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ORIGINAL ARTICLE

Effects of macamides on endurance capacity and anti-fatigue property in prolonged swimming mice

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ABSTRACT

Context: *Lepidium meyenii* Walp. (Brassicaceae), most commonly known as ''maca'', has been used as a food or folk medicine to improve vitality in Peru. Previous research demonstrated that lipid-soluble extract from maca improved swimming endurance capacity. Macamides are considered the typical lipid-soluble markers for maca and proved to have several pharmacological properties, such as improving sexual performance and neuroprotective activities.

Objective: The present study investigates the effects of macamides on endurance capacity and anti-fatigue property in prolonged swimming mice.

Materials and methods: The Balb/c mice were divided into seven groups: a control group, low-dose groups of *N*-benzyllinoleamide, *N*-benzyloleamide, and *N*-benzylpalmitamide, high-dose groups of these macamides. The macamides groups received the commercial products (12 and 40 mg/kg, ig), while the control group received vehicle for 21 d. On the 14th day, the mice were given the weight-loaded swimming test. On the 21st day, the mice were sacrificed immediately after 90 min swimming, and some biochemical parameters were measured.

Results and discussion: Compared with the control group, exhaustive swimming time was significantly prolonged in high-dose group of *N*-benzyloleamide ($p < 0.05$); the levels of lactic acid (LD), blood ammonia (BA), and lactate dehydrogenase (LDH) were significantly decreased ($p < 0.05$), whereas the levels of liver glycogen (LG) and non-esterified fatty acid (NEFA) were significantly increased ($p < 0.05$) in high-dose group of *N*-benzyloleamide. The malondialdehyde (MDA) contents in the brain, muscle, and liver were significantly decreased ($p < 0.05$), whereas superoxide dismutase (SOD) and glutathione peroxidase (GSH-PX) activities in the brain, muscle, and liver were significantly increased in high-dose group of *N*-benzyloleamide ($p < 0.05$).

Conclusion: The results indicate that *N*-benzyloleamide has pharmaceutical property against exercise-induced fatigue, and this effect can be explained by the modulated energy metabolism and improved antioxidant status.

KEYWORDS
Antioxidant, energy metabolism, *N*-benzyloleamide

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Introduction

Fatigue is known to be accompanied by a feeling of extreme physical or mental tiredness, resulting from severe stress and hard physical or mental work (Huang et al. 2011). Fatigue can be classified as physical and mental fatigue. Physical fatigue is thought to be accompanied by deterioration in exercise performance (Tanaka et al. 2008). The mechanisms of physical fatigue are complicated, metabolic dysregulation, and free radicals damage can result in fatigue (McArdle et al. 2005; Zhang et al. 2010). Metabolic dysregulation includes the exhaustion of energy sources such as glucose and glycogen, and the accumulation of metabolic products such as the lactic acid and ammonia (Pedersen et al. 2004). Free radicals can be generated and induced as a result of the large amount of oxygen consumed during exercise (Wang et al. 2008). When proteins and lipids in the muscle are oxidised by free radicals that accumulate in the skeletal muscle, muscle force production is decreased and may result in fatigue (Gomez-Cabrera et al. 2008). Moreover, mental fatigue impairs physical activity, and this decreased performance is caused by the neural mechanisms (Marcora et al. 2009; Tanaka et al. 2014). There are many methods to attenuate fatigue, and supplementation with natural active products is an effective method (Kim et al. 2002).

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Lepidium meyenii Walp. (Brassicaceae), most commonly known as "maca", is a traditional food or folk medicine of the indigenous people in the Peruvian Andes, and grows exclusively at altitudes over 3500 m (Wang et al. 2007). Maca has recently been studied profusely because of the diverse pharmacological properties, such as fertility enhancer, sexual performance effects, and endurance capacity enhancer (Cicero et al. 2001; Bustos-Obregón et al. 2005; Ruiz-Luna et al. 2005). The maca root contains high nutritional value component (Dini et al., 1994) and several unique secondary metabolites, such as macamides, macaene, alkaloids, glucosinolates, and sterols (Ganzera et al. 2002; Muhammad et al. 2002; Piacente et al. 2002; Cui et al. 2003).

