Original Article *J. Clin. Biochem. Nutr.*, **44**, 168–177, March 2009

Effects of Short-Term Dietary Change from High-Carbohydrate Diet to High-Fat Diet on Storage, Utilization, and Fatty Acid Composition of Rat Muscle Triglyceride during Swimming Exercise

Masaru Ochiai* and Tatsuhiro Matsuo

Faculty of Agriculture, Kagawa University, Ikenobe, Miki, Kagawa 761-0795, Japan

Received 9 October, 2008; Accepted 4 November, 2008

Summary **The purpose was to examine the effects of a 3-day dietary change from a high-carbohydrate (C) to high-fat (F) diet on muscle triglyceride (MTG) storage and utilization during the swimming exercise in rats. Rats were meal-fed on either the F diet or the C diet for 11 days. For an additional 3 days, half of the rats in each group were fed the same diets and the other rats were switched to counterpart diets. On the final day, half of the rats in each group were killed before the exercise and the others were killed after the exercise. Serum concentrations of glucose and free fatty acids (FFA) were higher in the post-exercise groups than in the pre-exercise groups. The tissue glycogen contents were lower in the post-exercise groups. However, the MTG contents and fatty acid (FA) compositions were not influenced by the exercise and dietary change. The F diet increased the FFA concentration and slightly increased the MTG content. Moreover, the dietary FA composition influenced the FA composition of the MTG. These results suggest that the exercise did not affect the contents and FA composition of MTG, but that the F diet had an effect on the MTG contents and FA composition.**

Key Words**: muscle triglyceride, rat swimming exercise, fatty acid composition, high-fat diet**

Introduction

Adequate carbohydrate stored as glycogen in muscles and liver are well-known to be absolutely essential for endurance exercise. Glycogen loading is considered to be an important and essential strategy for athletes. However, endogenous glycogen stores provided little energy and might be exhausted during prolonged exercise. On the other hand, fat, a main energy during endurance exercise, is largely stored in several tissues, especially subcutaneous and interperitoneal fat tissues. On average, humans have 80,000–140,000 kcal of potential energy stored as triglyceride (TG) in those tissues. This amount of energy is at least 40 times larger than that of glycogen in muscles and liver [*1*].

*To whom correspondence should be addressed.
Tel: +81-90-5250-6846 Fax: +81-87-891-3021
E-mail: masaru_141981@yahoo.co.jp

Intramuscular triglyceride (IMTG), corresponding to 2,000–3,000 kcal, is stored in intramuscular fat tissues, and is one of the sources of energy supply during moderate endurance exercise [*2–4*]. Exercise intensity, duration, and training status control the degree to which IMTG is utilized as a fuel source [*1*, *4*]. During moderate exercise, muscle triglyceride (MTG) including IMTG and free fatty acids (FFA) are well known to be utilized more effectively and easily as fuel sources [*5*, *6*]. For example, during endurance exercise at 65%VO_2max, about half of the total energy expenditure was derived from IMTG [*5*]. Many scientists have been studying the physiological function of MTG [*1*, *7*].

Accumulating and utilizing much MTG more properly and efficiently would suppress glycogen consumption, because the fat oxidative enzymes in muscles are activated during endurance exercise. If appropriate ways of fat loading in the muscles are discovered, the fuel source during endurance exercise would be more strongly dependent on fat, resulting in muscle glycogen saving. The most appro-

priate way to store lipid in working muscles has not yet been clarified, but increments of the blood free fatty acid (FFA) level and FFA uptake into working muscles are likely to be critical and indispensable [*7*]. It is known that lipoprotein lipases (LPL) are closely related with the FFA uptake into working muscles [*8*]. Therefore, stimulating the muscle LPL activity, FFA uptake and MTG synthesis are desirable ways to store lipids in working muscles. Many studies have been performed on how to fulfill these conditions, using high-fat diets [*9*] and endurance training [*10*].

High-fat diets could stimulate the enzyme activities related to fat oxidation, as well as increasing MTG accumulation and enhancing endurance performance. Changing the fat ingestion pattern might also be a good way to increase FFA uptake into working muscles. After the glycogen is fully stored in the liver and muscles by high-carbohydrate diets, the ingestion of high-fat diets might activate higher muscle LPL activities. It is possible that this dietary switch from a high-carbohydrate to a high-fat diet simultaneously meets the required glycogen and lipid in muscles.

Exercise training has also been reported to increase the capacity to store TG and glycogen as energy fuels in skeletal muscles [*10*], and has a subtle effect on the skeletal muscle fatty acids (FA) profile. Some studies have reported alterations in muscle FA composition after a long-term exercise training program [*11*], whereas others reported no significant changes [*12*]. Moreover, several reports of a temporary endurance exercise on skeletal muscle fatty acid profile are present [*13*].

In the present study, we examined the effects of a short term dietary change from a high-carbohydrate to a high-fat diet on the storage, utilization, and FA composition of MTG during 1h-endurance exercise in rats.

Materials and Methods

Animal care and experimental design

All procedures involving the rats were approved by the Experimental Animal Care Committee of Kagawa University.

