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Effects of Leu-Asp-Gln-Trp-enriched whey protein hydrolysate on mood and fatigue in healthy adults: A randomized, double-blind, placebo-controlled trial

Mayu Nakatsuka[1*], Kazumi Nabeshima[1], Ryo Sakiyama[1], Soichiro Sato[1], Manabu Nakano[1], Miyuki Tanaka[1], Masahiko Nakamura[2]

[1]Food Function Research Institute, R&D Division, Morinaga Milk Industry Co., Ltd., 5-1-83, Higashihara, Zama, 252-8583, Kanagawa, Japan; [2]Department of Neurosurgery, Matsumoto City Hospital, 4417-180, Hata, Matsumoto, 390-1401, Nagano, Japan.

***Corresponding Author:** Mayu Nakatsuka, Food Function Research Institute, R&D Division, Morinaga Milk Industry Co., Ltd., 5-1-83, Higashihara, Zama, 252-8583, Kanagawa, Japan.

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ABSTRACT

Background: Mental health concerns and fatigue are increasingly prevalent among healthy adults.

Objective: This study evaluated the effects of whey protein hydrolysate (WPH) enriched with the functional tetrapeptide Leu-Asp-Gln-Trp (LDQW) on mood state and fatigue.

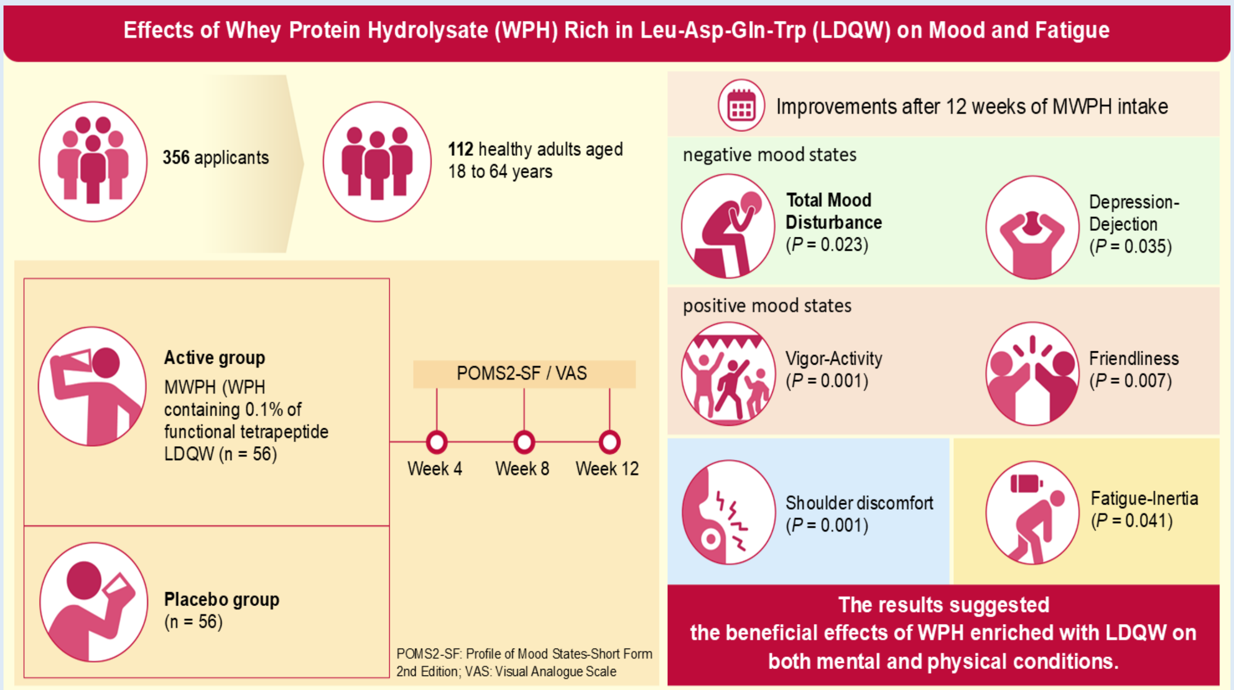
Methods: A randomized, double-blind, placebo-controlled, parallel-group comparative study was conducted on 112 healthy adults aged 18 to 64 years. Participants were randomly assigned to receive either an active food containing 1.0 g of MWPH (WPH with 0.1% of LDQW) or a placebo food daily for 12 weeks. The primary outcome was mood state using the Profile of Mood States-Short Form 2nd Edition (POMS2-SF), and the secondary outcome was fatigue, evaluated using the Visual Analog Scale (VAS).

Results: The active group showed significant improvements in several POMS2-SF scores (Total Mood Disturbance, Depression-Dejection, Fatigue-Inertia, and Friendliness) compared with the placebo group at 12 weeks. Notably, the active group showed significant improvements in the Vigor-Activity score of POMS2-SF compared to the placebo group, at as early as 4 weeks, which persisted throughout the study. The VAS score for shoulder discomfort also significantly improved in the active group compared with the placebo group at 4 weeks and beyond. No adverse events related to the test foods were observed.

Conclusions: Daily intake of the WPH enriched with LDQW improved mood state, reduced fatigue, and relieved shoulder discomfort, providing benefits for both mental and physical health.

Trial registration: The study protocol was registered with UMIN-CTR (ID: UMIN000054349).

