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Relationship between the Effect of Dietary Fat on Swimming Endurance and Energy Metabolism in Aged Mice

Guihua Zhang[a] Nobuya Shirai[a, b] Hiramitsu Suzuki[c]

National Agriculture and Food Research Organization, at [a]National Food Research Institute, Tsukuba, and at [b]National Institute of Vegetable and Tea Sciences, Shimada, and [c]Kagawa Nutrition University, Sakado, Japan

Key Words

Endurance · Fish oil · Glycogen sparing · Interleukin-6 · Lard · Minerals · Safflower oil

Abstract

Aim: The aim of this study was to investigate the effect of different dietary fats on alterations in endurance, energy metabolism, and plasma levels of interleukin-6 (IL-6) and minerals in mice. ***Method:*** Male mice (aged 58 weeks) were fed diets containing 6% safflower oil, fish oil, or lard for 12 weeks. Swimming time to exhaustion, energy metabolism, and plasma IL-6 levels were subsequently determined. ***Results:*** Mice fed safflower oil exhibited a marked increase in swimming time compared to the baseline level. Mice fed lard exhibited a significant decrease in swimming time, while mice on a fish oil diet exhibited a small decrease in swimming time. The final swimming time of mice fed safflower oil was significantly longer than that of animals fed lard. This improvement in endurance with dietary safflower oil was accompanied by decreased accumulation of lactate and less glycogen depletion during swimming. In the safflower oil group, muscle carnitine palmitoyltransferase activity increased significantly after swimming, while the plasma nonesterified fatty acid concentration decreased significantly. A trend to increased plasma IL-6 levels was observed in sedentary animals on a safflower oil diet compared to those on a lard diet. ***Conclusions:*** These results suggest that dietary safflower oil improves the swimming endurance of aged mice to a greater extent than lard, and that this effect appears to involve glycogen sparing through increased fatty acid utilization.

Introduction

Lipids are one of the three major nutrients and are an important fuel for endurance exercises. Increasing evidence suggests that different types of dietary lipids (such as safflower oil, sea-snake oil, conjugated linoleic acid, and medium-chain triglycerides) have varying effects on endurance [1–6]. Our previous studies [1–3] indicated that n–3 polyunsaturated fatty acids (PUFAs) do not actively improve endurance in mice. This finding has been supported by human studies in which supplementation with n–3 PUFA-rich fish oil failed to increase performance in endurance athletes [7] and well-trained soccer players [8]. Conversely, a study by Ayre and Hulbert [6] indicated that young rats (aged 21–23 days) subjected to graded treadmill exercise had greater endurance when administered a diet rich in n–6 PUFAs for 9 weeks compared to a diet rich

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Nobuya Shirai
National Agriculture and Food Research Organization
National Institute of Vegetable and Tea Sciences
2769 Kanaya, Shimada, Shizuoka 428-8501 (Japan)
Tel. +81 54 745 4101, E-Mail nshinya@affrc.go.jp

in n–3 PUFAs. However, these authors studied only the phospholipid fatty acid composition of muscle membrane, and hence mechanisms by which dietary n–6 PUFAs enhance endurance remain unclear. Furthermore, it is unknown whether consumption of n–6 PUFAs will result in improved endurance in older animals.

Interleukin-6 (IL-6), a polypeptide messenger, is mainly produced by T cells and macrophages, and its signaling pathways may be associated with lipid and glucose metabolism [9, 10]. Other studies suggest that dietary n–6 PUFAs (safflower oil and corn oil) may increase plasma IL-6 levels compared to dietary fish oil [11, 12]. Furthermore, different types of dietary lipids have varying effects on the regulation of IL-6 secretion from adipocytes [13]. Several studies have reported that IL-6 is associated with improved endurance performance in humans [14–16], suggesting a possible role for IL-6 in the biochemical pathways that determine endurance. Moreover, minerals such as magnesium, calcium, phosphorus, and iron are present in animal bodies and act as cofactors for enzymes involved in energy production, muscle contraction, and nerve excitation as well as, in the case of iron, the oxygen-carrying capacity of blood [17]. Many studies [18–21] have reported a relationship between the status of minerals and exercise capacity in animals and humans. These factors may therefore influence alterations in swimming endurance caused by dietary fat.

