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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

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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

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Table of Contents

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 post-menopausal 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 was measured. A blood draw was administered to assess sleep and metabolic blood markers.

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, CA) 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.

Introduction and Background

The older adult population (≥65 years) in the United States is projected to double between 2017 and 2060 [2]. By 2030, it is projected that one out of every five Americans [3], or about 72 million people [4], will be of retirement age. The growing older adult population pays 95% of its health care costs to treat chronic diseases such as cardiovascular disease, diabetes, and chronic lower respiratory disease [4]. Chronic disease affects most older adults, and two out of three suffer multiple chronic diseases [4]. Furthermore, in 2006, the Behavioral Risk Factor Surveillance System (BRFSS) indicated that in the state of Arkansas, 8.5-9.8% of adults 50 years of age or older experienced frequent mental distress (FMD) and 8.6-12.4% experienced depression [5]. FMD and depression can prevent successful treatment of chronic disease [5] and negatively impact overall diet through inducing unhealthy cravings [6].

As the older adult population doubles, evidence-based recommendations are needed to establish effective preventative nutrition recommendations for adults transitioning into the older adult life stage. It is especially important to develop recommendations for the subgroup of post-menopausal women, as projections estimate that by 2030, 25% of the United States population will be women ages 45 and up [2]. The study population of post-menopausal women has not yet been thoroughly studied. As the onset of menopause occurs typically between ages 42-55 [7] and chronic diseases are often first diagnosed around age 50 [4], the post-menopause time period is crucial for identifying and managing existing chronic disease. Post-menopausal women experience shifts in body composition, including an average 44% increase of visceral fat mass during menopause [7, 8], which may affect the way nutrients, such as tryptophan, are utilized in the body. This population also experiences disrupted sleep cycles and fluctuating mood, partially as a result of hormone levels [8].

Risk of developing chronic diseases such as obesity, type 2 diabetes, and cardiovascular disease increases with age. This is partially due to aging-associated changes in body composition and hormone levels [9, 10], but may also be due to an imbalance in macronutrient intake[11]. It is recommended to consume an evenly distributed, moderate amount of high-quality protein at each meal[12], but it is established that Western eating patterns are disproportionate, with lunch and dinner providing 31% and 41% of daily protein intake, respectively, snacks providing 12%, and breakfast providing a mere 16%[13]. Furthermore, protein-containing foods consumed at breakfast are often of low protein quality [14] resulting in inadequate consumption of essential amino acids (EEA) at the breakfast meal. Low consumption of EAA, such as tryptophan, may contribute to low tryptophan levels and increased mood disturbances[15]. It may also result in lower quality of life and increased risk for chronic disease and stress related mental disorders [15]. Recent studies suggest that these factors may be reduced by whey protein supplementation [16], thereby improving overall diet and mood regulation, potentially through increased tryptophan in the diet.

Whey protein isolate (WPI, 85-90% proteins) [17] has been associated with reduced desire to eat and increased feelings of fullness [11], indicating that WPI may be useful in regulating appetite. This is congruent with the theory that the satiety effect can be manipulated by changing the macronutrient composition of diets [18]. The Recommended Dietary Allowance (RDA) of tryptophan for adults 19 years and older is 0.005 g/kg/d [19]. As the average weight of this study's intervention participants is 73.4 kg ± 18.1 kg, the RDA for this participant pool would be approximately 0.3 g/d – 0.4 g/d – 0.5 g/d for participants weighing 55.3 kg – 73.4 kg – 91.5 kg, respectively. The Instantized BiPRO supplement used in this study contains approximately 0.8 g

of tryptophan per 25 g daily serving. Therefore, this supplement provides tryptophan in amounts greater than the RDA for these participants.

Overall diet is influenced by sleep efficiency[6]. Tryptophan is the precursor to melatonin, which is involved in regulating the sleep-wake cycle [20]. Low levels of melatonin are reflected by disturbances in the sleep-wake cycle and by the inability to sustain restful sleep. A lack of sleep increases food intake, snacking, and psychological distress [6]. Insufficient sleep also increases consumption of energy-rich foods in order to reduce psychological distress or relieve negative mood [6]. An increase in food intake, particularly high sodium- and carbohydrate-containing foods, contributes to the development of cardiovascular disease, obesity, and metabolic syndrome: hypertension, dyslipidemia, hyperglycemia, and abdominal obesity [21]. Research suggests the risk for chronic disease and metabolic syndrome can be lowered by high quality-protein via supplementation [9].

