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Short communications

Supplementation of standardized lipid-soluble extract from maca (*Lepidium meyenii*) increases swimming endurance capacity in rats

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ABSTRACT

The effect of lipid-soluble extract from maca (*Lepidium meyenii*), which contains maca-mides, on swimming endurance capacity, as an indicator of fatigue, in weight-loaded forced swimming rats was investigated. The swimming times to exhaustion of rats supplemented for 3 weeks with 30 and 100 mg/kg of maca extract increased by 25% and 41%, respectively. Supplementation with 100 mg/kg of maca extract reduced serum lactate dehydrogenase activity and muscle lipid peroxidation, and increased hepatic and muscle total glutathione compared with those values in controls. The levels of energy sources and serum lactate remained unchanged despite the longer swimming time in the supplemented rats than those in controls. These results suggest that supplementation with lipid-soluble maca extract improved swimming endurance capacity and this effect can be explained partly by attenuation of exercise-induced oxidative stress.

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1. Introduction

Maca (*Lepidium meyenii*) belongs to the Brassicaceae family and grows only in the high plateaus of the central Andes of Peru. Maca has been used by Aean Indians for thousands of years as a foodstuff and traditional medicine, especially as an aphrodisiac to enhance sexual drive and fertility, and as a tonic for Inca warriors to increase their energy and vitality (Wang, Wang, McNeil, & Harvey, 2007). Maca has recently attracted interests as a dietary supplement because of its potential positive effects on physical and sexual activity. Dietary supplements contain-

ing maca are available throughout the United States and South America (Gonzales et al., 2001). Recently, several studies have been conducted to examine its biological activity, but most scientific studies of the biological activity of maca have focused on enhancing sexual performance or fertility. Although two reports showed the increased endurance capacity with supplementation with fermented maca powder (Shin et al., 2008) or aqueous maca extract (Zheng et al., 2002), those studies found that very high doses up to g/kg were needed.

At the start of exercise, the energy sources such as glucose and glycogen are used first and may become exhausted, and

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then metabolites such as lactate and blood urea nitrogen may be accumulated, causing metabolic dysregulations (Pedersen, Nielsen, Lamb, & Stephensen, 2004). Excessive reactive oxygen species (ROS) can be generated and induced as a result of the large amount of oxygen consumed during exercise, and can cause muscle fatigue or oxidative damage (Wang et al., 2008). Post-exercise nutrition or antioxidant supplementation may be a strategy for recovering from exercise-induced fatigue. Delay in the point of exhaustion during prolonged exercise has been observed after administration of medicinal herb extracts or their constituents possessing antioxidant activity *in vivo*, such as green tea extract (Murase, Haramizu, Shimotoyodome, Nagasawa, & Tokimitsu, 2005) and ferulic acid (You et al., 2009). However, few studies have focused on the ability of maca to increase endurance capacity, and various biological activities of maca are still needed to determine and better characterize the properties of this plant.

Therefore, we investigated the effect of standardized lipid-soluble extract obtained by supercritical fluid extraction of maca on swimming endurance capacity, serum biochemical parameters, and antioxidant status in a weight-loaded forced swimming rat model.

2. Materials and methods

2.1. Plant materials and preparation

Yellow maca powder was obtained from Ecoandino SAC Co. (Lima, Peru). Lipid-soluble extract was prepared by supercritical fluid extraction from maca powder and two typical macamides as described previously (Lee et al., 2008). The extraction was performed using only supercritical carbon dioxide as a solvent.

2.2. Chemical properties and standardization of the lipid-soluble maca extract

Fatty acids and phytosterol contents in maca extract were analyzed by gas chromatography according to the modified method of Parcerisa, Richardson, Refecas, Codony, and Boatella (1998). Total phenolic content of lipid-soluble extract was measured by the Folin-Ciocalteu method with some modification (Singleton, Orthofer, & Lamuela-Raventós, 1998), and expressed as mg gallic acid equivalent (GAE) per gram extract. The product was standardized in the content of two macamides, *N*-benzylhexadecanamide and *N*-benzyl-5-oxo-6E,8E-octadecadienamide. The macamide contents were measured by high-performance liquid chromatography (HPLC) using standard compounds of the two macamides. These two macamides were isolated from maca according to the method of Ganzera, Zhao, Muhammad, and Khan (2002) and identified by LC/mass spectrometry (MS) and NMR in a previous study (Lee et al., 2008).