Previous research discovered that lipid-soluble extract from maca powder improved swimming endurance capacity, and the major components in the maca lipid-soluble extract were macamides, fatty acids, sterols, and phenolic (Choi et al. 2012). Macamides which are lipid-soluble and long-chain, are considered the typical markers for maca, and proved to have several pharmacological properties, such as improving sexual performance and neuroprotective effects (Zheng et al. 2000; Pino-Figueroa et al. 2010; Vu 2012; Wu et al. 2013). In previous experiment, we discovered that maca lipid-soluble extract by petroleum ether extraction could significantly extend the weight-loaded swimming time to exhaustion. In this study, we prepared maca petroleum ether extract (MPE), and analyzed the kinds and contents of macamides using high-performance liquid chromatography (HPLC) system, then investigated the anti-fatigue effect of the major macamides, as well as the possible mechanisms.

Materials and methods

Preparation of botanical extracts

Maca was provided from Lijiang BaiSuiFang Biotechnology Development Co. Ltd. (Yunnan, China). It was identified by Prof. Jun Xiang (College of Life Science, Huanggang Normal University, Hubei, China). The root of maca was rinsed clean and sliced. The slices were lyophilised, smashed into powder, and filtered through an 80-mesh sieve. Dried maca powder was reflux extracted by 12 volumes of petroleum ether at 50 °C for 30 min, and ultrasonically extracted for 15 min. The filtrate was collected by vacuum suction filtration. The above steps were repeated, and the two filtrates were merged. The total filtrate was concentrated under reduced pressure at 48–52 °C. The yield of maca petroleum ether extract (MPE) from dried maca powder was 1.96%.

Chemical constituents and contents of maca petroleum ether extract

The MPE was dissolved in methanol and filtered through a 0.45 μm membrane filter. The sample was standardised by using an HPLC system with a TC-C18 column (250 mm × 4.6 mm × 5 μm, Agilent Technologies, Inc., Santa Clara, CA). The column was operated at room temperature. The mobile phase was 90% acetonitrile with a flow rate of 0.6 mL/min. The sample and the macamides standards were put into HPLC vials and 20 μL automatically injected into the HPLC system, detected at 210 nm. The macamides (HPLC ≥ 95%) used as the reference standards for HPLC were provided from Wuhan Huashite Industrial Biotechnology Development Co. Ltd. (Hubei, China). The MPE was standardised based on the retention time of the macamides standards, and the contents of macamides in MPE were estimated by calculation up on a calibration curve.

Animals and housing

The Balb/c mice were supplied by Experimental Animal Research Center of Hubei Province. Five-week-old male mice were housed in an animal facility under light/dark cycles of 12 h and at an ambient temperature of 23 ± 1 °C. All mice were allowed free access to distilled water and a rodent chow diet throughout the experimental period. This study was approved by the committee for animal experiments of Huazhong University of Science and Technology (authorisation #2018112), and the animals were cared for in accordance with the Guide for the Care and Use of Laboratory Animals.

Groups and treatment

After 5 d of adaptation, the mice were allowed to swim for 10 min; those who could not swim were removed from the study. The mice were then randomly divided into seven groups with similar body weight, i.e. a control group and six macamide groups ($n = 10$/group): a control group (vehicle), low-dose group of *N*-benzyllinoleamide (12 mg/kg), high-dose group of *N*-benzyllinoleamide (40 mg/kg), low-dose group of *N*-benzyloleamide (12 mg/kg), high-dose group of *N*-benzyloleamide (40 mg/kg), low-dose group of *N*-benzylpalmitamide (12 mg/kg), and high-dose group of *N*-benzylpalmitamide (40 mg/kg). The macamides were suspended in a 1% aqueous solution of Tween-80. The control groups were treated with a similar volume of vehicle. The dose selections for the macamide groups were based on the contents of macamides in MPE and the appropriate dose of MPE

in the previous experiment. The mice in macamide groups were received the macamides intragastrically in a volume of 0.2 mL/10 g once per day for 21 consecutive days. The control group was treated with a similar volume of vehicle. *N*-benzyllinoleamide, *N*-benzyloleamide, and *N*-benzylpalmitamide were provided from Wuhan Huashite Industrial Biotechnology Development Co. Ltd. (Hubei, China).