Tryptophan, also a precursor to the neurotransmitter serotonin, influences mood regulation, emotional processing, and alertness [22]. Mood regulation is important because it influences three factors that contribute to quality of life: choice of daily activities, choice of diet, and choice of sleep habits. In a 19-day randomized-controlled trial, a test drink containing tryptophan was administered twice per day; researchers found significant improvements in emotional processing and reduced sensitivity to negative stimuli [22]. The results of this study suggest that high levels of tryptophan supplementation improve mood and mitigate depressive episodes [22].

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-grams 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

Subject Recruitment and Participation

Prior to subject recruitment, this study was submitted to the University of Arkansas' Institutional Review Board for approval 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 to the COVID-19 epidemic. Participants were recruited voluntarily through advertisement in the University of Arkansas Newswire, social media, word-of-mouth, and flyers posted throughout the area. Eligibility required that participants were postmenopausal women with a last reported menstrual cycle of 12 months or more, were absent of hormone replacement therapy (HRT), have no known food allergies, regularly eat breakfast (<5 times per week), not taking medications that impact appetite, blood coagulation, metabolism, or blood lipids, not regularly consuming protein supplements, not consuming more than 0.8g/kg/day of protein (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.

Intervention

This research was conducted using a randomized experimental design. A total of 13 post-menopausal women (12+ months after last reported menstrual cycle) were recruited and allocated to one of two dietary intervention (DI) groups. The demographic of post-menopausal 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 randomly assigned to 1 of 2 dietary intervention (DI) groups. The DI groups are 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, 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 completed the Pittsburgh Sleep Quality Index (PSQI) and Profile of Moods Questionnaire. Dietary logs were reviewed with participants. Height, weight, and waist-to-hip ratio was measured. A blood draw was administered by a certified phlebotomist to assess sleep and metabolic blood markers. Sleep was assessed at baseline, 8-, and 16-weeks.

Educational Materials

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

Body Composition, Anthropometrics, and Food Intake

Body composition was measured via DEXA at baseline and week 16. Height, weight, and waist-to-hip ratio was measured at baseline, 4-, 8-, 12-, and 16-weeks. Height was measured to the nearest 0.01 cm using a stadiometer (Detecto, St. Louis, MO) with participants barefoot, in the free-standing position. Weight was measured to the nearest 0.01 kg using calibrated balance scales (Detecto, St. Louis, MO), 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. Participants were trained to accurately record amounts of food using provided food scales (Greater Goods, LLC) 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, MIN) analysis software was used to analyze the energy, micronutrient, and macronutrient composition of the 3-day food records.

Mood

Mood was assessed at baseline, 8-, and 16-weeks, via the Profile of Mood States (POMS) questionnaire. The POMS questionnaire has been validated in post-menopausal women[1]. 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.

To score each affective state subscale, the sum of the responses for each adjective is calculated. 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 total mood disturbance (TMD) is calculated by the following equation:

$$TMD = (Tension - Anxiety) + (Depression - Dejection) + (Anger – Hostility)$$

$$+ (Fatigue - Inertia) + (Confusion – Bewilderment) – (Vigor – Activity)$$

Measurement of Tryptophan

Tryptophan (and all amino acids) was measured using the commercially available EZ Faast amino acid analysis kit via GC/MS.

Statistical Analysis

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, CA) was used for all analyses. All measurements are reported as the mean ± standard deviation.

