**Sasa borealis** Extract Efficiently Enhanced Swimming Capacity by Improving Energy Metabolism and the Antioxidant Defense System in Mice

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(Received May 27, 2015)

**Summary** This study was conducted to determine the effects of 50% ethanolic extract from *Sasa borealis* leaves (SBE) on swimming capacity and oxidative metabolism in mice. The mice were divided into 2 groups with similar swimming times and body weights: Ex-Control and Ex-SBE were orally administered with distilled water and 250 mg/kg body weight/d of SBE. Exhaustive swimming times were prolonged by 1.5-fold in the Ex-SBE group compared to the Ex-Control. The Ex-SBE group displayed lower lactate and higher non-esterified fatty acid levels 15 min after swimming and the hepatic and muscle glycogen levels were significantly higher than that in the Ex-Control. SBE potentially enhanced mRNA expression of citrate synthase (CS), carnitine palmitoyltransferase (CPT-1), and β-hydroxyacyl coenzyme A dehydrogenase (β-HAD) in skeletal muscle. The activities and mRNA expression of catalase (CAT), glutathione peroxidase (GPx), and superoxide dismutase (SOD) were elevated in the Ex-SBE compared with the Ex-Control after exhaustive swimming. These results suggest that SBE might be used as an effective agent to enhance swimming capacity by utilization of energy substrates and might ameliorate physical exhaustion by facilitating energy-generating metabolic genes and enhancing endogenous antioxidants.

**Key Words** *Sasa borealis*, swimming exercise, lactate, non-esterified fatty acid, glycogen

Improving exercise capacity is important both for athletic performance and active healthy lifestyles. The function of skeletal muscle to activate energy metabolism, plays a key role in exercise capacity (1, 2). During exercise, skeletal muscle utilizes glucose, glycogen, and fatty acid as energy sources, but the quantity of each energy source is limited in skeletal muscle. Numerous studies have reported that the appropriate selection of exogenous supplements may favorably improve exercise capacity (3) by sparing glycogen and increasing fatty acid utilization (4, 5), facilitating fatty acid oxidation (3, 4, 6) and enhancing mitochondrial functions (3, 6).

Exercise is known to promote good health and prevent various diseases. However, strenuous and exhaustive exercise can cause overwhelming production of reactive oxygen species (ROS), which leads to an imbalance between ROS production and antioxidant defense by cellular antioxidants and enzymes such as catalase (CAT), glutathione peroxidase (GPx), and superoxide dismutase (SOD), as well as the non-enzymatic antioxidant, reduced glutathione (7). This imbalance eventually damages biological molecules, key cellular components, and processes such as lipid peroxidation and enzyme inactivation, in addition to causing oxidative DNA damage (8), leading to deteriorated cellular function. Deteriorating function in skeletal muscle is tightly related to lower exercise capacity (9–12). Exogenous antioxidants may prevent exercise-induced oxidative damages, since they are able to detoxify certain free radicals and peroxides with enhancing endogenous antioxidants (12–14). In addition, antioxidants from plant-leaf may improve physiological conditions by promoting and interacting with endogenous antioxidants (3, 10, 12). Thus, antioxidants can be key elements to combat and reduce the degree of exhaustion.

Bamboo is well known for its extensive uses such as for building construction, handicrafts, food, and folk remedy. Its leaves, culm, and shoot are considered edible in Asian countries. Bamboo leaves are used for medicinal purposes and as food materials, including additives and wrappings (10). In medical protocols, they have been adopted as a common remedy for treating fever and for detoxification (15, 16). Over the past decade,
the biologically active components and health benefits of bamboo leaves have been widely studied (16). Many investigations have shown that an ethanol/water extract of bamboo leaves mainly contains phytochemicals such as polyphenols and flavones, and the phytochemicals are mostly responsible for the multiple health benefits due to biological activities such as anti-oxidative, anti-bacterial, and anti-viral effects (11, 15, 17). Sasa borealis (S. borealis) is a bamboo species widely grown in the southern area of Korea. S. borealis has been reported to exhibit anti-diabetic (18) and anti-oxidant activities (19).

In our previous study, an 80% ethanolic extract of bamboo leaves from Pseudosasa japonica (P. japonica) extended exhaustive swimming time and decreased blood lactate after swimming exercise in mice (10). Although several studies supported the beneficial effects of bamboo leaves on exercise capacity (10, 20), the effects of bamboo leaves on energy metabolism and the antioxidant defense system during exercise remain to be established. Therefore, the purpose of this study was to investigate the effects of a 50% ethanolic extract from S. borealis leaves (SBE) on swimming capacity and to examine the possible mechanisms, with a focus on energy substrate changes. Additionally, the mechanism of action relevant to the extract’s anti-oxidative activity was determined on exhaustive exercise.

