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RESEARCH NOTE

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L-Lactic Acid's Improvement of Swimming Endurance in Mice

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The aim of this study was to investigate the effect of L-lactic acid on swimming endurance of mice. Mice (n = 50) were injected intraperitoneally with saline, then with L-lactic acid (either 25 mg/kg or 50 mg/kg body weight), then after 2 days with the same doses of glucose, and after another 2 days again with L-lactic acid at the same doses. Swimming times to exhaustion were determined at 30 min after each injection, in a tank filled with 25 cm of water maintained at 23 °C. After another week, mice were given either saline, L-lactic acid, or glucose (25 or 50 mg/kg) dissolved in saline and sacrificed after 30 min for biochemical analyses. The ratios of swimming times of L-lactic acid or glucose injections to saline injection were calculated as an index for endurance changes. Swimming-time ratios for mice injected with L-lactic acid were significantly higher at either dose than for those injected with the corresponding doses of glucose ($p < .05$). The ratio of swimming time was greater in those given a dose of 50 mg/kg than in those given 25 mg/kg for mice in the L-lactic acid groups ($p < .05$) but not in the groups given glucose. There were no marked differences in biochemical parameters of plasma and muscle lactate, muscle and liver glycogen, or plasma glucose and nonesterified fatty acid between the L-lactic acid, glucose, and saline injection groups. These results suggest that L-lactic acid can enhance swimming endurance of mice and that this action is dose dependent.

Keywords: fatigue, endurance performance, skeletal muscle

In many early studies, lactic acid (2-hydroxypropanoic acid) or lactate was considered a metabolic end product of glycolysis and a potential candidate for inducing fatigue (Fletcher & Hopkins, 1907; Hill & Kupalov, 1929; Hill & Lupton, 1923). Some studies (Chase & Kushmerick, 1988; Fuchs, Reddy, & Briggs, 1969; Sutton, Jones, & Toews, 1981) indicated that when lactic acid builds up in myocytes, intracellular pH drops, contributing to the onset of fatigue by several mechanisms. These included inhibition of key enzymes in the glycolytic pathway (Sutton et al.) and inhibition of calcium release from the sarcoplasmic reticulum, thereby reducing binding of calcium to troponin C (Fuchs et al.), which in turn results in a reduction

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in the contractility of muscle fibers (Chase & Kushmerick). Other studies showed that preexercise ingestion of alkalizers such as sodium bicarbonate (Bird, Wiles, & Robbins, 1995; Lindh, Peyrebrune, Ingham, Bailey, & Folland, 2008; McNaughton, Backx, Palmer, & Strange, 1999) or sodium citrate (Linossier, Dormois, Bregere, Geyssant, & Denis, 1997; McNaughton, 1990) improved exercise performance, and this is probably the result of increased capacity for buffering H^+ ions during exercise.

In contrast, the review of Robergs, Ghiasvand, and Parker (2004) presented clear evidence that there is no biochemical support for lactate production's causing acidosis and insisted that lactate production retards a developing metabolic acidosis, as well as helping to remove H^+ ions from the cell. Furthermore, Pate, Bhimani, Franks-Skila, and Cooke (1995) indicated that acidosis (decreased pH) does not significantly affect force generation by mammalian muscle at the closer to physiological temperature of 30 °C and suggested that the data from in vitro studies that acidosis led to muscle fatigue were obtained at physiologically unrealistic temperatures. Again, the protective effect against fatigue of intracellular acidosis had been observed in K^+-depressed (Kristensen, Albertsen, Rentsch, & Juel, 2005; Nielsen Paoli, & Overgaard, 2001; Pedersen, Clausen, & Nielsen, 2003) or depolarized (Pedersen, Nielsen, Lamb, & Stephenson, 2004) muscles isolated from rats. In addition, some works have reported that lactate is also an important metabolic intermediate that contributes to gluconeogenesis and can act as a substrate for oxidative metabolism in both skeletal and cardiac muscle (Brooks, 1998, 2000, 2002, 2007; Miller et al., 2002a, 2002b; Miller et al., 2005), as well as in brain neurons as energy substrate (Medina & Tabernero, 2005; Tabernero, Bolanos, & Medina, 1993). Again, Azevedo, Tietz, Two-Feathers, Paull, and Chapman (2007) indicated that consumption of a leading sports drink containing lactate polymer improved exercise performance in cyclists during a high-intensity trial. Although these observations suggest that lactic acid or lactate may be beneficial for promoting exercise capacity, most of these data were obtained from in vitro or high-intensity exercise. The existence of an interaction between lactic acid and endurance performance in vivo is still controversial. To help clarify whether lactic acid can be used as an ergogenic aid for improving endurance, we injected mice fed MF, a commercial chow, with L-lactic acid, as well as glucose, intraperitoneally to avoid the effect of gastrointestinal digestion caused by oral administration and tested the endurance of the mice in a forced swimming test.