Results

Participant Characteristics

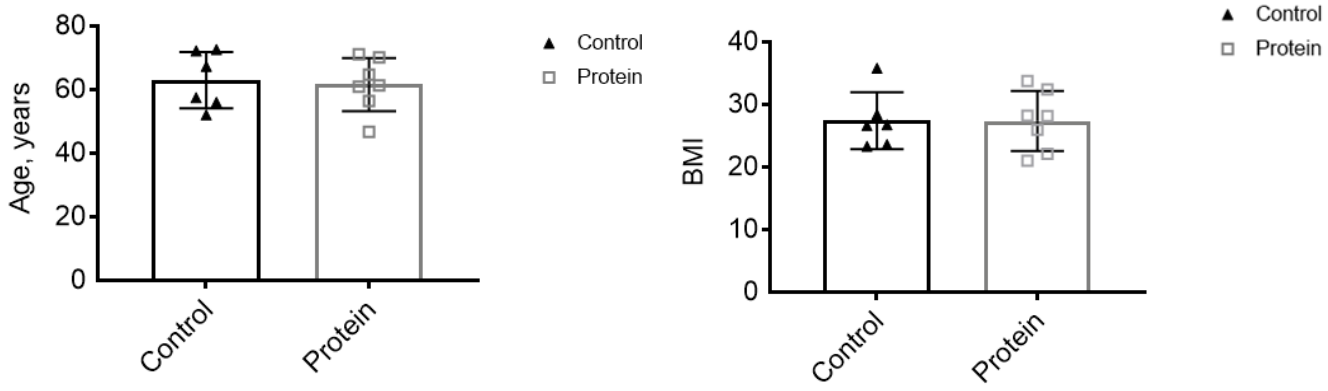
A total of 13 participants completed the study. **Table 1** shows baseline participant characteristics, including: age, height, weight, body mass index (BMI), waist-to-hip ratio, and total mood disturbance POMS score. There were no differences in anthropometrics (**Figure 1).** **Figure 2** indicates no change in BMI over the course of the intervention.

TABLE 1. Baseline anthropometric characteristics of the study population by treatment group.[1]

Baseline Characteristics	Control (n = 6)	PRO (n = 7)
Age, y	63.0 ± 8.9	61.6 ± 8.4
Anthropometrics		
Height, m	1.62 ± 0.08	1.63 ± 0.06
Weight, kg	72.4 ± 15.2	73.4 ± 18.1
BMI, kg/m^2	27.4 ± 4.6	27.4 ± 4.8
WHR	0.85 ± 0.05	0.85 ± 0.07
Mood		
POMS: TMD	6.3 ± 19.5	18.3 ± 33.0

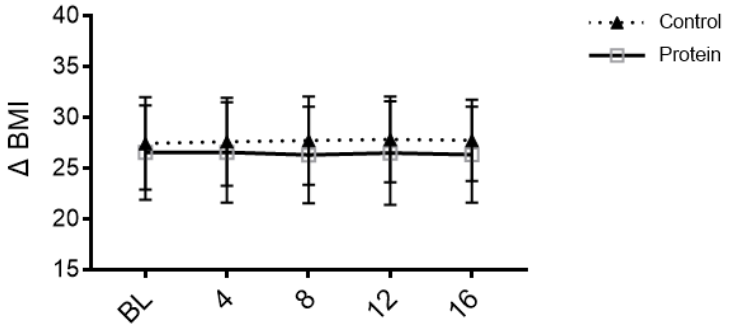
[1]Data are mean ± SDs. Significant differences: * $P < 0.05$. POMS: TMD = Total Mood Disturbance (Sum of all subscales except Vigor minus Vigor), TMD score range (-32) to 200.

FIGURE 1. Baseline anthropometrics for treatment groups.[1]



[1] Control, no intervention and free living, CON, n=6; whey protein isolate, PRO, n=7. (A) Baseline age in years. CON 63.0 ± 8.9; PRO 61.6 ± 8.4. (B) BMI in kg/m^2. CON 27.4 ± 4.6; PRO 27.4 ± 4.8.

FIGURE 2. BMI changes over the course of the intervention.[1]



[1]BMI in kg/m^2.

<u>Tryptophan</u>

Tryptophan (and all amino acids) was measured using the commercially available EZ Faast amino acid analysis kit via GC/MS. **Table 2** lists the nutrient composition of the whey protein isolate supplement. Notably, the WPI supplement contains approximately 0.8 g of tryptophan per serving. **Table 3** indicates dietary tryptophan intake in the groups. Significant differences in tryptophan intake were found, with the WPI intervention group having a higher intake than the control. **Figure 3** indicates a slight "U" shaped pattern of plasma tryptophan

levels over the course of the study. The trend for the control group starts high, decreases, then increases slightly but does not reach levels at baseline. The trend for the protein group indicates a decrease, then increase in plasma tryptophan levels.

