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Original Article

DIETARY FAT AND TRAINING ENHANCE UTILIZATION OF INTRAMUSCULAR TRIACYLGLYCEROL DURING EXERCISE

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The aim of this study was to investigate the effects of dietary fat levels combined with exercise training on the adaptation of muscle to use intramuscular triacylglycerol during exercise. Sixty male rats were randomly divided into three groups and were fed a normal fat diet (11% fat, NF) or moderate fat diet (20% fat, MF) or high fat diet (75% fat, HF) over 10 wk exercise training. Each dietary group was then assigned to three subgroups: endurance, exercise, and resting. The triacylglycerol storages in soleus and vastus lateralis muscles increased proportionally with increasing dietary fat levels. Fat diet consumption increased the activities of citrate synthase (CS) and β-hydroxyacyl-CoA dehydrogenase (3-HAD). The magnitude of increased activities of both enzymes was lesser in MF group compared to HF group. Adaptations to fat diets increased fat oxidation in soleus and vastus lateralis muscles during exercise. The average utilization rate of triacylglycerol in both muscles during exercise test in trained rats fed fat diets was significantly higher compared to those fed normal fat diets ($p<0.05$). Endurance performance was enhanced following fat diets. The endurance time was in order of HF>MF>NF. The highest utilization rate of triacylglycerol in muscles in HF group suggested that enhanced endurance performance was related to the magnitude of fat adaptation. In conclusion, muscle adaptation after fat diet consumption enhances utilization of intramuscular triacylglycerol (IMTG) as the energy source during exercise. The degree of adaptation is dependent on the dietary fat levels.

Key words: Fat, diet, muscle, triacylglycerol, exercise

The principal substrates that fuel aerobic ATP synthesis in skeletal muscle during exercise are fat and carbohydrate. Fat represents a major fuel for skeletal muscle at rest and during exercise at low and moderate intensities whereas CHO metabolism becomes quantitatively more important with increasing exercise intensity (Asmussen, 1971). Prolonged exercise of moderate intensity has been shown to result in a time dependent increase in fat oxidation and a decrease in carbohydrate oxidation (Brooks and Mercier, 1994; Phillips et al., 1996). The dependency of fat oxidation becomes even more pronounced after exercise training. In addition, consumption of a high fat, low carbohydrate-diet leads to increased rate of fat oxidation to maintain body composition (Flatt, 1995). The rate of adaptation to a high fat diet depends on the level of physical activity (Schrauwen et al., 2000; Smith et al., 2000) and aerobic fitness (Smith et al., 2000). Previous studies have shown that in

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those who exercise regularly, this adaptation is rapid and is manifest at rest (Schrauwen et al., 2000) and during exercise (Schrauwen et al., 2000; Starling et al., 1997).

IMTG contents have been shown to be increased with fat diet consumption, (Coyle et al., 2001; Kiens et al., 1987; Starling et al, 1997) and IMTG concentration is positively related to IMTG breakdown (Steffensen et al., 2002). It has been proposed that an increase in IMTG concentration and subsequent utilization contribute to the increase in fat oxidation following a high fat diet (Zderic et al., 2004). During exercise, the sources of oxidized fat are from circulating fatty acids derived from adipose tissue lipolysis, very low density lipoprotein triacylglycerol and IMTG. Among the sources of fat, the IMTG pool offers a readily available pool of fatty acid during exercise conditions. However, there has been much controversy on the contribution of IMTG oxidation to total energy expenditure during exercise. Studies in endurance-trained subjects, a decrease in IMTG content following exercise seems to be time dependent (Watt et al., 2002). IMTG content decreases as much as a 50% after more prolonged moderate- to high-intensity exercise (2-7 h) (Costill et al., 1973; Froberg et al., 1984) whereas smaller, nonsignificant decreases (~10-20%) have been reported after only 1-2 h of exercise (Watt et al., 2002; Roepstorff et al., 2002). Some studies has also been reported that IMTG stores do not play a significant role during moderate-intensity exercise in endurance-trained men as opposed to trained women (Roepstorff et al., 2002; Steffensen et al., 2002) but rather form an important substrate source during postexercise recovery (Kiens and Richter, 1998). It could be argued that this discrepancy is related to the heterogeneity in the research protocols used (mode, duration, and/or intensity of the exercise) and/or the selected subject population training status and/or nutritional status).