Keywords: whey protein hydrolysate; mood; fatigue; shoulder discomfort; LDQW; functional peptides



Graphical Abstract: Effects of leu-asp-gln-trp-enriched whey protein hydrolysate on mood and fatigue in healthy adults: A randomized, double-blind, placebo-controlled trial

INTRODUCTION

The global prevalence of mental disorders has been increasing steadily each year. According to a 2025 World Health Organization report, one in seven people worldwide is affected by mental disorders such as depression and anxiety, rendering them a significant health issue [1]. These disorders are frequently associated with stress-related mental and physical symptoms [2-3] and cause long-term discomfort and a significant decline in the quality of life. Additionally, individuals without mental disorders may experience deteriorating health and reduced quality of life due to everyday mental stress [4]. Hence, practicing daily mental health care helps prevent mental disorders and maintain quality of life.

A fundamental approach to sustaining mental health involves adopting daily habits that are healthy, such as a nutritious diet, adequate sleep, and regular physical activity. Recent research and development on the functionality of food ingredients have also progressed as a means of supporting the prevention of various diseases, including mental illness [5-7]. For example, fermented dairy products have been linked to a reduced risk of depression [8], and bioactive peptides derived from milk proteins offer health benefits such as blood pressure reduction and antidiabetic activities [9-11].

Previous studies on Leu-Asp-Gln-Trp (LDQW) derived from whey protein hydrolysate (WPH) have reported its antioxidant [12] and xanthine oxidase (XO) inhibitory activities [13]. Excessive oxidative stress from external stressors can lead to both mental and physical symptoms, including mood decline and shoulder stiffness [14-15]. LDQW may alleviate these effects by reducing oxidative stress through the inhibition of reactive oxygen species (ROS) production. A previous study has exploratively examined the effects of MWPH (WPH containing 0.1% LDQW) intake on mood and fatigue in adults with a higher body mass index (BMI), showing potential effectiveness [16]. However, robust clinical evidence supporting the mood-enhancing effects of LDQW-enriched WPH in a general adult population remains scarce. Establishing these benefits would offer a valuable strategy for promoting mental health.

Valid mental health assessment methods include the Profile of Mood States Second Edition-Adult (POMS2), a valid mental health questionnaire that measures total mood disturbance, with subscales covering a wide range of mood states, including fatigue, dejection, and vigor [17]. POMS2 Short Form (POMS2-SF) has equivalent validity to the POMS2 and has been used worldwide because of its simplicity. This study employed the POMS2-SF to evaluate the impact of LDQW-enriched WPH on mood improvement and fatigue reduction in healthy adults.

MATERIALS AND METHODS

Study Design: This randomized, double-blind, placebo-controlled, parallel-group study was conducted in Matsumoto City (Nagano, Japan). This study was conducted from July to December 2024. The study was approved by the Ethics Review Committee of Matsumoto City Hospital (approval code: 06-2, approval date: April 23, 2024), and the study protocol was registered in the UMIN-CTR (ID: UMIN000054349). All participants were fully informed about the purpose, procedures, and rights prior to participation, and informed consent was obtained. This study was carried out following the Declaration of Helsinki (2013 Fortaleza Revision) and the Ethical Guidelines for Life Science and Medical Research Involving Human Subjects (Notice No. 1 of 2021 issued jointly by the Ministry of Education, Culture, Sports, Science and Technology, the Ministry of Health, Labour and Welfare, and the Ministry of Economy, Trade, and Industry). No protocol changes were made after the approval of the Ethics Review Committee.

Participants: Participants who met the following inclusion criteria were selected: age 18–64 years. Exclusion criteria were as follows: (1) those who have severe diseases or life-style-related illnesses affecting the heart, liver, kidneys, respiratory system, and digestive system (e.g., diabetes, hyperlipidemia), or those with a history of such conditions; (2) those who regularly consume food or medication that may affect mental state; (3) those who have depression or other mental disorders (including suspected cases) or a history of such conditions; (4) those with chronic fatigue syndrome; (5) those who have severe allergies to medication or food; (6) those who have a BMI of 30.0 kg/m^2 or higher; (7) those with heavy alcohol consumption (60 g of alcohol or more per day); (8) those currently enrolled in other studies, or have participated in another study involving medication or food within one month prior to obtaining informed consent; (9) those who were pregnant, lactating, or intended to conceive during the course of the study; (10) those who were deemed unsuitable by the principal investigator.

Sample Size: The sample size was set at 200 participants (100 participants per group), reflecting the upper limit of participants that could be accommodated for testing at the study site.

Randomization and Blinding: An allocation manager, independent of the study conduct, assigned participants to the active and placebo groups in a 1:1 ratio using a computer-generated allocation table based on a permutated block method with block sizes of four. The allocation manager ensured the confidentiality of the allocation by randomly assigning participants to test food numbers using computer-generated random numbers. The allocation table was sealed and kept in a secure location until the study was completed.

Intervention: Active foods contained MWPH (WPH containing 0.1% LDQW [18]) manufactured by Morinaga Milk Industry, Tokyo, Japan, and were previously used in other clinical studies [16, 19]. In this study, the active food was prepared as a powder containing 1.0 g MWPH. The placebo food was prepared by replacing the MWPH with 1.0 g of dextrin. Flavorings, sweeteners, and acidulants were adjusted to ensure no perceptible differences in taste. The nutritional values and energy per package were as follows: active food—protein 0.8 g, fat 0.0 g, carbohydrates 0.4 g, sodium equivalent 0.02 g, and energy 4.8 kcal; placebo food—protein 0.0 g, fat 0.0 g, carbohydrates 1.2 g, sodium equivalent 0.04 g, and energy 4.9 kcal. The allocation manager, who was not directly involved in this study, confirmed that the active and placebo foods could not be distinguished by shape, color, taste, odor, or packaging. Participants were instructed to dissolve one package of the active or placebo food in approximately 100 mL of water and drink it once a day for 12 weeks. The timing of test food intake was not specified.