Forty-two male Wistar rats (age 5 weeks) were purchased from Japan SLC (Shizuoka, Japan). All rats were housed individually at 22 ± 1°C with light from 8:00–20:00 h and free access to water. Throughout this experimental period, the energy intakes of the rats in each dietary group were equivalent, and the body mass and food intakes were monitored daily. All rats were fed commercial rat chow (CE-2, Japan CLEA, Tokyo, Japan) for 3 days ad libitum, and were then fed meals twice a day (9:00–10:00, 21:00–22:00 h) for 21 days. During the initial 7 days of the meal-feeding period, all rats were fed CE-2. At the end of the 7 days, all rats were divided by body mass into 2 groups, a high-carbohydrate (C) diet group and a high-fat (F) diet group. During the next 11 days, the rats of each dietary group were fed each diet in the same manner as described above. The composition of each diet is shown in Table 1. The C diet provided 5, 72, and 23% of energy as fat, carbohydrate, and protein, respectively; the F diet provided 40, 37, and 23%, respectively. The composition of predominant fatty acids of the test oil (soybean oil, Nacalai Tesque, Inc., Kyoto, Japan) was as follows: palmitic acid 1.7, stearic acid 4.1, oleic acid 26.6, linoleic acid 52.5, and linolenic acid 5.1%.

Both dietary groups of rats were then divided by body mass into two subgroups; half of the rats were fed the same diets (C-C and F-F), and the other half were switched to the counterpart diets (C-F and F-C) for the final 3 days in the manner described above.

During these 21 days, all the rats were subjected to swimming exercise between 12:00 and 13:00. The swimming

Table 1. Composition of experimental diets

	High-carbohydrate-diet[1]		High-fat-diet[2]	
Ingredients	g·kg^{-1}	% kcal/diet	g·kg^{-1}	% kcal/diet
Casein	203.0	22.3	260.0	22.3
DL-methionine	3.0	0.4	4.0	0.4
Cornstarch	585.0	60.2	314.0	25.2
Sucrose	93.0	10.5	120.0	10.5
Cellulose	50.0	0.0	50.0	0.0
Soybean oil	18.8	5.0	192.0	40.1
Mineral mixture	35.0	0.5	45.0	0.5
Vitamine mixture	10.0	1.1	13.0	1.1
Choline chloride	2.0	0.0	2.0	0.0
Butylhydroxytoluene	0.01	0.0	0.01	0.0
Total	1000	100	1000	100

[1] The High-carbohydrate-diet provided 5, 72 and 23% of calories as fat, carbohydrate and protein, respectively.

[2] The High-fat-diet provided 40, 37 and 23% of calories as fat, carbohydrate and protein, respectively.

exercise was performed in a plastic barrel (80 × 56 × 48 cm) filled with water at 32–36°C (35 cm deep). During the initial 7 days, all rats were adjusted to the swimming exercise without any load. During the next 14 days, all rats were exercised with a sinker corresponding to 2% of their body mass.

On the final day, the rats of each dietary group were fed the previous meal (equivalent energy) between 9:00 and 10:00, and rested 2 h. The rats of each dietary group were again divided by body mass into two subgroups; half of the rats in each group were killed at 12:00 before the swimming exercise (pre-exercise groups) and the other half were killed at 13:00 after 1 h-swimming (post-exercise groups).

Sampling of blood and tissues

The rats were sacrificed by decapitation. Blood was collected and centrifuged at 3,000 rpm for 15 min in order to obtain serum. The serum was stored at −20°C. The liver, intra-abdominal (perirenal, mesenteric, and epididymal) fat, and muscles (soleus, plantarius, gastrocnemius, tibialis anterior, and extensor digitorum longus (EDL) muscle) were rapidly removed, weighted and frozen in liquid nitrogen. All of the tissues were then stored at −80°C until analysis.

Analysis

The serum glucose concentration was determined according to the method of Bergmeyer *et al.* [*14*]. Serum FFA and TG concentrations were measured using kits (NEFA C-Test, Triglyceride G-Test Wako, Wako Pure Chemical Industries, Ltd., Osaka, Japan). The serum lactate concentration was determined by the method described by Gutmann *et al.* [*15*] and Wieland *et al.* [*16*]. The glycogen content of the liver, gastrocnemius muscle, and EDL muscle was determined according to Lo *et al.* [*17*]. Total lipid in the liver and muscles was extracted by the method of Folch *et al.* [*18*]. The TG concentration of total lipid was determined using a kit (Triglyceride G-Test Wako, Wako Pure Chemical Industries, Ltd., Osaka, Japan).

The fatty acid compositions of muscles TG was determined by using gas chromatography. The TG extract liquid was vaporized by nitrogen gas and then transmethylated by using methanol-sulfuric acid (230:2, v/v). The fatty acid methyl esters were extracted with hexane and separated in a gas chromatograph (model GC-2014, Shimadzu Co., Kyoto, Japan) equipped with a 30-m long capillary column (VLBON HR-20M, Shimadzu Co., Kyoto, Japan). The column temperature was set at 210°C. The carrier gas was helium at a flow rate of 0.65 ml·min^{-1}. Methyl esters of individual fatty acids were identified in the chromatograms by comparing their retention times to those of pure methyl esters, and were quantified by comparing the area under their peaks.

Statistical analysis

The values are expressed as means ± SD. Data were evaluated by three-way ANOVA, and Fisher's PLSD test was used to determine specific mean differences [*19*]. In the case of significant difference by exercise in ANOVA, the comparison of mean values between the pre- and post-exercise groups was determined by Student's unpaired *t* test [*19*]. Statistical significance was considered a *p* value of $p<0.05$. All analyses were performed with a commercially available statistical package (StatView J-5.0, SAS Institute Inc., Cary, NC).

Results

Body mass and energy intake

The body mass and energy intakes of the rats are shown in Table 2. The final body mass and body mass gain during the final 3 days were not significantly different among groups. The energy intake among each group was also not significantly different.

