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Effect of Dietary Lipid Type on the Enhancement of Swimming Endurance of Mice by L-Lactic Acid

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Mice fed either a diet containing 6% lard, fish oil or Erabu sea snake lipids for 16 weeks were intraperitoneally injected L-lactic acid or glucose (25 mg/kg) following injection of physiological saline and subjected to swimming tests at 30 min after each injection. The swimming time ratio of lactic acid to saline injections was greater in mice fed sea snake lipids than those fed lard ($p < 0.01$) or fish oil ($p < 0.05$), and significantly greater than that of glucose to saline injections only in mice fed sea snake lipids ($p < 0.01$) but not those fed lard or fish oil. Increased endurance by injected glucose was not observed in mice fed any of the diets. These results indicate that dietary lipid type affects the endurance of mice injected with L-lactic acid, and Erabu sea snake lipids may contain factors that regulate lactic acid metabolism effectively.

Keywords: sea snake lipids, lactic acid, glucose, n-3 PUFAs

Introduction

Recent studies (Brook, 2007; Miller *et al.*, 2002a, 2002b) have revealed that lactate is an important metabolic intermediate contributing to gluconeogenesis in liver and also acting as an important substrate for oxidative metabolism in slow-twitch fibers of skeletal muscle and cardiac muscle. In a human study, consumption of a sports drink containing lactate polymer significantly improved exercise performance in cyclists (Azevedo *et al.*, 2007), implying a preferential role of lactate as a substrate for oxidative energy production. In addition, our previous explorative study (Zhang *et al.*, 2009a) demonstrated that intraperitoneally injected L-lactic acid enhanced the swimming endurance of mice fed MF, a commercial chow, suggesting that lactic acid improved swimming endurance without causing fatigue in vivo. Similar results were obtained with in vitro studies (De Paoli *et al.*, 2007; Nielsen *et al.*, 2001), where lactic acid exhibited a protective effect against fatigue at the closer to physiology temperature of 30°C. Moreover, Pate *et al.* (1995) suggested that the data from early in vitro studies, (Fuchs *et al.*, 1970; Sutton *et al.*, 1981) which argued a relationship between lactic acid or decreased pH and muscle fatigue, were obtained at physiologically unrealistic temperatures. These new observations indicate a novel role of lactic acid/lactate on endurance performance.

Erabu sea snake (*Laticauda semifasciata*) is a marine reptile distributed throughout the Pacific Ocean and along the coast of the South China Sea. Lipids extracted from a fat sack (fat body) in the viscera of Erabu sea snake have been used as a food supplement to promote health in Japan (Shirai *et al.*, 2002). Our previous studies (Zhang *et al.*, 2007a, b) have revealed that intake of a diet containing 6% sea snake lipids for 16 weeks markedly improved the swimming endurance of mice, and this was accompanied by lower lactate levels during swimming compared with those fed 6% lard or fish oil diet. This observation implies that dietary lipids may give different regulation of lactic acid/lactate metabolism, and this action may affect endurance performance.

Therefore, the present study conducted a manipulation of intraperitoneal administration of lactic acid accompanied with a forced swimming test, and explored whether dietary

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sea snake lipids could affect the swimming endurance of mice injected with L-lactic acid differently from dietary lard or fish oil. In addition, our previous studies (Zhang *et al.*, 2007a, b) have investigated the energy source levels during swimming. However, the effects of long term intake of dietary lipids on energy metabolism parameters before exercise are still unknown. Therefore, we also examined the effects of dietary lipids on body composition, energy source deposition, and enzymes and proteins associated with energy metabolism.

Materials and Methods

Animals 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 06:45 a.m. to 6:45 p.m.) for 23 weeks. Mice were switched from laboratory chow, MF (Oriental Yeast Co., Ltd., Tokyo, Japan), to three different experimental diets (lard, fish oil and sea snake lipid diets) at 27 weeks of age. Thirty-six mice were randomly divided into three diet groups (12 in each group, one cage was divided into two sections by a steel plate in which one section held 4 mice) with similar body weights and initial swimming times to exhaustion. They were fed one of three experimental diets for 19 weeks. Animals had free access to the experimental diets and water until they were sacrificed. The body weights of mice were determined at initial and final feeding trials. All animal procedures were reviewed and approved by the Animal Research Committee of the National Food Research Institute, National Agriculture and Food Research Organization (NARO), Japan.