Background information: Height and weight information was collected at the time of the screening. Other background information was collected using a web-based questionnaire.

Efficacy Evaluation: The primary outcome was the standardized score assessed using the POMS2-SF [17]. Secondary outcome was the Visual Analogue Scale (VAS) for fatigue, shoulder discomfort, and lower back discomfort. The exploratory outcome was the Norm-Based Scorings (NBS) of the 36-Item Short Form Health Survey (SF-36) v2 Japanese version [20].

Profile of Mood States-Short Form, 2nd Edition (POMS2-SF): POMS2-SF was administered on Thursday mornings at participant’s own homes at baseline, 4, 8, and 12 weeks. The time frame for evaluation was defined as “how you felt during the past week, including today.” Raw scores were converted to standardized scores (T scores) and evaluated using the Standardized Total Mood Disturbance Score (including overall mood state) and seven subscales: Anger-Hostility, Confusion-Bewilderment, Depression-Dejection, Fatigue-Inertia, Tension-Anxiety, Vigor-Activity, and Friendliness [17]. The Standardized Total Mood Disturbance score was calculated as follows: Total Mood Disturbance = (Anger-Hostility + Confusion-Bewilderment + Depression-Dejection + Fatigue-Inertia + Tension-Anxiety) − Vigor-Activity.

Visual Analog Scale: VAS scores were administered on Thursday morning at participant’s own homes at baseline, 4, 8, and 12 weeks. In accordance with fatigue guideline [21], participants were asked to indicate their current condition on a 100-mm line, where 0 mm meant “no fatigue at all” and 100 mm meant “extreme fatigue, unable to do anything.” The questionnaire included three items: fatigue, shoulder discomfort, and lower-back discomfort.

36-Item Short Form Health Survey: SF-36 was administered at the test site at baseline, 4, 8, and 12 weeks. The scoring results were converted to NBS scores, and evaluated using the physical component summary scores, mental component summary scores, role/social component summary scores, and their eight subscales [22].

Participant Management: During the study, participants were instructed to avoid excessive exercise, overeating, dieting, and alcohol consumption, which would substantially change their baseline lifestyle. They were also instructed to avoid medications and functional foods that could influence mood or fatigue. Participants recorded their daily lifestyle in a diary on their test food intake, sleep patterns, exercise, alcohol consumption, health conditions, hospital visits, intake of medications or functional foods, work status, and any unusual events. The test food intake rate was calculated using the records in the lifestyle diary and the number of test food items returned. If the intake values calculated from the lifestyle diary and the remaining test food quantities did not match, the lower value was adopted as the actual intake value. At baseline and week 12, a dietary survey was conducted at the test site using the Food Frequency Questionnaire ver.1.0 (FFQ NEXT, Kenpakusha, Tokyo, Japan). Energy, protein, fat, and carbohydrate contents were calculated using EiyoPlus ver.1.1 (Kenpakusha, Tokyo, Japan).

Safety: Safety analyses included all participants who consumed the test food, regardless of the frequency of consumption. Adverse events occurring during the study period were recorded based on the lifestyle diaries and test-site interviews. Severity was assessed based on the NCI-CTCAE Version 5.0 and the Japanese translation of the Common Terminology Criteria for Adverse Events Version. 5.0 (JCOG Edition). The causal relationship between adverse events and test food was judged by the principal investigator (physician) based on these records.

Statistical Analysis: An independent data manager oversaw all data management. Participants included in the efficacy analysis were those meeting the criteria for the per protocol set (PPS). Background information and measurement values were summarized by group, and mean values and standard deviations were calculated for each group. Student's *t*-tests were performed for quantitative data. Frequencies were summarized for ordinal and categorical data analyses. Ordinal data were analyzed using the Wilcoxon rank-sum test, and categorical data were analyzed using the Fisher's exact test. The incidence rates of adverse events were analyzed using Fisher's exact test, and *P*-values were calculated. The efficacy analysis was conducted with the primary outcome defined as the Total Mood Disturbance score on the POMS2-SF, and the secondary outcome defined as the VAS score. Statistical analysis was performed using the value obtained at week 12 as the main endpoint of this study. Comparisons between groups were made using analysis of covariance (ANCOVA), with the value from week 12 as the response variable, the intervention group as the explanatory variable, and the baseline values as the covariates. Multiple comparisons at the measurement time points were accounted for using a closed procedure [23]. Specifically, two-group comparisons were performed in the order of 12, 8, and 4 weeks, with subsequent comparisons conducted only if the previous comparison was significant. The adjustment for multiplicity between secondary and exploratory outcomes and the POMS2-SF subscales was not considered.

Statistical analysis was performed using IBM SPSS Statistics 29.0.0 (IBM Japan, Ltd., Tokyo, Japan), and the significance level for all tests was set at less than 5% for two-tailed tests. Data that were unavailable were treated as missing values without applying imputation methods.

RESULTS

Participants: A flowchart of the participant is presented in Figure 1. Screening tests were conducted on 356 applicants who consented to participate in this study. Based on the eligibility criteria, 112 participants (56 in the active group and 56 in the placebo group) were selected. They met the inclusion criteria and none of the exclusion criteria. The principal investigator, who is a physician, confirmed that none of the participants had any health issues. During the study period, one participant in the active group and one in the placebo group were lost to follow-up due to personal reasons. Thus, the safety analysis population comprised 110 participants (55 in the active group and 55 in the placebo group), excluding the two participants who were lost to follow-up and had no data after randomization. The PPS analysis included 83 participants (41 in the active group and 42 in the placebo group) after excluding 27 participants: 4 with changes in lifestyle, 1 with a food intake rate below 80%, 15 with inappropriate evaluation conditions, and 7 who used prohibited medications or functional foods.