MATERIALS AND METHODS

Reagents. Anthrone, bovine serum albumin (BSA), bovine liver glycogen, Bradford, potassium chloride (KCl), potassium hydroxide (KOH), nicotinamide adenine dinucleotide phosphate reduced form (NADPH), malondialdehyde (MDA), and phosphate buffered saline (PBS) were purchased from Sigma-Aldrich (St. Louis, MO).

Materials. Organic cultured S. borealis leaves were collected in Damyang (Jeollanamdo, Korea). The fresh S. borealis leaves were cleaned by washing thoroughly with water and rewashing with water. The freshly-washed leaves were steamed and dried under shade in a clean, dust-free environment then packed and stored at −4°C before extraction. Two kilograms of dried S. borealis leaves were cut into small pieces, mixed with 20 L of fermented ethanol and refluxed at 85°C for 3 h. The solution was filtered and concentrated with a vacuum evaporator at 65°C. The concentrate was lyophilized and kept at −20°C until needed. The final weight of the lyophilized powder for the SBE was 0.3 kg.

Animals. Four-week-old male ICR mice (19.2±0.2 g in body weight (b.w.)) were supplied by Orient Bio (Seongnam, Korea) and housed in cages under automatically controlled conditions of temperature (22±2°C), humidity (about 60%), and lighting (an alternating 12-h cycle of light and dark). The mice were allowed access to the AIN-76 diet (G-Bio, Seongnam, Korea) and water ad libitum. Chonnam National University’s Institutional Animal Care and Use Committee approved the protocol for the animal study (CNU-IACUC-YB-R-2014-2), and the animals were cared for in accordance with the “Guidelines for Animal Experiments” established by the university.

Exhaustive swimming capacity. An acryl plastic pool (90×45×45 cm) was used to determine the swimming capacity (21). The pool was filled with water to a depth of 38 cm and the temperature was maintained at 34±1°C. The current strength in the swimming pool was adjusted to 8 L/min by controlling the voltage in the pool pump, and monitored using a water flow meter.

Mice were acclimatized for 1 wk prior to use in experiments. The mice were forced to swim twice with 3 d-intervals to measure exhaustive swimming time. The mice were divided into two groups (n=20 per group) with similar mean swimming capacities (22.1±2.8 min vs. 22.0±3.3 min) and b.w. (27.7±0.4 g vs. 27.5±0.5 g): Ex-Control and Ex-SBE. Each group was administered distilled water or SBE for 21 d. On day 14, the two groups were administered distilled water or SBE (250 mg/kg b.w./d) 120 min before swimming and exhaustively exercised without any load. The experiments were conducted from 13:00 to 15:00, a period of in which the minimal variation of endurance capacity has been confirmed in rodents (21). The mice were determined to be exhausted when they failed to rise to the surface to breathe within a 5–7 s period (9, 21). On day 21, each group was divided into two subgroups (n=10 per group) for evaluation in energy metabolic parameters and endogenous antioxidant activities. The glucose, lactate, and non-esterified fatty acid (NEFA) levels were evaluated before and 15 min after swimming and glycogen was measured after the swimming in one sub-group. Biochemical parameters such as activity and mRNA expression of antioxidant enzymes and mRNA expression of enzymes on oxidative energy metabolism were estimated in the other sub-group.

Analysis of energy metabolic parameters on swimming. Blood glucose, lactate, and NEFA levels were measured to investigate the influence of SBE on the energy deficit and source status at the same swimming time for Ex-Control and Ex-SBE. On day 21, blood for glucose, lactate, and NEFA were collected from the tail 120 min after final administration and then collected again 15 min after swimming. The blood glucose and lactate levels were measured by the enzymatic method with commercial test strips (ARKRAY, Kyoto, Japan). The plasma used for NEFA was collected by centrifugation at 1,000×g at 4°C for 10 min and assayed by the enzymatic method with a commercial kit (Wako Pure Chemical Industries, Ltd., Osaka, Japan). Immediately after the exercise, the mice were sacrificed under light ether anesthesia to collect tissues, which were stored at −70°C. The hepatic and gastrocnemius muscle glycogen concentrations were determined in accordance with previous reports (22, 23), with some modifications. Briefly, liver and muscle pieces were washed twice, homogenized, restored to 10-fold PBS of their weight, and the homogenates put into 1.5 mL test tubes. After the tubes were centrifuged at 10,000×g for 10 min, the supernatant was removed completely. The pellet was re-suspended in 30% KOH of tissues to solubilize the glycogen. Each tube was sealed
and heated at 100°C for 30 min. Then the tubes were taken out and cooled to room temperature. Afterward, 0.1 mL of hydrolyzed tissues was transferred to a test tube and 0.4 mL of distilled water was added. 1 mL of 0.2% anthrone solution (2 g anthrone/L H2SO4) was added to each test tube and mixed by stirring. The mixture was maintained at room temperature for 15 min and the absorbance was measured at 620 nm. Glycogen standard (calibration) curves were generated by determining the absorbance of bovine liver glycogen at a range of 0–200 μg/mL. The values are expressed as mg/g tissue.