In the present study, we compared the effects of n–6 PUFA-rich safflower oil and n–3 PUFA-rich fish oil with lard on swimming endurance in aged mice, and examined the relationship between altered endurance caused by dietary fat and energy metabolism, plasma IL-6 level, and plasma mineral levels.

Materials and Methods

Animals

Male Crlj:CD-1 (ICR) mice (aged 4 weeks) were purchased from Charles River Japan, Inc. (Atsugi, Japan) and housed in cages under automatically controlled conditions of temperature (mean ± SD: 24 ± 0.5°C), relative humidity (65 ± 5%), and illumination (light on from 6:45 to 18:45 h). The mice were fed laboratory chow (MF; Oriental Yeast Co., Ltd., Tokyo, Japan) and switched to one of the three experimental diets (lard, fish oil, or safflower oil) at 58 weeks of age. Animals had free access to the experimental diets and water for 12 weeks, after which they were sacrificed. Animals were handled according to the guidelines of the Ministry of Agriculture, Forestry and Fisheries for laboratory animal studies. All animal procedures were reviewed and approved by the Animal Care and Use Committee of the National Food Research Institute, National Agriculture and Food Research Organization of Japan.

Table 1. Fatty acid composition (%) of experimental diets

	Dietary group		
	lard	fish oil	safflower oil
SFAs			
14:0	1.8	4.6	0.2
15:0	–	1.4	–
16:0	26.2	25	7.4
17:0	0.4	1.4	–
18:0	15	6.2	2.4
MUFAs			
16:1	2.6	5.7	0.1
18:1n–9	37.1	17.5	12.9
18:1n–7	2.8	2.2	0.7
20:1n–9	0.9	1.2	0.1
PUFAs			
18:2n–6	10.7	1.5	74.6
18:3n–3	0.7	0.9	–
20:4n–6	–	1.6	–
20:5n–3	–	4.9	–
22:5n–3	–	1.1	–
22:5n–6	–	1.5	–
22:6n–3	–	19.9	–

SFAs = Saturated fatty acids; MUFAs = monounsaturated fatty acids; PUFAs = polyunsaturated fatty acids.

Experimental Diets

Lard was purchased from the Oriental Yeast Co., Ltd., and safflower oil from MP Biomedicals, LLC (Solon, Ohio, USA). Fish oil was supplied by Nippon Chemical Feed Co., Ltd. (Hakodate, Japan). Each experimental diet contained 6% oil or fat and other components as follows: 47.8% corn starch, 20% casein, 15% granulated sugar, 5% cellulose, 4% mineral mixture, 2% vitamin mixture, and 0.2% L-methionine. The mineral and vitamin mixtures were obtained from Oriental Yeast Co., Ltd.

The fatty acid composition of the experimental diets is given in table 1. The lard diet contained mainly saturated fatty acids (SFAs, 43.5%) and monounsaturated fatty acids (MUFAs, 43.4%), with the other components including 18:2n–6 (10.7%) and 18:3n–3 (0.7%). The fish oil diet was rich in n–3 PUFAs (26.8%) and also included n–6 PUFAs (4.6%), SFAs (38.6%), and MUFAs (26.6%), while the safflower oil contained enriched 18:2n–6 (74.6%) and included SFAs (10%) and MUFAs (13.8%). Each experimental diet was stored at a temperature of –40°C or below to prevent oxidative changes in fatty acid composition.