Serum substrates

The serum concentrations of glucose, FFA, TG, and lactate are shown in Table 3. The serum glucose concentration was significantly influenced by the antecedent F diet and by the exercise. The serum glucose concentration of the F-C group was significantly higher than that of the C-F and F-F groups in the pre-exercise groups. However, significant differences in the serum glucose concentration were not seen in the post-exercise groups. In the C-F group, the

Table 2. Body mass at initial, dietary change (DC) and final day, and energy intake

	Dietary group			
	C-C	C-F	F-C	F-F
Body mass (initial) (g)	135 ± 5	138 ± 9	136 ± 8	138 ± 5
Body mass (DC) (g)	155 ± 10	154 ± 14	157 ± 17	158 ± 7
Body mass (final) (g)	163 ± 10	162 ± 14	169 ± 7	169 ± 6
Energy intake (kcal·day^{-1})	44.5 ± 0.4	44.6 ± 0.1	44.5 ± 0.5	44.7 ± 0.0

Values are means ± SD (n = 5–6 rats per group).

C: High-carbohydrate diet, F: High-fat-diet, C-F: Half C group switched to F diet for final 3 days, F-C: Half F group switched to C diet for final 3 days, C-C and F-F: The other half of C and F group remained on the same diet as before over these 3 days.

Table 3. Serum concentration of glucose, triacylglycerol (TG), free fatty acids (FFA), and lactate

		Dietary group			
		C-C	C-F	F-C	F-F
Glucose (mg·dl^{-1})	pre	137 ± 14^{bc}	129 ± 5^{c}	149 ± 3^{a}	144 ± 8^{ab}
	post	153 ± 17	148 ± 12*	166 ± 24	156 ± 18
TG (mg·dl^{-1})	pre	177 ± 35^{b}	237 ± 86^{a}	183 ± 31^{b}	221 ± 85^{a}
	post	155 ± 28	256 ± 73	162 ± 32	278 ± 84
FFA (mEq·l^{-1})	pre	0.23 ± 0.08^{b}	0.58 ± 0.09^{a}	0.29 ± 0.07^{b}	0.63 ± 0.15^{a}
	post	0.87 ± 0.13^{bc}*	1.15 ± 0.27^{a}*	0.70 ± 0.16^{c}*	1.06 ± 0.18^{ab}*
Lactate (mmol·l^{-1})	pre	1.50 ± 0.19	1.35 ± 0.19	1.26 ± 0.19	1.29 ± 0.32
	post	1.68 ± 0.26	1.78 ± 0.42	2.03 ± 0.33*	1.81 ± 0.26*

ANOVA

	Antecedent diet (A)	Current diet (B)	A × B	Exercise (C)	A × B × C
Glucose	$p<0.05$	NS	NS	$p<0.01$	NS
TG	NS	$p<0.001$	NS	NS	NS
FFA	NS	$p<0.01$	NS	$p<0.001$	NS
Lactate	NS	NS	NS	$p<0.001$	NS

Values are means ± SD (n = 5–6).

C: High-carbohydrate diet, F: High-fat-diet, C-F: Half C group switched to F diet for final 3 days, F-C: Half F group switched to C diet for final 3 days, C-C and F-F: The other half of C and F group remained on the same diet as before over these 3 days.

Pre: The group sacrificed before exercise, Post: The group sacrificed after exercise; Different letter(s) within a row are significantly different at $p<0.05$ calculated by three-way ANOVA and fisher's PLSD test. *Significant difference ($p<0.05$) from the pre-exercise group (Student's t test).

serum glucose concentration of the post-exercise group was significantly higher than that in the pre-exercise groups. The serum TG concentration was significantly influenced by the current F diet. The serum TG concentrations of the C-F and F-F groups were significantly higher than those of the C-C and F-C groups in the post-exercise groups. However, no significant difference was seen in the serum TG concentration among the pre-exercise groups. The serum FFA concentration was largely influenced by the current F diet and the exercise. The serum FFA concentrations in both the C-F and F-F groups were significantly higher than those of the C-C and F-C groups. The serum FFA concentrations of each dietary group were significantly higher in the post-exercise groups than in the pre-exercise groups. The serum lactate concentration was influenced by exercise, and the result was that the values in the F-C and F-F groups were significantly higher than in the pre-exercise groups.

Tissue glycogen contents

The glycogen contents of the liver and muscles (gastrocnemius and EDL) are shown in Table 4. The glycogen contents of the liver, gastrocnemius muscle, and EDL muscle were significantly influenced by the current diets and exercise. The liver glycogen contents of the C-C and F-C groups were higher than those of the C-F and F-F groups in the pre-exercise groups. The liver glycogen content of the C-C in the post-exercise groups was the highest, and that of the F-C group was significantly higher than that of the F-F group. The glycogen contents were significantly lower in each post-exercise group than in each pre-exercise group. The gastrocnemius muscle glycogen content of the F-F group was significantly higher than that of the C-F group in the post-exercise groups. The gastrocnemius muscle glycogen contents of the C-C, C-F, and F-C groups were significantly decreased by the exercise. The EDL muscle glycogen content of the F-C group was significantly higher than that of the C-F and F-F groups in the pre-exercise groups, but no significant differences were found in the post-exercise groups. The exercise significantly decreased the EDL muscle glycogen content of the F-C group.