2.3. Experimental protocol

Male Sprague Dawley rats, 6-week-old, were purchased from Orient Bio Inc. (Seongnam, Korea). Rats were housed under standard laboratory conditions (12 h light: 12 h dark cycle

and 22 ± 2 °C). All rats were allowed free access to tap water and a rodent chow diet (Harlan 2018S, 18.6% protein, 6.2% fat, and 44.2% carbohydrate, Harlan Teklad Premier Laboratory Diets, Madison, WI, USA), throughout the experimental period. This study was approved by the Institutional Animal Care and Use Committee of Korea Food Research Institute and the animals were cared for in accordance with the Guide for the Care and Use of Laboratory Animals. After a week adaptation, rats were divided randomly into three groups with similar swimming capacity ($n = 20$ per group): a control group which received the vehicle only (sterile water), and two treatment groups which received 30 or 100 mg/10 ml/kg body weight of maca extract administered by gavage once a day for 3 weeks. Each sample was suspended and diluted in sterile water before administration.

2.4. Measurement of swimming endurance capacity

The swimming endurance capacity of rats was assessed using an adjustable-current swimming pool with slight modifications (Matsumoto, Ishihara, Tanaka, Inoue, & Fushiki, 1996). Briefly, an acrylic plastic pool (80 × 50 × 70 cm) was filled with water to a depth of 40–50 cm, and the water was maintained at a flow rate of 6–8 l/min, at a temperature of 32 ± 2 °C. The rats were subjected to exhaustive swimming with a load attached to the tail corresponding to 6% of their body weight on day 21, averaging 344.0 ± 2.4 g, and the time range of swimming performed was from 10:00 am to 6:00 pm. Swimming time to exhaustion was used as the index of swimming endurance capacity. The rat was considered to be exhausted when it failed to rise to the surface to breathe for 10 s. All rats were made to swim for 15 min twice per week without loads to be accustomed to swimming.

2.5. Measurement of serum biochemical parameters and tissue glycogen

Each rat was sacrificed immediately after completing the final exhaustive swim. Blood was collected from the abdominal aorta under anesthesia and serum was prepared by centrifugation at 1000g for 15 min. The serum levels of glucose and lactate dehydrogenase (LDH) were estimated using commercial kits (Siemens Healthcare Diagnostics, Deerfield, IL, USA). Serum free fatty acids (FFA) and lactate concentrations were measured using a NEFA HR 2 kit (Wako Diagnostics, Richmond, VA, USA) and lactate assay kit (Eton bioscience Inc., San Diego, CA, USA), respectively. Liver and muscle glycogen contents were measured colorimetrically using an anthrone reagent (Chun & Yin, 1998).

2.6. Measurement of antioxidant status in liver and skeletal muscle

The liver and skeletal muscle from the hind limb were removed and weighed. Each tissue was homogenized in ice-cold 0.1 M phosphate buffer (pH 7.4) containing 1 mM EDTA. These tissue homogenates were centrifuged at 10,000g for 15 min at 4 °C, and the supernatants were assessed for the antioxidant status. As an indicator of lipid peroxidation, the level of thiobarbituric acid reactive substances (TBARS) was

measured as described previously (Choi, Park, Kim, & Chun, 2010) and total glutathione (GSH) content was measured according to the GSH-recycling method (Shaik & Mehvar, 2006). Catalase activity was measured according to the method of Johansson and Borg (1988) using Purpald as a chromogen. Superoxide dismutase (SOD) activity was measured using the pyrogallol autoxidation method (Nandi & Chatterjee, 1988).