Materials and Methods

Animal and Diet

Four-week-old male Crlj:CD-1 (ICR) mice were purchased from Charles River Japan, Inc. (Atsugi, Kanagawa, Japan), and housed in cages under automatically controlled conditions of temperature (24 ± 0.5 °C), relative humidity (65% ± 5%), and light (light from 6:45 a.m. to 6:45 p.m.). All mice had free access to MF, a commercial chow (Oriental Yeast Co., Ltd., Tokyo, Japan), and water until they were sacrificed. All mice were maintained according to the guidelines for experimental animals of the National Food Research Institute, National Agriculture and Food Research Organization, Japan.

Materials

L-lactic acid was purchased from Wako Pure Chemical Industries, Ltd., and glucose (dextrose, anhydrous), from Hikotaro Shudzui Co., Ltd. L-lactic acid and glucose were dissolved in saline (0.9% NaCl) at concentrations of 2.5 mg/ml and 5.0 mg/ml, respectively, and saline was used as a control. The pH of each solution is as follows: saline, 6.4; 25 mg/ml L-lactic acid, 2.5; 50 mg/ml L-lactic acid, 2.3; and glucose (25 or 50 mg/ml), 5.7. All solutions were stored at 0–4 °C.

Experimental Design

Hojo, Matsui, Watari, Kawazoe, and Mizutani (2000) reported an induction of hepatotoxicity by intraperitoneal administration of DL-lactic acid at a dose of 1.2 mmol/kg (about 108 mg/kg) body weight in mice. Therefore in the current study, the doses of L-lactic acid were set at 25 or 50 mg/kg body weight. By way of comparison, glucose was set in the same doses. Accordingly, the mice were injected with 1% body weight of L-lactic acid or glucose solution, as well as saline (control), as follows. The tails of the mice were colored with a magic marker for individual recognition. A flowchart for the intraperitoneal injections of the mice in this study is shown in Figure 1. Fifty male ICR mice (11 weeks old) were selected and injected intraperitoneally with saline (1% of body weight), and the swimming times to exhaustion of mice with 1% loads attached to their tails (see below for details) were determined 30 min after injection. The mice were then divided into five groups (Group 1 to Group 5) with similar body weights (41 ± 0.4 g) and swimming times to exhaustion (285 ± 12 s). To ensure similar fatigue stress (caused by a swimming test) in each experimental group, each group was divided into two subgroups (Sub 1 and Sub 2 for each group). After 2 days of physical recovery from the initial swimming exercise, all groups were subjected to an injection of L-lactic acid or glucose interspersed with swimming exercise (Figure 1). Groups 1–5 Sub 1 were initially intraperitoneally injected with 25 mg/kg, and Groups 1–5 Sub 2, with 50 mg/kg of L-lactic acid. The swimming times to exhaustion were then determined 30 min after intraperitoneal administration. After 2 days of rest the same groups of mice received either 25 mg/kg or 50 mg/kg body weight of glucose followed by exhausted swimming exercise and 2 days rest as previously. L-lactic acid administration was then repeated after 2 days of rest to investigate the reproducibility of any effect of L-lactic acid on swimming endurance. The ratios of swimming times of L-lactic acid or glucose injections to saline injection were calculated as the index for endurance changes to investigate the effects of injected L-lactic acid or glucose on swimming endurance.