Total TG contents of tissue lipids

The total TG contents of the liver and muscles (soleus, plantarius, gastrocnemius, and tibialis anterior) are shown in Table 5. The liver TG content was influenced by the current diets, and the contents of the F-C group were the highest among the pre-exercise groups. However, the exercise had no effect on the liver TG content. The soleus TG content was influenced by the antecedent diets, but no significant difference in each dietary group was seen in the pre- and post-exercise groups. The soleus TG contents of the F-C and F-F groups tended to increase, but the values were not significant. Furthermore, the soleus TG content was not affected by the exercise. The TG content in the tibialis

Table 4. Tissue glycogen contents of liver, gastrocnemius and EDL muscle

		Dietary group			
		C-C	C-F	F-C	F-F
Liver (mg·g^{-1})	pre	38.9 ± 6.0^{a}	30.1 ± 5.6^{b}	41.8 ± 3.3^{a}	24.0 ± 7.4^{b}
	post	23.5 ± 8.8^{a}*	12.2 ± 7.9^{bc}*	21.1 ± 8.9^{ab}*	8.7 ± 3.5^{c}*
Gastrocnemius (mg·g^{-1})	pre	0.43 ± 0.07	0.27 ± 0.09	0.38 ± 0.18	0.20 ± 0.15
	post	0.19 ± 0.03^{ab}*	0.13 ± 0.02^{b}*	0.17 ± 0.05^{ab}*	0.20 ± 0.09^{a}
EDL (mg·g^{-1})	pre	0.57 ± 0.26^{ab}	0.38 ± 0.12^{b}	0.69 ± 0.08^{a}	0.39 ± 0.07^{b}
	post	0.28 ± 0.11	0.25 ± 0.07	0.29 ± 0.10*	0.17 ± 0.12

ANOVA

	Antecedent diet (A)	Current diet (B)	A × B	Exercise (C)	A × B × C
Liver	NS	p<0.001	NS	p<0.001	NS
Gastrocnemius	NS	p<0.05	NS	p<0.001	NS
EDL	NS	p<0.01	NS	p<0.001	NS

Values are means ± SD (n = 5–6 rats per group).
C: High-carbohydrate diet, F: High-fat-diet, C-F: Half C group switched to F diet for final 3 days, F-C: Half F group switched to C diet for final 3 days, C-C and F-F: The other half of C and F group remained on the same diet as before over these 3 days.
Pre: The group sacrificed before exercise, Post: The group sacrificed after exercise; Different letter(s) within a row are significantly different at p<0.05 calculated by three-way ANOVA and fisher's PLSD test. *Significant difference (p<0.05) from the pre-exercise group (Student's t test).

Table 5. Triglyceride concenrations of liver, soleus, gastrocnemius, plantarius and tibia anterior muscle

		Dietary group			
		C-C	C-F	F-C	F-F
Liver (mg·g^{-1})	pre	11.9 ± 3.0^{b}	10.0 ± 1.6^{b}	15.9 ± 3.4^{a}	8.9 ± 2.1^{b}
	post	11.1 ± 4.1	10.7 ± 3.3	14.7 ± 4.1	10.0 ± 0.7
Soleus (mg·g^{-1})	pre	8.5 ± 2.1	10.6 ± 4.0	12.2 ± 5.2	13.2 ± 3.2
	post	9.9 ± 3.4	10.2 ± 3.7	12.1 ± 4.9	12.0 ± 3.9
Gastrocnemius (mg·g^{-1})	pre	2.3 ± 1.0	2.6 ± 1.3	2.9 ± 1.0	2.7 ± 1.6
	post	2.3 ± 1.2	2.4 ± 1.1	2.0 ± 0.9	2.4 ± 1.0
Plantarius (mg·g^{-1})	pre	2.4 ± 0.6	3.1 ± 1.3	2.6 ± 0.7	2.8 ± 0.3
	post	2.8 ± 0.9	2.7 ± 0.1	2.8 ± 0.6	2.4 ± 0.6
Tibialis anterior (mg·g^{-1})	pre	3.0 ± 0.9	2.4 ± 0.5	2.4 ± 0.2	3.0 ± 0.6
	post	2.0 ± 1.0	2.3 ± 0.7	2.4 ± 0.5	2.0 ± 0.6*

ANOVA

	Antecedent diet (A)	Current diet (B)	A × B	Exercise (C)	A × B × C
Liver	NS	p<0.01	NS	NS	NS
Soleus	p<0.05	NS	NS	NS	NS
Gastrocnemius	NS	NS	NS	NS	NS
Plantarius	NS	NS	NS	NS	NS
Tibialis anterior	NS	NS	NS	p<0.05	p<0.05

Values are means ± SD (n = 5–6 rats per group).
C: High-carbohydrate diet, F: High-fat-diet, C-F: Half C group switched to F diet for final 3 days, F-C: Half F group switched to C diet for final 3 days, C-C and F-F: The other half of C and F group remained on the same diet as before over these 3 days.
Pre: The group sacrificed before exercise, Post: The group sacrificed after exercise; Different letter(s) within a row are significantly different at p<0.05 calculated by three-way ANOVA and fisher's PLSD test. *Significant difference (p<0.05) from the pre-exercise group (Student's t test).

Table 6. Palmitic, stearic, oleic, and linoleic acids composition of soleus muscle triglyceride

Fatty Acid		Dietary group			
		C-C	C-F	F-C	F-F
Palmitic acid (16:0)	pre	32.1 ± 1.0[a]	29.2 ± 1.1[a]	29.7 ± 5.0[a]	23.8 ± 1.2[b]
(%)	post	33.4 ± 0.8[a]	29.6 ± 1.7[b]	27.2 ± 1.1[c]	23.6 ± 2.8[d]
Stearic acid (18:0)	pre	12.3 ± 2.3	12.9 ± 3.6	9.9 ± 2.3	12.8 ± 3.2
(%)	post	10.9 ± 1.1	12.7 ± 4.2	11.6 ± 2.3	13.8 ± 3.2
Oleic acid [18:1 (n-9)]	pre	28.5 ± 2.3	26.0 ± 4.4	24.8 ± 4.5	24.5 ± 4.5
(%)	post	29.4 ± 0.7[a]	27.0 ± 5.6[ab]	25.2 ± 3.8[ab]	24.0 ± 3.8[b]
Linoleic acid [18:2 (n-6)]	pre	27.1 ± 0.9[d]	31.9 ± 1.3[d]	35.6 ± 2.4[b]	38.9 ± 0.9[a]
(%)	post	26.3 ± 0.8[d]	30.7 ± 2.2[c]	36.0 ± 2.2[b]	38.6 ± 2.2[a]