2.7. Statistical analysis

All values were expressed as mean \pm SEM. All statistical analyses were performed using SPSS software (SPSS Inc., Chicago, IL, USA). The data were analyzed using Student's *t* test, and differences at $p < 0.05$ were considered as significant.

3. Results

3.1. Chemical properties and macamide content in the lipid-soluble extract

The yield of lipid-soluble extract from maca powder was 5%. The product contained 29.7% water and 10.8% fatty acids, which were composed of 2.58% palmitic acid, 1.85% oleic acid, 3.55% linoleic acid, and 1.75% linolenic acid, mainly. Other minor constituents were 0.7% sterols (β -sitosterol and campesterol). Total phenolic content of lipid-soluble extract was 26.5 mg/g extract. Macamides and macaene are considered the typical markers for maca and thought to represent the group of biologically active compounds in maca. Two macamides from the standardized product, *N*-benzylhexadecanamide and *N*-benzyl-5-oxo-6E,8E-octadecadienamide, were analyzed by HPLC, and their chromatograms are shown in Fig. 1. The contents of these macamides were 7.8 mg/g of lipid-soluble extract, and 0.3–0.4 mg/g of the original maca powder.

3.2. Effect of lipid-soluble extract from maca on swimming endurance capacity

The swimming time to exhaustion of the rats was measured to investigate the antifatigue activity of the lipid-soluble maca extract. As shown in Fig. 2, the swimming times of the rats after supplementation for 3 weeks with 30 or 100 mg/kg of lipid-soluble maca extract, were 25% and 41% higher, respectively, than that in the control group ($p < 0.05$).

3.3. Effect of lipid-soluble maca extract on serum biochemical parameters and glycogen storage

The levels of energy sources including glycogen storage are shown in Table 1. Serum concentrations of glucose, and FFAs, as well as the content of glycogen in liver and muscle did not differ significantly between two treatment groups and control group. The serum lactate level did not change, but serum LDH activity was significantly lower in rats given 100 mg/kg of lipid-soluble maca extract than that in the control group (300 ± 12 U/l vs. 426 ± 53 U/l, $p < 0.05$). LDH activity was lower in the rats fed 30 mg/kg of lipid-soluble maca extract (316 ± 16 U/l), but was not statistically significant (Table 1).

3.4. Effect of lipid-soluble maca extract on antioxidant status after exhaustive swimming in rats

To investigate whether supplementation of lipid-soluble maca extract reduces exhaustive exercise-induced oxidative stress, we evaluated the effect of lipid-soluble extract on lipid peroxidation (TBARS), total GSH, catalase, and SOD in liver and skeletal muscle of rats swum to exhaustion. The results are presented in Table 2. The muscular TBARS level as an index of lipid peroxidation was significantly lower in rats supplemented with 100 mg/kg of lipid-soluble maca extract than that in the controls ($p < 0.05$), but the hepatic TBARS level did not differ among groups. One of the most important antioxidants, total GSH in both liver and skeletal muscle, was significantly greater in rats supplemented with 100 mg/kg lipid-soluble maca extract than that in the control rats ($p < 0.05$). Catalase activity was significantly higher in liver but not in muscle tissue of rats supplemented with 100 mg/kg lipid-soluble maca extract compared with that of the control rats. SOD activity did not differ between the supplemented and control rats.

4. Discussion

In this study, we examined the effects of supercritical lipid-soluble extract from maca on swimming time to exhaustion in rats. Supplementation with the lipid-soluble maca extract for 3 weeks markedly improved endurance capacity in rats subjected to weight-loaded forced swimming in a dose-dependent manner. Several serum biochemical parameters, glycogen storages, and antioxidant status were measured in the rats immediately after exhaustive swimming to explain the reasons for this increased endurance capacity. The serum levels of glucose, FFA, and lactate and tissue glycogen storages did not differ between the supplemented and the control rats. Serum LDH, an indicator of muscle damage, and muscle TBARS, a lipid peroxidation product, were significantly lower in rats treated with 100 mg/kg of lipid-soluble maca extract than those in the control. Also, a critical nonenzymatic antioxidant GSH in muscle and liver and catalase activity in liver were significantly higher in the rats supplemented with the higher dose (100 mg/kg) of maca extract.