Experimental diets The lard was purchased from Oriental Yeast Co., Ltd. The fish oil was supplied by Nippon Chemical Feed Co., Ltd. (Hakodate, Japan), and the sea snake lipids by Fuji Pharmaceutical Inc. (Tokyo, Japan). Lard contains 16:0 (25.0%), 18:0 (12.5%), 16:1 (3.5%), 18:1n-9 (41.7%), 18:1n-7 (3.0%) and 18:2n-6 (8.0%). Fish oil is rich in n-3 PUFAs, e.g., 22:6n-3 (19.8%) and 20:5n-3 (5.3%), and also contains 16:0 (21.5%), 18:0 (5.6%), 16:1 (5.5%), 18:1n-9 (17.2%), 18:1n-7 (2.6%), 18:2n-6 (1.6%), 20:4n-6 (1.6%). Sea snake lipids are rich in 22:6n-3 (12.0%) and 20:5n-3 (1.5%), and the other fatty acids are primarily 16:0 (30.4%), 18:0 (8.6%), 16:1 (5.6%), 18:1n-9 (22.6%), 18:1n-7 (2.4%), 18:2n-6 (1.4%), and 20:4n-6 (1.6%). As sea snake lipids are crude lipids, they may contain some unknown trace constituents other than fatty acids. Each experimental diet contains 6% fat or oil and the following components: 47.8% corn starch, 20% casein, 15% granulated sugar, 5% cellulose, 4% mineral mixture, 2% vitamin mixture, and 0.2% L-methionine. Mineral mixture (per 100 g: $CaHPO_4 \cdot 2H_2O$, 14.56 g; KH_2PO_4, 25.72 g; NaH_2PO_4, 9.35 g; NaCl, 4.66 g; Ca-lactate, 35.09 g; Fe-citrate, 3.18 g; $MgSO_4$, 7.17 g; $ZnCO_3$, 0.11 g; $MnSO_4 \cdot 4H_2O$, 0.12 g; $CuSO_4 \cdot 5H_2O$, 0.03 g; and KI, 0.01 g) and vitamin mixture (per 100 g: retinyl acetate, 0.1 g; cholecalciferol, 0.00025 g; α-tocopheryl acetate, 0.5 g; menadione, 0.52 g; thiamin·HCl, 0.12 g; riboflavin, 0.4 g; pyridoxine·HCl, 0.08 g; cyanocobalamine, 0.00005 g; ascorbic acid, 3 g; biotin, 0.002 g; folic acid, 0.02 g; calcium pantothenate, 0.5 g; *p*-aminobenzoic acid, 0.5 g; niacin, 0.6 g; inositol, 0.6 g; choline chloride, 20 g; and cellulose powder, 73.0577 g) were obtained from Oriental Yeast Co., Ltd. Each experimental diet was stored below -40°C to prevent oxidative changes in fatty acid composition during storage.

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 physiological saline (0.9% NaCl) at a concentration of 2.5 mg/mL, and physiological saline was used as a control. The pH of each solution is as follows: physiological saline, 6.4; L-lactic acid, 2.5; and glucose, 5.7. Mice were injected with 1% body weight of L-lactic acid or glucose solution, as well as physiological saline as the control. All solutions were stored at 4°C.

Experimental design Hojo *et al.* (2000) have 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. Our explorative study (Zhang *et al.*, 2009a) has also indicated that intraperitoneally injected L-lactic acid at a dose of 25 mg/kg body weight enhances the swimming endurance of mice. Therefore, in the present study, the dose of lactic acid was set at 25 mg/kg body weight. By way of comparison, glucose was also set at the same dose. At the end of 16 weeks of feeding, the mice fed lard, fish oil and sea snake lipid diets were injected intraperitoneally with physiological saline (PSI) at a dose of 1 mL/100 g body weight, and the swimming times of mice attached with 1% loads corresponding to their body weights, were determined at 30 min after injection in a forced swimming system which has been described in our previous studies (Zhang *et al.*, 2007a, b, 2009a, b). Briefly, 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 assessed as exhausted when they failed to rise to the surface of water to breathe within a 7-s period (Matsumoto *et al.*, 1996). The swimming test was carried out between 11 a.m. and 5 p.m. to avoid circadian variations in physical activity (Matsumoto *et al.*, 1996). A 10-min swim-training session was carried out once with no loads attached

to the tails of mice on the day before the swimming test, to accustom mice to swimming exercise.

Since the injection order of lactic acid and glucose may affect the swimming endurance, the mice receiving intraperitoneal injection of physiological saline were divided into two manipulation procedures (MP1 and 2), with the injection sequence of MP1 as L-lactic acid injection (LI), followed by glucose injection (GI), and then 1 week of fatigue recovery; that of MP2 as GI followed by LI (Fig. 1). Swimming time to exhaustion was determined 30 min after LI or GI. There were 2 days of rest between two injection tests in each manipulation procedure. To eliminate the effect of injection order on swimming endurance, the mean swimming times of individual mice injected by lactic acid or glucose in MP1 and MP2 were used to calculate the ratios of swimming times of LI or GI to PSI. These ratios in each diet group were used to evaluate the effect of injected L-lactic acid or glucose on the swimming endurance in mice following the lard, fish oil or sea snake lipid diet.

