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The Effects of A High-Fat Diet on Pro- and Macro-Glycogen Accumulation and Mobilization During Exercise in Different Muscle Fiber Types and Tissues in Rats*

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We investigated the effects of diet manipulation on pro- and macro-glycogen accumulation and mobilization during exercise in different kinds of muscle fiber and tissue. Thirty-two Sprague-Dawley rats were divided into groups representing one of two dietary conditions: high fat (HF, n=16) or standard chow (CHOW, n=16). Each dietary group was further divided into control (REST, n=8) and exercise (EXE, n=8). After an eight-week dietary intervention period, the animals in EXE swam for 3 hours while the animals in REST remained at rest. Skeletal muscle (soleus, red gastrocnemius and white gastrocnemius) and liver samples were then dissected out and used for analyses. There was no statistical difference in body weight between the animals in the HF and CHOW groups (p>.05). Three hours of exercise significantly increased plasma free fatty acid (FFA) concentration in the animals in the CHOW group but not in the animals in the HF group. Both citrate synthase (CS) and β-hydroxyacyl dehydrogenase (β-HAD) activities in skeletal muscles were higher in the HF group than in the CHOW group. CS and β-HAD activities were also the highest in red gastrocnemius and the lowest in white gastrocnemius. At both time points (i.e., rest and immediately after exercise) intramuscular triglyceride (IMTG) and liver TG concentrations were significantly higher in the HF compared to the CHOW. IMTG and liver TG changed selectively in the CHOW. Except in white gastrocnemius muscle, there was no significant difference in total glycogen content between HF and CHOW at rest. Although exercise significantly lowered total glycogen content in all groups and tissues (p<.05), the degree of reduction was markedly greater in the CHOW than in the HF. Whereas changes in proglycogen concentration showed a trend similar to those of total glycogen, alterations in macroglycogen concentrations clearly differed from those of total glycogen. Specifically, the degree of reduction of macroglycogen following three hours of exercise was substantially greater in the CHOW than in the HF. These results suggest that metabolic alterations induced by a long-term high fat diet may be caused by macro-glycogen rather than pro-glycogen.

Key Words: Proglycogen, Macroglycogen, High fat fed, Exercise

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INTRODUCTION

Glycogen is an important energy source during prolonged strenuous exercise and exercise must be abandoned if glycogen stores are depleted. Glycogen storage in the body is limited (300~500 gram).[1] As exercise continues, stored glycogen in the skeletal muscle and liver becomes depleted rapidly and exercise performed at 65~75% of maximal oxygen consumption can be maintained only for 90~120 min.[2] Therefore, amelioration of glycogen depletion during prolonged strenuous exercise can be a major determinant of endurance.[3,4] A number of studies have tried to maximize glycogen storage in muscle and the liver.[1,5] Recently, some studies have been undertaken with the aim of identifying the morphologic construction and individual function of glycogen.[6-8]

Since early studies have suggested that the size of glycogen molecules is not uniform,[9,10] recent biochemical and electron microscopic studies have revealed that glycogen exists in two pools (so-called proglycogen [PG] and macroglycogen [MG]) on the

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basis of solubility in acid.[11-13] These two forms of glycogen have similar protein compositions but vary in their carbohydrate content. PG has been found to precipitate in trichloracetic acid because of its 10% protein component[12] but MG, with a protein content of only 0.35%, is soluble.[14] MG, also called "classic glycogen," is a larger molecule (10,000 kDa) than PG (~400 kDa), considered to be a precursor of MG, but serves as a stable intermediate on the pathways to and from the MG depot.[11] Evidence has been presented that the molecular mass of PG may reach close to 1,000 kDa when muscle glycogen concentration is high.[15,16] It has also been suggested that glycogen oscillates between the macroglycogen and proglycogen forms[7,11] in accordance with glucose supply and energy demand.