Researchers studying the antifatigue effects of maca reported the increased endurance capacity in mice administered with an aqueous extract of maca and benzylglucosinolate, one of the active compounds from maca. They suggested that the increased endurance capacity reflected increased fatty acid utilization as an energy source and increased clearance of accumulated lactic acid (Ikeuchi, Koyama, Takei, Kino, & Yazawa, 2009; Zheng et al., 2002). In the present study, supplementation with lipid-soluble maca extract did not affect the levels of energy fuels and serum lactate, but influenced antioxidant status. Generally, exhaustive exercise decreases the amount of energy sources, but increases metabolites such as lactate. According to Wilber (1959), violent swimming to exhaustion significantly elevated blood lactate level, and the rate of the lactate accumulation in the blood was related inversely to swimming time. In our preliminary data, serum level of lactate was increased by 325% in weight-loaded forced

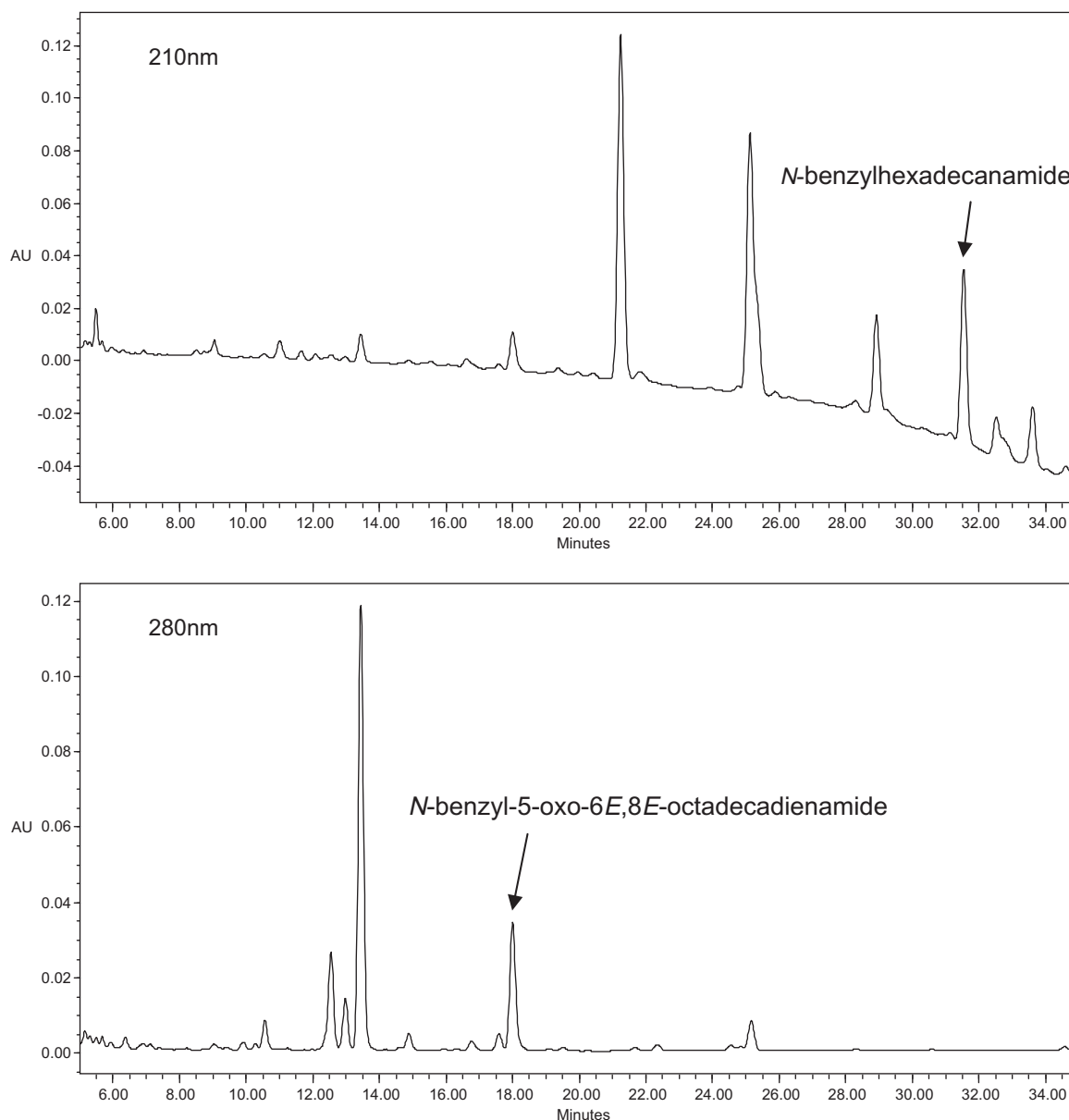


Fig. 1 – Analysis of macamides in the lipid-soluble maca extract measured by HPLC. The lipid-soluble maca extract was prepared by supercritical fluid extraction from maca powder and standardized in the content of two macamides, *N*-benzylhexadecanamide and *N*-benzyl-5-oxo-6*E*,8*E*-octadecadienamide.

swimming rats after swimming to exhaustion. Therefore, this observation of the unchanged accumulation of the toxic metabolite, lactate, despite the longer swimming time in rats supplemented with lipid-soluble maca extract than that in the control group, is meaningful.