Measurement of the weight-loaded swimming capacity

Mice were made to swim without loading for 10 min, twice a week, to accustom them to swimming. On the 14th day of the experiment, the weight-loaded swimming test was employed in our study to evaluate the effects of macamides on the endurance capacity of mice. The procedure used was described previously (Huang et al. 2011) with some modifications. Briefly, 30 min after the oral administration, the mice were dropped individually into acrylic plastic pool (80 cm × 45 cm × 40 cm) containing fresh water maintained at 27 ± 1 °C, approximately 35 cm deep. The mice were loaded with a lead block, weighting approximately 5% of their body weight, attached to the tail. The swimming time to exhaustion was used as the index of the degree of fatigue (Tanaka et al., 2003). The mice were assessed to be exhausted when they cannot keep their nose out of the water within a 10 s period.

Measurement of serum parameters and liver glycogen

After the weight-loaded swimming test, the mice were orally administered for 7 more days. Thirty minutes after the last dosing on the 21st day, each mouse was sacrificed immediately after 90 min swimming without load. The selection of swimming time that leads to fatigue was according to the previous research (Wang et al. 2005). The blood was then collected from the retrobulbar vessels under ethyl carbamate anesthesia. Blood samples were cooled for about 0.5 h at 4 °C, and the serums were prepared by centrifugation at 2000 rpm at 4 °C for 10 min and stored at -80 °C in a deep-freezer. The levels of serum glucose, lactate (LD), lactate dehydrogenase (LDH), blood urea nitrogen (BUN), non-esterified fatty acid (NEFA), blood ammonia (BA), and the contents of liver glycogen were measured using commercially available kits (Nanjing Jiancheng Biology Engineering Institute, China) according to the manufacturer's instructions.

Measurement of antioxidant enzymes and lipid peroxidation in the brain, liver and muscle

Immediately after the blood had been collected, the brain, liver, and left gastrocnemius muscle were quickly dissected out, frozen in liquid nitrogen, and kept at −80 °C until analysis. Each tissue was homogenised in ice-cold normal saline. These tissue homogenates were centrifuged at 3000 rpm for 10 min at 4 °C, and the supernatants were assessed for the antioxidant status. The activities of superoxide dismutase (SOD) and glutathione peroxidase (GSH-PX), the contents of lipid peroxidation product malondialdehyde (MDA), and the tissue proteins were determined using commercially available kits (Nanjing Jiancheng Biology Engineering Institute, China) according to the manufacturer's protocol.

Statistical analysis

Data are presented as means ± standard deviation (SD). The data were analyzed by one-way analysis of variance, followed by LSD-*t*-test for multi-group comparisons. The value of $p < 0.05$ was considered as statistically significant. All statistical analyses were conducted using the SPSS statistical package for Windows version 17.0 (SPSS Inc., Chicago, IL).

Results

Kinds and contents of macamides in MPE analyzed from HPLC

In this study, the yield of maca petroleum ether extract (MPE) from maca powder was 1.96%. Figure 1 shows the HPLC analysis of MPE. The sample was standardised based on the retention time of the macamides standards, and the contents of macamides in MPE were estimated by calculation up on a calibration curve. MPE contained about 20% macamides, and the macamides with higher contents were 6.63% *N*-benzylpalmitamide, 4.59% *N*-benzyloleamide, and 4.22% *N*-benzyllinoleamide.

Effects on body weights of mice

Body weights were recorded before the experiment (initial), and on the 14th day (final). As presented in Table 1, no significant difference was observed on body weights of the mice between the control group and each treatment group. However, the increase of the body weight in high-dose group of *N*-benzyloleamide was lower than that in the control group.

