Research Article **Open Access**

FFHD
Functional Foods in Health and Disease

The improvement of daily fatigue in women following the intake of maca (*Lepidium meyenii*) extract containing benzyl glucosinolate

Atsushi Honma[1*], Yumi Fujiwara[2], Shin Takei[1], Takao Kino[1]

[1]Kinos, Inc., 1-3-17 Shinkawa, Chuo-ku, Tokyo, 104-0033, Japan; [2]Department of Kampo Pharmaceutical Science, Nihon Pharmaceutical University, 10281 Komuro, Ina-machi, Kitaadachi-gun, Saitama 362-0806, Japan

***Corresponding author:** Atsushi Honma, Kinos, Inc., 1-3-17 Shinkawa, Chuo-ku, Tokyo, 104-0033, Japan

Submission Date: March 4th, 2022; **Acceptance Date:** March 29th, 2022; **Publication Date:** April 8th, 2022

Please cite this article as: Honma A., Fujiwara Y., Takei S., Kino T. The improvement of daily fatigue in women following the intake of maca (Lepidium meyenii) extract containing benzyl glucosinolate. *Functional Foods in Health and Disease*. 2022;12(4): 175-187. DOI: https://www.doi.org/10.31989/ffhd.v12i4.912

ABSTRACT

Background: Daily fatigue is a problem for many people because of its association with other illnesses. Maca has lately attracted considerable attention as a food for recovering from fatigue. Maca is high in benzyl glucosinolate which improves endurance capacity by preventing glycogen depletion. However, its effect on fatigue has not been investigated in clinical trials on humans.

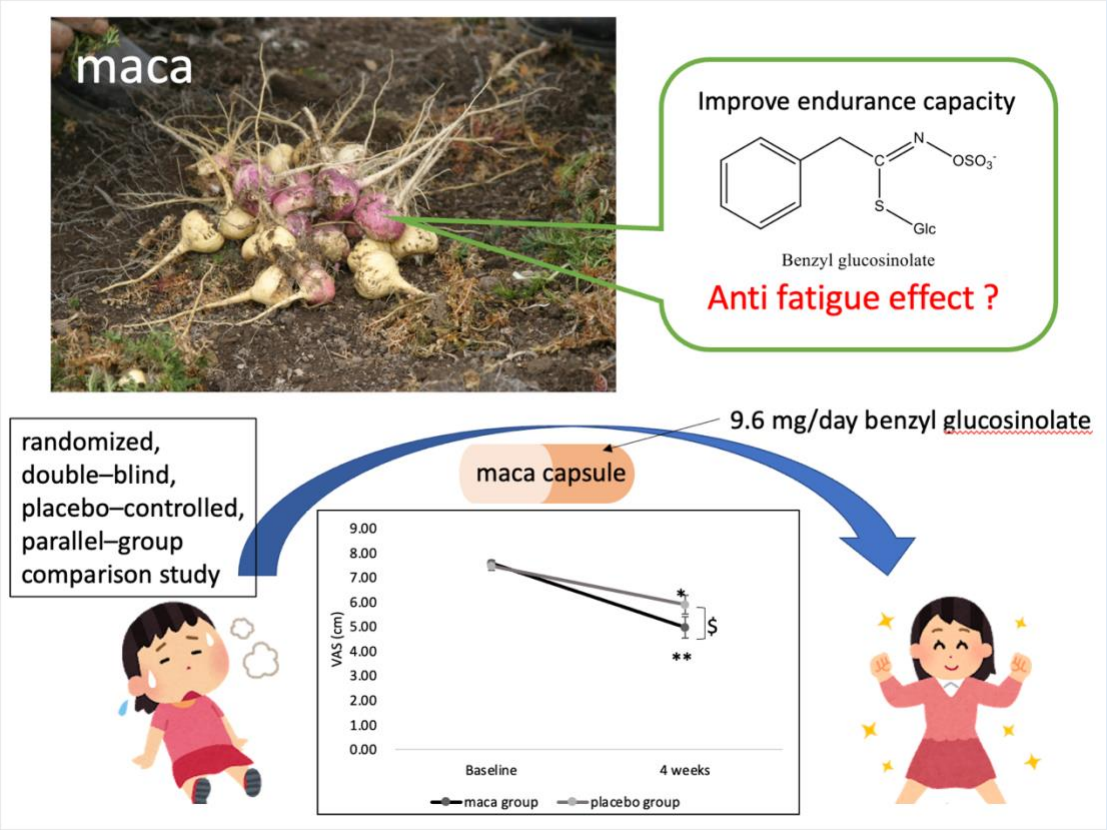
Objective: The main objective of this study is to investigate the effects of maca supplements on reducing fatigue.

Methods: This study evaluated the effect of the consumption of maca extract containing benzyl glucosinolate (9.6 mg/day) on fatigue in adult women. This randomized, placebo–controlled, parallel–group double–blind study enrolled 60 subjects who were allocated to consume maca extract or placebo for 4 weeks. A visual analogue scale (VAS) for fatigue was used to evaluate the results. Blood variables and adverse events were used to assess safety.

Results: In both groups, the VAS of fatigue was decreased after 4 weeks compared with that before consumption. On stratification analysis, in subjects younger than 45 years, the VAS was significantly lower in the maca group, and the change between before and after supplementation was significantly larger in this group.