It has been well accepted that an increase in fat oxidation results in a reduction in glycogen and blood glucose utilization, thereby sparing muscle glycogen. Such glycogen sparing effects have additional benefits for physical performance (Burke and Hawley 2000; Costill et al., 1973). However, one of the drawbacks in promoting fat diet as an ergogenic aid is the strong relationship between high fat diet and fat body deposition (Miller et al., 1984 and Oscai et al., 1987). Most studies have focused on the optimal fat intake to induce positive adaptation in energy metabolism and endurance performance. Previous study of Veerapun et al. (2002) demonstrated that trained rats fed with either high carbohydrate (79.9% E) or high fat diet (74.85% E), followed by diet containing fat 20.14% E showed a significantly enhanced endurance performance compared with those received only high carbohydrate or high fat diet. Furthermore, data of blood borne substrates indicated a shift of energy substrates from CHO to fat during exercise in trained rats received only HF (74.85% E) and moderate fat (20.14%) diets. However, the substrate utilization in muscle has not been described. We hypothesized that muscle substrate storage play an important role as a substrate source during exercise following fat diets. The purpose of this study was to determine the effects of increasing dietary fat consumption together with training on the utilization of IMTG as the energy source during exercise, and whether these effects are dependent on the dietary fat levels.

Materials and Methods

Animals

Sixty male Wistar rats, weighing between 120-150 g, were purchased from the National Animal Center, Salaya Campus, Mahidol University. All animals were housed individually in rodent cages in the animal room where the temperature was maintained approximately at 24-25 ^{o}C with 12:12 hour dark-light cycle. After being acclimatized for at least 5 days, the animals were randomly divided into 3 groups: normal fat (NF), moderate fat (MF) and high fat (HF) groups. The NF group was provided with a normal fat diet, the MF group consumed a moderate fat diet, and the HF group received a high fat diet during 10 weeks of experimental period. The composition of

diets is shown in Table 1. Food and water were provided *ad libitum*. All animals were enrolled in the exercise-training program during the period of dietary intervention.

At the end of 10 wk training, the animals were allowed to rest for 2 days. On the final day of the experiment, animals in each group were assigned to three subgroups: endurance, exercise, and resting subgroups. The animals in endurance subgroup were subjected to endurance test and endurance times were determined. The animals in exercise subgroup received similar endurance test protocol and the animals in resting subgroup served as resting control. All animals were immediately sacrificed post-exercise for collection of blood and tissue samples.

Table 1. Composition of experiment diets

Ingredients	NF		MF		HF	
	g	%kcal	g	%kcal	g	%kcal
Cornstarch	680	68.51	590	59.44	49.62	5
Lard	50	11.34	90	20.14	330.2	74.85
Casein	200	20.15	200	20.15	200	20.15
Vitamins and minerals	70	0.0	70	0.0	70	0.0

Diets ingredients and nutrient analyses were modified from Conlee et al. (1990). Energy (Kcal) per gram: carbohydrate 4; fat 9; protein 4. All diets were isocaloric except that the dietary fat contents increased in the order of NF<MF<HF. NF, normal fat diet; MF, moderate fat diet; HF, high fat diet.

Exercise training

The exercise-training program was modified from the training protocol of Mokelke et al. (1997). All animals were trained on a motorized rodent treadmill, 5 days/week for a period of 10 weeks. Initially, the animal ran for 10 min at 20 m/min with 5% grade for 3 weeks. The duration and treadmill speed were progressively increased until the animal was running for 60 min/day at 28 m/min with a 10% grade; this exercise intensity was achieved after 6 week, and they were then maintained on this exercise training until the end of training period.

Endurance test

The submaximal running endurance was determined in trained rats, according to method of Mercier et al. (1995), by having them run at 30 m/min at 10% grade until they could no longer keep pace with the treadmill or they were exposed to electric grid at the rear of the treadmill five times in two minutes. At that point, the time to exhaustion or endurance time was recorded and the rats were immediately anesthetized for surgery.

Exercise test

To investigate whether IMTG contributed to energy metabolism during the initial stage of exercise, the rats were subjected to exercise test protocol. The fundamental procedure of the exercise test was similar to endurance test protocol except for the duration of exercise. The speed of treadmill was 30 m/min at 10% grade and the duration of exercise running in each subgroup was only 1/3 of the individual average endurance time. Consequently, all rats received approximately the same relative exercise intensity.