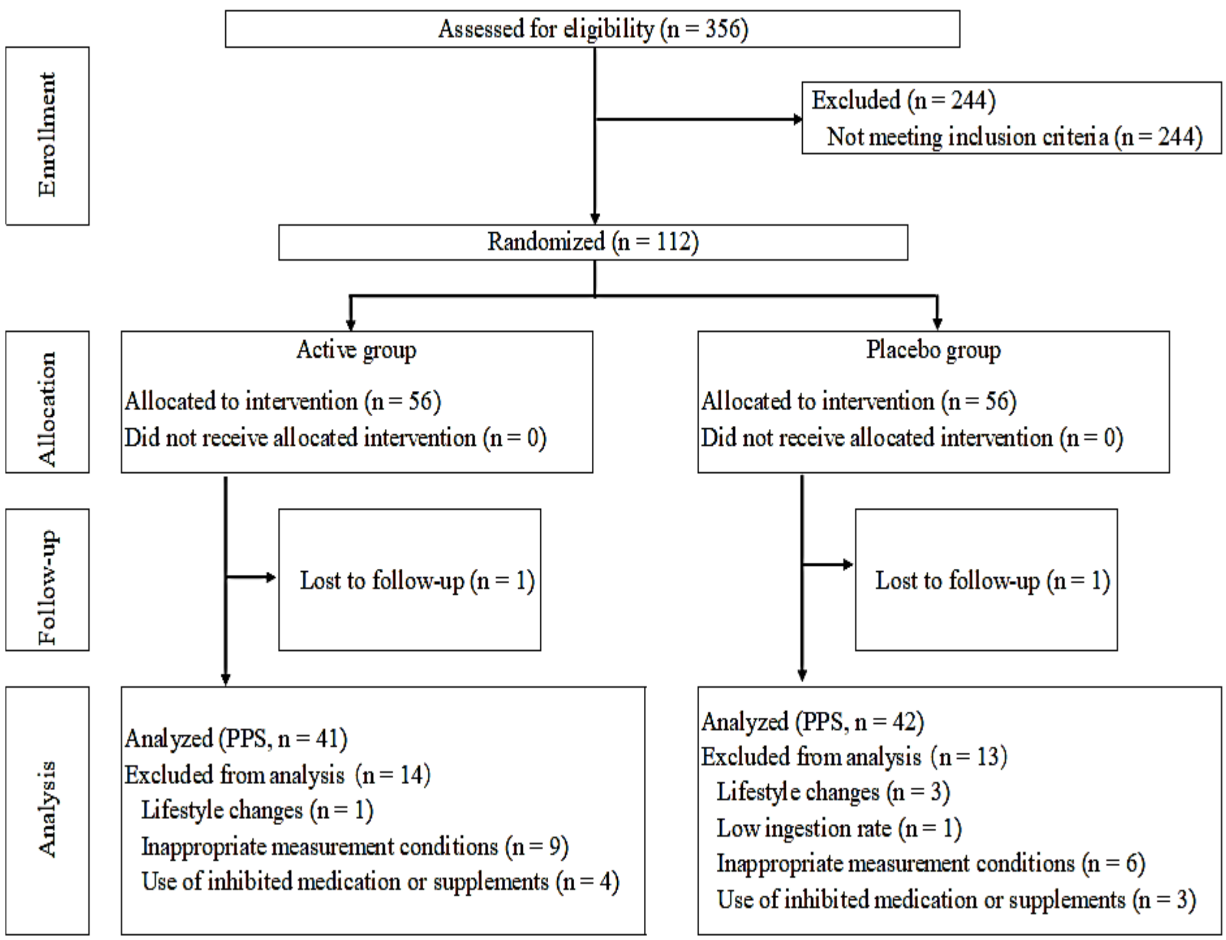


Figure 1. Flowchart of the study, which shows participant enrollment, allocation, follow-up, and analysis.

Baseline characteristics of participants are summarized in Table 1. The two groups did not differ significantly in age, sex, weight and BMI at baseline, in either the intention-to-treat (ITT) or PPS populations. The average intake rate of the test food in the PPS was 96.8 ± 4.4% in the active group and 96.3 ± 5.1% in the placebo group, with no significant difference (P = 0.46).

Table 1. Baseline characteristics of participants.

	All Participants (n = 112)	ITT Population			PPS Population		
		Active (n = 56)	Placebo (n = 56)	*P* value	Active (n = 41)	Placebo (n = 42)	*P* value
Male / Female (n)	48 / 64	24 / 32	24 / 32	1.000[1]	16 / 25	20 / 22	0.509[1]
Age (years)	45.1 ± 10.6	44.7 ± 11.0	45.5 ± 10.3	0.697[2]	44.1 ± 11.4	46.4 ± 10.2	0.351[2]
Weight (kg)	57.8 ± 9.9	58.2 ± 9.9	57.5 ± 9.9	0.697[2]	57.1 ± 9.7	58.1 ± 10.9	0.656[2]
BMI (kg/m^2)	21.4 ± 2.8	21.7 ± 3.1	21.2 ± 2.5	0.335[2]	21.4 ± 3.0	21.1 ± 2.7	0.722[2]

Values are means ± standard deviations (SD). ITT: intention-to-treat, PPS: per protocol set, BMI: body mass index.