Conclusions: It was suggested that intake of maca extract containing benzyl glucosinolate may have anti–fatigue effects in young women. So, maca supplements containing benzyl glucosinolate represent a food and drug candidate for reducing fatigue.

Keywords: maca, Lepidium meyenii, benzyl glucosinolate, daily fatigue, visual analogue scale



INTRODUCTION

Lepidium meyenii Walp (maca) is a biennial herbaceous plant belonging to the Brassicaceae family that was discovered more than 2,000 years ago in the Andes highlands of Peru. The plant grows almost exclusively in a restricted area exceeding 4,000 m above sea level in Central Peru. Maca has traditionally been used by indigenous Andean people as a nutritional supplement and as a traditional folk medicine to increase fertility and sexual function and enhance physical endurance. Scientific evidence suggests that both acute and chronic oral maca consumption significantly improves sexual performance parameters in male rats [1]. Another report found that black maca improved sperm counts in the epididymis as early as 1 day after treatment [2]. Some pharmacological research on sexual desire or athletic performance has been reported. Maca root invigorates the initial stage of spermatogenesis in male rats [3]. Furthermore, an investigation in humans reported that 4 months of oral treatment with maca tablets in nine adult normal men aged 24–44 years old resulted in increases in the seminal volume, motile sperm count, and sperm motility [4]. The components of maca are classify into these groups: macamides, glucosinolates, amino acids,

fatty acids, polyunsaturated fatty acids, saccharides, imidazoles [5-6]. Some components have been reported biological activity. Benzyl glucosinolate (Fig 1) is the active ingredient responsible for the effect of maca on physical endurance. Benzyl glucosinolate increases the utilization of fatty acids as an energy source, resulting in decreased glycogen depletion [7]. Polysaccharides could be responsible for the anti–fatigue effect. Polysaccharides effectively elongate swimming durations and accelerate average swimming speeds of mouse weight–loaded swimming model. They suggested that polysaccharides elicited its anti–fatigue effects by enhancing the serum antioxidant activity and by eliminating metabolic products [8]. These results suggested that maca has potential as an anti–fatigue medicine. However, clinical trials on human of the anti–fatigue effects of maca have not been previously reported. In an epidemiological survey on fatigue in the general population in the region of Nagoya, Japan conducted in 1999, 59% of the respondents to the survey on were aware of fatigue, and 35% of the respondents had daily fatigue that lasted for more than 6 months. Furthermore, in a survey of the same general area in 2012, 38.7% of the respondents were aware of daily fatigue that lasted for at least 6 months [9-10]. A survey of employee health was conducted in 1997 and 2002 by the Japanese Ministry of Health, Labour and Welfare, finding that 72.0% of workers in 1997 and 72.2% in 2002 were aware of fatigue from their daily work [11-12]. Chronic Fatigue Syndrome (CFS) is a disease concept proposed by the Centers for Disease Control and Prevention (CDC) in 1988 [13]. CFS is characterized by severe unexplained fatigue lasting at least 6 months, as well as pain throughout the body, impaired thinking, depression, and sleep disturbances, which interfere with daily life and social activities [14]. The latest epidemiological studies show a high prevalence of 3.3% in the general population, and it is most common in adults [15-17]. Women account for 80% of affected individuals [18]. There is no established cure for CFC, and patients may experience a reduced quality of life and the occupational inability for years [19]. As a result, CFS is an economic burden to society and the medical establishment [20]. The average annual medical costs for patients with CFS have been reported to be as high as $6,000 [21], and the annual global productivity loss due to CFS is reported to be approximately $7 billion [22]. The development of anti–fatigue products and scientific evidence of the efficacy are desired. As maca derivatives have become increasingly popular as dietary supplements, evidence of their anti–fatigue effects are highly awaited. The current study investigated the anti–fatigue effects of maca extract supplementation in a placebo–controlled clinical trial. Given the reported higher rates of fatigue among women compared to men, only women were included in this study [18].

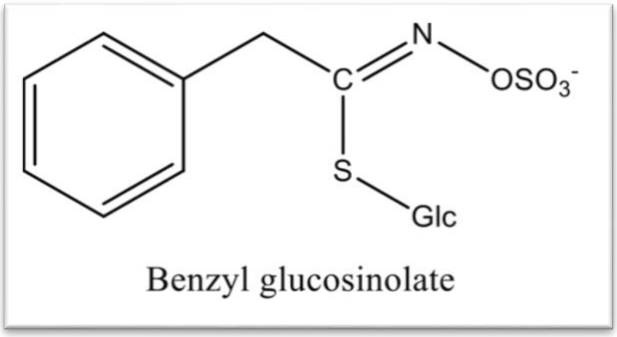


Fig 1. The structure of benzyl glucosinolate.