Animal sacrifice and tissue preparation

All animals were anesthetized with Pentobarbital sodium (50 mg/kg body weight) administered intraperitoneally. Once the anesthesia took effect, both hind legs were skinned and samples of the soleus and vastus lateralis muscles were surgically removed, trimmed of connective tissue and adherent adipose tissue and immediately frozen in liquid nitrogen. The abdominal cavity was opened and one lobe of liver was excised and similarly frozen. Blood was drawn from the heart, placed into glass tubes containing sodium fluoride and potassium oxalate anticoagulant, and centrifuged. The plasma and tissue samples were stored at -70^{o}C until time for assay.

Biochemical analysis

Glucose concentration in plasma was determined by enzymatic colorimetric method using commercial kits (Biosub GLU, Biocon Diagnostik, Germany).

The plasma triacylglycerol or glycerol concentration was analyzed using a commercial enzymatic colorimetric kit (Fluitest TG, Biocon Diagnostik, Germany).

Total cholesterol level in plasma was analysed by enzymatic colorimetric method using commercial kits (Cholesterol Des, Erba Diagnostics Mannheim, Germany).

Determination of muscle glycogen content

The glycogen was determined as glucose residues after hydrolysis by amylo-α-1, 4-α1, 6-glucosidase (AG) enzyme. Tissue homogenates were prepared for glycogen assay by a modification of the method of Passonneau and Lauderdale (1974). A 100-200 mg portion of liver or muscle was minced and placed in 3-5 ml of 0.03 N HCl. The homogenate was placed in a boiling water bath for 5 min and was centrifuged at 4,000 rpm for 15 minutes to obtain clear supernatant. A 150 µl of supernatant was added into a glass tube containing 150 µl of 0.4 M acetate buffer (0.2 M acetic acid: 0.2 M sodium acetate, pH 4.7) and 50 µl of AG. The reagent was then mixed and incubated at 37^{o}C in a water bath for 2 hours. After that, the glucose formed was determined by enzymatic colorimetric method using commercial kits (Biosub GLU, Biocon Diagnostik, Germany). The glycogen utilization rate was calculated as the average rate of muscle glycogen utilization (µg/gm tissue/min) = (Resting muscle glycogen – Muscle glycogen after exercise test or exhaustion)/ Duration of exercise test or endurance time (Lapachet et al., 1996).

Determination of tissue triacylglycerol content

Tissue homogenates were prepared for triacylglycerol assay by a modified method of Frayn and Maycock (1980). A 100-200 mg portion of liver or muscle was minced and put into a glass tube containing 3 ml of chloroform-isopropanol 7:11 (v/v). The homogenate was left at room temperature for at least 16 hours. Then, a 1 ml of homogenate was pipetted into a glass tube and evaporated to dryness at 40 ^{o}C for 16 hours. The dried residue was dissolved and mixed in 10% bovine serum albumin. The triacylglycerol concentration was analyzed with a commercial enzymatic colorimetric kit (Fluitest TG, Biocon Diagnostik, Germany). The triacylglycerol utilization rate was calculated as the average rate of muscle triacylglycerol utilization (µg/gm

tissue/min) = (Resting muscle triacylglycerol – Muscle triacylglycerol after exercise test or endurance exercise)/Duration of exercise or endurance time.

Statistical analysis

Data were presented as means + SE. A one-way analysis of variance (ANOVA) followed by Fisher's post-hoc test was used to analyse the group differences and significance was taken at $p<0.05$.

Results

Body weight and energy intake

At the end of week 10, body weights were similar in all dietary groups and the average weight gains were about 47, 45 and 49% in NF, MF and HF groups, respectively (Table 2). Correspondingly, an analysis of dietary record showed that the total energy intakes over 10 wk-exercise were not significant differences between the dietary groups ($p>0.05$).

Table 2. Body weights and energy intakes of trained rats on diets of increasing dietary fat levels

	Group		
	NF	**MF**	**HF**
Body wt. (g)			
Initial wt.			
Final wt.	37.23 ± 2.75	38.61 ± 3.47	81.93 ± 2.91 [a, b]
Energy intake			
kcal/wk	1767.78 ± 10.65	1742.46 ± 9.95	1749.67 ± 9.38

Each value represents mean ± SE from 24 rats. NF, normal fat diet; MF, moderate fat diet; HF, high fat diet. [*], significantly different from initial, $p < 0.01$.