The forced-swimming system, constructed to assay the endurance of mice, has been described previously (Zhang, Higuchi, Shirai, Suzuki, & Shimizu, 2007; Zhang, Shirai, Higuchi, Suzuki, & Shimizu, 2007). A soapless soap solution (1% neutral kitchen detergent, Kao, Tokyo, Japan) was used to wash the bodies and thoroughly moisten the hair of the mice before the swimming exercise to prevent any buoyancy difference (Kitagawa & Iwashiro, 1963; Tamaki, Hayashi, & Kowa, 1961). The swimming times to exhaustion of mice with 1% loads attached to their tails were determined in an acrylic plastic tank (26 × 22 × 29 cm) filled with water to a depth of 25 cm at a temperature of 23 °C. The mice were deemed exhausted

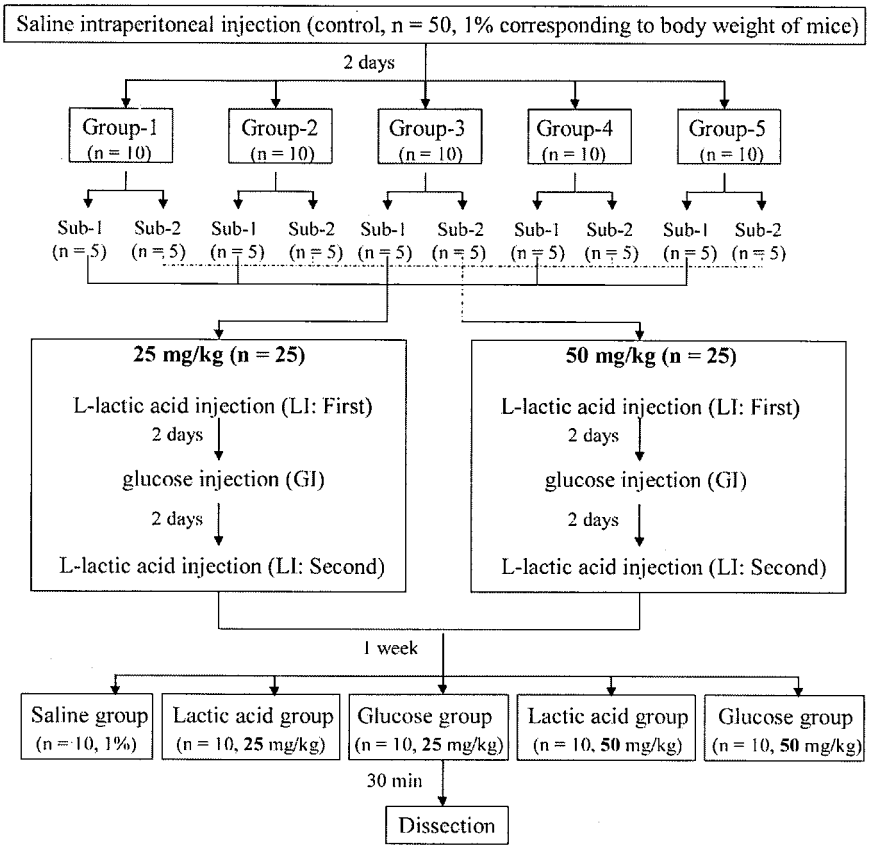

Figure 1 — Protocol of intraperitoneal injections of saline as control and L-lactic acid or glucose in doses of 25 or 50 mg/kg body weight in mice fed MF, a commercial chow.

when they failed to rise to the surface of the water to breathe within a 7-s period; at this point they were immediately rescued from the tank (Matsumoto, Ishihara, Tanaka, Inoue, & Fushiki, 1996). The swimming test was carried out between 11 a.m. and 5 p.m. to avoid circadian variations in physical activity, because it has been confirmed that the minimal variation of endurance occurs in mice at these times (Matsumoto et al.). A 10-min swim-training session was carried out once with no loads attached to the tails of the mice the day before the swimming test was conducted to accustom the mice to the swimming exercise, thereby eliminating any possibility of influencing swimming times that may occur with increased swim training (Favier, Constable, Chen, & Holloszy, 1986; Kim, Park, Han, & Park, 2003).