MATERIALS AND METHODS

Study design: This randomized, double–blind, placebo–controlled, parallel–group comparison study of Japanese women was conducted at Higash–Koganei Sakura Clinic (Tokyo, Japan). The study received approval from the Healthcare systems (Nagoya, Japan) on December 06, 2019 (no. 1915). This study was conducted from January 18, 2020 to February 29, 2020 according to the Declaration of Helsinki (2013) and the ethical guidelines for medical research for humans in Japan (2015). Equal numbers of subjects were allocated to the two groups. A randomized, controlled designed was used to prevent a difference in medical backgrounds between the two groups following allocation. Subjects were allocated to the two groups based on a visual analogue scale (VAS) for fatigue at screening and age. The 60 subjects with the highest VAS values were enrolled. Subjects were instructed to maintain their lifestyles, including their normal dietary and exercise habits, and to not use medicines or "food for specified health uses" or other foods affecting physical or mental fatigue. One day before examination or screening, subjects were instructed to avoid excessive exercise, finish dinner by 9 pm, avoid the consumption of excessive amounts of alcohol and food, and to go to bed early. On the day of examination or screening, participants were told to avoid exercise and the intake of food and drink excluding water. In the case of poor physical condition on the day of examination, such as fever, diarrhea, or vomiting, the examination was discontinued. Participants were instructed to not disclose any information related to this study to a third party. The primary endpoint was the subjective fatigue score as assessed by a VAS in line with the guideline published by the Japanese Society of Fatigue Science [23]. Safety was evaluated by blood tests, laboratory measurements (e.g., weight, height, body mass index), adverse event assessments, and a survey by a physician.

Participants: The inclusion criteria were as follows: the provision of written informed consent after receiving a full explanation of the study; healthy; age of at least 20 years old but younger than 65 years old; and presence of daily fatigue. The exclusion criteria were as follows: current medical treatment for chronic disease including chronic fatigue syndrome or a history of serious illness; allergy to the test supplement; use of medicines or foods for specified health uses or other foods affecting physical or mental fatigue; participation in another clinical trial within the prior month before the start of this trial or plans to participate in another clinical trial after agreeing to participate in this trial; deemed inappropriate for this study by the attending physician or principal investigator; and pregnancy, lactation, or planned pregnancy during the study period. The sample size was set at 60 subjects based on the maximum number of subjects who can be examined at this facility because there is no previous research on fatigue improvement following maca extract intake.

Screenings: Seventy–eight people participated in the screening test. All participants provided written informed consent prior to screening. Screening was conducted in terms of laboratory measurements, blood tests, VAS for fatigue, questionnaires about lifestyle questionnaires about exercise, diet, and physical condition on the day of and day before screening, and physician's questions. The 60 subjects with the highest VAS values were enrolled.

Supplements: The test food capsules contained 200 mg of dextrin or 200 mg of concentrated maca extract, which contained 4.8 mg of benzyl glucosinolate (Kinos Inc. Tokyo, Japan). Both capsules included crystalline cellulose, starch, calcium stearate, and silicon dioxide as

a filler. The color of the capsules was deep color, and subjects could not see the contents of the capsules. Subjects were instructed to take two capsules every day for 4 weeks. The dosage time was not designated.

Primary outcome: The primary outcome was VAS for fatigue [24]. This method is concise, and thus, subjects require only a little reading skill and time [25]. Subjects were asked the score their fatigue using a 10–cm VAS, with 0 indicating no fatigue and 10 indicating the worst fatigue imaginable.

Safety evaluation: A blood test and laboratory assessment were conducted. The physician asked each woman about the presence or absence of adverse events and her physical condition during the period of examination. The examination items of the blood and laboratory tests were total protein, albumin, neutral fat, total cholesterol, high–density lipoprotein cholesterol, low–density lipoprotein cholesterol, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, gamma–glutamyltransferase, creatine phosphokinase, total bilirubin, uric acid, urea nitrogen, creatinine, Na, K, Cl, Ca, P, Mg, lactate dehydrogenase, body weight, and body mass index (BMI).

Statistical analysis: Continuous data were evaluated for normality using the Shapiro–Wilk test. A paired *t*–test was performed for paired data when normality was confirmed, whereas the Wilcoxon signed–rank test was used for non–normally distributed data. If normality was demonstrated for unpaired data, variance was tested using the F–test followed by Student's *t*–test (with homoscedasticity) or Aspin–Welch's *t*–test (without homoscedasticity). If normality was not observed, Mann–Whitney's U test was used. For ordered data with several stages, paired data were analyzed Wilcoxon's signed–rank test, and unpaired data were analyzed using Mann–Whitney's U test. For ordered data with few stages and nominal data, McNemar's test was performed for paired data, and Fisher's direct standardization test or the chi–squared test was performed for unpaired data depending on the amount of data. If multiple comparisons were required, Bonferroni's test, Tukey's test, or Dunnett's test was applied depending on the data format. In addition, analysis of variance and coefficient analysis were performed as necessary. Regarding safety evaluation items, generally, only within–subgroup comparisons and intergroup comparisons between before and after supplementation were performed. In a two–sided test, a significance level of less than 5% was judged as statistically significant, and a significance level of at least 5% but less than 10% was judged as tendency. All statistical analyses were performed using SPSS Statistics Version 25.0 (IBM Japan, Ltd).

RESULTS

Participants: Sixty subjects were equally allocated to the maca and placebo groups after screening. One subject declined to participate before the start of supplementation, and two other women withdrew after the start of supplementation. Thus, 57 subjects completed the test. One woman with an intake rate of 90% or less and a second woman with CPK levels of at 1,000 U/L at screening who was believed to have changed her exercise habits were excluded. Ultimately, 55 subjects (27 in the maca group and 28 in the placebo group) were included in the efficacy analysis. The safety evaluation included all 57 participants who completed 4 weeks of supplementation. The background characteristics of the subjects are presented (Table 1).