Blood parameters and muscle substrate storage

As shown in Table 3A, the plasma triacylglycerol level was significantly higher in HF group than in NF and MF groups (both $p<0.01$). There were no differences in total cholesterol levels among the dietary groups ($p>0.05$) whereas plasma glycerol concentrations were significantly increased in animals received diets with increasing fat contents (MF and HF groups)(both $p<0.01$). The plasma glucose concentrations were also similar in all groups regardless of diet composition.

The effects of increasing dietary fat levels on glycogen and triacylglycerol storage in muscles are presented in Table 3B. The glycogen concentrations in vastus lateralis and soleus muscles were significantly low in HF group compared with MF and NF groups (both $p<0.01$). In addition, the glycogen concentration in both muscles were also significantly lower in MF than in NF groups ($p<0.01$). In contrast to glycogen, the triacylglycerol storages in vastus lateralis and soleus muscles were highest in HF group being significantly different from NF and MF group (both $p<0.01$). Compared with NF group, muscle TG concentrations in MF group was significantly higher ($p<0.01$).

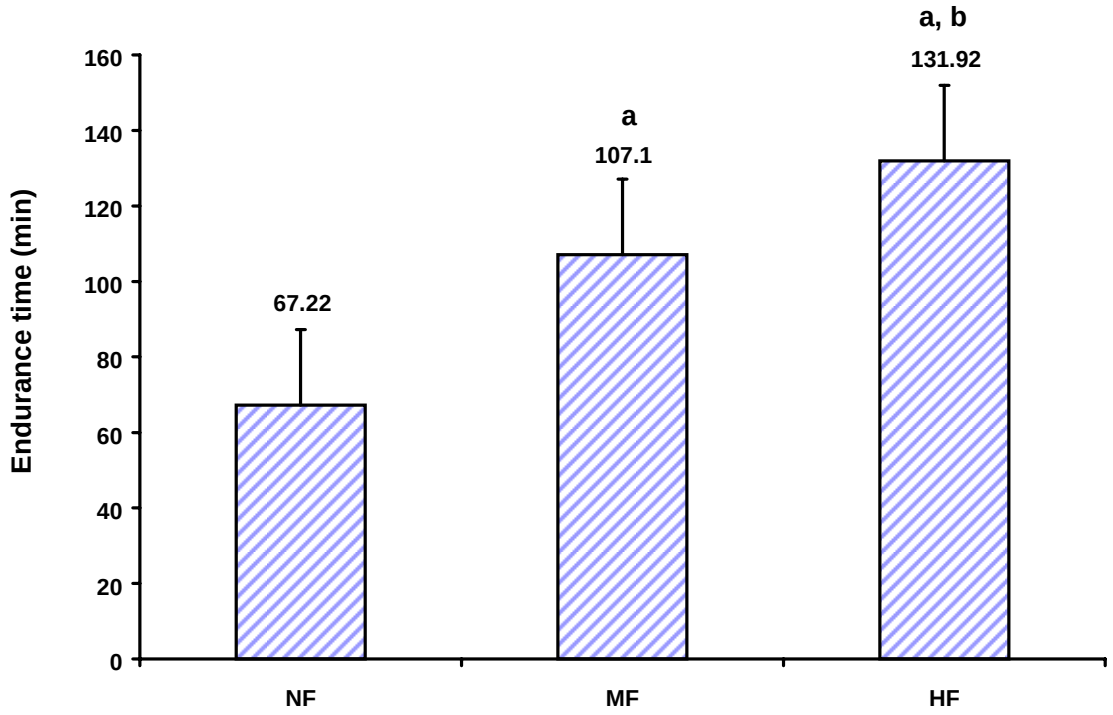

Figure 1. Endurance time of trained rats on diets of increasing dietary fat levels. Each value represents mean ± SE for 8 rats in each group. NF, normal fat diet; MF, moderate fat diet; HF, high fat diet. [a] , significantly different from NF group, $p<0.01$; [b] , significantly different from MF group, $p<0.01$.

Endurance performance

Mean endurance time was significantly longest in the HF group ($p<0.01$)(Figure 1). The HF group ran longer than the MF group by 19% and ran longer than the NF group by 96%. Compared with the NF group, however, endurance time in the MF group was significantly longer by 59% ($p<0.01$).