Sample collection and processing After 1 week of recovery from the injection and swimming test, nonfasted 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, heart and muscle tissue from the hind limbs were removed, and rapidly frozen using liquid nitrogen. Then epididymal and perirenal adipose tissues were dissected and weighed. 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

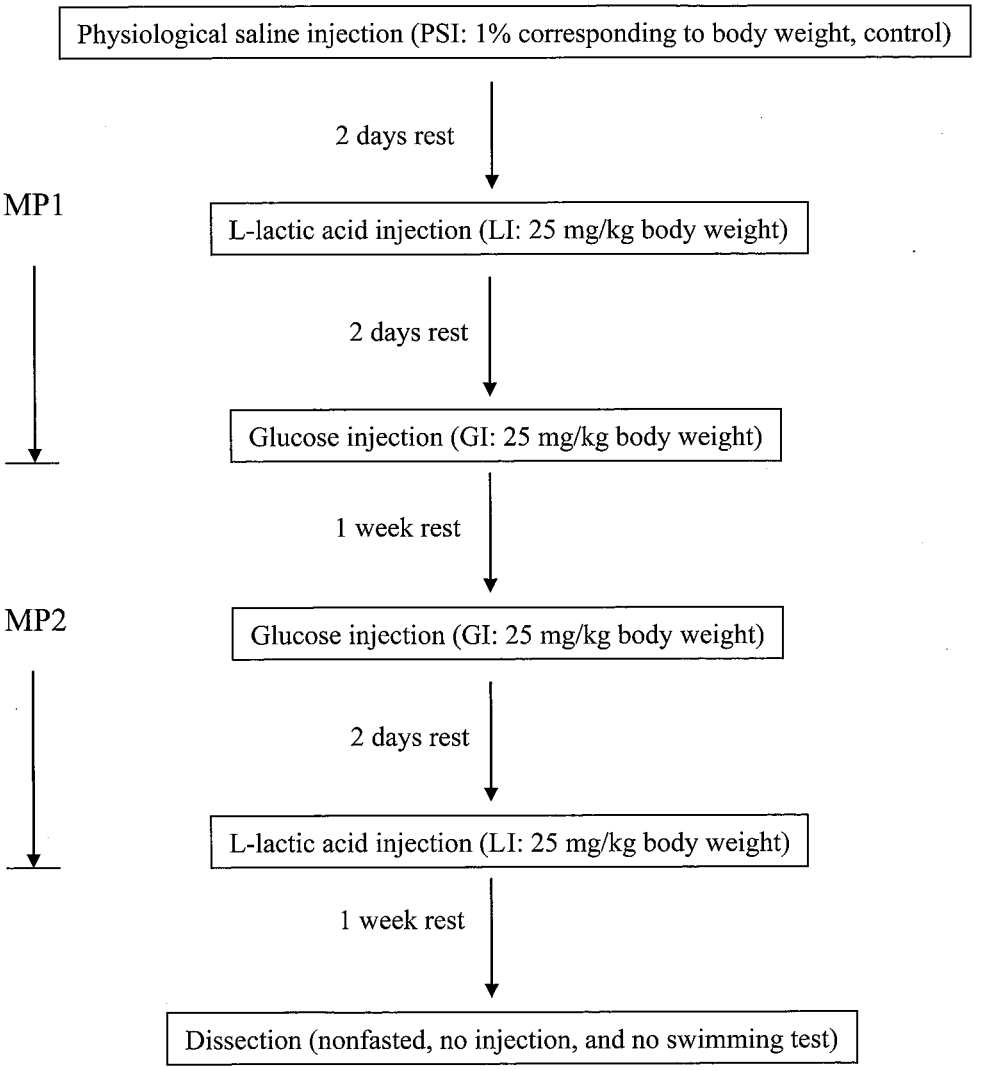

Fig. 1. Flow chart of L-lactic acid and glucose injections (25 mg/kg body weight), with physiological saline as the control, in mice fed lard, fish oil and sea snake lipid diets (n = 12 for each diet group). The swimming times to exhaustion of mice were determined 30 min after each injection. The injection order was L-lactic acid followed by glucose in manipulation procedure (MP)1, and glucose followed by L-lactic acid in MP2.