Among the various fatigue mechanisms, the radical theory has attracted increasing interests. During exercise, a large amount of oxygen is consumed, and the rate of free radical generation is accelerated as an outcome of electron transport chain activity (Kanter, 1994). Many reports have shown that muscular exercise increases the production of ROS, which contribute to oxidative muscle damage and muscle fatigue (Davies, Quintanilha, Brooks, & Packer, 1982; Liu et al., 2000). In this study, the administration of lipid-soluble maca extract reduced the TBARS level in skeletal muscle, and increased total

GSH in liver and muscle in rats swum to exhaustion. In the present study, the antioxidant enzyme activities were not changed much by the supplementation of lipid-soluble maca extract, except for the hepatic catalase. It may be partially caused by oxidant and antioxidant balance, intensity and duration of exercise, and adaptation of training. Recent studies have demonstrated that maca showed radical scavenging activities and protective effects against radical-induced apoptosis (Sandoval et al., 2002). Serum LDH is an indicator of muscle damage because LDH normally exists in the muscle cells and is released into the bloodstream as a result of muscle damage. Thus, reduction of serum LDH and muscle TBARS levels in rats treated with lipid-soluble maca extract may reflect its protective action against lipid peroxidation and subsequent muscle damage induced by exhaustive exercise.

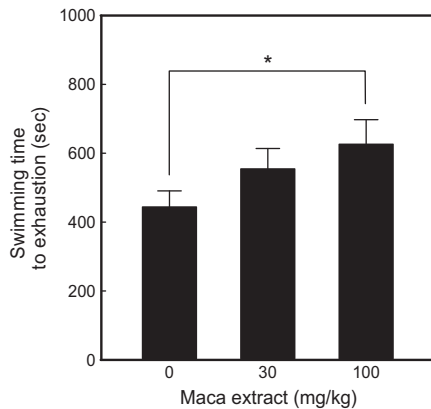


Fig. 2 – Effect of standardized lipid-soluble maca extract on swimming time to exhaustion in rats. The rat was subjected to exhaustive swimming with a load attached to the tail corresponding to 6% of the body weight, and was considered exhausted when it failed to rise to the surface to breathe for 10 s. The values are expressed as mean \pm SEM ($n = 19$ – 20). $^* p < 0.05$ compared with the vehicle-treated control group.