Sample Collection and Processing

After 1 week of physical recovery from the injection and swimming test, nonfasted mice in Groups 1–5 were injected intraperitoneally with saline (control), 25 mg

L-lactic acid/kg, 25 mg glucose/kg, 50 mg L-lactic acid/kg, or 50 mg glucose/kg, and after 30 min, the mice were sacrificed by dislocation of the neck. Blood samples were collected from the caudal vena cava with a heparinized syringe and put into ice-cold tubes. Samples of liver and muscle tissue from the hind limbs were then removed and stored at –40 °C. A blood sample (0.2 ml) was immediately deproteinized with perchloric acid (0.6 mol/L) and centrifuged at 3,000 *g* for 10 min for determination of plasma lactate. The remaining blood sample was centrifuged at 900 *g* for 15 min, and the supernatant was stored at –40 °C for determination of plasma glucose and nonesterified fatty acid (NEFA) concentrations. Muscle samples were used to measure L-lactate and glycogen content, and liver tissue, for glycogen content.

Biochemical Analyses

The plasma glucose and NEFA were assayed by glucose oxidase and acyl-CoA oxidase methods using commercial kits (Glucose CII test Wako, and NEFA C-test Wako, Wako Pure Chemical Industries, Ltd., Osaka, Japan). The plasma and muscle L-(+)-lactates were measured using an enzymatic analysis with lactate dehydrogenase and NAD^+ (Gutmann & Wahlefeld, 1974). Liver and muscle glycogen were determined using an enzymatic method with amyloglucosidase (Keppler & Decker, 1974).

Statistical Analyses

The data are expressed as $M \pm SEM$. Comparisons of the ratios of the swimming times of L-lactic acid or glucose injections to saline injection and the biochemical parameters in the five treated groups were assayed using one-way analysis of variance (ANOVA) and the Spjotvoll/Stoline test using the Statistica statistical program package (StatSoft Inc., Tulsa, OK). A level of $p < .05$ was used as the criterion for statistical significance.

Results

Ratios of Swimming Times of L-Lactic Acid or Glucose Injections to Saline Injection

The ratios of swimming times of mice injected with 25 or 50 mg/kg of L-lactic acid or glucose to those of mice injected with saline are shown in Figure 2. The ratios of mice injected with lactic acid in any regimen to those injected with saline were greater than 1.1 (25 mg/kg: first injection 1.15 ± 0.04, second injection 1.20 ± 0.05; 50 mg/kg: first injection 1.29 ± 0.05, second injection 1.34 ± 0.06), whereas the ratios of those injected with glucose were less than 1.0. For the mice injected both once and twice with 25 mg/kg lactic acid, the ratio obtained was significantly higher than that obtained from mice injected with glucose ($p < .05$). Similar differences were seen between the mice injected with 50 mg/kg of lactic acid or glucose ($p < .05$). After the mice had received a second injection of either 25 mg/kg or 50 mg/kg of lactic acid, the ratios were not significantly different from those observed after the first injection.