blood sample was centrifuged at 900 × *g* for 15 min, and the supernatant was stored at -40°C for determination of plasma glucose and non-esterified fatty acid (NEFA) concentrations. Skeletal muscle samples were used to determine lactate and glycogen; the activities of carnitine palmitoyltransferase (CPT), lactate dehydrogenase (LDH), and citrate synthase (CS); and the content of monocarboxylate transporter 1 (MCT1). Additionally, cardiac muscle was used for measurement of MCT1 protein, and liver for glycogen. Skeletal and cardiac muscle, and liver tissues were stored at -60°C prior to analysis. For determining CPT and LDH activities, frozen muscle samples were homogenized in buffer A (0.25 M sucrose, 1 mM EDTA, and 3 mM Tris-HCl, pH 7.2) and the homogenates were centrifuged at 500 × *g* for 10 min. Half of the resulting supernatant was used for determination of CPT activity, and the other half was again centrifuged at 10,000 × *g* for 10 min to yield a supernatant for measurement of LDH activity. For CS activity, frozen muscle was homogenized in buffer B (5.4 M glycerol, 5 mM 2-mercaptoethanol, 0.5 mM EDTA, 0.02% bovine serum albumin and 20 mM phosphate buffer, pH 7.4), and the homogenates were centrifuged at 600 × *g* for 10 min to obtain the supernatant for determination of CS activity.

Analysis of biochemical parameters Plasma glucose and NEFA concentrations 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-(+)-lactate were measured using an enzymatic analysis with LDH and NAD^+ (Gutmann and Wahlefeld, 1974). Muscle and liver glycogen levels were determined using an enzymatic method with amyloglucosidase (Keppler and Decker, 1974). CPT activity was determined using palmitoyl-CoA as substrate (Markwell *et al.*, 1973). LDH activity was determined by a spectrophotometric method with pyruvate and NADH (Kaczor *et al.*, 2005). CS activity was assayed spectrophotometrically according to the method of Stitt (1984). For calculations of CPT, LDH, and CS activities, total protein in supernatant was determined using the Lowry method (Lowry *et al.*, 1951).

Measurement of MCT 1 in skeletal and cardiac muscles The MCT-1-enriched fraction of skeletal or cardiac muscle in mice was prepared according to the method of Baker *et al.* (1998). Antibodies used for western blotting of MCT1 were goat anti-mouse primary antibody (1:500, Santa Cruz Biotechnology, Inc.) and rabbit anti-goat secondary antibody (1:4000, Chemicon). MCT1 protein was detected using an alkaline phosphatase conjugate substrate kit (Bio-Rad). Quantification of the MCT1 protein was performed by scanning the PVDF membrane and analyzing the band optical density with Scion Image (free software) as described in our comparative study (Zhang *et al.*, 2009b).

Statistical analysis Data were expressed as mean ± SE. Comparisons of swimming times at 30 min after physiological saline injection among the lard, fish oil, and sea snake lipid diet groups were assessed using one-way analysis of variance (ANOVA). Comparisons of the ratios of swimming times of LI or GI to PSI among the three experimental diet groups were assessed using two-way ANOVA. The body weight, tissue weights, and biochemical parameters among the three experimental diet groups were assessed using one-way ANOVA, and the Spjotvoll/Stoline test using the Statistica statistical program package (StatSoft Inc., Tulsa, OK). A level of $p < 0.05$ was used as the criterion for statistical significance.

Results

Food intake, body and tissue weights The food intake was 4.3 g/d for each diet group. There were no statistically significant differences in body weight (lard: 45.7 ± 1.3 g; fish oil: 48.6 ± 1.4 g; sea snake lipids: 47.7 ± 1.4 g), adipose tissue (lard: 2.16 ± 0.21 g; fish oil: 2.23 ± 0.21 g; sea snake lipids: 2.14 ± 0.15 g), hind limb muscle (lard: 2.39 ± 0.11 g; fish oil: 2.19 ± 0.07 g; sea snake lipids: 2.37 ± 0.06 g), liver (lard: 2.04 ± 0.05 g; fish oil: 2.18 ± 0.05 g; sea snake lipids: 2.01 ± 0.04 g), and heart (0.20 ± 0.01 g each) among the mice fed lard, fish oil and sea snake lipid diets.

Swimming endurance The swimming times to exhaustion of mice fed lard, fish oil and sea snake lipid diets, 30 min after intraperitoneal injection of physiological saline, are shown in Fig. 2. The swimming times of mice fed the sea snake lipid diet were significantly higher than those of mice fed the fish oil diet ($p < 0.05$), and tended to increase