Muscle substrate utilization

As shown in Table 4, the glycogen concentrations in vastus lateralis and soleus muscles after exercise test significantly decreased in NF and MF groups as compared with its resting value (both $p<0.01$). The average utilization rate of glycogen in either vastus lateralis or soleus muscle was significantly higher in NF than in MF groups ($p<0.01$). When expressed as the total utilization of glycogen in both muscles, there was a decrease of intramuscular glycogen utilization with increasing dietary fat content. The total utilization rate of muscle glycogen was apparently depressed in HF group compared with NF group ($p<0.05$). A decrease of IMTG concentration, either in vastus lateralis or soleus muscle, in response to exercise test was observed in MF and HF groups (both $p<0.05$). In NF group, the triacylglycerol concentration after exercise test significantly decreased in vastus lateralis muscle ($p<0.05$) while it slightly changed in soleus muscle. The total utilization rate of muscle triacylglycerol was highest in HF group and lowest in NF group. Likewise, the total utilization rate of muscle triacylglycerol was significantly higher in MF than in NF groups ($p<0.01$).

After endurance exercise, both glycogen and TG concentrations in vastus lateralis and soleus muscles significantly depleted in all dietary groups (all $p<0.05$) (Table 5). The total utilization rate of muscle glycogen was lowest in HF group while it was highest in NF group. In MF group, the total utilization rate of muscle glycogen was significantly different from NF and HF groups (both $p<0.01$). There were significant decreases of IMTG, both in vastus lateralis and soleus muscles, in all exhausted groups (all $p<0.05$). The IMTG utilization during endurance exercise was enhanced in animals received diets with increasing fat contents. The total utilization rate of intramuscular triacylglycerol in HF group was higher compared with MF and NF groups (both $p<0.01$). In MF group, the total utilization of intramuscular triacylglycerol in muscle appeared to be higher than NF group although there was no significant difference ($p<0.05$).

Table 3. Blood parameters (A) and muscle substrate storages (B) of trained rats on diets of increasing dietary fat levels

A.

	Group		
	NF	**MF**	**HF**
Triacylglycerol (mg/dl)	37.23 ± 2.75	38.61 ± 3.47	81.93 ± 2.91 [a, b]
Total cholesterol (mg/dl)	31.00 ± 1.82	34.50 ± 0.50	39.17 ± 4.94
Glycerol (mg/dl)	8.28 ± 0.77	12.42 ± 0.48 [a]	16.44 ± 0.73 [a, b]
Glucose (mg/dl)	169.79 ± 5.28	164.60 ± 2.08	170.85 ± 4.92

B.

Substrate	Group		
	NF	**MF**	**HF**
Glycogen (mg/gm tissue)			
Vastus lateralis muscle	2.22 ± 0.03	1.87 ± 0.06 [a]	1.33 ± 0.09 [a, b]
Soleus muscle	3.00 ± 0.08	2.04 ± 0.07 [a]	1.46 ± 0.03 [a, b]
Triacylglycerol (mg/gm tissue)			
Vastus lateralis muscle	1.82 ± 0.04	3.34 ± 0.03 [a]	9.49 ± 0.21 [a, b]
Soleus muscle	5.34 ± 0.09	6.87 ± 0.22 [a]	11.15 ± 0.32 [a, b]

Each value represents mean ± SE from 8 rats. NF, normal fat diet; MF, moderate fat diet; HF, high fat diet. [a], significantly different from NF group, $p < 0.01$; [b], significantly different from MF group, $p < 0.01$.

Table 4. Muscle substrate utilization during exercise test of trained rats on diets of increasing dietary fat levels

A. Intramuscular glycogen

	Group		
	NF	**MF**	**HF**
Vastus lateralis muscle			
at rest (mg/gm tissue)	2.22 ± 0.03	1.87 ± 0.06[a]	1.33 ± 0.09 [a, b]
after exercise test (mg/gm tissue)	1.70 ± 0.01*	1.68 ± 0.02*	1.33 ± 0.02
utilization rate (μg/gm tissue/min)	25.92 ± 0.59	5.33 ± 0.60[a]	0.01 ± 0.42 [a, b]
Soleus muscle			
at rest (mg/gm tissue)	3.00 ± 0.08	2.04 ± 0.07[a]	1.46 ± 0.03 [a, b]
after exercise test (mg/gm tissue)	2.53 ± 0.04*	1.80 ± 0.02*	1.46 ± 0.02
utilization rate (μg/gm tissue/min)	23.51 ± 1.86	6.72 ± 0.48[a]	1.25 ± 0.44 [a, b]
Total utilization (μg/gm tissue/min)	49.42 ± 2.05	12.04 ± 0.64 [a]	0.73 ± 0.56 [a, b]