*Endogenous antioxidant activities.* Antioxidant enzyme activities and metabolic gene expression were determined to investigate the influence of SBE on endogenous antioxidant systems and oxidative metabolic changes after exhaustive swimming. After exhaustive swimming on day 21, the mice were sacrificed under light ether anesthesia to collect tissue, which was stored at −70°C. To evaluate the antioxidant enzyme activities, the livers were homogenized in 50 mM phosphate buffer containing 1.13% KCl. The suspension was then centrifuged at 13,000 × g for 15 min at 4°C, and the supernatant was used for the measurement. The activity of CAT was determined as described by Aebi (24). The GPx activity was estimated by the spectrophotometric method reported by Thomson et al. (25) based on the oxidation rate of NADPH at 340 nm. SOD activity was measured using an adaptation of the method described by McCord and Fridovich (26). Each enzyme activity was expressed.

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*Fig. 1.* Effect of SBE on swimming capacity in mice. Data are expressed as the mean±SE for 20 mice in each group. *p<0.05, compared to Ex-Control. The Ex-Control and Ex-SBE groups were given distilled water or SBE 120 min before exercise and then forced to swim until they were exhausted. The Ex-SBE group was orally given SBE at a dose of 250 mg/kg/d for 14 d. SBE, 50% ethanolic extract from Sasa borealis leaves.

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*Fig. 2.* Effects of SBE on the levels of blood lactate (A) and NEFA (B) before and after swimming. Data are expressed as the mean±SE for 10 mice in each group. *p<0.05, compared to Ex-Control, #p<0.05, compared to Pre-Exercise. The Ex-Control and Ex-SBE groups were given distilled water or SBE 120 min before exercise and the levels of lactate and NEFA were evaluated before and 15 min after forced swimming with a current flow of 8 L/min. The Ex-SBE group was orally given SBE at a dose of 250 mg/kg/d for 21 d. SBE, 50% ethanolic extract from Sasa borealis leaves.
as U/mg protein. The level of total glutathione (TGSH) was estimated by the method described by Akerboom and Sies (27). The concentration of malondialdehyde (MDA) was assayed by monitoring thiobarbituric acid reactive substance (TBARS) formation as described by Draper and Hadley (28). TGSH and MDA values were expressed as nmol/mg protein and μmol/mg protein. The amount of protein was measured using the Bradford assay (29).

Real-time polymerase chain reaction. The gastrocnemius muscle was homogenized with a pestle under liquid nitrogen. Total RNA was isolated by easy-BLUETM (INtRON Biotechnology, Seongnam, Korea) according to the manufacturer’s instructions. Real-time polymerase chain reaction (PCR) was performed using selective primer sets with Quantifast SYBR Green PCR Master Mix (Qiagen, Valencia, CA) according to the manufacturer’s instructions. The sequences were as follows: CAT (009804): 5'-TCA GAA GAA AGC GGT CAA GAA TT-3', 3'-GGT GCC GGG CCC CAT A-3', GPx (008160): 5'-CCC CAC TGC GCT CAT GA-3', 3'-GCG ACA CCG GAG ACC AAA-3', CuZn-SOD (M35725): 5'-GCC CGG CCG ATG AAG-3', 3'-CCT TTC TCC CAG CAG TCA CAT TGC-3', MnSOD (X04972): 5'-TTA AGC CGC AGA TCA TGC A-3', 3'-TCA CGT AGG CCG CAT GGT-3', citrate synthase (CS) (MN_026444): 5'-TGT CCT GCC CCT CCT CAT C-3', 3'-GTG CTG GAG TTG GGT TCC AT-3', carnitine palmitoyltransferase (CPT-1) (MN_013495): 5'-GTG ACT GGT GGG AGG AAT AC-3', 3'-GAG CAT CTC CAT GGC GTA G-3', β-hydroxyacyl coenzyme A dehydrogenase (β-HAD) (MN_145558): 5'-GAC AGG GTC ATG CTA TGA TTG TG-3', 3'-TCG GTC GCC TGC TCC TTC TAG AG-3', β-actin (NM_007393): 5'-AGG TGG AAA AGA GAC ATA-3', 3'-ACA CAA AAG GAG CCC TAA TGA-3'. Quantification of the mRNA expression was calculated using the ΔΔCT method.