[1] Comparisons between groups were performed using Fisher's exact test.

[2] Comparisons between groups were made using unpaired Student's t-tests.

Nutrient intake assessed using the FFQ NEXT for energy, protein, fat, and carbohydrates (Supplementary Material A1) showed no significant differences between groups at baseline or at 12 weeks.

Profile of Mood States-Short Form 2nd Edition: The results of the POMS2-SF are summarized in Table 2. Standardized scores for Total Mood Disturbance showed significant improvement in the active group compared with the placebo group at 12 weeks (*P* = 0.023) and tended to improve in the active group at 8 weeks (*P* = 0.084, Figure 2A). The effect size for Total Mood Disturbance in the POMS2-SF at 12 weeks was moderate (Cohen's d = 0.51).

Among the subscales, Depression-Dejection (*P* = 0.035), Fatigue-Inertia (*P* = 0.041), Vigor-Activity (*P* = 0.001), and Friendliness (*P* = 0.007) improved significantly in the active group compared to the placebo group at 12 weeks. Additionally, Vigor-Activity showed significant improvement in the active group at 4 weeks (*P* = 0.018) and 8 weeks (*P* = 0.002, Figure 2B). Friendliness improved in the active group at 4 (*P* = 0.084) and 8 weeks (*P* = 0.067).

Table 2. POMS2-SF T-scores in the active and placebo groups during the intervention.

		0 week				4 weeks				8 weeks				12 weeks			
	Group	LS mean		SE		LS mean		SE		LS mean		SE		LS mean		SE	
Total Mood Disturbance (TMD)	Active	50.8	±	0.6		46.8	±	1.1		45.0	±	1.0		43.4	±	1.1	†
	Placebo	50.8	±	0.6		48.4	±	1.1		47.4	±	0.9		46.9	±	1.0	
Anger-Hostility (AH)	Active	48.6	±	0.8		46.5	±	0.9		45.2	±	0.9		44.7	±	1.0	
	Placebo	48.6	±	0.8		48.1	±	0.9		47.2	±	0.9		46.2	±	1.0	
Confusion-Bewilderment (CB)	Active	50.8	±	0.7		48.4	±	1.3		47.7	±	1.0		46.5	±	1.0	
	Placebo	50.8	±	0.7		48.1	±	1.3		47.0	±	1.0		48.0	±	1.0	
Depression-Dejection (DD)	Active	48.6	±	0.7		47.4	±	1.1		45.8	±	0.8		45.2	±	1.0	†
	Placebo	48.6	±	0.7		48.4	±	1.1		47.4	±	0.8		48.1	±	0.9	
Fatigue-Inertia (FI)	Active	53.0	±	0.7		47.1	±	1.2		46.0	±	1.0		44.0	±	1.2	†
	Placebo	53.0	±	0.7		48.8	±	1.1		47.3	±	1.0		47.4	±	1.1	
Tension-Anxiety (TA)	Active	51.5	±	0.8		48.8	±	1.2		46.5	±	1.1		44.9	±	1.2	
	Placebo	51.5	±	0.8		48.3	±	1.2		47.6	±	1.0		46.1	±	1.2	
Vigor-Activity (VA)	Active	48.9	±	0.8		53.1	±	1.1	†	54.0	±	1.0	††	55.9	±	1.2	††
	Placebo	48.9	±	0.8		49.4	±	1.1		49.5	±	1.0		50.3	±	1.2	
Friendliness (F)	Active	49.7	±	1.0		53.3	±	1.1		54.4	±	1.1		55.5	±	1.1	††
	Placebo	49.7	±	1.0		50.6	±	1.1		51.4	±	1.1		51.2	±	1.1	

POMS2-SF: Profile of Mood States-Short Form 2nd Edition, LS mean: least squares mean, SE: standard error, ANCOVA: analysis of covariance

Multiple comparisons at the measurement time points were accounted for using closed procedures. Difference between the active and placebo groups determined to be significant using ANCOVA adjusted for the baseline (†; $P < 0.05$, ††; $P < 0.01$).