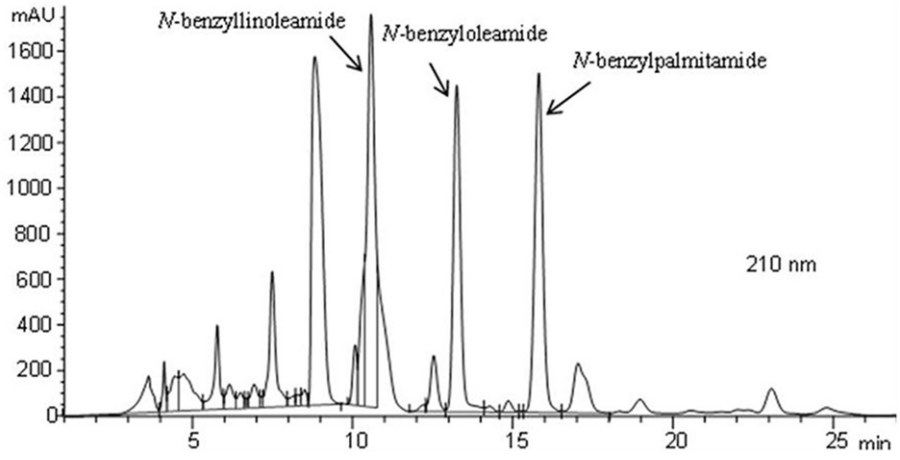

Figure 1. High performance liquid chromatograms of macamides.

Table 1. Effects of macamides on body weights of mice.

Groups	Initial (g)	Final (g)	Increased (g)
Control	20.8 ± 0.8	23.9 ± 1.0	3.1 ± 1.0
A	20.4 ± 1.0	24.0 ± 1.4	3.7 ± 1.5
B	20.9 ± 0.8	24.1 ± 1.5	3.1 ± 1.4
C	20.8 ± 1.2	23.9 ± 1.3	3.1 ± 1.2
D	20.5 ± 0.9	23.1 ± 1.1	2.7 ± 1.1
E	20.8 ± 0.8	24.4 ± 1.1	3.6 ± 1.1
F	20.6 ± 0.8	24.2 ± 1.2	3.5 ± 1.3

A: low-dose group of *N*-benzyllinoleamide; B: high-dose group of *N*-benzyllinoleamide; C: low-dose group of *N*-benzyloleamide; D: high-dose group of *N*-benzyloleamide; E: low-dose group of *N*-benzylpalmitamide; F: high-dose group of *N*-benzylpalmitamide. All values are expressed as mean ± SD (n = 10/group).

Effects on weight-loaded swimming capacity

Figure 2 shows the weight-loaded swimming capacities. The high-dose group of *N*-benzyloleamide significantly improved the swimming time to exhaustion in mice compared with the control group ($p < 0.05$). Although no significant difference was observed, the high-dose group of *N*-benzyllinoleamide and the low-dose group of *N*-benzyloleamide could extend the swimming time to exhaustion.

Effects on serum biochemical parameters and glycogen storage

The serum biochemical parameters and glycogen storage are presented in Table 2. The levels of LD were significantly decreased in both dose groups of *N*-benzyloleamide and the high-dose group of *N*-benzyllinoleamide ($p < 0.05$). The LDH activity was significantly decreased in the high-dose groups of three kinds of macamides compared with the control group ($p < 0.05$). The NEFA levels were significantly increased in the low-dose group of *N*-benzyloleamide and the high-dose group of three kinds of macamides ($p < 0.05$). The level of BA was significantly decreased in the high-dose group of *N*-benzyloleamide ($p < 0.05$), whereas the content of LG was significantly increased ($p < 0.05$). The levels of BUN and glucose were not significantly different in all groups.

Effects on antioxidant enzymes and lipid peroxidation

The results are presented in Table 3. Compared with the control group, the MDA contents in the brain were significantly decreased in the high-dose groups of *N*-benzyloleamide and *N*-benzyllinoleamide ($p < 0.05$, $p < 0.01$), whereas SOD and GSH-PX activities in the brain were significantly increased in both dose groups of *N*-benzyloleamide and *N*-benzyllinoleamide ($p < 0.05$, $p < 0.01$). The MDA contents in muscle were significantly decreased, whereas SOD and GSH-PX activities in muscle were significantly increased in the high-dose groups of *N*-benzyloleamide and *N*-benzyllinoleamide ($p < 0.05$). In the liver, the MDA content was significantly decreased in the high-dose group of *N*-benzyloleamide, whereas SOD and GSH-PX activities were significantly increased in the high-dose groups of *N*-benzyloleamide and *N*-benzyllinoleamide ($p < 0.05$). The results indicated that supplementation with *N*-benzyloleamide and *N*-benzyllinoleamide could improve the antioxidant enzyme activities and reduce the lipid peroxidation in the brain, muscle, and liver of mice. Moreover, the high-dose group of *N*-benzyloleamide had more significant influence on the antioxidant status.