Statistical analysis. Data are presented as mean± standard errors (SE). The data were statistically evaluated using one-way analysis of variance (ANOVA) with Tukey’s test to compare significant differences between the groups at p<0.05.

RESULTS

Effect of SBE on swimming capacity

Endurance exercise capacity was evaluated by measuring the total swimming time until exhaustion of the mice in an adjustable-current water pool. As shown in Fig. 1, the exhaustive swimming capacity of the Ex-SBE group was significantly enhanced over that of the Ex-Control on day 14 of the experiment (+56%, p<0.05). The increased exercise capacity of the Ex-SBE group was not associated with a significant change in body weight, food intake, or tissue weight, which suggests that the prolonged time to exhaustion was mainly due to the...
Effect of SBE on energy substrates on swimming

Exercise is known to induce blood biochemical changes (30). Generally, it is interpreted that a decrease in glucose and an elevation in free fatty acids indicate energy consumption whereas increased lactate is indicative of fatigue during prolonged exercise. Glucose, lactate, and NEFA were measured before and 15 min after swimming on day 21 to determine energy status. The glucose level was not significantly different between the Ex-Control and the Ex-SBE groups (data not shown). As shown in Fig. 2, the blood lactate and NEFA levels in the Ex-Control and Ex-SBE groups were similar before swimming. The blood lactate level was lower in the Ex-SBE group than in the Ex-Control (−25%, p<0.05). The blood NEFA level was higher in the Ex-SBE than in the Ex-Control (+22%, p<0.05).

Depletion of muscle glycogen during prolonged exercise is a factor in fatigue and exhaustion (31). As shown in Fig. 3, a relative increase in liver and muscle was observed in the Ex-SBE group as compared with the Ex-Control. The liver glycogen level in the Ex-SBE group was significantly higher than in the Ex-Control (+14%, p<0.05). The muscle glycogen level in the Ex-SBE group was also significantly higher than in the Ex-Control (+63%, p<0.05).

Effect of SBE on endogenous antioxidants in liver and muscle after swimming

The antioxidant activities of SBE on exercise-induced oxidative stress were evaluated by evaluating antioxidant enzyme activities and antioxidant contents such as CAT, GPx, SOD, TGSH, GSSH, and TBARs. As shown in Table 1, hepatic antioxidant enzyme activities in the Ex-SBE group were significantly higher than those in the Ex-Control. The activities of CAT, SOD and GPx in

Table 1. Effects of SBE on antioxidant enzyme activities in liver.

| Group   | CAT (U/mg protein) | GPx (U/mg protein) | SOD (U/mg protein) |
|---------|--------------------|--------------------|--------------------|
| Ex-Control | 98.8±3.1           | 66.4±0.6           | 11.9±0.4           |
| Ex-SBE   | 114.9±5.5*         | 71.9±1.1*          | 14.2±0.3*          |

Data express the mean±SE for 10 mice in each group. *p<0.05 compared with Ex-Control.

SBE, 50% ethanolic extract from Sasa borealis leaves; CAT, catalase; GPx, glutathione peroxidase; SOD, superoxide dismutase.

Fig. 4. Effects of SBE on the levels of TGSH (A) and MDA (B) after exhaustive swimming. Data are expressed as the mean±SE for 10 mice in each group. *p<0.05, compared to Ex-Control. The Ex-Control and Ex-SBE groups were given distilled water or SBE 120 min before exercise and then sacrificed 2–3 h after exhaustive swimming with a current flow of 8 L/min. The Ex-SBE group was orally given SBE at a dose of 250 mg/kg/d for 21 d. SBE, 50% ethanolic extract from Sasa borealis leaves; TGSH, total glutathione; MDA, malondialdehyde.
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the Ex-SBE group were significantly enhanced by 16%, 12%, and 18% compared with the Ex-Control, respectively. The hepatic levels of TGSH were increased by 14% in the Ex-SBE groups (Fig. 4A) while the hepatic concentration of MDA was significantly decreased by 22% in comparison to the Ex-Control, as shown in Fig. 4B.