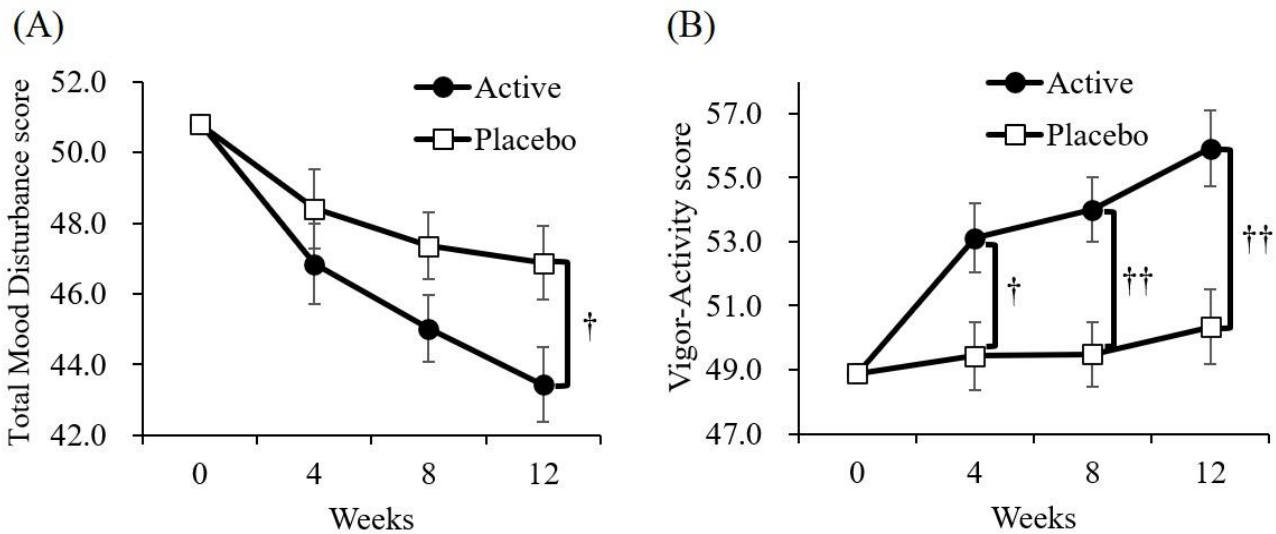


Figure 2. Total Mood Disturbance (A) and Vigor-Activity (B) score changes over time in POMS2-SF.

Values are the least square means ± standard error. Significant difference between active group and placebo group determined by ANCOVA adjusted for the baseline (†; *P* < 0.05, ††; *P* < 0.01).

Visual Analog Scale: The VAS results are summarized in Table 3. Shoulder discomfort significantly improved in the active group compared with the placebo group at 4 weeks (*P* = 0.035), 8 weeks (*P* < 0.001), and 12 weeks (*P* = 0.001) in Figure 3. Fatigue tended to improve in the active group compared to the placebo group at 12 weeks (*P* = 0.064).

Table 3. VAS scores in the active and placebo groups during the intervention.

		0 week				4 weeks				8 weeks				12 weeks			
	Group	LS mean		SE		LS mean		SE		LS mean		SE		LS mean		SE	
Feeling fatigue	Active	542.2	±	24.7		436.6	±	26.8		369.2	±	29.2		375.4	±	31.8	
	Placebo	542.2	±	24.7		449.4	±	26.5		430.0	±	28.9		459.2	±	31.4	
Shoulder discomfort	Active	402.9	±	29.9		328.9	±	34.6	†	260.2	±	32.5	††	274.5	±	32.3	††
	Placebo	402.9	±	29.9		433.1	±	34.2		444.3	±	32.2		424.1	±	32.0	
Lower back discomfort	Active	365.3	±	29.6		285.5	±	33.0		285.5	±	34.6		286.4	±	33.7	
	Placebo	365.3	±	29.6		316.8	±	32.6		310.2	±	34.2		315.2	±	33.3	

VAS: Visual Analog Scale, LS mean: least squares mean, SE: standard error, ANCOVA: analysis of covariance.
Multiple comparisons at measurement time points were accounted for using a closed procedure. Difference between the active and placebo groups determined to be significant by using ANCOVA adjusted for the baseline (†; *P* < 0.05, ††; *P* < 0.01)

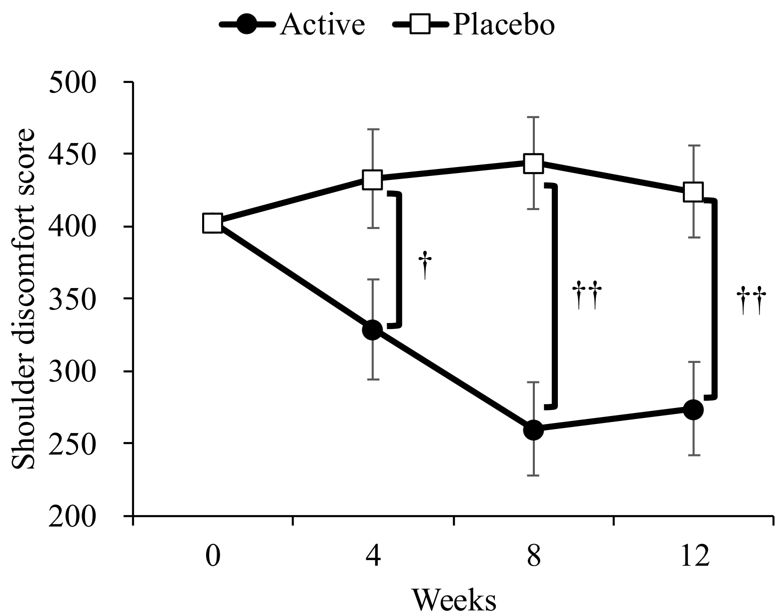


Figure 3. Shoulder discomfort score changes over time in each group.

Values are the least square means ± standard error. Significant difference between active group and placebo group determined by ANCOVA adjusted for the baseline (†; $P < 0.05$, ††; $P < 0.01$).

36-Item Short Form Health Survey: The results of SF-36 are presented in Table 4. In the active group, the mental component summary score improved significantly compared to that in the placebo group at week 8 ($P < 0.001$) and week 12 ($P = 0.004$). The vitality score improved significantly in the active group compared to the placebo group at 4 weeks ($P = 0.047$), 8 weeks ($P = 0.001$), and 12 weeks ($P = 0.005$). The Role/Social component summary score significantly improved in the placebo group compared to the active group at 4, 8, and 12 weeks.

Safety: A safety assessment was conducted for 110 participants whose consumption of the test food was confirmed, regardless of intake frequency. The incidence rate of adverse events was 76.4% in the active group and 74.5% in the placebo group, with no significant difference between the two groups ($P = 1.00$). All adverse events were mild and transient, and none were judged by the principal investigator (physician) to be related to the test food.