TABLE 2. Nutrient composition of dietary supplement.[1]

	PRO
Energy content, kcal	106.4
Protein, g	25.5
Tryptophan, g	0.812
Fat, g	0.4

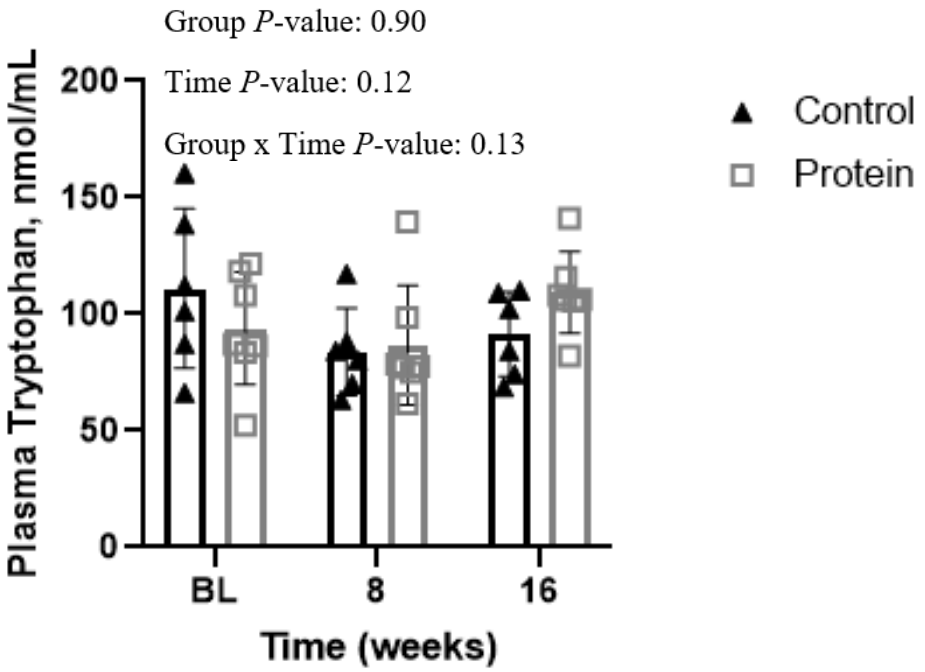
[1]The PRO represents a single dose (25 g) of whey protein isolate which participants consumed prior to 10:00 AM with breakfast daily. Whey protein isolate, PRO.

TABLE 3. Dietary tryptophan profile in intervention and control groups.[1]

Weeks of Intervention				Treatment Effect[2]			ANCOVA *P*[3]	
	0	8	16	Δ16 wk	*P*	Group	Time	Group X Time
Tryptophan, g/d					<0.01	<0.01	<0.01	<0.01
CON	0.83 ± 0.16	0.83 ± 0.21	0.89 ± 0.23	0.06 ± 0.20				
PRO	1.03 ± 0.37	1.58 ± 0.45$^{\#\$}$	1.64 ± 0.41$^{\#\$}$	0.61 ± 0.46*				

[1]Values are mean ± SD. Control, no intervention and free living, CON, n=6; whey protein isolate, PRO, n=7.
[2]Treatment effect between groups were tested by one-way ANOVA with baseline measurements subtracted from 16-week values. LS-means significant differences: * $P < 0.05$
[3]Differences in raw data were analyzed at 0, 8, and 16 using ANCOVA with age and BMI as covariates. P-values are indicated for main effects of group and time and an interaction effect of group X time. * P < 0.05 for main effect of intervention; # significantly different from control within time point following LS-means; $ significantly different compared to baseline follow LS-means.

FIGURE 3. Plasma tryptophan levels over the course of the intervention.[1]



[1]Control, no intervention and free living, CON, n=6; whey protein isolate, PRO, n=7. Significant differences: * P < 0.05. Results insignificant.

Diet

Table 4 indicates the overall diet of both the WPI supplement group and the control group over the course of the study. Significant increases in protein intake, both g/kg/bw and %, were found in the PRO group. The combined value for CHO, g/d, Time was also found to be significant.