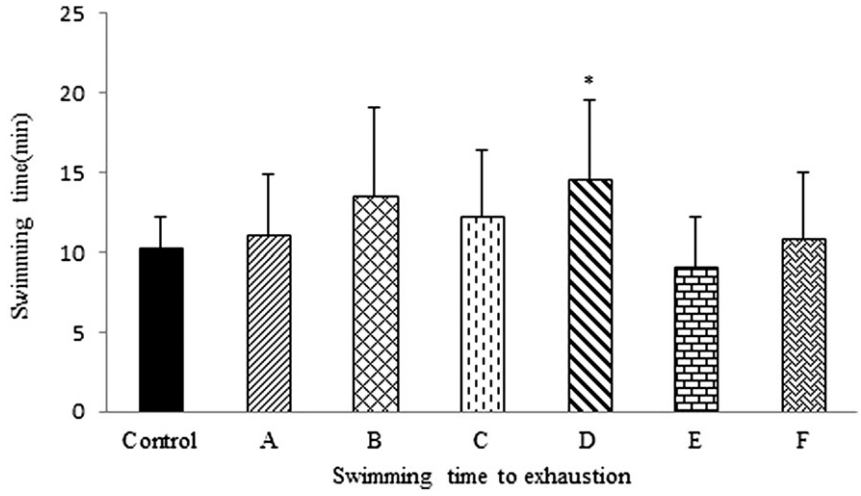

Figure 2. Effects of macamides on the swimming time to exhaustion in weight-loaded mice. A: low-dose group of *N*-benzyllinoleamide; B: high-dose group of *N*-benzyllinoleamide; C: low-dose group of *N*-benzyloleamide; D: high-dose group of *N*-benzyloleamide; E: low-dose group of *N*-benzylpalmitamide; F: high-dose group of *N*-benzylpalmitamide. All values are expressed as mean ± SD ($n = 10$/group). *$p < 0.05$, compared with the control group.

Table 2. Effect of macamides on serum parameters and glycogen storage in mice.

Parameter	Control	A	B	C	D	E	F
LD (mmol/*L*)	5.38 ± 0.50	5.01 ± 0.67	4.82 ± 0.59*	4.87 ± 0.46*	4.69 ± 0.55*	5.62 ± 0.48	5.51 ± 0.98
LDH (U/*L*)	3182.35 ± 290.11	2998.77 ± 231.22	2785.25 ± 357.2*	2980.67 ± 334.91	2793.74 ± 210.59*	3051.26 ± 293.14	2821.05 ± 236.97*
NEFA (μmol/L)	823.11 ± 80.97	856.02 ± 73.25	896.43 ± 77.41*	898.06 ± 83.24*	926.08 ± 74.94*	840.42 ± 76.32	900.11 ± 80.22*
BUN (mmol/*L*)	6.99 ± 0.76	6.41 ± 0.66	6.67 ± 0.83	6.44 ± 0.99	6.79 ± 1.00	6.82 ± 0.84	7.13 ± 0.94
BA (μmol/*L*)	148.84 ± 13.68	145.49 ± 15.42	138.67 ± 16.72	149.94 ± 18.71	131.60 ± 16.28*	141.20 ± 14.35	136.45 ± 19.13
Glucose (mmol/*L*)	5.59 ± 0.78	5.34 ± 0.64	5.96 ± 0.95	5.56 ± 0.74	5.92 ± 0.83	5.34 ± 0.86	5.77 ± 0.70
LG (mg/g)	6. 45 ± 0.80	6.22 ± 0.81	6.65 ± 0.78	6. 88 ± 0.90	7.36 ± 1.02*	6.11 ± 0.69	6.32 ± 0.77

All values are expressed as mean ± SD ($n = 10$/group). A: low-dose group of *N*-benzyllinoleamide; B: high-dose group of *N*-benzyllinoleamide; C: low-dose group of *N*-benzyloleamide; D: high-dose group of *N*-benzyloleamide; E: low-dose group of *N*-benzylpalmitamide; F: high-dose group of *N*-benzylpalmitamide; LA, serum lactic acid; BUN, blood urea nitrogen; LDH, lactate dehydrogenase; NEFA, non-esterified fatty acid; BA, blood ammonia; LG, liver glycogen. *$p < 0.05$ compared with the control group.

Table 3. Effect of macamides on antioxidant enzyme and lipid peroxidation in mice.