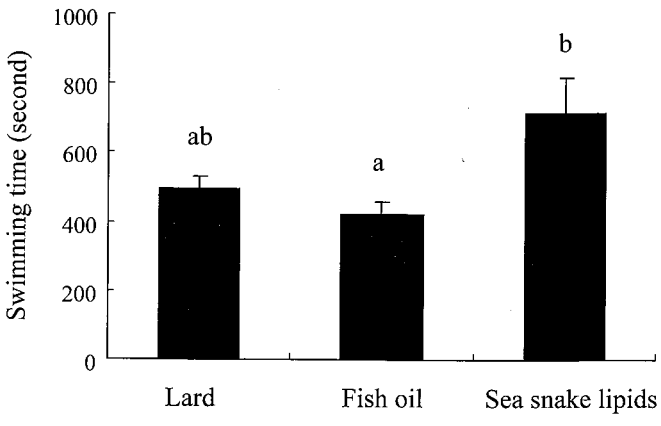

Fig. 2. Effects of lard, fish oil and sea snake lipids on swimming endurance (30 min after physiological saline injection) of mice (n = 12 for each group). Significant difference among three experimental groups is shown by a and b ($p < 0.05$); for sea snake lipid diet group versus lard diet group, $p = 0.07$.

compared with those of mice fed the lard diet ($p = 0.07$); this difference approached statistical significance. The fish oil diet group had slightly lower swimming times than lard diet group ($p = 0.71$).

Ratios of swimming times of mice injected with L-lactic acid or glucose to those injected with physiological saline

The ratios of swimming times of LI or GI to PSI using the mean swimming time of individual mice in MP1 and MP2, in lard, fish oil and sea snake lipid diet groups are shown in Fig. 3. The ratios of swimming times of LI to PSI in sea snake lipid diet group were significantly higher than that those in lard ($p < 0.01$) and fish oil diet groups ($p < 0.05$). However, significant differences between lard and fish oil diet groups in the ratios of LI to PSI were not observed. Moreover, the ratios of swimming times of LI to PSI were significantly higher than those of GI to PSI in sea snake lipid diet group ($p < 0.01$), but such a difference was not observed in lard or fish oil diet group. The mean ratio of swimming time of LI to PSI was greater than 1.0 only in sea snake lipid diet group. The mean ratio of GI to PSI was less than 0.9 in all three diet groups. These data revealed that an additional increase on the swimming endurance by intraperitoneally injected L-lactic acid was only observed in mice receiving sea snake lipid diet but not those fed lard or fish oil diet; in contrast, glucose administration before swimming showed a decreased endurance in all mice fed three experimental diets.

Components of energy metabolism in sedentary mice

The levels of glycogen in muscle and liver, plasma glucose and NEFA, lactate in plasma and muscle in sedentary mice fed lard, fish oil, and sea snake lipid diets are given in Table 1. No significant differences in these parameters except plasma NEFA among three diet groups were observed. The plasma NEFA concentrations of sea snake lipids and fish oil diet groups were significantly lower than those of the lard diet group ($p < 0.05$). Similar NEFA concentrations were observed between the sea snake lipids and fish oil diet groups.

In addition, no significant difference was observed for muscle enzyme activities including LDH (lard: 18.1 ± 0.5