TABLE 4. Energy and macronutrient intake in dietary intervention and control groups.[1]

Weeks of Intervention				Treatment Effect[2]			ANCOVA *P*[3]	
Energy & Macronutrients	0	8	16	Δ16 wk	*P*	Group	Time	Group X time
Energy, kcal/d					0.27	0.60	0.73	0.84
CON	1757.3 ± 573.5	1963.3 ± 858.3	1844.6 ± 490.9	87.3 ± 170.8				
PRO	2193.9 ± 1403.0	1560.9 ± 447.1	1560.2 ± 332.1	-124.5 ± 409.9				
Protein, g/day					0.51	<0.01*	0.04*	0.12
CON	71.3 ± 18.6	69.9 ± 16.2	2.7 ± 19.7	1.4 ± 18.7				
PRO	87.1 ± 35.9[$]	90.8 ± 32.8	93.9 ± 31.5	6.8 ± 36.5				
Protein, %					0.29	<0.01*	<0.01*	<0.01*
CON	16.2 ± 1.6	15.4 ± 2.1	16.1 ± 3.2	-0.07 ± 4.22				
PRO	18.9 ± 6.7	26.6 ± 9.0[$#]	23.6 ± 4.2[$#]	4.70 ± 6.13				
Protein, g/kg/bw					0.14	0.01	0.05	0.14
CON	0.99 ± 0.23	1.00 ± 0.027	1.01 ± 0.29	0.018 ± 0.274				
PRO	1.23 ± 0.54	1.32 ± 0.62	1.6 ± 0.54	0.128 ± 0.548				
CHO, g/d					0.71	0.98	0.03*	0.49
CON	183.8 ± 70.3	204.6 ± 75.1	194.2 ± 46.3	10.3 ± 21.7				
PRO	272.3 ± 202.7	178.2 ± 45.7	175.8 ± 42.3	-96.5 ± 192.8				
CHO, %					0.29	0.72	.15	0.27
CON	41.4 ± 6.1	42.3 ± 3.7	41.8 ± 9.9	0.36 ± 11.89				
PRO	45.8 ± 11.4	44.5 ± 8.7	47.6 ± 7.3	1.79 ± 6.11				
Fat, g/d					0.79	0.65	0.27	0.14
CON	79.0 ± 30.7	83.1 ± 35.7	83.6 ± 33.8	4.6 ± 1.2				
PRO	89.3 ± 64.0	55.8 ± 22.8	55.6 ± 17.6	-33.8 ± 60.4				
Fat, %					0.79	0.05	0.38	0.27
CON	39.0 ± 8.3[$]	40.0 ± 5.3	38.2 ± 8.3	-0.82 ± 10.87				
PRO	34.8 ± 6.5	30.0 ± 7.2	30.4 ± 4.6	-4.40 ± 3.89				

[1]Values are mean ± SD. Control, no intervention and free living, CON, n=6; whey protein isolate, PRO, n=6.

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

[3]Differences in raw data were analyzed at 0, 8, and 16 using ANCOVA with age and BMI as covariates. P-values are indicated for main effects of group and time and an interaction effect of group X time. * $P < 0.05$ for main effect of intervention; # significantly different from control within time point following LS-means; $ significantly different compared to baseline follow LS-means.

Mood

Table 5 indicates POMS data over the course of the study. Results were not significant. **Figures 4** and **5** specifically illustrate POMS depression and TMD data. Results were not significant.

TABLE 5. Negative and positive affect states in the dietary intervention and control groups.[1]