The mRNA expressions of antioxidant enzymes in muscle are shown in Fig. 5. The mRNA levels of CAT, GPx, CuZn-SOD, and Mn-SOD were significantly increased by 4.5-fold, 2.4-fold, 1.8-fold, and 1.9-fold in the Ex-SBE group.

**Effects of mRNA expression of energy metabolism-related enzymes and transcription factors in skeletal muscle after swimming**

The mRNA expression of energy metabolism-related molecules in the muscle were determined by monitoring CS, CPT-1, and β-HAD. As shown in Fig. 6, the expression levels of CS were significantly increased in the gastrocngeminus muscle of the Ex-SBE group by 2.2-fold. The levels of CPT-1 and β-HAD in the muscle of the Ex-SBE group were significantly upregulated by 2.1-fold and 2.3-fold compared with the Ex-Control, respectively.

**DISCUSSION**

Plants are abundant sources of biologically active molecules that have played a critical role in human growth and development for thousands of years. Many plants have been demonstrated to have medicinal attributes based on their traditional therapeutic applications (32). In recent years, these medicinal plants and their extracts have been studied to understand their physiological actions and elucidate their biochemical mechanisms. They are also marketed as functional materials and touted as beneficial for health (33, 34).

Bamboo leaves have been used as medicinal plants for over 1,000 y (17). Their biologically active components...
and health benefits were recently discovered and their health benefits have been widely demonstrated in scientific studies. *S. borealis* is an edible dwarf bamboo that belongs to the Gramineae family. Its ethnologic extract has been reported to have several health-promoting properties, such as preventing chronic hyperglycemia-induced oxidative stress and modulating inflammatory cytokines in obese mice (35). However, little is known about the effect of the ethnologic extract from *S. borealis* leaves on swimming capacity. Although the beneficial effect of 80% ethnologic extract of *P. japonica* leaves on extended swimming has been reported, the action mechanism of the extract on energy metabolism and endogeneous antioxidants is not well understood. In this study, the stimulatory effects of SBE, a Korean medicinal plant, was investigated using the forced swimming pool. In order to clarify the effects during exercise and after exercise, biochemical parameters in the blood and tissues were measured after administration of SBE.

A swimming capacity test using an adjustable-current water pool was chosen over other exercise tests, because it allows for reliable and reproducible evaluation of physical work capacity in mice (21). In the present study, exhaustive swimming capacity was significantly enhanced in the Ex-SBE group compared to the Ex-Control group. Similar swimming capacity results were observed in the previous study using *P. japonica* leaves (10). Purified compounds and extracts from natural sources have been reported to increase exercise capacity under similar models (12, 36, 37). Thus, SBE was identified as possessing performance-enhancing activity.

The blood lactate level was determined primarily as an index of anaerobic metabolism during swimming (30, 38) and is a key indicator of the degree of fatigue after exercise (6, 10). Many organs, especially the liver, heart, and skeletal muscle, help remove lactate from the blood, but intense exercise can increase lactate production to a point that exceeds the rate of lactate removal, which results in fatigue (10, 13). If supplements can inhibit the accumulation and accelerate the clearance of lactate, the result will be less fatigue. Lactate accumulation in the blood showed an inverse relationship with the swimming time of the mice after administration (9, 10, 39). In this study, Ex-SBE effectively retarded the blood lactate production, postponing the appearance of fatigue and increasing swimming time. Hence, we speculate that the SBE-treated mice experienced a reduction in lactate production, an increased rate of lactate removal or both.

NEFA mobilized from adipose tissue was initialized in contractive muscle during moderately intense endurance exercise (9, 34). The blood NEFA is a major fuel source for skeletal muscle metabolism during prolonged exercise (9). Although the output of NEFA from adipose tissue cannot be evaluated, the blood NEFA level of SBE-treated mice was higher than that of the Ex-Control after swimming. It is possible that the blood NEFA increased by SBE administration was utilized in skeletal muscle as an energy source, and thus swimming capacity was improved.

Enhancement of exercise capacity can be attributed to a reduced rate of hepatic and muscle glycogen breakdown. After swimming to exhaustion, severe depletion of liver glycogen was noted (6, 34). Glycogen depletion in liver and muscle might be an important factor in the development of exhaustion because glycogen is depleted during exercise and results in the inability to maintain the blood glucose level (35). Several reasonable results showed that exercise capacity can be improved by increasing the availability of fatty acid and this effect is mediated by a glycogen depletion (9, 40). In this study, hepatic and muscle glycogen levels in the Ex-SBE group were much higher than in the Ex-Control group after similar swimming exercise. The findings indicated that the rate of glycogen storage increased after administration of SBE and the utilization of glycogen was delayed in the Ex-SBE mice during exercise.