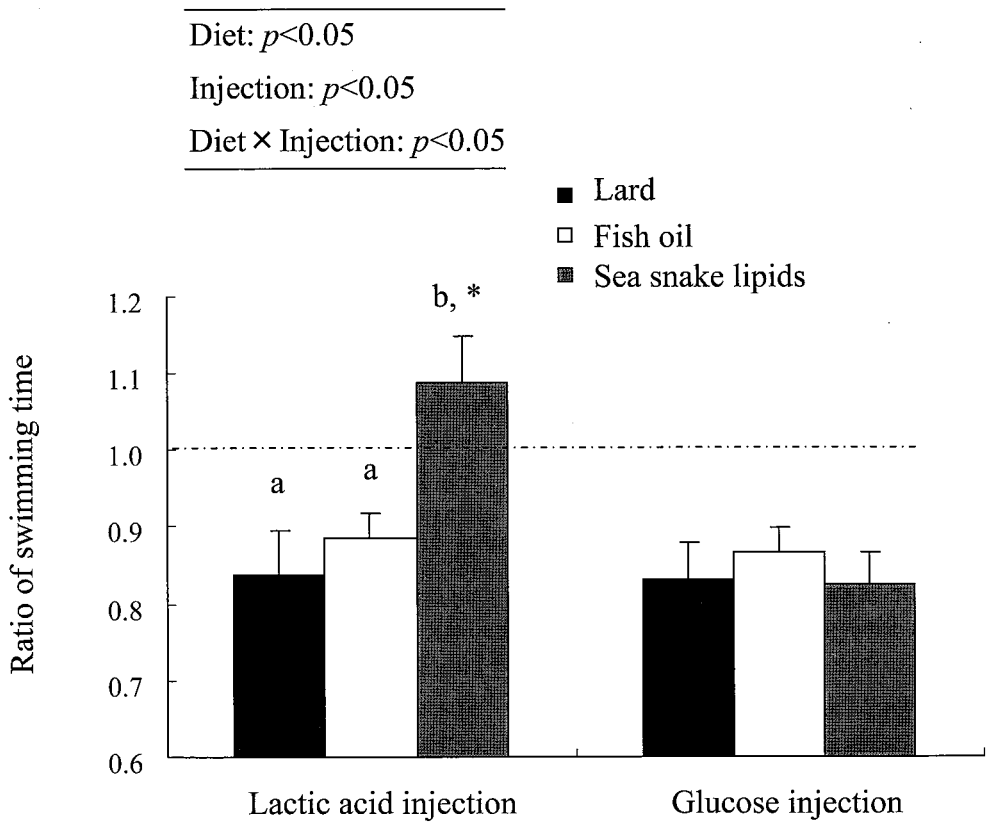

Fig. 3. Effects of intraperitoneally injected L-lactic acid and glucose on the swimming endurance of mice fed lard, fish oil and sea snake lipid diets ($n = 12$ for each group, and the mean swimming times of individual mice injected by lactic acid or glucose in MP1 and MP2 were used to calculate the ratios of swimming times for injected lactic acid or glucose to injected physiological saline). Statistically significant differences in the ratios of swimming times of LI to PSI among lard, fish oil and sea snake lipid diet groups are shown by a and b ($p < 0.05$); significant difference between the ratios of swimming times of LI or GI to PSI in the sea snake lipid diet group is denoted by * ($p < 0.05$).

Table 1. The levels of glycogen in muscle and liver, plasma glucose and non-esterified fatty acid (NEFA), and lactate in plasma and muscle in sedentary mice fed lard, fish oil and sea snake lipid diets.

	Lard	Fish oil	Sea snake lipids
Glycogen, plasma glucose and NEFA			
Muscle glycogen (mg/g)	2.11 ± 0.10	2.28 ± 0.11	2.10 ± 0.13
Liver glycogen (mg/g)	56.3 ± 3.6	59.4 ± 4.3	50.1 ± 3.5
Plasma glucose (mg/dL)	205.5 ± 10.0	200.9 ± 5.5	211.9 ± 10.6
Plasma NEFA (mEq/L)	0.68 ± 0.03 [a]	0.31 ± 0.02 [b]	0.36 ± 0.03 [b]
Lactate			
Plasma lactate (mg/dL)	59.3 ± 2.0	60.5 ± 3.0	56.6 ± 3.6
Muscle lactate (mg/100 g)	292.4 ± 10.5	281.5 ± 12.3	281.6 ± 9.7

Values are mean ± SE ($n = 12$). Significant difference among three diet groups is shown by a and b ($p < 0.05$).

U/mg protein; fish oil: 17.7 ± 0.5 U/mg protein; sea snake lipids: 17.4 ± 0.3 U/mg protein), CS (lard: 0.61 ± 0.01 U/mg protein; fish oil: 0.61 ± 0.01 U/mg protein; sea snake lipids: 0.60 ± 0.01 U/mg protein), and CPT (lard: 1.61 ± 0.07 U/mg protein; fish oil: 1.74 ± 0.08 U/mg protein; sea snake lipids: 1.63 ± 0.09 U/mg protein) in sedentary mice.

The western blots and arbitrary optical density of MCT1 in skeletal and cardiac muscle samples in sedentary mice fed each of the three diets are shown in Fig. 4. No significant differences in MCT1 levels in skeletal or cardiac muscle were observed among the three diet groups.

Discussion

Our previous studies indicated that intake of a diet containing 6% sea snake lipids improves the swimming endurance of mice (Zhang *et al.*, 2007a, b). The present confirmed our previous reports, showing that mice fed a sea snake lipid diet could swim longer (after intraperitoneal injection of physiological saline) than those following fed a lard or fish oil diet. As the body and adipose tissue weights were similar following intake of one of the three experimental diets, it would appear that the improved endurance is mainly due to a specific effect of sea snake lipids, and is not a secondary effect caused by the difference in buoyancy of body fat.

In this study, lower levels of plasma NEFA were observed in sedentary mice fed sea snake lipids and fish oil diets. This may be due to the suppression of fatty acid synthesis by n-3 PUFAs contained in these diets (Kim *et al.*, 1999; Nakatani *et al.*, 2003). CPT activity in muscle, which is associated with long-chain fatty acid oxidation, was similar in sedentary mice fed dietary lipids. As Rustan *et al.* (1993) reported more carbohydrate and less fat oxidation for energy production in animals fed dietary n-3 PUFAs than those fed dietary lard, improved endurance by dietary sea snake lipids does not appear to be related to increased fatty acid oxidation. In contrast, long-term intake of dietary lipids showed no different effect on glycogen storage. In our previous study of young mice (Zhang *et al.*, 2007a), both muscle glycogen and plasma glucose levels at 5 min of swimming were greater in mice fed the sea snake lipid diet than those fed the lard diet, suggesting that glycogen sparing may have contributed to the improved endurance in mice fed sea snake lipids. However, this is not reflected in changes of citrate synthase (CS, a pace-maker enzyme of tricarboxylic acid (TCA) cycle) and LDH activities in sedentary mice. Higuchi *et al.* (2008) have reported lower activities of glycolytic enzymes, e.g. hexokinase and phosphofructokinase, in mice fed fish oil compared with those fed lard, which would reduce glycolytic activity, and possibly resulted in the slightly lower swimming times in this diet group. It is still to be determined whether the mice fed the sea snake lipid diet have glycolytic enzymes with higher activity than those fed the lard or fish oil diet. Moreover, our previous studies (Zhang *et al.*, 2007a, b) have indicated lower levels of lactate during swimming in mice