Tissue	Parameter	Control	A	B	C	D	E	F
Brain	SOD (U/mgprot)[a]	180.71 ± 30.31	211.40 ± 31.99*	207.49 ± 20.46*	201.66 ± 18.77*	217.01 ± 25.89**	197.51 ± 36.01	201.54 ± 30.00
	GSH-PX (U/mgprot)[b]	33.23 ± 10.11	44.20 ± 9.89*	45.35 ± 11.44*	42.73 ± 9.20*	50.12 ± 9.73**	33.70 ± 9.51	37.32 ± 8.20
	MDA (nmol/mgprot)	3.20 ± 0.74	3.03 ± 0.69	2.58 ± 0.52*	3.04 ± 0.49	2.43 ± 0.64**	3.06 ± 1.49	3.29 ± 0.83
Muscle	SOD (U/mgprot)[a]	47.29 ± 9.21	46.39 ± 10.40	60.29 ± 9.20*	50.11 ± 10.33	58.48 ± 10.19*	51.35 ± 8.87	53.78 ± 13.94
	GSH-PX (U/mgprot)[b]	8.47 ± 0.97	9.34 ± 1.02	11.13 ± 1.07*	9.25 ± 1.13	10.88 ± 0.74*	8.88 ± 0.87	9.14 ± 0.78
	MDA (nmol/mgprot)	2.83 ± 0.26	2.68 ± 0.35	2.39 ± 0.32*	2.61 ± 0.38	2.43 ± 0.31*	3.05 ± 0.43	2.92 ± 0.35
Liver	SOD (U/mgprot)[a]	110.75 ± 28.68	131.73 ± 26.62	142.72 ± 27.18*	123.22 ± 31.45	145.50 ± 29.49*	112.51 ± 24.41	118.64 ± 31.19
	GSH-PX (U/mgprot)[b]	152.60 ± 28.66	160.06 ± 21.8	176.84 ± 19.34*	157.14 ± 17.10	180.21 ± 20.33*	149.46 ± 23.68	161.46 ± 27.11
	MDA (nmol/mgprot)	1.36 ± 0.22	1.28 ± 0.26	1.20 ± 0.18	1.25 ± 0.24	1.10 ± 0.32*	1.34 ± 0.31	1.31 ± 0.43

All values are expressed as mean ± SD ($n = 10$/group). *$p < 0.05$. **$p < 0.01$ compared with the control group. A: low-dose group of *N*-benzyllinoleamide; B: high-dose group of *N*-benzyllinoleamide; C: low-dose group of *N*-benzyloleamide; D: high-dose group of *N*-benzyloleamide; E: low-dose group of *N*-benzylpalmitamide; F: high-dose group of *N*-benzylpalmitamide.

[a]One unit of SOD is defined as the amount of enzyme required to cause 50% inhibition of superoxide anion free radicals that is produced by xanthine oxidase reaction system/mL reaction liquid/mg protein.

[b]One unit of GSH-PX is expressed as the reduction of GSH concentration for 1 μmol/L/min/mg protein, excluding the effect of non-enzyme in reaction system.

Discussion

The present study was designed to evaluate the effects of macamides on endurance capacity and anti-fatigue property in prolonged swimming mice. The results indicated that supplementation with *N*-benzyloleamide (40 mg/kg) significantly extended the weight-loaded swimming time to exhaustion, improved swimming endurance capacity.

The endurance capacity of the body depends on the levels of energy sources including glycogen storage and clearance of accumulated metabolic products (Tan et al. 2012). Glycogen as the important resource of energy is used to complement the consumption of blood glucose during exercise, and maintains the blood glucose in the physiologic range. Increase in glycogen consumption has been related to elevated fatigue (Ren et al. 2011). By contrast, increase in fatty acid metabolism during exercise, leads to a decreased glycogen depletion rate and enhanced endurance exercise performance (Azevedo et al. 1998; Favier & Koubi 1988). The enhanced availability of free fatty acids is thought to cause greater fat mobilisation in the active muscles (Oh & Ohta 2003). Our results showed that supplementation with high dose of *N*-benzyloleamide could significantly increase LG and NEFA ($p < 0.05$), while the glucose levels were not significantly different in all groups. The results suggested that *N*-benzyloleamide decreased glycogen utilisation and increased fatty acid metabolism as an energy source during exercise.