Experimental Design

Forty mice (aged 58 weeks) were assigned randomly to the three experimental diet groups (lard and safflower oil groups, n = 13; fish oil group, n = 14). Individual mice were identified by ear punches. At baseline, the mice had similar body weights and swimming time to exhaustion, as measured by the forced-swimming system described in our previous studies [1–3, 22, 23]. The mice were then fed the experimental diets for 12 weeks, and the

swimming times were reassessed using the same system. In brief, the swimming time to exhaustion of mice supporting loads corresponding to 1% of their body weights was determined in a tank (26 × 22 × 29 cm) filled to a depth of 25 cm with water maintained at 23°C. The mice were classified as exhausted when they failed to rise to the surface of the water to breathe within a 7-second period [24]. Exhausted mice were immediately sacrificed by cervical dislocation.

To obtain biochemical parameters before the swimming exercise, 36 control mice of similar age to those described above were divided into three groups (12 mice per group) and fed one of the same diets. After 12 weeks, these sedentary mice were sacrificed by cervical dislocation without being subjected to a swimming test.

Sample Collection and Processing

Blood samples were collected from the caudal vena cava. Samples of liver and hind limb muscles were immediately flash frozen in liquid nitrogen and stored at −80°C until analysis. The epididymal and perirenal adipose tissues were then dissected and weighed. A blood sample (0.2 ml) was deproteinized with perchloric acid (0.6 M) and centrifuged at 3,000 *g* for 10 min to determine the plasma lactate level. The remaining blood sample was centrifuged at 900 *g* for 15 min, and the supernatant was stored at −60°C for the measurement of plasma glucose, non-esterified fatty acid (NEFA), IL-6, and minerals (Mg, Ca, P, and Fe).

Biochemical Analysis

Plasma glucose, NEFA, and plasma mineral (Mg, Ca, P, and Fe) levels were measured using commercial kits (glucose, CII-test Wako; NEFA, C-test Wako; magnesium, B-test Wako; calcium, E-test Wako; phosphorus, C-test Wako; Fe, C-test Wako; Wako Pure Chemical Industries, Osaka, Japan). Plasma IL-6 was assayed using an enzyme-linked immunosorbent assay (ELISA) kit (mouse IL-6 ELISA; Bender Medsystems GmbH, Vienna, Austria). Plasma and muscle L-(+)-lactate levels [25] and muscle and liver glycogen contents [26] were measured using published enzymatic methods. Muscle and liver carnitine palmitoyltransferase (CPT) activities were determined using palmitoyl-CoA as the substrate [27].

Statistical Analysis

Data are expressed as means ± SE. All statistical analyses were performed using the Statistica program package (StatSoft Inc., Tulsa, Okla., USA). A one-way analysis of variance with Dunnett's test was used to compare the fish and safflower oil groups with the lard group. A paired Student's t test was used to compare the initial and final swimming times, while an independent Student's t test was used to compare biochemical parameters between sedentary and exhausted mice in the three diet groups. A p value <0.05 was used as the criterion for statistical significance.

Results

Food Intake and Body and Adipose Tissue Weights

The average food intake for aged mice was 4.1 g/day in each diet group. No significant differences were observed between the diet groups for mean final body weights (lard group, 49.7 ± 2.4 g; fish oil group, 51.3 ± 1.5 g, and safflower oil group, 50.9 ± 1.3 g) or epididymal fat weight (lard group, 2.4 ± 0.4 g; fish oil group, 2.4 ± 0.1 g, and safflower oil group, 2.4 ± 0.2 g).

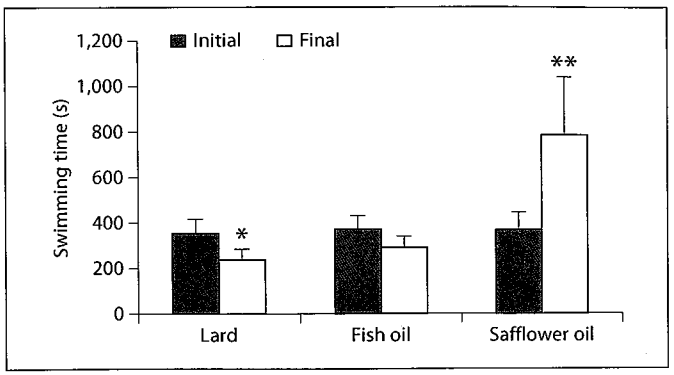

Fig. 1. Swimming time in aged mice fed lard, fish oil, or safflower oil at the initial and final trials. * $p < 0.05$, significant difference between initial and final trials analyzed by Student's t test; ** $p < 0.05$, significant difference for lard at each treatment analyzed by Dunnett's test.