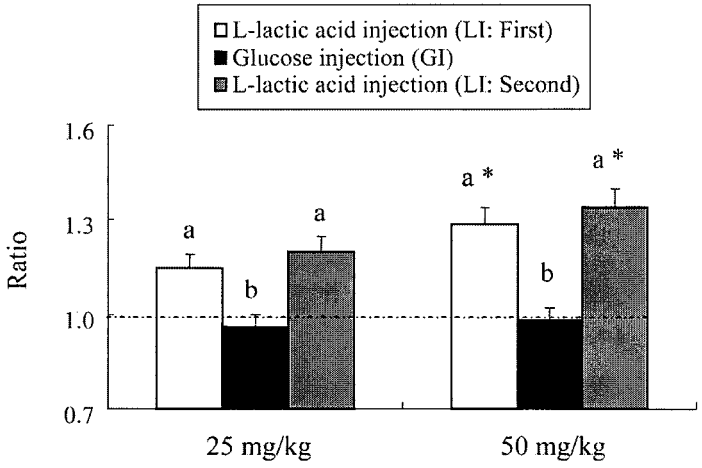

Figure 2 — Effects of L-lactic acid or glucose injections in doses of 25 or 50 mg/kg body weight on the ratios (lactic acid or glucose injection to saline injection) of swimming times of mice. Values are $M \pm SEM$, $n = 25$. Significant differences in ratios of swimming times between L-lactic acid and glucose injections to saline injection are indicated by *a* and *b* ($p < .05$) at each injection dose. *Significant differences in ratios of swimming times of mice receiving the L-lactic acid injections at between doses of 25 and 50 mg/kg ($p < .05$).

The mice that had received first or second injections of 50 mg/kg lactic acid had a significantly higher ratio than those that had received similar injections of 25 mg/kg ($p < .05$). In contrast, there were no significant differences in the ratios between the mice that had received either 25 mg/kg or 50 mg/kg of glucose.

Biochemical Parameters

There were no significant differences between the mice receiving any of the five different treatments in plasma (Figure 3A) or muscle (Figure 3B) lactate levels, muscle (Figure 4A) or liver (Figure 4B) glycogen levels, and plasma glucose (Figure 5A) or NEFA (Figure 5B) concentrations.

Discussion

L-lactic acid exists intrinsically in the bodies of animals and humans. The intraperitoneal injection doses (25 or 50 mg/kg) of L-lactic acid used in the current study are considerably lower than the LD_{50} for lactic acid (54.1 mmol/kg, about 4,873 mg/kg body weight) in mice (Hojo et al., 2000) and were expected to be safe for these experimental observations.

The current results suggest that L-lactic acid injected at a concentration of either 25 or 50 mg/kg can enhance the swimming endurance of mice, indicating that L-lactic acid did not cause fatigue. However, it appeared that an injection of glucose had no beneficial effect on the swimming endurance of mice. Indeed, there was a tendency for swimming endurance to decrease. The ratios of swimming times of

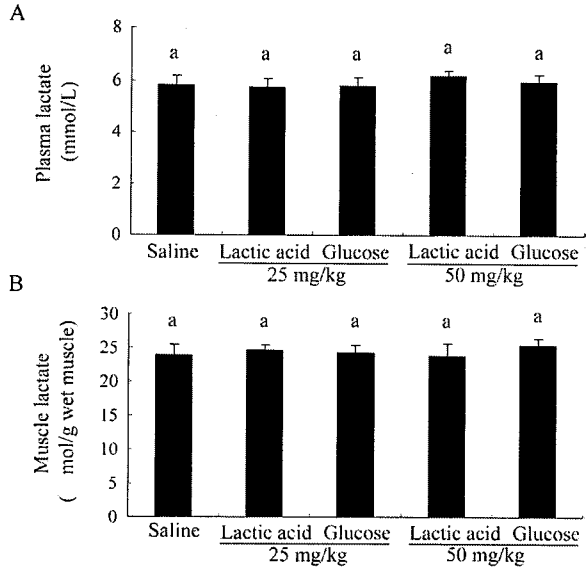

Figure 3 — Effects of intraperitoneal injections of saline (1% corresponding to body weight) and L-lactic acid or glucose in doses of 25 or 50 mg/kg on the lactate levels of plasma or muscle after 30 min of injection in mice. Values are $M \pm SEM$, $n = 10$. Significant differences among the five treated groups are shown by a and b ($p < .05$).