Energy sources for prolonged exercise are available through intramuscular glycogen and triacylglyceride, plasma glucose, and free fatty acid. In addition, energy substrates are utilized by energy-generating metabolic pathways, including glucose utilization, glycogenolysis, lipolysis, the tricarboxylic acid (TCA) cycle, and fatty acid oxidation (1, 41). CS is one of the key regulatory enzymes in the TCA cycle (6) and its activity is routinely used as a metabolic marker for the oxidative and respiratory capacity in skeletal muscle. In this study, the mRNA expression of CS was significantly upregulated in SBE-treated mice. CPT-1 and β-HAD are the rate-limiting enzymes of β-oxidation (3). In this study, the enzymes of β-oxidation were estimated by the mRNA expression of oxidative metabolic enzymes. CPT-1 and β-HAD in Ex-SBE were significantly upregulated compared with the Ex-Control. This indicated that their increased mRNA expression during swimming may be attributable to the energy generation substrates. SBE may enhance the utilization of energy substrates in serum and skeletal muscle and upregulate oxidative capacity by activation of energy-generating metabolic pathways in skeletal muscle. Therefore, we speculated that SBE improves the energy metabolic efficiency and its related molecules may contribute to improving swimming capacity.

Strenuous and exhaustive exercise may manifest an imbalance between ROS and antioxidant defense, resulting in an oxidative stressful environment in the body (42). During prolonged exercise, energy-generating systems, including fatty acid oxidation and energy production by the mitochondrial electron transport chain (ETC) act as a powerful source of ROS (43–45). During exhaustive exercise, fat is typically used as the primary energy source, thus sparing glycogen stores, which in turn retards fatigue. However, substantial production of ROS occurs via fatty acid oxidation during the utilization of fat (8, 36, 41). Excessive oxygen consumption by strenuous and exhaustive exercise generates O$_2^•$ and H$_2$O$_2$ during the intermediate metabolism, which eventually fatigues the body due to an imbalance between ROS and antioxidant defense systems (7, 14, 43). The antioxidant defense system in the body plays an important role in the protection against ox-
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Inactivation of hepatic antioxidants during exhaustive swimming is caused by exposure to lipid peroxides or excess ROS (11, 14). Exercise-induced ROS may rapidly attack lipids in the cell, leading to lipid peroxidation chain reactions and the production of peroxidation byproducts including MDA. In previous studies, increased MDA levels during exhaustive swimming were confirmed to increase oxidative stress (8, 11, 16). In the present study, the administration of SBE decreased MDA levels after exhaustive swimming, suggesting that SBE can inhibit the lipid peroxidation induced by exhaustive exercise. GSH is an important non-enzymatic antioxidant and is involved in various enzymatic processes that reduce peroxides and free radicals (16). In previous studies, a decreased GSH level during exhaustive swimming was confirmed to increase oxidative stress (8, 11, 16). In the present study, the level of hepatic GSH in the Ex-SBE group increased after exhaustive exercise. Endogenous free radicals are removed by a set of antioxidant enzymes including CAT, GPx, and SOD. Each of these enzymes is capable of producing other less reactive species or neutralizing reactive oxygen metabolites. CAT and GPx work together to decompose the toxic H$_2$O$_2$ into water and oxygen. SOD promotes the dismutation of the superoxide anion radical (O$_2^-$) and forms hydrogen peroxide (H$_2$O$_2$) and oxygen. The systems are important for scavenging free radicals and their metabolic products as well as in maintaining normal cellular physiology (13). Previous studies reported decreased activities in each enzyme after exhaustive exercise (11, 12, 16). In this study, the SBE-treated mice showed increased activities in all three enzymes. The stimulating effects of exogeneous molecules on exercise may differ in the levels of protein and gene (44). In the present study, the levels of CAT, GPx, CuZn-SOD (cytosolic SOD), and Mn-SOD (mitochondrial SOD) in muscle had higher expression after exhaustive swimming. This data indicates that administration of SBE for 21 d effectively enhanced antioxidant defense systems in cellular organelles. In addition, we speculated that the increased ROS in energy-generating systems following prolonged swimming might be prevented by SBE administration.