Swimming Endurance

The swimming times at the initial and final trials of mice fed the experimental diets are depicted in figure 1. Mice fed safflower oil had significantly longer swimming times than mice fed lard at the end of the feeding trial, although no significant differences were observed between the two groups at the start of the study. Dietary lard significantly shortened swimming time in the initial trial. The fish oil group did not exhibit any significant difference in swimming times at the initial and final trials, with the times being comparable to those measured for the lard group at the final trial.

Biochemical Parameters

The muscle and liver glycogen contents and plasma glucose levels in aged mice are depicted in figure 2. No significant differences were observed in muscle or liver glycogen content or plasma glucose levels between the fish oil or safflower oil groups and the lard group in sedentary or exhausted mice. Exhausted mice fed the three lipid diets had significantly decreased muscle glycogen content compared with the corresponding sedentary mice. The plasma glucose levels in mice fed lard or safflower oil were significantly lower after exhaustive swimming.

The plasma and muscle lactate levels in aged mice fed the above-mentioned experimental diets are depicted in

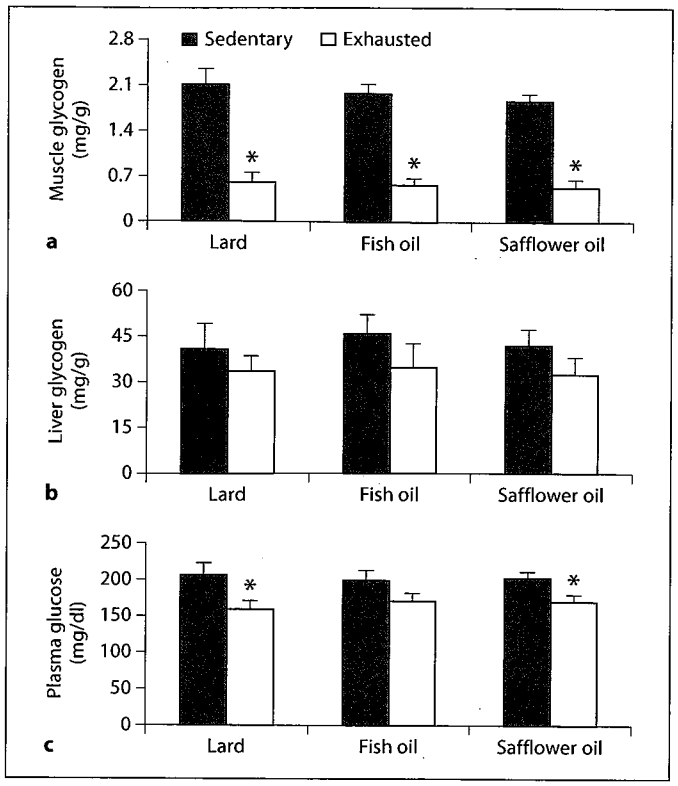

Fig. 2. Muscle (**a**) and liver glycogen (**b**), and plasma glucose levels (**c**) in aged sedentary and exhausted mice fed lard, fish oil, or safflower oil. * $p < 0.05$, significant differences between the initial and final trials analyzed by Student's t test.