ANOVA

	Antecedent diet (A)	Current diet (B)	A × B	Exercise (C)	A × B × C
Palmitic acid (16:0)	$p<0.001$	$p<0.001$	NS	NS	NS
Stearic acid (18:0)	NS	$p<0.05$	NS	NS	NS
Oleic acid [18:1 (n-9)]	$p<0.05$	NS	NS	NS	NS
Linoleic acid [18:2 (n-6)]	$p<0.001$	$p<0.001$	NS	NS	NS

Values are means ± SD (n = 5–6 rats per group).
C: High-carbohydrate diet, F: High-fat-diet, C-F: Half C group switched to F diet for final 3 days, F-C: Half F group switched to C diet for final 3 days, C-C and F-F: The other half of C and F group remained on the same diet as before over these 3 days.
Pre: The group sacrificed before exercise, Post: The group sacrificed after exercise; Different letter(s) within a row are significantly different at $p<0.05$ calculated by three-way ANOVA and fisher's PLSD test. *Significant difference ($p<0.05$) from the pre-exercise group (Student's t test).

anterior muscle was influenced by the exercise and the TG content of the F-F group in the post-exercise groups was lower than that in the pre-exercise groups. The contents of the other muscles were not influenced by the antecedent and current diets, or by the exercise.

The fatty acids composition of MTG

The fatty acids compositions of the soleus and gastrocnemius muscles TG are shown in Tables 6 and 7, respectively. The sum of the percentages of palmitic, stearic, oleic, and linoleic acids was assumed to be 100% because other fatty acids in the MTG were not fully detected. The percentages of palmitic and linoleic acids in the soleus muscle TG were significantly and strongly influenced by the antecedent and current diets (Table 6). The percentage of palmitic acid in the soleus muscle of the F-F group was significantly lower than those of the other dietary groups in the pre-exercise groups. The palmitic acid percentage in the post-exercise groups was the following: C-C < C-F < F-C < F-F. The percentage of the linoleic acid was a contrast to that of the palmitic acid (F-F < F-C < C-F < C-C). The percentage of oleic acid was influenced by the antecedent diets, and that of the C-C group was significantly higher than the F-F group for the post-exercise groups. The percentage of stearic acid was influenced by the current diets, but significant differences in each group were not seen in the pre- and post-exercise groups. In comparison between the pre- and post-exercise groups, the exercise did not affect the fatty acids composition. The percentages of palmitic and linoleic acids in the gastrocnemius muscle were significantly influenced by the antecedent and current diets, and the percentage of oleic acid was influenced by the antecedent diets (Table 7). However, no effect was found on the fatty acids composition by the exercise. The percentage of palmitic acid was higher in the C-C group and lower in the F-F group. On the other hand, the result of the percentage of linoleic acid was in contrast to that of the palmitic acid.

Discussion

The major findings of the present study were that MTG made little contribution as an energy fuel to endurance exercise, and that serum glucose FFA and muscle glycogen were mainly utilized during the exercise. The short-term dietary change from the C diet to the F diet did not have an influence on the MTG contents, and the contents and FA composition of MTG were influenced by the ingestion period of the F diet, not by the exercise. On the other hand, muscle glycogen, the main energy for endurance exercise, was fully stored by the short term ingestion of the C diet.

MTG utilization during the endurance exercise

Significant decreases by the exercise did not occur in most of the dietary groups except for the F-F group of the

Table 7. Palmitic, stearic, oleic and linoleic acids composition of gastrocnemius muscle triglyceride

Fatty Acid		Dietary group			
		C-C	C-F	F-C	F-F
Palmitic acid (16:0)	pre	39.4 ± 0.7^{a}	35.9 ± 1.3^{b}	34.9 ± 1.4^{b}	31.6 ± 0.6^{c}
(%)	post	40.3 ± 0.9^{a}	36.6 ± 1.3^{b}	34.9 ± 1.4^{c}	31.9 ± 0.7^{d}
Stearic acid (18:0)	pre	18.4 ± 0.6	17.7 ± 2.1	19.7 ± 1.0	18.6 ± 2.0
(%)	post	19.4 ± 3.7	18.4 ± 1.3	18.6 ± 1.8	19.6 ± 0.6
Oleic acid [18:1 (n-9)]	pre	19.6 ± 0.7^{a}	19.2 ± 1.9^{ab}	17.5 ± 0.4^{b}	17.6 ± 1.5^{b}
(%)	post	19.0 ± 2.1	18.4 ± 1.2	18.0 ± 1.5	17.9 ± 0.7
Linoleic acid [18:2 (n-6)]	pre	22.6 ± 0.4^{c}	27.2 ± 1.9^{b}	17.6 ± 1.5^{b}	32.2 ± 1.9^{a}
(%)	post	21.3 ± 3.3^{c}	26.7 ± 1.3^{b}	17.9 ± 0.7^{b}	30.7 ± 0.9^{a}

ANOVA

	Antecedent diet (A)	Current diet (B)	A × B	Exercise (C)	A × B × C
Palmitic acid (16:0)	$p<0.001$	$p<0.001$	NS	NS	NS
Stearic acid (18:0)	NS	NS	NS	NS	NS
Oleic acid [18:1 (n-9)]	$p<0.01$	NS	NS	NS	NS
Linoleic acid [18:2 (n-6)]	$p<0.001$	$p<0.001$	NS	NS	NS

Values are means ± SD (n = 5–6 rats per group).