Table 1. The background of the analysis subjects

(a) All subjects					
	Maca group (n=27)		Placebo group (n=28)		*p*-value
					Group comparison
Age (years)	43.26	±9.88	41.11	±9.06	0.403
Body height (cm)	157.71	±5.68	158.08	±5.44	0.805
Body weight (kg)	53.99	±9.52	54.03	±7.63	0.989
Body mass index	21.65	±3.40	21.64	±3.05	0.992
Data were presented as mean ± standard deviation and were analyzed by Student's *t* test.					
(b) Subjects of younger than 45 years					
	Maca group (n=15)		Placebo group (n=17)		*p*-value
					Group comparison
Age (years)	35.93	±5.82	35.29	±5.83	0.759
Body height (cm)	157.00	±6.38	158.84	±5.01	0.370
Body weight (kg)	53.59	±11.34	54.59	±9.05	0.785
Body mass index	21.67	±4.11	21.65	±3.60	0.992
Data were presented as mean ± standard deviation and were analyzed by Student's *t* test.					
(c) Subjects of 45 years of older					
	Maca group (n=12)		Placebo group (n=11)		*p*-value
					Group comparison
Age (years)	52.42	±4.80	50.09	±4.57	0.248
Body height (cm)	158.59	±4.78	156.91	±6.11	0.468
Body weight (kg)	54.49	±7.06	53.15	±4.96	0.608
Body mass index	21.63	±2.42	21.63	±2.12	0.995
Data were presented as mean ± standard deviation and were analyzed by Student's *t* test.					

VAS for fatigue: The results of the VAS questionnaire are presented (Table 2 and Fig. 2). In the analysis of all subjects, the VAS of fatigue after 4 weeks of intake and the mean change in VAS between before and after supplementation were smaller and larger, respectively, in the maca group than in the placebo group. On stratification analysis, age 45 was decided as the boundary between two groups. Menopause comes when she is about 45. Women in perimenopausal stage often experience negative physiological and psychological symptoms by hormone imbalance. This discomfort felt symptoms might be expressed as fatigue. We should consider that point, therefore age 45 was set as a borderline in present study. The VAS of fatigue among women younger than 45 years was significantly lower in the maca group after 4 weeks of supplementation, and the mean change in the VAS between before and after supplementation was significantly larger in this group. There were no significant differences in the VAS of fatigue and the mean change after 4 weeks among subjects 45 years old or older. In addition, a subgroup analysis was performed of subjects with baseline scores of ≥6.6, and the VAS of fatigue was significantly lower in the maca group after 4 weeks of supplementation. In all analyses, the VAS for fatigue was significantly lower than the baseline value after 4 weeks of supplementation in both groups.

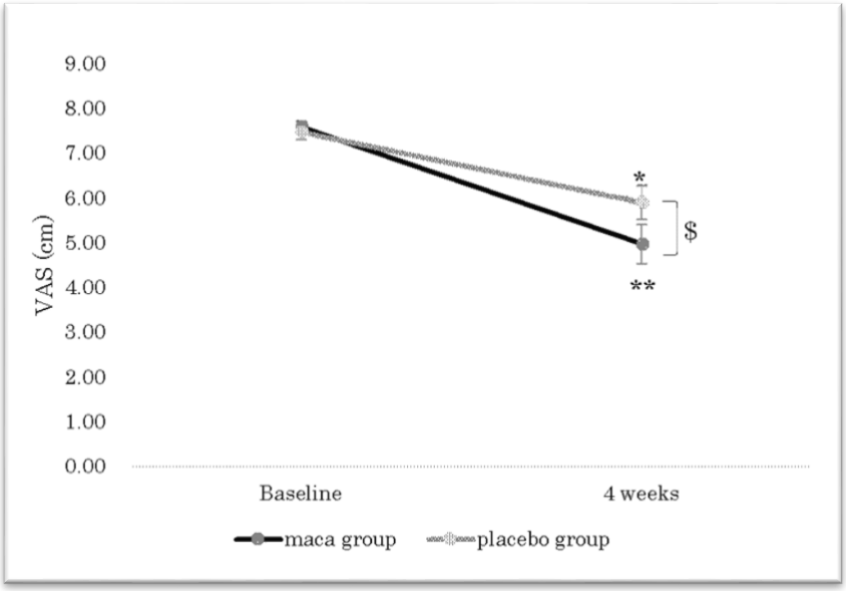


Fig 2. The fatigue-reducing effect of those younger than 45 years

Data were presented as mean ± standard error. Statistical differences of baseline and after 4 weeks were calculated using Wilcoxon signed-rank test. *$p < 0.01$ and **$p < 0.001$. Statistical differences of intergroup comparison were calculated using Mann–Whitney U test. $$p < 0.05$.

Safety evaluation: The results of laboratory assessments and blood tests are presented (Table 3). There were no significant differences in body weight or BMI between the maca and placebo groups and before and after maca or placebo supplementation. Although significant changes were observed for some blood variables, these changes were not considered problematic because the fluctuation did not exceed the normal values. No event was judged to have an association with the test supplement.