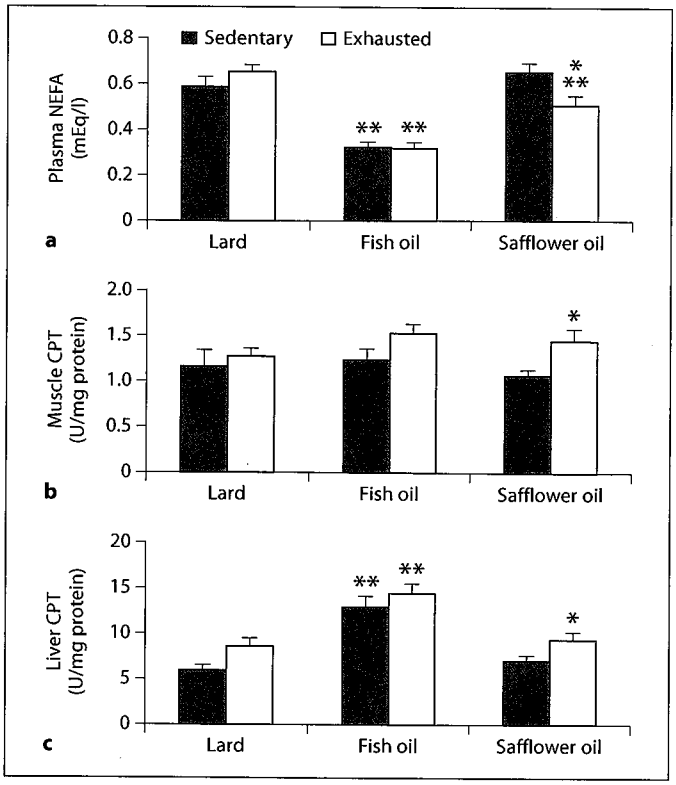

Fig. 4. Plasma NEFA (**a**), and muscle (**b**) and liver CPT (**c**) in aged sedentary and exhausted mice fed lard, fish oil, or safflower oil. * $p < 0.05$, significant differences between the initial and final trials analyzed by Student's t test. ** $p < 0.05$, significant difference for lard at each treatment analyzed by Dunnett's test.

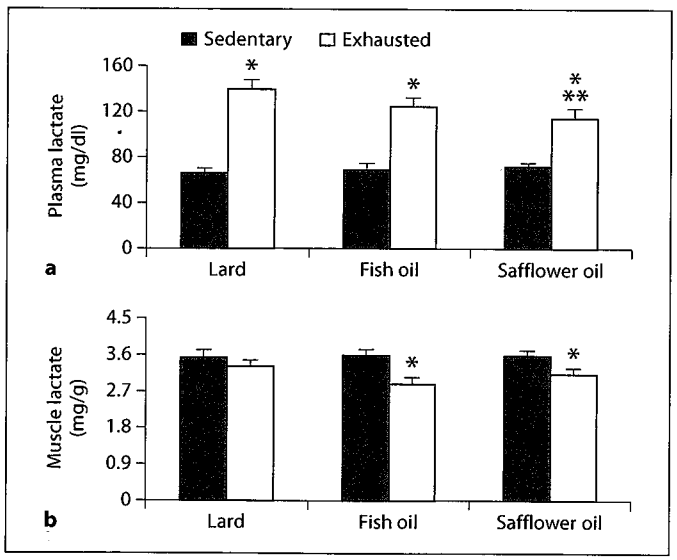

Fig. 3. Plasma (**a**) and muscle lactate (**b**) in aged sedentary and exhausted mice fed lard, fish oil, or safflower oil. * $p < 0.05$, significant differences between the initial and final trials analyzed by Student's t test; ** $p < 0.05$, significant difference for lard at each treatment analyzed by Dunnett's test.