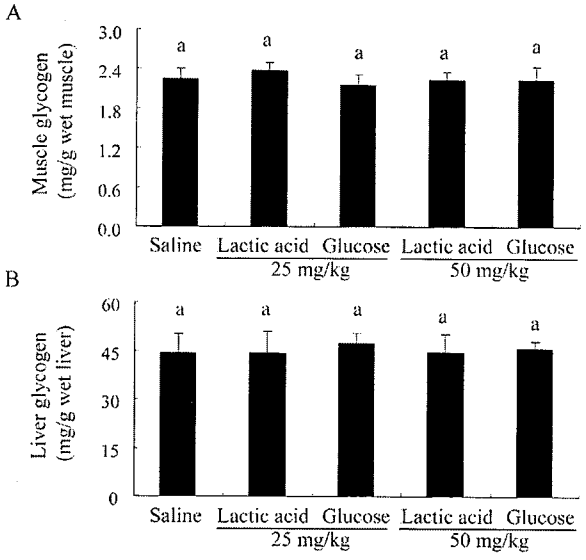

Figure 4 — Effects of intraperitoneal injections of saline (1% corresponding to body weight) and L-lactic acid or glucose in doses of 25 or 50 mg/kg on the glycogen levels of muscle or liver after 30 min of injection in mice. Values are $M \pm SEM$, $n = 10$. Significant differences among the five treated groups are shown by a and b ($p < .05$).

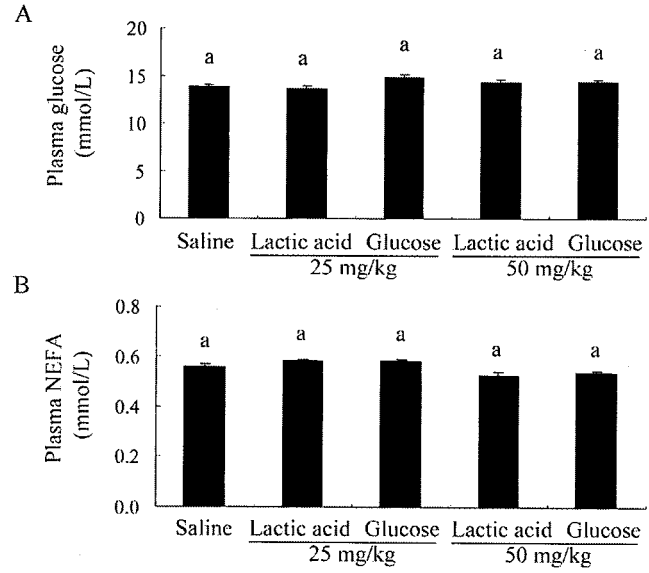

Figure 5 — Effects of intraperitoneal injections of saline (1% corresponding to body weight) and L-lactic acid or glucose in doses of 25 or 50 mg/kg on the plasma glucose or nonesterified fatty acid (NEFA) concentrations after 30 min of injection in mice. Values are $M \pm SEM$, $n = 10$. Significant differences among the five treated groups are shown by *a* and *b* ($p < .05$).

mice administered 50 mg/kg were significantly higher than those administered 25 mg/kg in the first or second injections of L-lactic acid, suggesting a dose-dependent increase in endurance. Furthermore, there were similar increases in the ratio of swimming time between the first and second injection of L-lactic acid at either dose, indicating the reproducibility of data using this technique.

L-lactic acid and glucose administration (25 or 50 mg/kg body weight) did not markedly influence plasma or muscle lactate levels 30 min after intraperitoneal injection. In addition, the other biochemical parameters, for example, plasma glucose and NEFA and muscle and liver glycogen levels, were not markedly influenced after administration of either substance. Results from other research (Jung et al., 2008; Morioka et al., 2007; Yin et al., 2008) indicate that intraperitoneally injected glucose (1.8 or 2 g/kg body weight) induces an increase of plasma glucose 30 min after administration in mice. The absence of such changes after glucose administration in the current study may be because of lower doses or a more rapid metabolism in these mice.