C: High-carbohydrate diet, F: High-fat-diet, C-F: Half C group switched to F diet for final 3 days, F-C: Half F group switched to C diet for final 3 days, C-C and F-F: The other half of C and F group remained on the same diet as before over these 3 days.

Pre: The group sacrificed before exercise, Post: The group sacrificed after exercise; Different letter(s) within a row are significantly different at $p<0.05$ calculated by three-way ANOVA and fisher's PLSD test. *Significant difference ($p<0.05$) from the pre-exercise group (Student's t test).

tibialis anterior muscle (−32%). This observation does not indicate that MTG was a critical source of energy in the exercise. Our observation did not agree with the studies reporting that the IMTG contents were decreased during endurance exercises [*3*]. It was reported that in rodents, exercising muscles relied mainly on FA derived from outside the muscle as a fuel source and did not oxidize intracellular lipids, such as triglycerides, to any great extent [*20*].

The intensity and time of endurance exercise are largely related with the degree of utilization of MTG as an energy source. According to the report [*6*], IMTG contributes a lot as a source of energy during endurance exercise at intensities between 40 and 55% of maximal oxygen uptake (VO_2max). Also, it has been stated that IMTG oxidation is an important portion (about 50%) of total fat oxidation at an exercise intensity of 65%VO_2max [*2*]. In contrast, at a weaker intensity (30%VO_2max), the main substrate was blood FFA and TG [*2*]. Therefore, these reports show that MTG contributes more largely as an energy fuel at low-moderate prolonged exercise. We could not clearly determine how intensity 2% sinker of body mass used in the present study was during the swimming exercise, but the load (2%) has been used in many similar studies [*19*, *21*]. The serum lactate concentration was significantly increased by the swimming exercise, but the values in the post-exercise groups were not much higher than those in the pre-exercise groups (less twice). These results indicated that the produced lactate was returned to be pyruvate acids and transported into the tricarboxylic acid (TCA) cycle for ATP production. The serum FFA concentration was remarkably elevated among each group by the exercise. The higher FFA was supposed to be derived from the adipose tissues TG. The high concentration of FFA might it possible to be transported into working muscle, resulting that the FFA could be utilized effectively as a main energy source during endurance exercise. From the results of the FFA levels in the present study, fat metabolism was supposed to be greatly enhanced. In moderate exercise (40–65% VO_2max), fat oxidation reaches maximal rates and generally contributes between 40 and 60% of total energy expenditure [*1*]. About 50–70% of total fat oxidation is reported to be derived from plasma FFA, leaving muscle-and/or lipoprotein-derived TG to provide the other part of total fat oxidation [*1*]. Therefore, the intensity of the swimming exercise was considered to be low-moderate.

Many researchers reported that endurance time was also closely involved in MTG utilization [*1*, *3*, *4*, *22*]. Watt *et al.* (2002) investigated the IMTG utilization during 240 min-endurance exercise at 55%VO_2max by using human muscle biopsies [*3*]. The IMTG content was significantly decreased during the initial 120 min, and the content was not significantly decreased during the latter 120 min. These findings indicated that the stimulation of the TG degradation in the peripheral tissues and the increment of blood FFA level from

0 min to 120 min inhibited the IMTG utilization during the latter 120 min. The study also showed that glycogen and blood glucose were half occupied as the main energy, and that IMTG made little contribution during the exercise between 0 min and 120 min. Also in the present study, the muscle glycogen contents were significantly lower in the post-exercise groups than in the pre-exercise groups, indicating that muscle glycogen was one of the main energy fuels. We investigated the interaction between the FFA concentration and swimming exercise time (0, 60, 120, 180, and 240 min) in rats given a commercial diet and the F diet using the same exercise program as in the present study (unpublished observation). The levels of FFA and liver TG in both dietary groups were increased with increases in exercise time. The increased FFA was mainly deprived from the TG in adipose, and FFA was likely to be oxidized during the exercise. The excess FFA was transported into the liver for TG resynthesis. We recognize that a continual swimming enhances fat oxidation, resulting in the decrease of MTG content. In addition, from the many results of MTG contents using biochemical analysis, even a 120 min endurance exercise did not decrease the MTG contents [*23*].

FA composition of muscles

The percentage of linoleic acid of the F-F group was significantly higher than that of the other dietary groups, especially the C-C group. This high percentage was caused by the FA composition of the soybean oil, which contained a high amount of linoleic acid (53%), and agreed with the many reports that the FA composition of dietary oil influences the FA composition of skeletal muscles [*24*, *25*]. Ayre and Hulbert [*25*] reported that the turnover of FA in rodent skeletal muscle is rapid, with major changes observed after 2–3 days. Therefore, much MTG oxidation might be caused by the ingestion of dietary oil which oxidizes easily and effectively, such as the ingestion of oil containing medium-chain fatty acids. The swimming exercise did not change the FA composition among four long-chain fatty acids in skeletal muscles in the present study. So far, significant changes of skeletal muscles FA composition by contemporary endurance exercise have not been conclusively reported. In the case of endurance training for more than 4 weeks, significant alterations were reported by many studies [*7*, *11*], but some reports also found that exercise training had little effect on the skeletal muscle FA profile [*13*, *26*]. Therefore, the FA compositions of MTG were influenced by the dietary fat contents more easily than the endurance exercise.