Phenolic compounds such as capsaicin, ferulic acid, and ferulic acid from extract from bamboo leaves were reported to improve swimming capacity in rodents (11, 16, 46). Green tea extract and red grape leaf extract, which contain a lot of phenolic compounds, were beneficial for improving exercise capacity (3, 36). Extracts from plant leaves contain abundant amounts of phenolic compounds, which act as antioxidants, and may have beneficial effects on exercise performance (3, 10, 12). Based on these observations, the improved swimming capacity by the SBE-treated mice may be at least in part due to a protective effect against oxidative stress with oxidative metabolism. Therefore, the phenolic compounds of SBE as active molecules may be responsible for enhancing swimming capacity.

In summary, supplementation of SBE (250 mg/kg b.w./d) markedly improved swimming capacity by delaying the accumulation of plasma lactate in addition to increasing fat utilization and the capacity for glycogen storage. The beneficial effects on swimming capacity may be related to the enhanced metabolic capacity through the upregulation of energy-generating and supporting metabolic genes, increasing glucose utilization by cellular glucose transport, facilitating oxidative metabolism and enhancing antioxidant defense systems. These findings suggest that SBE can be used to design functional supplements aimed at enhancing swimming capacity by upregulating energy-generating metabolic pathways and improving the endogenous antioxidant system.

Acknowledgments

This research was financially supported by the Ministry of Trade, Industry and Energy (MOTIE) and the Korea Institute for Advancement of Technology (KIAT) through the Promoting Regional Specialized Industry program (R00024642).

REFERENCES

1) Holloszy JO, Kohrt WM, Hansen PA. 1998. The regulation of carbohydrate and fat metabolism during and after exercise. *Front Biostat* 3: D1011–D1027.
2) Hunter GR, Larson-Meyer DE, Sirikul B, Newcomer BR. 2006. Muscle metabolic function and free-living physical activity. *J Appl Physiol* 101: 1356–1361.
3) Minegishi Y, Hiramitsu S, Hase T, Murase T. 2011. Red grape leaf extract improves endurance capacity by facilitating fatty acid utilization in skeletal muscle in mice. *Eur J Appl Physiol* 111: 1983–1989.
4) Davies KJ, Packer L, Brooks GA. 1981. Biochemical adaptation of mitochondria, muscle, and whole-animal respiration to endurance training. *Arch Biochem Biophys* 209: 539–554.
5) Hawley JA, Brouns F, Jeukendrup A. 1998. Strategies to enhance fat utilisation during exercise. *Sports Med* 25: 241–257.
6) Kim YJ, Yoo SR, Chae CK, Jung UJ, Choi MS. 2014. Omija fruit extract improves endurance and energy metabolism by upregulating PGC-1α expression in the skeletal muscle of exercised rats. *J Med Food* 17: 28–35.
7) Aguiló A, Tauler P, Fuentespina E, Tur JA, Cordova A, Pons A. 2005. Antioxidant response to oxidative stress induced by exhaustive exercise. *Physiol Behavior* 84: 1–7.
8) Yu SH, Huang HY, Korivi M, Hsu MF, Huang CY, Hou CW, Chen CY, Kao CL, Lee RP, Lee SD, Kuo CH. 2012. Oral Rg1 supplementation strengthens antioxidant defense system against exercise-induced oxidative stress in rat skeletal muscles. *J Int Soc Sports Nutr* 9: 23.
9) Ikeuchi M, Yamaguchi K, Koyama T, Sono Y, Yawazawa K. 2006. Effects of fenugreek seeds (Trigonella foenum-graecum) extract on endurance capacity in mice. *J Nutr Sci Vitaminol* 52: 287–292.
10) You Y, Kim K, Heo H, Lee K, Lee J, Shim S, Jun W. 2006. Stimulatory effects of *Pseudosasa japonica* leaves on exercise performance. *Biosci Biotechnol Biochem* 70: 2532–2535.
11) You Y, Kim K, Yoon HG, Lee KW, Lee J, Chun J, Shin DH, Park J,Jun W. 2010. Chronic effect of ferulic acid from *Pseudosasa japonica* leaves on enhancing exercise activity in mice. *Phytother Res* 24: 1508–1513.
12) Zheng X, Long W, Liu G, Zhang X, Yang X. 2012. Effect of seabuckthorn (Hippophae rhamnoides ssp. sinesis) leaf extract on the swimming endurance and exhaustive exercise-induced oxidative stress of rats. *J Sci Food Agric* 92: 736–742.