B. Intramuscular triacylglycerol

	Group		
	NF	**MF**	**HF**
Vastus lateralis muscle			
at rest (mg/gm tissue)	1.82 ± 0.04	3.34 ± 0.03 [a]	9.49 ± 0.21 [a, b]
after exercise test (mg/gm tissue)	1.72 ± 0.02 *	2.80 ± 0.05 *	6.49 ± 0.11 *
utilization rate (μg/gm tissue/min)	4.86 ± 0.95	15.35 ± 1.45 [a]	66.65 ± 2.37 [a, b]
Soleus muscle			
at rest (mg/gm tissue)	5.34 ± 0.09	6.87 ± 0.22 [a]	11.15 ± 0.32 [a, b]
after exercise test (mg/gm tissue)	5.08 ± 0.08	5.89 ± 0.17 *	6.35 ± 0.16 *
utilization rate (μg/gm tissue/min)	17.15 ± 3.47	27.92 ± 4.94 [a]	106.68 ± 13.38 [a, b]
Total utilization (μg/gm tissue/min)	22.62 ± 3.14	43.27 ± 4.62 [a]	173.33 ± 3.97 [a, b]

Each value represents mean ± SE from 8 rats. NF, normal fat diet; MF, moderate fat diet; HF, high fat diet. The glycogen utilization rate in muscle is expressed as an average rate of use over time and does not necessarily reflect a linear relationship. *, significantly different from rest, $p < 0.05$; [a] ; significantly different from NF group, $p < 0.01$; [b], significantly different from MF group, $p < 0.01$.

Table 5. Muscle substrate utilization during endurance exercise of trained rats on diets of increasing dietary fat levels

A. Intramuscular glycogen

	Group		
	NF	**MF**	**HF**
Vastus lateralis muscle			
at rest (mg/gm tissue)	2.22 ± 0.03	1.87 ± 0.06 [a]	1.33 ± 0.09 [a, b]
after exhastion (mg/gm tissue)	1.31 ± 0.02 *	1.40 ± 0.01 *	1.16 ± 0.01 *
utilization rate (μg/gm tissue/min)	13.56 ± 0.25	4.37 ± 0.13 [a]	1.27 ± 0.09 [a, b]
Soleus muscle			
at rest (mg/gm tissue)	3.00 ± 0.08	2.04 ± 0.07 [a]	1.46 ± 0.03 [a, b]
after exhaustion (mg/gm tissue)	1.52 ± 0.03 *	1.01 ± 0.02 *	1.28 ± 0.02 *
utilization rate (μg/gm tissue/min)	22.07 ± 0.43	9.59 ± 0.21 [a]	1.34 ± 0.18 [a, b]
Total utilization (μg/gm tissue/min)	35.63 ± 0.55	13.96 ± 0.24 [a]	2.61 ± 0.20 [a, b]

B. Intramuscular triacylglycerol

	Group		
	NF	**MF**	**HF**
Vastus lateralis muscle			
at rest (mg/gm tissue)	1.82 ± 0.04	3.34 ± 0.03 [a]	9.49 ± 0.21 [a, b]
after exhaustion (mg/gm tissue)	1.43 ± 0.02 *	1.94 ± 0.04 *	2.50 ± 0.05 *
utilization rate (μg/gm tissue/min)	5.87 ± 0.36	13.04 ± 0.33 [a]	52.96 ± 0.36 [a, b]
Soleus muscle			
at rest (mg/gm tissue)	5.34 ± 0.09	6.87 ± 0.22 [a]	1.15 ± 0.32 [a, b]
after exhaustion (mg/gm tissue)	2.41 ± 0.07 *	2.70 ± 0.06 *	3.41 ± 0.11 *
utilization rate (μg/gm tissue/min)	43.62 ± 1.06	38.89 ± 0.55 [a]	58.65 ± 0.80 [a, b]
Total utilization (μg/gm tissue/min)	49.50 ± 1.06	51.94 ± 0.65 [a]	111.61 ± 1.00 [a, b]

Each value represents mean ± SE from 8 rats. NF; normal fat diet; MF, moderate fat diet; HF, high fat diet. The triacylglycerol utilization rate in muscle is expressed as an average rate of use over time and does not necessarily reflect a linear relationship. Likewise, the liver triacylglycerol mobilization rate represents average rate of mobilization over time. * , significantly different from rest, $p < 0.05$; [a] , significantly different from NF group, $p < 0.01$; [b] , significantly different from MF group, $p < 0.01$.