Table 2. The results of the VAS questionnaire

(a) All subjects

	Maca group (n=27)						Placebo group (n=28)						p-value				
													Before and after comparison[a]		Group comparison[b]		
	Baseline		4 weeks		Mean change		Baseline		4 weeks		Mean change		Maca group	Placeb group	Baseline	4 weeks	Mean change
VAS (cm)	7.55	±0.65	5.20	±1.48	-2.35	±1.48	7.52	±0.80	5.67	±1.74	-1.85	±1.77	< 0.001	< 0.001	0.532	0.095	0.083

Data were presented as mean ± standard deviation.

[a] Data were analyzed by Wilcoxon signed-rank test; [b]Data were analyzed by Mann–Whitney U test; VAS, visual analogue scale.

(b) Subjects of younger than 45 years

	Maca group (n=15)						Placebo group (n=17)						p-value				
													Before and after comparison[a]		Group comparison[c]		
	Baseline		4 weeks		Mean change		Baseline		4 weeks		Mean change		Maca group	Placeb group	Baseline	4 weeks	Mean change
VAS (cm)	7.59	±0.48	4.97	±1.71	-2.61	±1.69	7.46	±0.69	5.90	±1.57	-1.56	±1.75	< 0.001[b]	0.001	0.57[d]	0.027	0.041

Data were presented as mean ± standard deviation.

[a] Data were analyzed by Wilcoxon signed-rank test; [b] Data were analyzed by Paired *t* test; [c] Data were analyzed by Mann–Whitney U test; VAS, visual analogue scale.

(c) Subjects of 45 years old or older

	Maca group (n=12)						Placebo group (n=11)						*p*-value				
													Before and after comparison[a]		Group comparison[b]		
	Baseline		4 weeks		Mean change		Baseline		4 weeks		Mean change		Maca group	Placeb o group	Baseline	4 weeks	Mean change
VAS (cm)	7.51	±0.83	5.48	±1.14	-2.03	±1.14	7.60	±0.98	5.31	±2.01	-2.29	±1.79	< 0.001	0.002	0.81[c]	0.798[c]	0.926

Data were presented as mean ± standard deviation.

[a] Data were analyzed by Paired t test; [b] Data were analyzed by Mann–Whitney U test; [c] Data were analyzed by Student's *t* test; VAS, visual analogue scale

(d) Subjects with baseline scores of ≥6.6

	Maca group (n=26)						Placebo group (n=25)						*p*-value				
													Before and after comparison[a]		Group comparison [b]		
	Baseline		4 weeks		Mean change		Baseline		4 weeks		Mean change		Maca group	Placeb o group	Baselin	4 weeks	Mean change
VAS (cm)	7.60	±0.62	5.24	±1.49	-2.35	±1.50	7.65	±0.74	5.80	±1.75	-1.85	±1.83	< 0.001	< 0.001	0.843	0.046	0.090

Data were presented as mean ± standard deviation.

[a] Data were analyzed by Wilcoxon signed-rank test; [b] Data were analyzed by Mann–Whitney U test; VAS, visual analogue scale.

Table 3 The results of laboratory assessments and blood test.

	Maca group (n=28)				Placebo group (n=29)				p-value			
									Before and after supplementation comparison[a]		Group comparison[c]	
	Baseline		4 weeks		Baseline		4 weeks		Maca group	Placebo group	Baseline	4 weeks
Body weight (kg)	54.51	±9.74	54.46	±9.66	54.56	±8.02	54.48	±8.36	0.754[b]	0.655[b]	0.986[d]	0.994[d]
Body mass index	21.83	±3.46	21.80	±3.41	21.70	±3.01	21.67	±3.15	0.753[b]	0.687[b]	0.885[d]	0.881[d]
Total protein (g/dL)	7.10	±0.29	7.09	±0.36	7.13	±0.31	7.14	±0.33	0.939	0.841[b]	0.487	0.540
Albumin(g/dL)	4.42	±0.27	4.40	±0.27	4.40	±0.27	4.39	±0.24	0.59[b]	0.722[b]	0.802[d]	0.878[d]
Total cholesterol (mg/dL)	216.46	±32.14	216.32	±36.15	218.90	±30.57	216.79	±36.15	0.972[b]	0.715	0.79[d]	0.593
Neutral fat (mg/dL)	75.43	±28.95	77.25	±24.97	60.41	±30.04	63.72	±20.07	0.694[b]	0.256	0.030	0.05[d]
High-density lipoprotein cholesterol (mg/dL)	76.50	±18.68	77.25	±19.02	78.72	±17.81	78.62	±17.56	0.61[b]	0.965	0.684	0.771[d]
Low-density lipoprotein cholesterol (mg/dL)	122.57	±29.54	121.04	±30.38	125.41	±29.97	122.55	±30.86	0.656[b]	0.217	0.955	0.943
Aspartate aminotransferase (U/L)	18.86	±6.47	17.96	±8.30	19.59	±4.03	20.03	±6.55	0.827	0.444	0.898	0.353
Alanine aminotransferase (U/L)	14.71	±8.19	13.71	±12.35	17.17	±4.33	17.86	±10.56	0.637	0.220	0.648	0.423
Alkaline phosphatase (U/L)	177.50	±55.93	175.32	±40.63	163.79	±55.98	167.21	±40.54	0.464[b]	0.258[b]	0.293[d]	0.532[d]
Lactate dehydrogenase (U/L)	174.25	±30.37	178.29	±31.17	178.41	±26.47	184.59	±24.60	0.252[b]	0.010	0.817	0.356[d]
Gamma-glutamyltransferase (U/L)	20.07	±15.56	19.71	±9.85	17.69	±15.68	17.90	±10.26	0.360	0.274	0.512	0.496
Creatine phosphokinase (U/L)	137.14	±233.81	96.75	±40.36	77.76	±57.60	86.97	±49.98	0.381	0.050	0.058	0.346
Total bilirubin (mg/dL)	0.55	±0.24	0.53	±0.15	0.52	±0.21	0.50	±0.13	0.321	0.158	0.909	0.863
Uric acid (mg/dL)	4.25	±0.86	4.31	±0.85	4.51	±1.05	4.46	±0.83	0.592[b]	0.616[b]	0.269[d]	0.557[d]
Urea nitrogen (mg/dL)	11.16	±3.28	11.97	±5.14	12.93	±2.60	12.67	±4.10	0.049	0.957	0.107	0.655
Creatinine (mg/dL)	0.61	±0.10	0.64	±0.08	0.62	±0.10	0.66	±0.10	0.007[b]	0.008[b]	0.717[d]	0.512
Na (mEq/L)	140.89	±1.29	140.64	±1.33	140.14	±1.47	140.59	±1.35	0.311	0.119[b]	0.056	0.849
K (mEq/L)	4.44	±0.31	4.18	±0.20	4.34	±0.24	4.16	±0.25	0.000	0.007[b]	0.265	0.764
Cl (mEq/L)	103.43	±1.45	103.32	±1.40	102.55	±1.68	102.76	±1.43	0.717[b]	0.512[b]	0.024[d]	0.178[d]
Ca (mg/dL)	9.27	±0.27	9.23	±0.29	9.28	±0.31	9.30	±0.36	0.481	0.723[b]	0.88[d]	0.569
P (mg/dL)	3.61	±0.47	3.52	±0.48	3.51	±0.51	3.50	±0.50	0.281[b]	0.891[b]	0.449[d]	0.894[d]
Mg (mg/dL)	2.18	±0.12	2.25	±0.15	2.20	±0.12	2.27	±0.13	0.015[b]	0.014	0.594	0.416