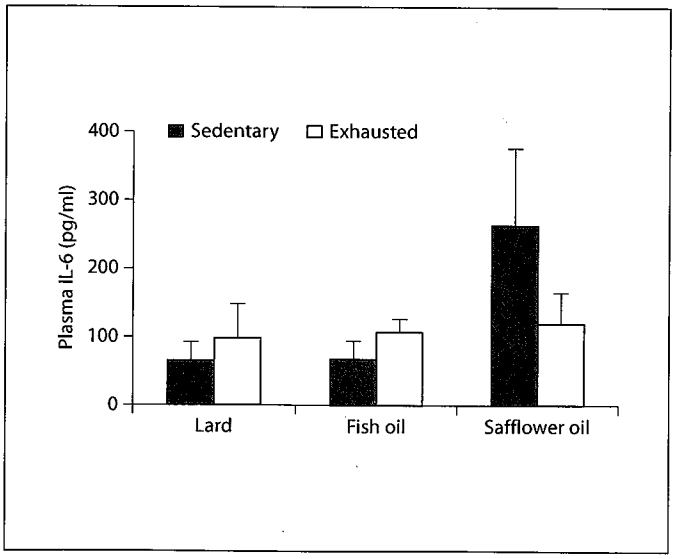

Fig. 5. Plasma IL-6 levels in aged sedentary and exhausted mice fed lard, fish oil, or safflower oil. The significant differences between the initial and final trials analyzed by Student's t test ($p < 0.05$) and the significant difference for lard at each treatment analyzed by Dunnett's test ($p < 0.05$) are not shown.

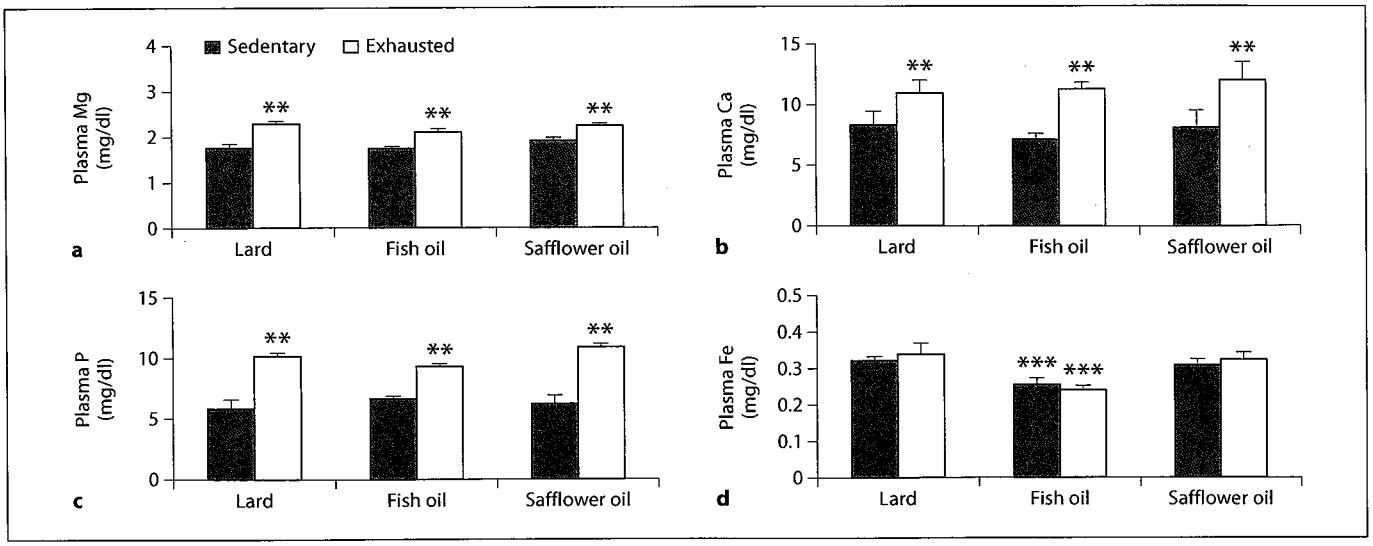

Fig. 6. Plasma Mg (**a**), Ca (**b**), P (**c**), and Fe (**d**) levels in aged sedentary and exhausted mice fed lard, fish oil, or safflower oil. ** $p < 0.01$, significant differences between sedentary and exhausted mice analyzed by Student's t test; *** $p < 0.05$, significant difference for lard at each treatment analyzed by Dunnett's test.

figure 3. The plasma lactate level was significantly higher in exhausted mice than in sedentary mice in all three diet groups. Although the plasma lactate levels in the fish oil and safflower oil groups did not significantly differ from those in the lard group in sedentary mice, the levels in exhausted mice fed safflower oil were significantly lower than in those fed lard. The muscle lactate level of the safflower and fish oil groups did not significantly differ from that of the lard group in sedentary or exhausted mice. Furthermore, the muscle lactate level in exhausted mice fed fish or safflower oil was significantly lower than that in the corresponding sedentary animals.