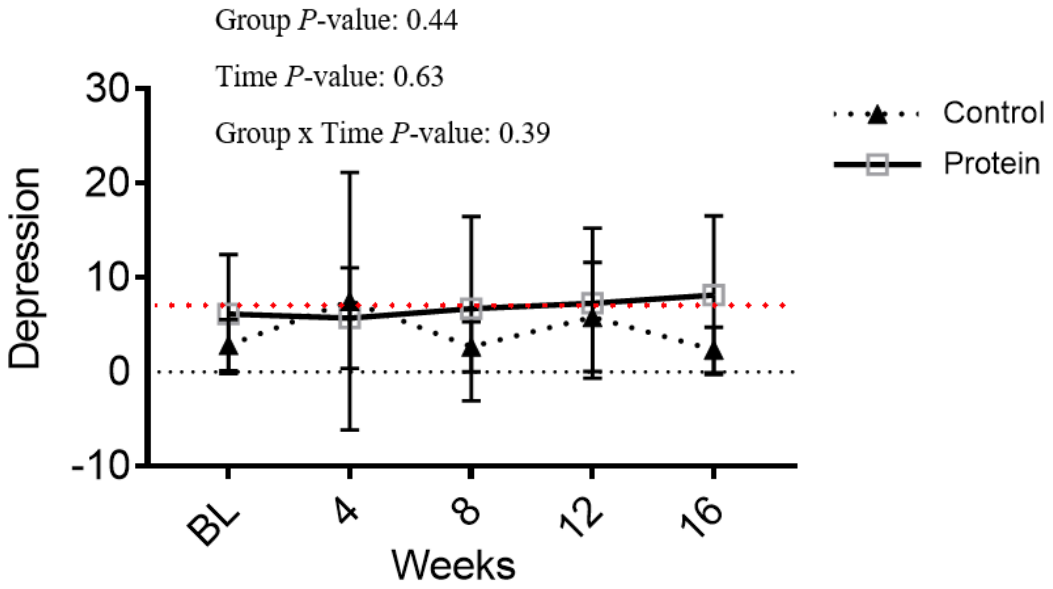
Weeks of Intervention				Treatment Effect[2]		ANCOVA *P*[3]		
POMS	0	8	16	Δ16 wk	*P*	Group	Time	Group X time
Tension/Anxiety					0.83	0.58	0.28	0.79
CON	4.0 ± 3.3	3.7 ± 3.9	4.0 ± 3.8	0.0 ± 1.9				
PRO	8.7 ± 6.9	7.0 ± 6.4	6.4 ± 5.5	-2.3 ± 6.5				
Depression					0.87	0.20	0.84	0.50
CON	3.0 ± 3.0	7.7 ± 13.6	2.7 ± 2.7	5.8 ± 5.8				
PRO	7.4 ± 8.3	6.7 ± 9.8	8.1 ± 8.4	0.7 ± 7.3				
Anger					0.89	0.65	0.56	0.12
CON	3.8 ± 5.5	1.5 ± 2.5	3.0 ± 4.0	-0.8 ± 5.3				
PRO	5.0 ± 3.4	5.9 ± 10.4	4.4 ± 7.9	-0.6 ± 6.7				
Fatigue					0.42	0.43	0.14	0.41
CON	6.5 ± 8.8	6.7 ± 6.1	8.5 ± 8.1	2.0 ± 4.6				
PRO	9.1 ± 7.2	8.0 ± 6.1	4.9 ± 4.5	-4.3 ± 4.6				
Confusion					0.27	0.76	0.93	0.02
CON	5.3 ± 3.0	5.2 ± 3.8	5.7 ± 4.3	0.3 ± 2.0				
PRO	6.6 ± 4.5	6.1 ± 4.1	3.6 ± 3.9	-3.0 ± 3.7				
Vigor					0.17	0.71	0.73	0.11
CON	16.3 ± 2.3	15.8 ± 5.5	12.3 ± 6.4	-4.0 ± 5.9				
PRO	18.6 ± 6.9	23.0 ± 7.3	20.6 ± 9.1	2.0 ± 6.5				
TMD					0.71	0.71	0.73	0.11
CON	6.3 ± 19.5	3.8 ± 18.1	11.2 ± 23.4	4.8 ± 15.6				
PRO	18.3 ± 33.0	10.7 ± 37.8	6.9 ± 32.6	-11.4 ± 26.1				

[1]Values are mean ± SD. Control, no intervention and free living, CON, n=6; whey protein isolate, PRO, n=7. POMS: TMD = Total Mood Disturbance (Sum of all subscales except Vigor minus Vigor), TMD score range (-32) to 200. All subscales are positive.