13) Kadiska MB, Gladen BC, Baird DD, Milak AE, Sohal RS, Hacht GB, Jones DP, Mason RP, Barret JC. 2000. Biomarkers of oxidative stress study: are plasma antioxidants markers of CCl4 poisoning? *Free Rad Biol Med* 28: 838–845.

14) Xu J, Li Y. 2012. Effects of salidroside on exhaustive exercise-induced oxidative stress in rats. *Mol Med Rep* 6: 1195–1198.

15) Lu B, Wu X, Shi J, Dong Y, Zhang Y. 2006. Toxicology and safety of antioxidant of bamboo leaves. Part 2: developmental toxicity test in rats with antioxidant of bamboo leaves. *Food Chem Toxicol* 44: 1739–1743.

16) You Y, Park J, Yoon HG, Hwang K, Lee J, Kim K, Lee KW, Shim S, Jun W. 2009. Stimulatory effects of ferulic acid on endurance exercise capacity in mice. *Biosci Biotechnol Biochem* 73: 1392–1397.

17) Kweon MH, Hwang HJ, Sung HC. 2001. Identification and antioxidant activity of novel chlorogenic acid derivatives from bamboo (Phyllostachys edulis). *J Agric Food Chem* 49: 4646–4655.

18) Nam JS, Chung HJ, Jang MK, Jung IA, Park SH, Cho SI, Jung MH. 2013. Sasa borealis extract exerts an antidiabetic effect via activation of the AMP-activated protein kinase. *Nutr Res Pract* 7: 15–21.

19) Park HS, Lim JH, Kim HJ, Choi HJ, Lee IS. 2007. Antioxidant flavone glycosides from the leaves of Sasa borealis. *Arch Pharm Res* 30: 161–166.

20) Zhang Y, Yao X, Bao B, Zhang Y. 2006. Anti-fatigue activity of a triterpenoid-rich extract from Chinese bamboo shavings (Caulis bamfusae in taeniam). *Phytother Res* 20: 872–876.

21) Matsumoto K, Ishihara K, Tanaka K, Inoue K, Fushiki T. 1996. An adjustable-current swimming pool for the evaluation of endurance capacity of mice. *J Appl Physiol* 81: 1843–1849.

22) Fadamiro HY, Chen L, Onagbola E, Graham L. 2005. Lifespan and patterns of accumulation and mobilization of nutrients in sugar fed porid Ily Pseudacteon tricuspis. *Physiol Entomol* 30: 212–224.

23) Gierus M, Rocha JB. 1997. Forage substitution in a grain-based diet affects pH and glycogen content of semimembranosus and semitendinosus rabbit muscles. *Physiol Entomol* 22: 212–224.

24) Dohm GL, Tappcott EB, Barakat HA, Kasperek GJ, Kasperek. 1983. Influence of fasting on glycogen depletion in rats during exercise. *J Appl Physiol* 55: 830–833.

25) Yin J, Zhang H, Ye J. 2008. Traditional Chinese medicine in treatment of metabolic syndrome. *Endocr Metab Immune Disord Drug Targets* 8: 99–111.

26) Borchers AT, Stern JS, Hackman RM, Keen CL, Gershwin ME. 1999. Mushrooms, tumors, and immunity. *Proc Soc Exp Biol Med* 221: 281–293.

27) Jung KA, Kim IH, Han DS. 2004. Effect of medicinal plant extracts on forced swimming capacity in mice. *J Ethnopharmacol* 93: 75–81.

28) Choi YJ, Lim HS, Choi JS, Shin SY, Bae JY, Kang SW, Kang IJ, Kang YH. 2008. Blockade of chronic high glucose-induced endothelial apoptosis by Sasa borealis bamboo extract. *Exp Biol Med (Maywood)* 233: 580–591.

29) Bradford MA. 1976. Rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. *Anal Biochem* 72: 248–254.

30) Moriura T, Matsuda H, Kubo M. 1996. Pharmacological study on Agkistrodon blomhoffii blomhoffii Boie. V. Anti-fatigue effect of the 50% ethanol extract in acute weight-loaded forced swimming-treated rats. *Biol Pharm Bull* 19: 62–66.

31) Wang JL, Tappcott EB, Barakat HA, Kasperek GJ, Kasperek. 1983. Influence of fasting on glycogen depletion in rats during exercise. *J Appl Physiol* 55: 830–833.

32) Jafarzadeh A, Li, Cai H, Chen H, Liu X, Zeng H, Xu J, Huang Y, Zhang Y. 2008. Traditional Chinese medicine in treatment of metabolic syndrome. *Endocr Metab Immune Disord Drug Targets* 8: 99–111.