The current results suggest that enhanced swimming endurance after L-lactic acid administration may be independent of the differences in these energy substrates; rather, it may be related to the mechanisms regarding the regulation of excitation-contraction coupling of skeletal muscle. In vitro, Nielsen et al. (2001) found that reduced pH by addition of lactic acid or propionic acid, as well as increased CO_2 tension, similarly led to an almost complete force recovery in K^+-depressed rat muscle. Then, Pedersen et al. (2004) showed that intracellular acidosis increases the excitability of the internal network of tubules in depolarized muscle fibers,

thus counteracting fatigue. Again, results from other studies have indicated that reduced pH or acidosis may elicit the release of catecholamine (Rico, Prieto-Lloret, Gonzalez, & Rigual, 2005; Rocher et al., 2005) and calcitonin gene-related peptide (Santicioli, Del Bianco, Geppetti, & Maggi, 1992; Wang & Fiscus, 1997) from nerve tissues. These hormones could stimulate the Na^+-K^+ ion pump, thereby contributing to excitation-induced force recovery (Cairns, Flatman, & Clausen, 1995; Clausen, Andersen, & Flatman, 1993; Clausen & Everts, 1991; Nielsen, Hilsted, & Clausen, 1998). It is known that more than 99% of lactic acid can be dissociated into protons (H^+) and lactate anions at physiological pH because of the relatively low pKa (pH = 3.87) of the carboxylic acid functional group of lactic acid (Gladden, 2004). Accordingly, dissociated H^+ from injected L-lactic acid may induce acidosis and then increase the excitability of muscle and/or trigger the release of catecholamine and calcitonin gene-related peptide. These actions may stimulate the excitation-contraction coupling of skeletal muscle against muscle fatigue and therefore contribute to the improved endurance of mice observed in this study.

Dissociated lactate from L-lactic acid may be used as precursor for gluconeogenesis. However, Miller et al. (2002a) demonstrated that increased lactate level did not increase glucose recycling during rest or exercise. The current study also showed the same levels of plasma glucose, as well as liver glycogen, 30 min after administrations of L-lactic acid as with saline or glucose. These results suggest that gluconeogenesis from dissociated lactate may not be an effective pathway for improved endurance of mice. Again, dissociated lactate can also be converted to pyruvate by the enzyme lactate dehydrogenase in the cytoplasm and enter into the TCA cycle in mitochondria for aerobic metabolism (Brooks, 2000, 2002, 2007). In vitro, Miller et al. (2002b) reported that lactate infusion increased lactate oxidation, spared blood glucose, and decreased glucose oxidation, implying that lactate is a useful carbohydrate source and preferentially oxidized during exercise. In a human study, the consumption of a lactate polymer improved exercise performance of cyclists during a high-intensity trial because of faster and greater oxidation of lactate than glucose (Azevedo et al., 2007). However, the low dose of L-lactic acid used in this study suggests that the oxidation of lactate dissociated from injected L-lactic acid as an energy source may be not a primary cause for the improved endurance seen in mice. In addition, it is possible that low levels of exogenous lactate dissociated from injected L-lactic acid may activate the oxidation pathways of endogenous lactate and therefore promote the oxidation of endogenous lactate produced by glycolysis during exercise, which ultimately improved the swimming endurance in mice. However, these assumptions still need further study to clarify.

In human studies, preexercise (30–45 min) glucose ingestion did not significantly affect exercise performance (Foster, Costill, & Fink, 1979; Tokmakidis & Volaklis, 2000), which is consistent with results from the current study. It may be that glucose administered before exercise induces an increase in insulin secretion (Costill et al., 1977; Geser, 1976; Newsholme & Dimitriadis, 2001), which could impede the mobilization of fatty acids during exercise (Foster et al.In summary, this study suggests that L-lactic acid administration enhances swimming endurance in mice, and this action is dose dependent. Further studies are required to elucidate the precise mechanisms for this action of lactic acid on endurance in mice. In addition, our results imply that L-lactic acid may be used as an ergogenic aid for improving endurance performance in humans, particularly in athletes, but further work is required.

Acknowledgments

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