It has been proposed that macaene and macamide might be the biologically active components in lipid-soluble extract

of maca improving sexual performance (Zheng et al., 2000). Pino-Figueroa, Nguyen, and Maher (2010) demonstrated that the pentane extract of maca, rich in lipid-soluble alkaloids, macamides, macaenes, and benzyl isothiocyanates, showed the neuroprotection in crayfish neurons subjected to H_2O_2 and the decrease in infarct volume in rats following focal ischemic stroke. Besides, phytosterols, glucosinolates and phenolic compounds in the extract having antioxidant activity may contribute at least in part to protective effects of lipid-soluble maca extract against oxidative damage induced by exhaustive exercise (Ganzera, Zhao, Muhammad, & Khan, 2002; Sandoval et al., 2002; Wang et al., 2007; Zheng et al., 2002). However, we cannot conclude which compounds are exactly responsible for these effects including antioxidant and other effects. The antioxidant activity of maca is collectively linked to these substances in the extract. Further isolation and systematic evaluation of isolated compounds such as macamides and isothiocyanates are required to elucidate the mechanisms for the beneficial effects of maca.

In summary, the supplementation with lipid-soluble maca extract increased swimming time to exhaustion in weight-loaded forced swimming rats. The lipid-soluble maca extract can contribute to the improvement in endurance capacity by activation of antioxidant defense system. Because a large number of complex mechanisms may be involved in the exer-

Table 1 – Effect of standardized lipid-soluble maca extract on biochemical parameters in rats swum to exhaustion.

Parameters	Maca extract (mg/kg)		
	0	30	100
Serum			
Glucose (mg/dl)	242 \pm 12	243 \pm 10	265 \pm 12
FFA (μ Eq/l)	429 \pm 18	415 \pm 23	420 \pm 26
Lactate (mmol/l)	21.5 \pm 0.7	21.4 \pm 0.9	21.7 \pm 0.9
LDH (U/l)	426 \pm 53	316 \pm 16	300 \pm 12 [*]
Muscle glycogen (mg/g)	23.7 \pm 1.8	21.6 \pm 1.8	25.6 \pm 1.3
Liver glycogen (mg/g)	2.21 \pm 0.23	2.21 \pm 0.23	2.28 \pm 0.17

Values are expressed as mean \pm SEM ($n = 19$ – 20).

^{*} $p < 0.05$ compared with the vehicle-treated control group.

Table 2 – Effect of standardized lipid-soluble maca extract on skeletal muscle and liver antioxidant status in rats swum to exhaustion.

Parameters	Skeletal muscle			Liver		
	Maca extract (mg/kg)			Maca extract (mg/kg)		
	0	30	100	0	30	100
TBARS (nmol/g)	19.8 \pm 0.8	19.6 \pm 1.0	17.3 \pm 0.7 [*]	40.5 \pm 2.0	41.5 \pm 1.6	41.4 \pm 0.6
GSH (μ mol/g)	1.02 \pm 0.03	1.08 \pm 0.03	1.12 \pm 0.02 [*]	7.03 \pm 0.17	7.04 \pm 0.20	7.65 \pm 0.16 [*]
SOD (U/mg) ^a	8.08 \pm 0.23	7.99 \pm 0.21	8.23 \pm 0.20	32.3 \pm 0.43	33.9 \pm 0.76	33.0 \pm 0.70
Catalase (μ mol/min/mg) ^b	0.019 \pm 0.001	0.021 \pm 0.001	0.019 \pm 0.001	1.65 \pm 0.06	1.76 \pm 0.03	2.09 \pm 0.05 [*]

Values are expressed as mean \pm SEM ($n = 19$ – 20).

^a One unit of SOD is defined as the amount of enzyme required to cause 50% inhibition of pyrogallol autoxidation.

^b Catalase activity is expressed as μ mol of formaldehyde formed/min/mg protein.

^{*} $p < 0.05$ compared with the vehicle-treated control group.

cise-induced fatigue, a further research including molecular study is required to evaluate its antifatigue mechanism. These results suggest that supplementation with lipid-soluble maca extract improved swimming endurance capacity and this effect can be explained partly by attenuation of exercise-induced oxidative stress.

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