Relationship between TG contents of skeletal muscles and diets

A high-fat diet is strongly related with MTG metabolism. The interaction between high-fat diets and endurance exercises has been investigated by many researchers [*8*, *9*, *27*, *28*]. Many reports suggested that long and/or short term adaptation of high-fat diets enhanced the fat oxidation, and that the adaptation of high-carbohydrate diets decreased the activities of fat metabolism [*9*, *29*]. Zderic *et al.* [*9*] showed that the increment of total fat oxidation after adaptation of high-fat diets was largely connected with IMTG utilization as an energy fuel. Thus, IMTG utilization is closely related with IMTG accumulations. In the case of the soleus (oxidative) muscle, TG contents were about 3–6 fold more than the other muscles in each dietary group. Similar results were reported in a review [*21*]. The results can be explained by the characteristic differences of the skeletal muscles. The soleus muscle, which has a high percentage (less than 88%) of type 1 fibers, is related more closely with fat oxidation [*7*] because it contains more mitochondria, suggesting that MTG can serve as a rapid available energy source for mitochondrial oxidation. In contrast, the tibialis anterior and gastrocnemius muscle have lower percentages of type 1 fibers (less than 70% and less than 50%, respectively). The soleus TG contents were slightly increased with the ingesting period of the F diet. It is well known that MTG contents are also closely associated with fat intake (contents and period). It was reported in the review [*1*] that the ingestion of a high-fat (40–65% fat as energy) diet increased the MTG contents (50–100%), while the ingestion of a high-carbohydrate (2–25% fat as energy) diet decreased the MTG contents (10–30%). The present study supports these findings, at least in part.

The short term F diet intake after adaptation to the C diet enhanced the serum concentrations of TG and FFA of the C-F group, just as it did in the F-F group. The soleus TG content of the C-F group was about 20% lower than that of the F-F group. These results suggested that the F diet ingestion for even a short term filled the adequate FFA in blood, and made it easy to stimulate the uptake of FFA into the tissues. Also, the adjacent F diet which was fed before killed would have much influence on blood substrates, such as FFA and TG. The liver TG content in the C-F group was equivalent to those in the C-C and F-F groups, indicating that the serum FFA and TG were not effectively utilized for TG synthesis. Adequate glycogen contents in muscles were met by the short-term ingestion of the C diet. In the C-F group, the glycogen contents were significantly lower than the adjacent C dietary groups, which is not a desirable condition for endurance exercise. The lack of muscle glycogen contents might cause poor endurance performance. These results also suggested that the ingestion of high-fat diets was necessary, but it was more important to consume high-carbohydrate diets before endurance exercise for better endurance performance. These results are in agreement with the fat- and glycogen-loading methods reported by Havemann *et al.* [*30*] In the future, new glycogen- and

fat-loading methods to stimulate fat oxidation and retain a lot of glycogen in working muscles are needed for better endurance exercise performance.

References

[1] Van Loon, L.J.C.: Use of intramuscular triacylglycerol as a substrate source during exercise in humans. *J. Appl. Physiol.*, **97**, 1170–1187, 2004.

[2] Romijin, J.A., Coyle, E.F., Sidossis, L.S., Gastaldelli, A., Horowitz, J.F., Endert, E., and Wolfe, R.R.: Regulation of endogenous fat and carbohydrate metabolism in relation to exercise intensity and duration. *Am. J. Physiol. Endocrinol. Metab.*, **265**, E380–E391, 1993.

[3] Watt, M.J., Heigenhauser, G.J., Dyck, D.J., and Spriet, L.L.: Intramuscular triacylglycerol, glycogen and acetyl group metabolism during 4 h of moderate exercise in man. *J. Physiol.*, **541**, 969–978, 2002.

[4] Watt, M.J., Heigenhauser, G.J., and Spriet, L.L.: Intramuscular triacylglyerol utilization in human skeletal muscle during exercise: is there a controversy?. *J. Appl. Physiol.*, **93**, 1185–1195, 2002.

[5] Romijin, J.A., Coyle, E.F., Sidossis, L.S., Rosenblatt, J., and Wolfe, R.R.: Substrate metabolism during different exercise intensities in endurance-trained women. *J. Appl. Physiol.*, **88**, 1707–1714, 2000.

[6] Van Loon, L.J.C., Greenhaff, P.L., Constantin-Teodosiu, D., Saris, W.H.M., and Wagenmakers, A.J.M.: The effects of increasing exercise intensity on muscle fuel utilization in humans. *J. Physiol.*, **536**, 295–304, 2001.

[7] Schrauewn-Hinderling, V.B., Hesselink, M.K.C., Schrauwen, P., and Kooi, M.E.: Intramyocellular lipid contant in human skeletal muscle. *Obesity*, **14**, 357–367, 2006.

[8] Kiens, B., Essen-Gustavsson, B., Gap, P., and Lithell, H.: Lipoprotein lipase activity and intramuscular triglyceride stores after long-term high-fat and high-carbohydrate diets in physically trained men. *Clin. Physiol.*, **7**, 1–9, 1987.

[9] Zderic, T.W., Davidson, C.J., Schenk, S., Byerley, L.O., and Coyle, E.F.: High-fat diet elevates resting intramuscular triglyceride concentration and whole body lipolysis during exercise. *Am. J. Physiol. Endcrinol. Metab.*, **286**, E217–E225, 2004.