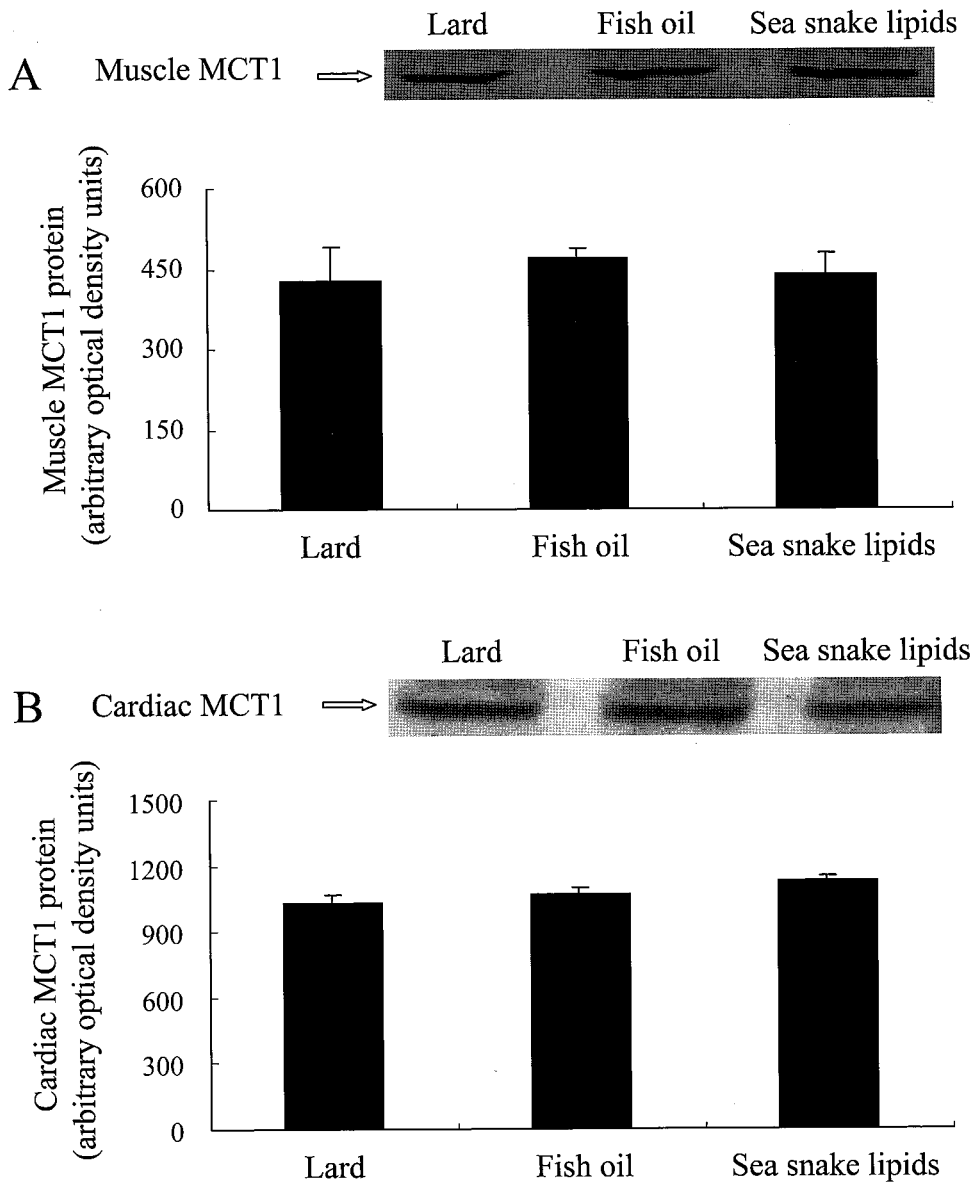

Fig. 4. Representative monocarboxylate transporter (MCT)1 blots in skeletal (A) and cardiac (B) muscle samples from mice fed lard, fish oil and sea snake lipid diets, and the effects of lard, fish oil and sea snake lipids on the skeletal and cardiac MCT1 protein content (quantification) in sedentary mice (n = 12 for each group). There are no significant differences in MCT1 protein content among the three experimental groups. The quantification of MCT1 protein was performed by scanning the PVDF membrane and analyzing arbitrary optical density of bands with Scion Image (free software).