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

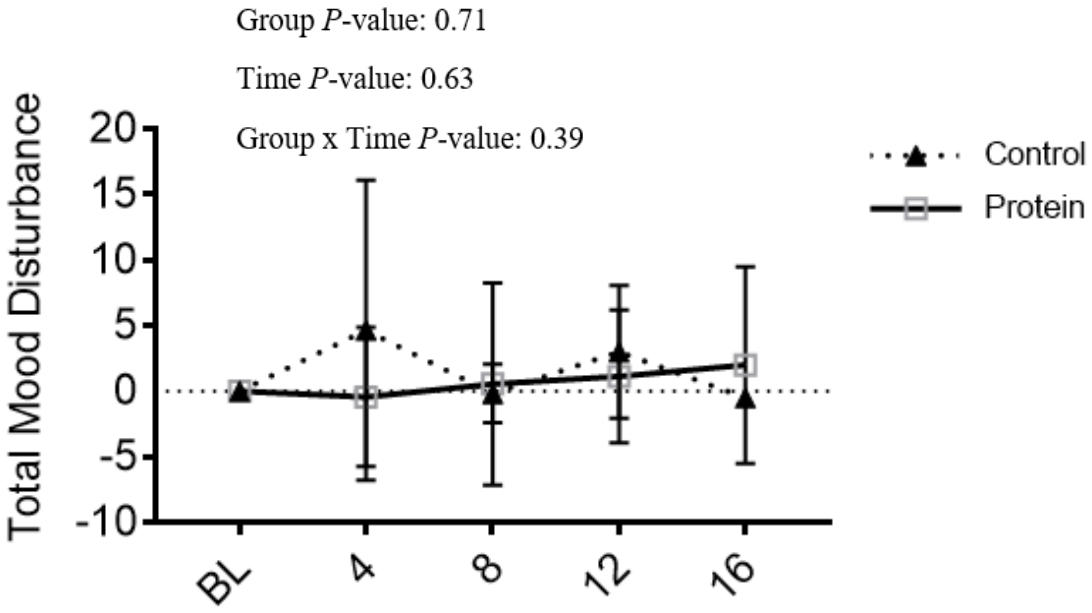
[3]Differences in raw data were analyzed at 0, 8, and 16 using ANCOVA with age and BMI as covariates. P-values are indicated for main effects of group and time and an interaction effect of group X time. * $P < 0.05$ for main effect of intervention; # significantly different from control within time point following LS-means; $ significantly different compared to baseline follow LS-means.

FIGURE 4. Depression from baseline to 16-weeks.[1]



[1]Control, no intervention and free living, CON, n=6; whey protein isolate, PRO, n=7. Significant differences: * $P < 0.05$.

FIGURE 5. Total Mood Disturbance from baseline to 16-weeks.[1]



[1]Control, no intervention and free living, CON, n=6; whey protein isolate, PRO, n=7. Significant differences: * $P < 0.05$.

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 post-menopausal 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 post-menopausal women.

There was no significant difference for body mass index, plasma tryptophan levels, and mood with 16-weeks of protein supplementation. Current research indicates that high-quality protein supplementation can reduce mood disturbances and stress related mental disorders[15,16]. Additionally, the literature suggests a positive impact of protein supplementation on overall mood, through improving sleep efficiency[6]. 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 is a whey protein isolate supplement. These results are inconsistent with current literature, as some studies are finding significant results in regulating mood with protein supplements[24]; however, the composition of said supplements may be different from the Instantized BiPRO supplement used in this study.

There are several notable strengths of this study. First, on laboratory days, shakes were delivered in opaque containers, with lids and straws. Second, we provided recipes to both intervention groups, with modifications for the protein group; this increased the probability of compliance by increasing palatability of the whey protein. Additionally, most of the 3-day food

record data was uploaded to the Nutrition Data System for Research (Nutrition Coordinating Center, University of Minnesota, Minneapolis, MIN) analysis software by the same researcher. Finally, there was low participant anthropometric variability.

However, there were several limitations to this study. First, the food records were not reviewed by a registered dietitian. This does increase risk of missing food record data. Second, food records are not 100% reliable, as information is recorded by the participant, not the researcher. Additionally, protein supplement compliance cannot be verified with absolute certainty, though biomarker trends will support compliance.

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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Appendices

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

UNIVERSITY OF ARKANSAS

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

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

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

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

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

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

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

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

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

Page 1 of 1

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

UNIVERSITY OF ARKANSAS

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

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

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

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

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

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Page 1 of 1