The plasma NEFA levels and muscle and liver CPT activities in aged mice are depicted in figure 4. The plasma NEFA level was significantly lower in exhausted mice than in sedentary mice in the safflower oil group but not in the fish oil or lard groups. The plasma NEFA levels of exhausted mice in the fish oil and lard groups were similar to those in the corresponding sedentary groups. The fish oil group had significantly lower plasma NEFA levels than the lard groups in both sedentary and exhausted mice. In contrast, plasma NEFA levels were not significantly different between the safflower oil and lard groups in sedentary mice but were significantly lower in the safflower oil group after exhaustion. Similar muscle CPT activities were observed in the sedentary and exhausted mice in the three groups. In contrast, in both the sedentary and exhausted groups, mice fed fish oil had significantly higher liver CPT activities than those fed lard. Liver CPT activities in sedentary or exhausted animals were similar for the safflower oil and lard groups. Muscle and liver CPT activities in mice fed safflower oil were significantly increased by exhaustive swimming ($p < 0.01$ for muscle and $p < 0.05$ for liver). A similar increase was not observed in mice administered the lard and fish oil diets.

The effect of dietary safflower oil on plasma IL-6 levels in aged mice is depicted in figure 5. No significant difference was observed in plasma IL-6 levels in sedentary or exhausted mice between the safflower oil, fish oil, and lard groups. Plasma IL-6 levels were similar following exhaustive swimming in each diet group. However, sedentary mice in the safflower oil group had higher IL-6 levels than the lard diet group, although this difference was not significant ($p = 0.098$). Apparent differences in IL-6 levels were observed between exhausted mice and the corresponding sedentary mice in each diet group, but these differences were also not significant. Interestingly, plasma IL-6 levels tended to decrease in exhausted mice in the safflower oil group compared with those in the sedentary group, whereas in the lard and fish oil groups levels tended to increase compared with those in the corresponding sedentary mice.

The plasma mineral levels in aged mice are depicted in figure 6. In sedentary and exhausted mice fed the safflower oil and fish oil, plasma levels of Mg, Ca, and P were

similar to those in the corresponding lard groups. However, dietary fish oil resulted in significantly lower plasma Fe levels in both sedentary and exhausted mice than in the lard group. Plasma Fe levels were similar in sedentary or exhausted mice in the lard and safflower oil groups. Exhaustive swimming significantly increased levels of plasma Mg, Ca, and P, but not plasma Fe, compared with levels in sedentary mice fed the three lipid diets.

Discussion

Our results suggest that dietary safflower oil (rich in n–6 PUFAs) improved swimming endurance in aged mice, whereas dietary fish oil (rich in n–3 PUFA) and lard (rich in SFAs and MUFAs) appeared to have negative effects on endurance. Dietary safflower oil did not significantly affect body or adipose tissue weight, suggesting that the improved endurance was a primary effect of diet and not a secondary effect of buoyancy differences because of body fat. Furthermore, fish oil and lard diets negatively affect endurance of aged mice, as observed in our previous study [2].

Similar glycogen storage and plasma glucose levels in sedentary mice in the three diet groups suggest that the improvement in swimming endurance with safflower oil intake is not influenced by these parameters prior to swimming. However, following swimming, the accumulation of plasma lactate was significantly lower in the safflower oil group than in the lard group. In addition, although the glycogen level of muscles did not differ significantly among dietary groups in sedentary and exhausted mice, the swimming time of the safflower oil group was the longest in the experimental diet group. These results imply that the improved endurance associated with dietary safflower oil may be due, at least in part, to glycogen sparing. The working skeletal muscle is not only the major site of lactate production but is also important for utilization of lactate, which is mainly removed by oxidation [28, 29]. The decreased accumulation of lactate observed in aged mice fed safflower oil could be due to increased lactate oxidation and subsequent utilization as an additional energy source during swimming.