Data were presented as mean ± standard deviation.

[a] Data were analyzed by Wilcoxon signed-rank test; [b] Data were analyzed by Paired *t*-test; [c] Data were analyzed by Mann–Whitney U test; [d] Data were analyzed by Student's *t*-test.

DISCUSSION

This randomized, double–blind, placebo–controlled, parallel–group comparative study assessed the effect of long–term intake of maca extract on daily fatigue in women. The VAS of fatigue was significantly smaller in the maca group than in the placebo group. The mean change versus baseline was larger in the maca group than in the placebo group. However, the VAS of fatigue was also decreased in the placebo group. A potential placebo effect attributable to the subjective evaluation method was suspected. Studies in perimenopausal women show effects of maca on mood and menopausal symptoms and the effects is not due to direct effects on hormones [26-27]. A pilot study conducted on early postmenopausal women, confirmed that through balancing hormones in the body, maca helped women to reduce discomfort which they experienced in early postmenopausal stage [26]. It appears that maca may act as a toner of hormonal processes and balancing levels of hormones (FSH, E2, PG and ACTH) and leading to alleviation of discomfort felt by perimenopausal women [27]. If they thinking a discomfort is synonymous with a fatigue, the results of our present study that the VAS of fatigue was significantly smaller in the maca group than in the placebo group in subjects 45 years old or older was agree with the previous pilot study that maca helped women to reduce discomfort which they experienced in early postmenopausal stage. Interestingly, the changes in the VAS for fatigue following maca extract intake were particularly prominent in women younger than 45 years. In addition, a significant difference was also found in subjects with a baseline VAS of ≥6.6. Therefore, maca extract's anti–fatigue effect was suggested to be remarkable in young women and subjects with extreme fatigue. Maca polysaccharides and benzyl glucosinolate are capable of effecting anti–fatigue [7-8]. Maca polysaccharides were investigating their anti–fatigue effect *in vivo* [8]. They obtained the purified maca polysaccharides with 0.106 g/kg yield on basis of maca powder in dry weight. In addition, mice weight–loaded swimming tests showed that maca polysaccharides high–dose group (100 mg/kg bw/d) and mid–dose group (50 mg/kg bw/d) not only significantly accelerated the average swimming speed of the mice, but also significantly prolonged the swimming duration of the mice in a dose–dependent manner. However, low–dose group (25 mg/kg bw/d) didn't show a significant difference between vehicles. Therefore, if they want to get anti–fatigue effects, they need about 500 g~1 kg/kg bw/d maca powder in dry weight. On the other hand, our research that investigate anti–fatigue effect of benzyl glucosinolate in mice swimming exercise tests showed significant anti–fatigue effect on the dose of 0.015 mg/kg and 0.03 mg/kg [7]. It suggested that benzyl glucosinolate can increase swimming endurance in mice and reduce fatigue by increasing the utilization of fatty acids as an energy source and reducing the rate of glycogen depletion. We predicted that benzyl glucosinolate will be a great contribution to maca's anti–fatigue effect because of significant anti–fatigue effect on small dose compared to maca polysaccharides. Therefore, we prepared a test food which a clear dose of benzyl glucosinolate in the present study. Each 400–mg dose of maca extract manufactured from 1.3 g of maca powder contained 9.6 mg of benzyl glucosinolate. If the content of polysaccharides is equal to previous report [8], it is 130 µg/400 mg. However, this dose is too small to expect anti–fatigue effect. In addition, it has been reported that benzyl glucosinolate has high antioxidant capacity based on *in vitro* findings [28]. Studies found that exercise has a significant effect on cellular oxidative stress [29-30], and oxidative stress contributes to fatigue [31]. The anti–fatigue effect of maca in women younger than 45 years old and in those with extreme fatigue in present study