[10] Schrauwen-Hinderling, V.B., Schrauwen, P., Hesselink, M.K.C., van Engelshoven, J.M.A., Nicolay, K., Saris, W.H.M., Kessels, A.G.H., and Kooi, M.E.: The increase in intramyocellular lipid content is very early response to training. *J. Clin. Endocrinol. Metab.*, **88**, 1610–1616, 2003.

[11] Andersson, A., Sjodin, A., Hedman, A., Olsson, R., and Vessby, B.: Fatty acid profile of skeletal muscle phospholipids in trained and untrained young men. *Am. J. Physiol. Endocrinol. Metab.*, **279**, E744–E751, 2000.

[12] Turner, N., Lee, J.S., Bruce, C.R., Mitchell, T.W., Else, P.L., Hulbert, A.J., and Hawley, J.A.: Greater effect of diet than exercise training on the fatty acid profile of rat skeletal muscle. *J. Appl. Physiol.*, **96**, 974–980, 2004.

[13] Sen, C.K., Atalay, M., Ågren, J., Laaksonen, D.E., Roy, S., and Hänninen, O.: Fish oil and vitamine E supplementation in oxidative stress at rest and after physical exercise. *J. Appl. Physiol.*, **83**, 189–195, 1997.

[14] Bergmeyer, H.U. and Bernt, E.: D-Glucose: Determination with glucose oxidase and peroxidase, *in Methods of Enzymatic Analysis*, ed. By Bergmeyer, H.U., Academic Press, New York, pp. 1205–1215, 1974.

[15] Gutmann, I. and Wahlefeld.: L-(+)-lactate dehydrogenase and NAD, *in Methods of Enzymatic Analysis*, ed. By Bergmeyer, H.U., Academic Press, New York, pp. 1464–1468, 1974.

[16] Wieland, O. and Jagow-Westermann, B.V.: L-(+)-lactate. Determination with Yeast Lactate Dehydrogenase, *in Methods of Enzymatic Analysis*, ed. By Bergmeyer, H.U., Academic Press, New York, pp. 1483–1491, 1974.

[17] Lo, S., Russel, J.C., and Taylor, A.W.: Determination of glycogen in small tissues. *J. Appl. Physiol.*, **28**, 234–236, 1970.

[18] Folch, J., Lees, M., and Sloane-Standley, G.H.: A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.*, **226**, 497–509, 1957.

[19] Saitoh, S., Matsuo, T., Tagami, K., Chang, H., Tokuyama, K., and Suzuki, M.: Effects of short-term dietary change from high fat to high carbohydrate diets on the storage and utilization of glycogen and triacylglycerol in untrained rats. *Eur. J. Appl. Pysiol.*, **74**, 13–22, 1996.

[20] Lee, J.S., Bruce, C.R., Tunstall, R.J., Cameron-Smith, D., Hugel, H., and Hawley, J.A.: Interaction of exercise and diet on GLUT-4 protein and gene expression in Type I and Type Π rat skeletal muscle. *Acta. Physiol. Scand.*, **175**, 37–44, 2002.

[21] Spriet, L.L., Peters, S.J., Heigenhauser, G.J.F., and Jones, N.L.: Rat skeletal muscle triacylglycerol utilization during exhaustive swimming. *Can. J. Physiol. Pharmacol.*, **63**, 614–618, 1985.

[22] Phillips, S.M., Green, H.J., Tarnopolsky, M.A., Heigenhauser, G.J., Hill, R.E., and Grant, S.M.: Effects of training duration on substrate turnover and oxidation during exercise. *J. Appl. Physiol.*, **81**, 2182–2191, 1996.

[23] Starling, R.D., Trappe, T.A., Parcell, A.C., Kerr, C.G., Fink, W.J., and Costill, D.J.: Effects of diet on muscle triglyceride and endurance performance. *J. Appl. Physiol.*, **82**, 1185–1189, 1997.

[24] Anderson, A., Nalsen, C., Tengblad, S., and Vessby, B.: Fatty acid composition of skeletal muscle reflects dietary fat composition in humans. *Am. J. Clin. Nutr.*, **76**, 1222–1229, 2002.

[25] Ayre, K.J. and Hulbert, A.J.: Dietary fatty acid profile influences the composition of skeletal muscle phospholipids in rats. *J. Nutr.*, **126**, 653–662, 1996.

[26] Helge, J.W., Wu, B.J., Willer, M., Daugaard, J.R., Storlien, L.H., and Kiens, B.: Training affects muscle phospholipids fatty acid composition in humans. *J. Appl. Physiol.*, **90**, 670–677, 2001.

[27] Helge, J.W., Watt, P.W., Richter, E.A., Rennie, M.J., and Kiens, B.: Fat utilization during exercise: adaptation to a fat-rich diet increases utilization of plasma fatty acids and very

low density lipoprotein-triacylglycerol in humans. *J. Physiol.*, **537**, 1009–1020, 2001.

[28] Johnson, N.A., Stannard, S.R., Mehalski, K., Trenell, M.I., Sachinwalla, T., Thompson, C.H., and Thompson, M.W.: Intramyocellular triacylglycerol in prolonged cycling with high- and low-carbohydrate availability. *J. Appl. Physiol.*, **94**, 1365–1372, 2003.

[29] Helge, J.W.: Long-term fat diet adaptation effects on performance, training capacity, and fat utilization. *Med. Sci. Sports Exerc.*, **34**, 1499–1504, 2002.

[30] Havemann, L., West, S.J., Goedecke, J.H., Macdonald, I.A., Gibson, A.S.C., Noakes, T.D., and Lambert, E.V.: Fat adaptation followed by carbohydrate loading compromises high-intensity sprint performance. *J. Appl. Physiol.*, **100**, 194–202, 2006.