Several studies have demonstrated that the type of dietary lipid can alter energy substrate utilization. For example, animals fed a safflower oil diet have increased fat oxidation compared with others fed fish oil or beef tallow rich in SFAs and MUFAs [30, 31]. Other studies have reported that the mobilization and utilization of fatty acids enhance endurance in experimental animals [32–34]. In our study, the significant increase in muscle and liver CPT activities and decrease in plasma NEFA levels observed following exhaustive swimming in mice fed safflower oil implies an upregulation of fatty acid metabolism in these mice. In contrast, muscle and liver CPT activities of the groups fed lard or fish oil did not change significantly before or after exhaustive swimming. These results suggest that the safflower oil group may have increased fatty acid utilization for energy than the other diet groups. Conversely, significantly increased activities of liver CPT were observed in sedentary or exhausted mice fed fish oil compared with animals fed lard. This result is consistent with the results of Ide et al. [35], who reported that sedentary rats fed fish oil exhibited increased liver CPT activity compared with others fed safflower or palm oil (SFA- and MUFA-rich diets). However, according to other reports, dietary n–3 suppresses fatty acid synthesis [36, 37] and lipid oxidation [30]. Furthermore, in our study, plasma NEFA levels were significantly lower in the fish oil group compared with those in the lard group. Haramizu et al. [38] have indicated that the metabolism of muscle lipid, but not liver lipid, contributes to endurance performance in mice. This is consistent with our finding that the fish oil group did not exhibit significantly improved swimming endurance.

Previous studies reported that the type of dietary lipid may influence plasma IL-6 levels [11, 12] and IL-6 secretion from adipocytes [13]. In our study in exhausted mice, the levels were similar in the three diet groups, although sedentary mice fed safflower oil tended to have non-significant increases in plasma IL-6 levels compared with those fed lard. IL-6 signaling may be associated with lipid and glucose metabolism [9, 10]. In a human study, an acute increase in IL-6 levels selectively stimulated lipolysis in skeletal muscles [39]. These findings imply that dietary safflower oil may influence plasma IL-6 levels, which in turn influence lipid metabolism.

The observation of significant increases in plasma Mg, Ca, and P levels after exhaustive swimming in aged mice is supported by studies that involved similar exercises in other animal experiments [19, 40, 41]. The swimming exercise may trigger the release of these minerals because of increased energy production and muscle contraction. However, in our study, similar plasma Mg, Ca, and P levels were observed in sedentary and exhausted mice in the three diet groups. Conversely, plasma Fe levels were lower in aged mice fed fish oil than in mice fed the lard diet. Rumberger et al. [42] demonstrated that transferrin and Fe may contribute to the lipolytic effect of serum in isolated adipocytes. In our study, plasma NEFA levels in the

fish oil group were similar to those in the sedentary and exhausted groups. These findings imply that decreased Fe levels in aged mice fed fish oil may affect endurance capacity by downregulating fatty acid utilization.

These results suggest that improved endurance with intake of safflower oil is associated with glycogen sparing as a consequence of increased fatty acid utilization. Future studies have to determine whether this increased fatty acid utilization is related to IL-6. Although the intake of n–3 PUFAs is essential for preserving the nervous system in elderly people, intake of n–6 PUFAs may be an important factor in maintaining physical strength in this population. However, further research is necessary to clarify whether the consumption of oils with a high content of n–6 PUFAs improves the endurance of humans, e.g. elderly people and athletes, and the detailed mechanisms of action in animals and humans.

In conclusion, the results of this study suggest that dietary n–6 PUFAs, such as safflower oil, improve the swimming endurance of aged mice to a greater extent than lard. This positive effect might be due to glycogen sparing through increased fatty acid utilization.

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