may be attributable to the mechanism of action of benzyl glucosinolate, which has anti–oxidant effects. Regarding the safety evaluation, blood tests and the physical examination did not produce any results indicating a danger of maca supplementation, and no adverse events with a causal relationship with the test extract were identified. Therefore, it appears than 400 mg of maca extract can be safety consumed for 4 weeks. Although the subjects of this study were limited to women, similar studies on men are needed in the future.

CONCLUSION

In the present study, it was suggested that intake of maca extract containing benzyl glucosinolate may have anti–fatigue effects in young women. So, maca supplements containing benzyl glucosinolate represent a food and drug candidate for reducing fatigue. There is a tendency to believe daily fatigue will resolve naturally, but if fatigue accumulates, it may lead to various diseases such as autonomic imbalance and depression and decrease quality of life. Therefore, foods that can improve daily fatigue represent a medically and socially significant need.

List of Abbreviations: chronic fatigue syndrome: CFS; Centers for Disease Control and Prevention: CDC; visual analogue scale: VAS; body mass index: BMI; creatine phosphokinase: CPK; follicle stimulating hormone: FSH; estradiol: E2; progesterone: PG; adrenocorticotrophic hormone: ATCH

Conflict of Interest: The authors declare no competing financial interest.

Authors' Contributions: Acquisition of data: A. H., S. T. and T. K. Analysis and/or interpretation of data: A. H., Y. F., S. T. and T. K. Drafting the manuscript: A. H. and Y. F. Revising the manuscript critically for important intellectual content: A. H. and Y. F. Approval of the version of the manuscript to be published: A. H., Y. F., S. T. and T. K.

Funding Information: No funding was received.

REFERENCES

1. Cicero AF, Bandieri E, Arletti R: Lepidium meyenii Walp. improves sexual behaviour in male rats independently from its action on spontaneous locomotor activity. J Ethnopharmacol 2001, 75(2–3):225–229. https://doi.org/10.1016/S0378-8741(01)00195-7
2. Gonzales GF, Nieto J, Rubio J, Gasco M: Effect of black maca (Lepidium meyenii) on one spermatogenic cycle in rats. Andrologia 2006, 38(5):166–172. https://doi.org/10.1111/j.1439-0272.2006.00733.x
3. Gonzales GF, Ruiz A, Gonzales C, Villegas L, Cordova A: Effect of Lepidium meyenii (Maca) roots on spermatogenesis of male rats. Asian J Androl 2001, 3:231–233. http://www.asiaandro.com/archive/1008-682X/3/231.htm
4. Gonzales GF, Cordova A, Gonzales C, Chung A, Vega K, Villena A: Lepidium meyenii (Maca) improved semen parameters in adult men. Asian J Androl 2001, 3:301–303. http://www.asiaandro.com/archive/1008-682X/3/301.htm
5. Zhou Y, Li P, Brantner A, Wang H, Shu X, Yang J et al.: Chemical profiling analysis of maca using UHPLC–ESI–Orbitrap MS coupled with UHPLC–ESI–QqQ MS and the neuroprotective study on its active ingredients. Scientific reports 2017, 7:44660. https://doi.org/10.1038/srep44660
6. Geng P, Sun J, Chen P, Brand E, Frame J, Meissner H et al.: Characterization of maca (Lepidium meyenii /Lepidium peruvianum) using a mass spectral fingerprinting, metabolomic analysis, and genetic sequencing approach. Planta Med 2020, 86(10):674–685. https://doi.org/10.1055/a-1161-0372