fed sea snake lipids compared with those fed lard or fish oil. In this study, similar levels of lactate in sedentary mice fed dietary lipids reveal that the lactate level before swimming was not differently affected by dietary lipids. These results suggest less lactate accumulation due to dietary sea snake lipid intake, although this different lactate accumulation by dietary lipids was not reflected in LDH activity in sedentary mice. It is well known that working skeletal muscle is not only the major site of lactate production but also the major site of its removal mainly via oxidation (Brooks *et al.*, 1991; Stanley *et al.*, 1986). A decreased lactate accumulation in mice fed sea snake lipids may be due to the effective oxidation for utilization of endogenous lactate during swimming. Lactate oxidation includes the processes of membrane transport which is mediated by MCT1 as transporter (Brooks *et al.*, 1999; McCullagh *et al.*, 1996) and subsequent oxidation in the TCA cycle. However, significant differences were not observed in MCT1 content and CS activity among sedentary mice fed dietary lipids. Moreover, it could not be excluded that different endogenous lactate metabolism between the

mice fed sea snake lipids and those fed lard or fish oil is related to other MCT isoforms. Taken together, long-term intake of dietary lipids does not appear to affect energy storage and energy metabolism parameters before swimming, and the improved endurance by dietary sea snake lipids is most likely not related to energy storage.

In order to explore whether dietary lipids affect lactate metabolism differently, we also examined the effect of intraperitoneal injection of L-lactic acid on the swimming endurance of mice fed dietary lipids. An additional increase of endurance by injected lactic acid was only observed in mice fed sea snake lipids and not those fed lard or fish oil, indicating that the type of dietary lipid affects the endurance of mice injected with L-lactic acid. Pagano *et al.* (1997) reported an increased storage of glycogen by exogenous lactate infusion. However, similar levels of glycogen and lactate 30 min after injection of lactic acid compared with physiological saline injection were observed in our explorative study (Zhang *et al.*, 2009a), indicating that considerably lower doses of lactic acid did not affect energy storage prior to exercise. These findings suggest that the mice fed dietary lipids have similar energy storage just before swimming, and different effects on endurance by injected lactic acid may be due to altered lactate metabolism by dietary lipids. Some studies have reported that the release of some hormones, e.g., calcitonin gene-related peptide and catecholamine, in response to lactic acid or decreased pH (Rico *et al.*, 2005; Wang and Fiscus, 1997) could stimulate the Na^+-K^+ ion pump of skeletal muscle in vitro, thereby counteracting fatigue (Nielsen *et al.*, 2001; Pedersen *et al.*, 2004). The lack of additional increase of endurance by injected lactic acid in mice fed lard or fish oil may depend on the difference in hormone secretion, although further studies need to clarify this. In addition, decreased endurance following glucose injection 30 min before swimming compared with physiological saline injection was similarly observed in mice fed all three experimental diets, which is consistent with the observations from some human studies where glucose ingestion before exercise (15-90 min) failed to improve exercise performance (Foster *et al.*, 1979; Tokmakidis and Volaklis, 2000; Wouassi *et al.*, 1997). Thus, glucose administration prior to exercise may have no beneficial effect on exercise performance, most likely due to an increase in insulin secretion (Tokmakidis and Volaklis, 2000; Wouassi *et al.*, 1997) and decrease in epinephrine response (Wouassi *et al.*, 1997) that are induced following glucose administration before exercise, thereby impeding the mobilization of fatty acids for energy production during exercise (Foster *et al.*, 1979).

In addition, dietary sea snake lipid intake resulted in different swimming endurance by L-lactic acid injection compared to intake of dietary fish oil, despite similar fatty acid compositions between sea snake lipids and fish oil. This finding suggests that this difference in endurance by injected lactic acid may be attributed to certain trace components other than fatty acids in sea snake lipids. Further study needs to identify these unknown constituents.

In conclusion, the present study demonstrates that the type of dietary lipid has an effect on swimming endurance of mice injected with L-lactic acid, and an additional increase in endurance by injected lactic acid is only observed in mice fed sea snake lipids. Erabu sea snake lipids may contain factors that effectively regulate lactic acid/lactate metabolism. Long-term intake of dietary lipids does not affect body composition, energy storage and biochemical parameters associated with energy metabolism before swimming differently. Further study is required to elucidate the effects of L-lactic acid administration on enzyme activities involved in energy metabolism, and secretion of hormones regarding excitation-contraction coupling of muscle during swimming in mice fed dietary lipids.

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