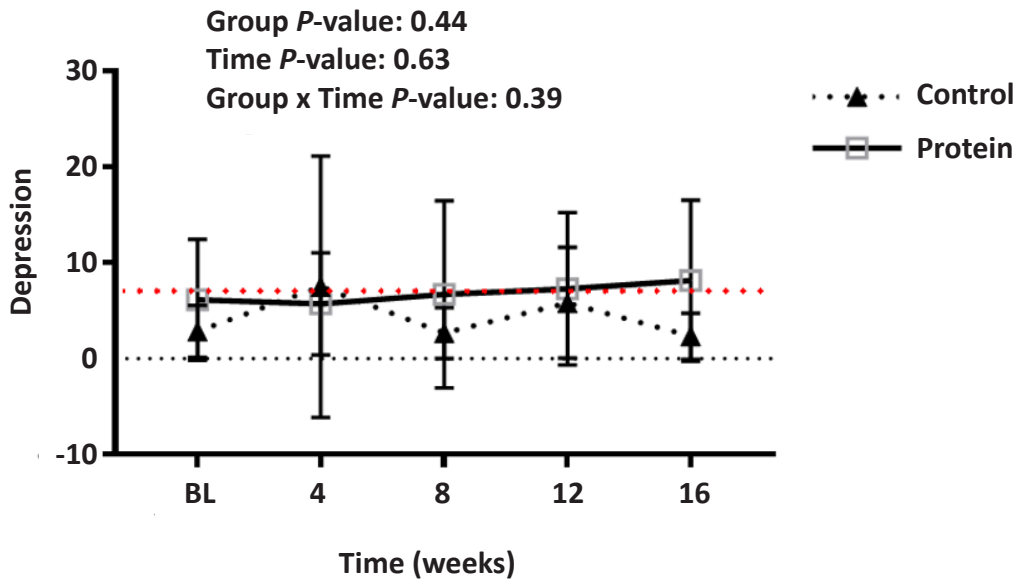

Fig. 2. Depression from baseline to 16 weeks. Control, indicated by black triangles, represents the no intervention and free living group, n = 6. Protein, indicated by the gray squares, represents the whey protein isolate intervention group, n = 7. Depression for the control group returned to baseline (BL) levels at weeks 8 and 16, rising at weeks 4 and 12. Depression for the protein group remained stable throughout the intervention. Significant differences: * $P < 0.05$. Results not significant

disorders (Hoglund et al., 2019; Wirunsawanya et al., 2018). Additionally, the literature suggests a positive impact of protein supplementation on overall mood through improving sleep efficiency (Chaput, 2014). Our results were incongruent, though the sample size may have been too small for statistically significant improvements. Aside from increasing sample size, the addition of exercise or nutrition education to the intervention may be necessary for more informative results.

Statistically significant results were found for protein intake; however, this would be expected since the intervention was a whey protein isolate supplement. These results are inconsistent with current literature, as some studies find significant results in regulating mood with protein supplements (Mohajeri et al., 2015); however, the composition of said supplements may be different from the Instantized BiPRO supplement used in this study.

Conclusions

Further research is necessary to determine a correlation between whey protein isolate and tryptophan levels, overall diet, and mood regulation in postmenopausal women. High-quality protein does appear to improve mood regulation; therefore, further research is needed.

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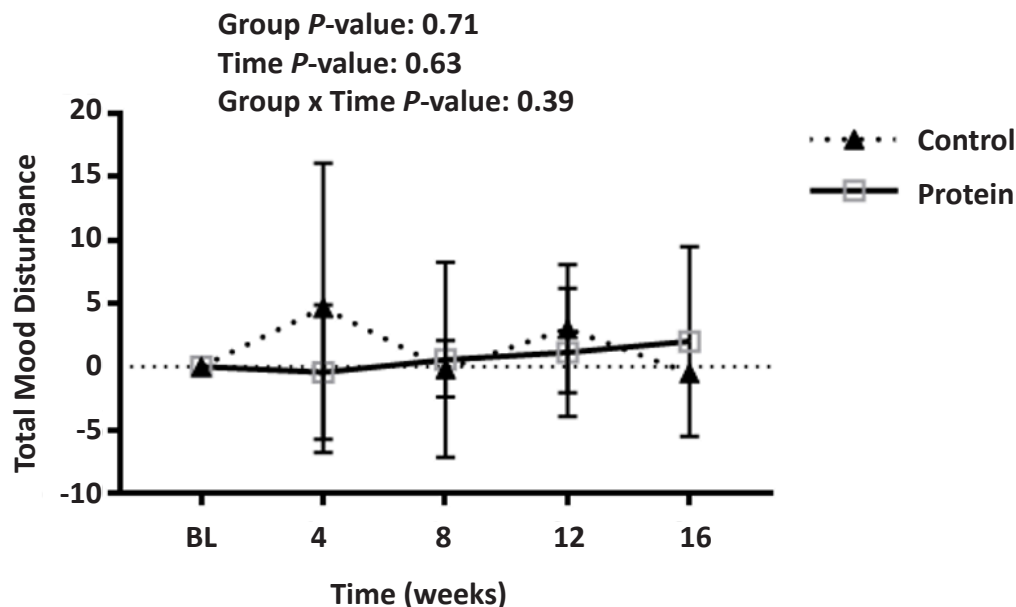
Group *P*-value: 0.71
Time *P*-value: 0.63
Group x Time *P*-value: 0.39

Fig. 3. Total Mood Disturbance from baseline to 16 weeks. Control, indicated by black triangles, represents the no intervention and free living group, n = 6. Protein, indicated by the gray squares, represents the whey protein isolate intervention group, n = 7. Total Mood Disturbance for the control group returned to baseline (BL) at weeks 8 and 16, rising at weeks 4 and 12. Total Mood Disturbance for the protein group remained mostly stable throughout, rising at week 16. Significant differences: * $P < 0.05$. Results not significant.

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