Table 6 Muscle enzyme activity of trained rats on diets of increasing dietary fat levels.

Tissue	Group		
	NF	MF	HF
Vastus lateralis muscle			
CS (mM/gm tissue/min)	9.44 ± 0.28	13.58 ± 0.56 [a]	17.14 ± 0.70 [a, b]
3-HAD (mM/gm tissue/min)	0.85 ± 0.02	0.99 ± 0.08	1.74 ± 0.11 [a, b]
Soleus muscle			
CS (mM/gm tissue/min)	15.67 ± 0.72	23.11 ± 0.73 [a]	27.33 ± 0.79 [a, b]
3-HAD (mM/gm tissue/min)	1.71 ± 0.05	1.96 ± 0.03 [a]	2.37 ± 0.08 [a, b]

Each value represents mean ± SE from 8 rats. NF, normal fat diet; MF, moderate fat diet; HF, high fat diet. CS; Citrate synthase activity, 3-HAD; 3-hydroxyacyl CoA dehydrogenase activity. [a], significantly different from NF group, $p < 0.01$; [b], significantly different from MF group, $p < 0.01$.

Muscle enzyme activity

Increasing dietary fat was associated with increased oxidative capacity of skeletal muscle. As shown in Table 6, the CS activity in vastus lateralis and soleus muscles significantly increased in HF and MF groups compared with NF group (both $p<0.01$). Of the two groups received fat diets, the CS activities were significantly higher in HF than in MF groups ($p<0.01$). The 3-HAD activity, an index of relative capacity for oxidative of fatty acid, was also enhanced by dietary fat. Increased 3-HAD activity in soleus muscle was in order of HF>MF>NF groups. In vastus lateralis muscle, 3-HAD activity was significantly high in HF group compared with MF and NF groups (both $p<0.01$) whereas its activity in MF group was comparable to NF group.

Discussion

The two main findings of this study were that IMTG storage was increased with increasing dietary fat contents. A metabolic adaptation induced by an increase of fat intake was associated with an increased utilization of muscle TG and this resulted in enhanced endurance performance.

The results obtained herein showed a substantial increase of plasma glycerol level in trained rats received HF and MF diets. As the plasma glycerol level is an indication of whole body lipolysis (Jensen, 2003), the increased lipolysis following increasing dietary fat appeared to compensate for lower CHO availability (William et al., 2000). This finding demonstrated that increasing dietary fat induced metabolic adaptations toward increased fat oxidation at rest. Insulin is known to be an important inhibitor of lipolysis (Cambell et al., 1992). A lower insulinemia at rest and during exercise has been found during fat diet (Maughan et al., 1997). Thus, an enhanced lipolysis in response to a lower insulinemia during increasing fat intake might be suggested. A lower blood insulin leads to less inhibition of lipolysis, resulting in increased plasma glycerol levels and higher circulating free fatty acids. In rats, glycerol is a highly gluconeogenic substrate (Maughan et al., 1997). It was likely that during fat diet with a low carbohydrate availability glycerol from adipose tissue lipolysis was directed toward gluconeogenesis. This was reflected by the similar resting plasma glucose level in all groups regardless of diet composition.

IMTG storage has been shown to increase following high fat diets (Kiens et al., 1987; Starling et al., 1997; Straczkowski et al., 2001). Consistently, the present study also showed that

the magnitude of elevated muscle storage increased proportionally with increasing dietary fat content. The IMTG concentration in HF group was significantly higher compared with MF group. It has been proposed that an increased muscle triacylglycerol concentration following increased fat intake is associated with the enzyme lipoprotein lipase (LPL) activity. Several investigators have demonstrated an increased activity of skeletal muscle LPL after several days (Jacobs et al., 1982) or weeks (Kiens et al., 1987) of fat diet. In addition, skeletal muscle LPL activity has been shown to increase after training (Oscai et al., 1990; Turcotte et al., 1995). An increased LPL activity following fat diet and exercise training indicates a higher capacity for uptake of fatty acids from circulating triacylglycerols into the muscle cell in association with greater capacity for TG storage in the muscle (Kiens et al., 1987).