7. Ikeuchi M, Koyama T, Takei S, Kino T, Yazawa K: Effect of benzylglucosinolate on endurance capacity in mice. J Health Sci 2009, 55(2):178–182. https://doi.org/10.1248/jhs.55.178
8. Weimin T, Lu J, Lianghua X, Huang J, Wang N, Chu B et al.: Structural characterization and antifatigue effect in vivo of maca (Lepidium meyenii Walp) polysaccharide. J Food Sci 2017, 82(3):757–764. https://doi.org/10.1111/1750-3841.13619
9. Kitani T: Survey of actual state of fatigue and the research on fatigue recovery methods for health promotion. [https://mhlw-grants.niph.go.jp/project/4765] Retrieved Jan 21, 2021.
10. Kuratsune H: The survey of actual state of chronic fatigue and the research on objective diagnostic methods. https://mhlw-grants.niph.go.jp/project/21431 Retrieved Jan 2021.
11. Japanese Ministry of Health, Labour and Welfare: Survey on State of Employees' Health. [https://www.jil.go.jp/jil/kisya/daijin/980623_03_d/980623_03_d.html] Retrieved Jan 21, 2021.
12. Japanese Ministry of Health, Labour and Welfare: Survey on State of Employees' Health. [https://www.mhlw.go.jp/toukei/itiran/roudou/saigai/anzen/kenkou02/index.html] Retrieved Jan 21, 2021.
13. Holmes GP, Kaplan JE, Gantz NM, Komaroff AL, Schonberger LB, Straus SE et al.: Chronic fatigue syndrome: a working case definition. Ann Intern Med 1988, 108(3):387–389. https://doi.org/10.7326/0003-4819-108-3-387
14. Afari N, Buchwald D: Chronic fatigue syndrome: a review. Am J Psychiatry 2003, 160(2): 221–236. https://doi.org/10.1176/appi.ajp.160.2.221
15. Johnston S, Brenu EW, Staines D, Marshall–Gradisnik S.: The prevalence of chronic fatigue syndrome/myalgic encephalomyelitis: a meta–analysis. Clin Epidemiol 2013, 5: 105–110. https://doi.org/10.2147/CLEP.S39876
16. Reyes M, Nisenbaum R, Hoaglin DC, Unger ER, Emmons C, Randall B et al.: Prevalence and incidence of chronic fatigue syndrome in Wichita, Kansas. Arch Intern Med 2003, 163(13): 1530–1536. https://doi.org/10.1001/archinte.163.13.1530
17. Prins JB, van der Meer JW, Bleijenberg G.: Chronic fatigue syndrome. Lancet 2006, 367(9507), 346–355. https://doi.org/10.1016/S0140-6736(06)68073-2
18. Jason LA, Richman JA, Rademaker AW, Jordan KM, Plioplys AV, Taylor RR, et al.: A community-based study of chronic fatigue syndrome. Arch Intern Med 1999, 159(18), 2129–2137. https://doi.org/10.1001/archinte.159.18.2129
19. Bombardier CH, Buchwald D.: Outcome and prognosis of patients with chronic fatigue vs. chronic fatigue syndrome. Arch Intern Med 1995, 155(19), 2105–2110. https://doi.org/10.1001/archinte.1995.00430190101014
20. Bombardier CH, Buchwald D.: Chronic fatigue, chronic fatigue syndrome, and fibromyalgia disability and health–care use. Med Care 1996, 34(9), 924-930. https://doi.org/10.1097/00005650-199609000-00005
21. Lin JM, Resch SC, Brimmer DJ, Johnson A, Kennedy S, Burstein N et al.: The economic impact of chronic fatigue syndrome in Georgia: direct and indirect costs. Cost Eff Resour Alloc 2011 9(1), 1. https://doi.org/10.1186/1478-7547-9-1
22. Alfredo AF, Alvaro PM, Maravillas IM, Mar AB, Javier BHF, Javier CL et al.: Chronic fatigue syndrome: aetiology, diagnosis and treatment. BMC Psychiatry 2009, 9(1), S1. https://doi.org/10.1186/1471-244X-9-S1-S1
23. Japanese Society of Fatigue Science: The guidelines of anti–fatigue clinical evaluation. [https://www.hirougakkai.com/index.html] Retrieved Jan 21, 2021.
24. Lee KA, Hicks G, Nino–Murcia G: Validity and reliability of a scale to assess fatigue. Psychiatry Res 1991, 36(3):291–298. https://doi.org/10.1016/0165-1781(91)90027-M
25. LaChapelle DL, Finlayson MA: An evaluation of subjective and objective measures of fatigue in patients with brain

injury and healthy controls. Brain Injury 1998, 12(8):649–659. https://doi.org/10.1080/026990598122214

26. Meissnera HO, Reich–Bilinskab H, Msciszc A, Kedziac B: Therapeutic effects of pre–gelatinized maca (Lepidium peruvianum Chacon) used as a non–hormonal alternative to HRT in perimenopausal women – clinical pilot study. Int J Biomed Sci 2006, 2(2):143–159. http://www.ijbs.org/User/All-by-date.aspx
27. Meissner HO, Mscisz A, Reich–Bilinska H, Mrozikiewicz P, Bobkiewicz-Kozlowska T, Kedzia B et al.: Hormone–balancing effect of pre–gelatinized organic maca (Lepidium peruvianum Chacon): (III) Clinical responses of early–postmenopausal women to maca in double blind, randomized, placebo–controlled, crossover configuration, outpatient study. Int J Biomed Sci 2006, 2(4):375–394. http://www.ijbs.org/User/All-by-date.aspx
28. Castro–Vargas HI, Baumann W, Parada–Alfonso F: Valorization of agroindustrial wastes: Identication by LC–MS and NMR of benzylglucosinolate from papaya (Carica papaya L.) seeds, a protective agent against lipid oxidation in edible oils. Electrophoresis 2016, 37(13):1930–1937. https://doi.org/10.1002/elps.201500499
29. Sen CK: Oxidants and antioxidants in exercise. J Appl Physiol 1995, 79(3):675–686. https://doi.org/10.1152/jappl.1995.79.3.675
30. Powers SK, Ji LL, Leeuwenburgh C: Exercise training–induced alterations in skeletal muscle antioxidant capacity: a brief review. Med Sci Sports Exercise 1999, 31(7):987–997. https://journals.lww.com/acsm-msse/Fulltext/1999/07000/Exercise_training_induced_alterations_in_skeletal.11.aspx
31. Reid MB, Stokic DS, Koch SM, Khawli FA, Leis AA: N–acetylcysteine inhibits muscle fatigue in humans. J Clin Investig 1994, 94(6):2468–2474. https://doi.org/10.1172/JCI117615