The excessive accumulation of metabolic products such as serum lactic acid is a factor causing fatigue (Pedersen et al. 2004; Jia & Wu 2008). The excess lactic acid production can induce a decrease of pH that inhibits muscle contraction. Thus, rapid removal of lactic acid is beneficial to attenuate fatigue. In the study, lower levels of LD were observed in the *N*-benzyloleamide and *N*-benzyllinoleamide groups compared with the control group ($p < 0.05$). LD is produced as a result of carbohydrate metabolism; therefore, these results are consistent with the decrease of glycogen utilisation as an energy source during exercise. The levels of BUN, which is the metabolism outcome of protein and amino acid, were not significantly different in all groups. Serum LDH is an indicator of muscle damage because LDH normally exists in the muscle cells and is released into the blood stream as a result of muscle damage (Kim et al. 2003). The results showed that supplementation with the three kinds of macamides significantly reduced the levels of LDH ($p < 0.05$), compared with the control group. Energy sources, such as glucose and glycogen, are used first and may become exhausted, and metabolic products, such as lactic acid, are accumulated, hence causing metabolic dysregulations (Pedersen et al. 2004; Zhang et al. 2010). Our results suggested that supplementation with *N*-benzyloleamide improved the endurance capacity because of the modulated energy metabolism, including the increase of fatty acid metabolism, decrease of glycogen depletion, and rapid removal of the metabolic products in prolonged exercise.

Exercise-induced ammonia accumulation may contribute to the inducement of mental fatigue (Schenker et al. 1967; Pathak 1969; Banister & Cameron 1990). Our results showed that the level of BA was significantly decreased in the high-dose group of *N*-benzyloleamide compared with the control group. The decreased BA protects the central nervous system to attenuate fatigue.

Many studies have shown that prolonged strenuous exercise accelerated the free radicals generation and caused lipid peroxidation *in vivo* (McArdle et al. 2005; Radak et al. 2008). Lipid peroxidation can reduce the integrity of biological membranes, such as the sarcolemma and mitochondrial membranes, which ultimately diminish the body's ability to work and thus induce fatigue (Alessio 1993). MDA is often used to reflect the degree of lipid peroxidation caused by free radicals (Alessio et al. 1998); meanwhile, SOD and GSH-PX are used to reflect the ability of cleaning free radicals (Aguiló et al. 2005). Recent studies demonstrated that maca showed radical scavenging activities and protective effects against radical-induced apoptosis (Sandoval et al. 2002). Our results showed that supplementation with *N*-benzyloleamide and *N*-benzyllinoleamide significantly reduced MDA contents and increased SOD and GSH-PX activities ($p < 0.05$) in the brain, liver, and muscle, while high-dose group of *N*-benzyloleamide had markedly significant influence ($p < 0.01$) in the brain. Supplementation with *N*-benzyloleamide and *N*-benzyllinoleamide should be beneficial to protect against oxidative damage induced by prolonged exercise. The results indicated that supplementation with *N*-benzyllinoleamide and *N*-benzyllinoleamide could improve the antioxidant enzyme activities and reduce the lipid peroxidation in the brain, muscle, and liver of mice. Moreover, *N*-benzyloleamide had more significant influence on the antioxidant status.

Conclusion

These results suggest that supplementation with *N*-benzyloleamide (40 mg/kg) extended the weight-loaded swimming time to exhaustion, improved swimming endurance capacity. *N*-Benzyloleamide has pharmaceutical property against exercise-induced fatigue. The effect can be explained by the modulated energy metabolism, including the increase of fatty acid metabolism, the decrease of glycogen depletion, and rapid removal of the metabolic products in prolonged exercise. And supplementation with the high dose of *N*-benzyloleamide could decrease BA, which was beneficial to protect the central nervous system to attenuate fatigue. Moreover, supplementation with the high dose of *N*-benzyloleamide could improve the antioxidant status in the brain, liver and muscle. Further research is needed

to characterise the anti-fatigue mechanisms at the molecular levels.

Declaration of interest

The authors declare that there are no conflicts of interest. This work was supported by National Natural Science Foundation of China (Grant No. J1103514), Nature Science Foundation of Hubei Province (No. 2012FFB02607).

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