It has been proposed that the increased TG storage in muscle after fat adaptation might be substitute as fuel source in association with the decreased glycogen deposition. Considering the observed substantial decreases of TG concentrations in both vastus lateralis and soleus muscles after exercise test and endurance exercise in MF and HF groups, it was suggested that IMTG significantly contributed to the energy source during exercise in trained rat with increasing fat intake. The most significant finding in this study was an increased utilization of intramuscular fat during exercise test. Although the substrate utilization rate per se was not measured periodically throughout the exercise period in the present study, the calculated average rate of substrate utilization can be used as an approximation of substrate utilization rate throughout the entire exercise period (Lapachet et al., 1996). The total utilization of intramuscular triacylglycerol during exercise test was in order of HF > MF > NF. These findings suggested the change of muscle substrate utilization toward higher fat oxidation following fat adaptation. Further evidence supported the increased intramuscular fat oxidation in MF and HF groups was the activities of two key mitochondria enzymes ; CS, a key enzyme in TCA cycle and 3-HAD, a key enzyme in β-oxidation. The current study found, in harmony with other reports (Miller et al., 1984), that activity of these two enzymes increased dramatically in soleus and vastus lateralis muscles with HF diet. The increased activities of both CS and 3-HAD in muscles also presented in MF group although these changes were apparent in soleus muscle. The lesser magnitude of increased activities of both enzymes might explain the lower utilization of IMTG in MF group compared with HF group.

The present study also showed the contribution of IMTG pool as a substrate source during endurance exercise. However, the total utilization of IMTG over the entire period of endurance exercise was prominent in HF group compared with MF group. An alternative mechanism for the increased fat oxidation in muscles of HF group was associated with the carnitine palmitoeyl transferase I (CPT-I) enzyme. CPT-I plays important role in the transport of fatty acid CoA via carnitine, the main-rate-limiting step in the utilization of fatty acids for energy production in muscle. A significant increase of CPT-I activity has been found in muscles of trained rats fed high fat diet (78%E) (Simi et al., 1991). However, the activity of CPT-I is reversibly inhibited by malonyl CoA (Maughan et al., 1997). Increased carbohydrate availability has been linked with increased acetyl CoA and malonyl CoA concentrations in muscle and liver (Saha et al 1995; Sidossis et al 1996). Thus, the increased carbohydrate amount in MF diet might partly inhibit CPTI activity that reduced uptake of fatty acids into the mitochondria and thus lowered triacylglycerol-derived fatty acid oxidation.

The dietary fat induced changes toward higher fat oxidation during exercise and concomitant glycogen sparing was considered to favor endurance performance. Previous studies regarding the effects of dietary fat on endurance performance provided a conflicting picture. Helge et al (1998) did not found enhanced endurance in trained or untrained rats consumed fat diets (65%E) despite of a shift toward increased fat utilization during exercise. The positive effect of fat diet on endurance performance in rats, however, was demonstrated when dietary fat content was

~ 75-85%E (Lapachet et al., 1996; Miller et al., 1984; Simi et al., 1991). The current study clearly showed that the endurance performance was enhanced in trained rats received either MF (20.14%E) or HF (74.85%E) diet (Figure 1). The most marked improvement in endurance performance was seen in the HF group whereas the increase of endurance performance in MF group was significantly different from the NF group. From the present data it could be speculated that the degree of enhanced endurance capacity in trained rats consumed diets with increasing fat content was related to the proportion of metabolic adaptations. Previous study demonstrated the great enhanced endurance performance was observed in trained rats primed with high fat diets (74.85%E) and then switched to moderate fat diets (20.14%E) (Veerapun et al., 2002). It might be proposed that after the metabolic adaptations induced by high fat diets in trained rats, an increase of carbohydrate availability in diets is an anaplerotic supply of oxaloacetate for fat metabolism in tricarboxylic acid cycle.

In summary, the present study increasing dietary fat intake for 10 wk-exercise training was associated with elevated muscle triacylglycerol storage. Adaptations of muscle to increase of dietary fat levels enhanced the activities of CS and 3-HAD enzymes. The magnitude of increased enzyme activities depended on the dietary fat levels. In addition, fat adaptations enhanced the utilization of IMTG as the substrate source during exercise. The enhanced endurance performance after increasing dietary fat intake was directly related to the utilization rate of IMTG.

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