DISCUSSION

This study is the first to demonstrate that daily intake of the WPH containing 0.1% LDQW leads to significant improvements in mood, reductions in fatigue, and alleviation of shoulder discomfort in healthy adults. POMS2-SF in the primary outcome confirms an improvement in overall mood. The Total Mood Disturbance score is an indicator of overall negative mood. In addition, the subscale Depression-Dejection indicates a state of depressed mood, while Fatigue-Inertia serves as an indicator of fatigue and reduced vitality [17]. In this study, significant improvements were observed in the active group. Therefore, the WPH enriched with LDQW may alleviate depressed mood, reduce fatigue, and improve negative emotional states.

Additionally, the Vigor-Activity and Friendliness

Table 4. SF-36 NBS-scores in the active and placebo groups during the intervention.

		0 week				4 weeks				8 weeks[1]				12 weeks			
	Group	LS mean		SE		LS mean		SE		LS mean		SE		LS mean		SE	
Physical component summary	Active	53.2	±	0.7		53.9	±	0.9		53.3	±	0.8		52.8	±	0.8	
	Placebo	53.2	±	0.7		52.5	±	0.9		52.5	±	0.8		52.6	±	0.8	
Mental component summary	Active	49.7	±	0.7		52.6	±	0.9		54.8	±	0.8	††	55.3	±	1.0	††
	Placebo	49.7	±	0.7		50.9	±	0.9		50.6	±	0.8		51.2	±	1.0	
Role/social component summary	Active	48.3	±	0.8		50.1	±	1.1		51.1	±	0.8		51.5	±	0.9	
	Placebo	48.3	±	0.8		53.7	±	1.1	†	54.3	±	0.8	††	54.4	±	0.9	†
Physical functioning	Active	53.6	±	0.5		54.9	±	0.4		55.0	±	0.4		54.8	±	0.4	
	Placebo	53.6	±	0.5		55.2	±	0.4		54.8	±	0.4		55.3	±	0.4	
Role physical	Active	51.2	±	0.8		53.8	±	0.8		53.4	±	0.8		53.3	±	0.7	
	Placebo	51.2	±	0.8		53.4	±	0.8		53.9	±	0.8		53.9	±	0.7	
Bodily pain	Active	47.6	±	1.0		49.1	±	1.1		50.9	±	1.0		50.1	±	1.1	
	Placebo	47.6	±	1.0		47.4	±	1.1		49.4	±	1.0		49.3	±	1.1	
General health	Active	53.7	±	0.8		56.4	±	0.8		57.3	±	0.9		57.7	±	0.9	
	Placebo	53.7	±	0.8		55.8	±	0.8		55.2	±	0.9		55.7	±	0.9	
Vitality	Active	48.0	±	0.9		52.6	±	1.0	†	53.8	±	1.0	††	54.4	±	1.1	††
	Placebo	48.0	±	0.9		49.7	±	1.0		48.9	±	1.0		50.0	±	1.0	
Social functioning	Active	49.5	±	0.9		52.7	±	1.0		54.9	±	0.7		54.3	±	0.7	
	Placebo	49.5	±	0.9		54.8	±	1.0		54.8	±	0.7		55.8	±	0.7	
Role emotional	Active	48.6	±	0.8		50.9	±	0.9		52.1	±	0.8		53.3	±	0.7	
	Placebo	48.6	±	0.8		53.3	±	0.9		53.7	±	0.8		53.8	±	0.7	
Mental health	Active	51.6	±	0.7		53.0	±	0.9		55.4	±	0.8		56.0	±	1.0	
	Placebo	51.6	±	0.7		53.6	±	0.9		54.0	±	0.8		54.1	±	0.9	

SF-36: 36-Item Short Form Health Survey, NBS: Norm-Based Scoring, LS mean: least squares mean, SE: standard error, ANCOVA: analysis of covariance.

Multiple comparisons at measurement time points were accounted for using a closed procedure. The difference between the active and placebo groups determined to be significant using ANCOVA adjusted for the baseline (†; $P < 0.05$, ††; $P < 0.01$). [1]One participant in the placebo group was excluded due to missing data.

subscales of the POMS2-SF are indicators of positive mood, such as feelings of liveliness, proactivity, and enjoyment of social interactions. This suggests that the WPH enriched with LDQW may also help maintain a positive mood. VAS fatigue score, which was a secondary outcome in this study, and the SF-36 vitality score, an indicator of fatigue and reduced vitality evaluated exploratively, also yielded results supporting these findings. As similar results have been obtained across multiple indicators, the reliability of this effect on mood state is considered highly reliable.

Furthermore, not only was its effect on mood states demonstrated, but the WPH enriched with LDQW also showed efficacy in improving QOL in the exploratory assessment of the SF-36. This questionnaire has been validated and widely used to measure health-related quality of life (HRQOL) [20]. The summary score is categorized into physical, mental, and social domains and calculated from the subscale scores. The WPH enriched with LDQW is considered to improve the mental quality of life (QOL) among these. Although the role/social component summary score was higher in the placebo group, this result may be influenced by external social factors beyond the scope of the intervention, such as work, school, and community activities.

Significant improvements in shoulder discomfort were also observed in the active group. Shoulder discomfort is a symptom of shoulder stiffness and may also appear as a stress response [24]. In other words, the WPH enriched with LDQW demonstrates improvement effects for both mental symptoms such as depressive states, reduced vitality, and fatigue, as well as physical symptoms like stiff shoulders (both of which are part of the stress response). This suggests that the WPH enriched with LDQW may influence overall stress-related symptoms.

Excessive oxidative stress is known to contribute to mental and physical discomfort, including worsening mood [14-15]. LDQW, contained in MWPH, has demonstrated antioxidant properties [12]. The WPH enriched with LDQW may reduce oxidative stress by removing ROS in the body through its antioxidant activity, thereby temporarily improving worsened mood states. Furthermore, previous studies have shown that psychological stress increases xanthine oxidase (XO) activity [25]. XO is an enzyme involved in purine metabolism that produces uric acid and reactive oxygen species (ROS) through a series of metabolic reactions. Therefore, under stress, increased XO activity leads to increased ROS production, resulting in increased oxidative stress in the body. It has been reported that administering allopurinol, an XO inhibitor, improves depression-like behavior in rats [26]. LDQW has been reported to possess not only antioxidant activity but also XO-inhibitory activity [13]. Supporting this, a clinical study reported that MWPH reduced serum uric acid levels in healthy adults with elevated uric acid levels, suggesting that it affects the body [19]. Therefore, it is considered that MWPH containing 0.1% LDQW reduces oxidative stress by removing ROS from the body through both its antioxidant and XO-inhibitory activity. Furthermore, antioxidant substances are known to improve blood flow [27]. Previous studies have reported that improvements in blood flow led to improvements in HRQOL [28]. The HRQOL-improving effect of the WPH enriched with LDQW may be partly attributed to its antioxidant properties. Reduced blood flow is also a contributing factor to shoulder discomfort. When oxidative stress persists in the muscles around the shoulders, it affects vascular endothelial cells, leading to reduced blood flow and discomfort [15]. In this study, it is thought that the antioxidant activity of LDQW reduces oxidative stress in the vascular endothelial cells around the shoulder, thereby increasing blood flow and relieving discomfort.

In this study, the incidence of adverse events after 12 weeks of continuous MWPH intake was evaluated. There were no significant differences in incidence rates by food type, and no events attributable to test food intake were identified. Previous reports have likewise shown that even when MWPH was consumed at five times the intake level used in this study for 12 weeks, no adverse events occurred [19]. These results support the safety of the continued intake of the WPH enriched with LDQW.

The test food used in this study was a water-soluble powder type containing only MWPH, flavorings, sweeteners, and adjusters. Based on these results, the digestibility of the test food and components other than functional components was unlikely to affect the WPH enriched with LDQW. Furthermore, since the timing of consumption was not specified in this study, it is assumed that the effects of the WPH enriched with LDQW are not influenced by the timing of consumption or by other food components. These findings suggest that the WPH enriched with LDQW can be incorporated into various types of food products. Moreover, this study showed that the WPH enriched with LDQW significantly improved positive mood and shoulder discomfort after 4 weeks of consumption, indicating that it may be possible to develop products that demonstrate effects relatively early. Therefore, MWPH is a beneficial food component that contributes to the maintenance and promotion of health.

The WPH enriched with LDQW has demonstrated potential beneficial effects on mood and fatigue in an exploratory study [16]. Due to the heterogeneous backgrounds of the participants, these findings were not considered in the sample size estimation for this study. Therefore, the study was planned with 200 participants based on the maximum number of participants that could be tested at the study facility. However, recruiting participants who met the eligibility criteria was challenging, and the study was eventually conducted with 112 participants. A post-hoc analysis confirmed a moderate effect size (Cohen’s d = 0.51) based on the primary outcome of this study, the TMD standardized score in the POMS2-SF at 12 weeks. The effects of the WPH enriched with LDQW on mood improvement observed in this study are considered reliable, as they have been consistently replicated across multiple studies with comparable effect sizes.

The limitations of this study should be noted. Only healthy adults were included in this study. Therefore, it is unclear whether the effects of the WPH enriched with LDQW in this study can be extrapolated to patients. A previous study has reported that XO activity significantly increases in patients with major depressive disorder, suggesting a potential association between patients with mental disorders and XO activity [29]. Future studies involving patients may help verify the potential of the WPH enriched with LDQW to alleviate the symptoms of mental disorders. Additionally, this study did not evaluate saliva or blood sample data. Assessing these parameters related to mood states could provide further insights regarding the effects of the WPH enriched with LDQW.

Scientific Innovations and Practical Implications: Our study is the first to demonstrate that WPH enriched with LDQW can simultaneously enhance psychological well-being and alleviate physical fatigue in healthy adults. This dual benefit suggests a promising approach to supporting overall health. Previous studies have reported that LDQW exhibits xanthine oxidase inhibitory activity and antioxidant properties, which may help explain the physiological effects observed in our study. These findings represent a significant advancement in peptide research. Furthermore, WPH is a highly safe ingredient suitable for diverse food applications, such as functional beverages or dietary supplements, offering a convenient strategy to promote health maintenance.

CONCLUSIONS

This study supports the efficacy of the LDQW-enriched WPH in improving mood, reducing fatigue, and alleviating shoulder discomfort in healthy adults. These findings suggest that the LDQW-enriched WPH may serve as a functional component for enhancing mental and physical well-being.

Abbreviations: ANCOVA: Analysis of covariance, FFQ NEXT: Food frequency questionnaire ver.1.0, HRQOL: Health-related quality of life, ITT: Intention to Treat, LDQW: Leu-Asp-Gln-Trp, POMS2-SF: Profile of Mood States-Short Form 2nd Edition, PPS: Per Protocol Set, ROS: Reactive Oxygen Species, SF-36: 36-Item Short Form Health Survey, VAS: Visual analog scale, WPH: Whey protein hydrolysate, XO: Xanthine oxidase

Competing Interests: M.N. (Mayu Nakatsuka), K.N., R.S., S.S., M.N. (Manabu Nakano), and M.T. are employed by Morinaga Milk Industry Co., Ltd. M.N. (Masahiko Nakamura) has no conflicts to declare.

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