In resting muscle containing normal levels of glycogen, only a small fraction (15~25%) of the total glycogen pool is found in the form of MG. In glycogen-supercompensated muscle, however, this MG supply may increase to 50% or more, becoming the dominant form.[11,17] Conversely, in glycogen-depleted muscle, very little glycogen (~10%) exists in the form of MG.[16] Lomako *et al.*[13] have suggested that glycogen synthase acts differently on PG and MG, depending on the level of glycogen in skeletal muscle. This would suggest that the two pools of glycogen are metabolically distinct.[13] Although these studies have tried to shed light on PG and MG metabolism, little is known about the physiological function and the metabolic differences between the two largely unknown forms of glycogen. In addition, it has not been reported whether PG and MG are differentially regulated following long-term administration of a high-fat diet. In this study, therefore, we examined the effects of a high-fat diet on PG and MG accumulation and mobilization during exercise in different types of muscle fiber and tissue. As long-term administration of a high-fat diet provokes metabolic changes leading to the more effective use of lipid sources to provide required energy during exercise, this study looks at which forms of glycogen are better for storage or mobilization as dietary manipulation is adopted.

MATERIALS AND METHODS

1. Animal Care

Thirty-two male Sprague-Dawley rats (initial body mass 90~110 g) were obtained from Samtako, Bio Korea (Seoul, Korea) and housed four per cage in an environmentally controlled laboratory (temperature 22±1℃, humidity 50±2%) with a 12:12 light:dark cycle (light, 7:00 AM to 7:00 PM). The animals were fed standard rodent chow (67.5 E% carbohydrate, 11.7 E% fat, 20.8 E% protein; Samtaco, Korea) and were acclimatized to laboratory conditions for one week. The animals were divided into groups representing one of two dietary conditions: high fat (HFD, n=16) and standard chow (CHOW, n=16). The animals under each dietary condition were further randomly assigned to one of two subgroups: control (REST, n=8) and exercise (EXE, n=8). The Animal Ethics Committee of Korea National Sport University approved all experimental procedures.

2. Dietary Treatments and Exercise Program

After being acclimatized to the environmental conditions, the animals in the CHOW group were fed a diet of standard rodent chow, while the animals in the HF group were put on a high-fat diet (52.8 E% fat, 22.9 E% protein and 24.3 E% carbohydrate). The nutritional composition and energy content of both diets are shown in Table 1. The high-fat diet was freshly prepared every 2 weeks and stored at 4 ℃. The vitamin and mineral content of both diets was in accordance with the guidelines of the

Table 1. Composition and energy content of the experimental diets

Ingredients	CHOW ($g \cdot 100g^{-1}$ of diet)	HFD ($g \cdot 100g^{-1}$ of diet)
Carbohydrate sources;		
Corn starch	48.3	
Rice starch	-	18.0
Sucrose	-	8.0
Fiber source; wheat bran	3.9	5.1
Fat sources;		
Lard	2.9	15.0
Coconut Oil	-	15.0
Vegetable Oil	3.0	-
Protein sources;	-	
Casein		23.0
Soybean Meal (48% protein)	20.0	-
Fish Meal (64% protein)	14.4	-
Gelatin	-	6.0
DL-Methionine[1)]	0.3	0.3
Mineral Mixtures	5.9	6.7
Vitamin Mixtures	1.3	1.3
$Kcal \cdot 100g^{-1}$ (% Kcal)	***337.9 (100.0)***	***568.0 (100.0)***
CARBOHYDRATE	**208.8 (61.8)**	**100.8 (17.8)**
FAT	**53.1 (15.7)**	**360.0 (63.4)**
PROTEIN	**76.0 (22.5)**	**107.2 (18.9)**

Standard CHOW was purchased from SAMTAKO (Bio Korea, Kyoung-ki, Korea).
1) DL-2-Amino-4-methylthiobutanoic acid.
All ingredients (including mineral and vitamin mixtures) for high-fat diet were prepared from ICN Biomedicals, Inc.

American Institute of Nutrition.[18] The rats were provided with food and water *ad libitum* throughout the experimental period. Immediately after the eight-week experimental period, the animals that had either been kept at rest or put through the three-hour exercise regimen were anesthetized with ether. As soon as the anaesthesia took effect, skeletal muscle (soleus, red gastrocnemius, and white gastrocnemius) and liver samples were dissected out, immediately frozen in liquid nitrogen and stored at -80 ℃ for later analyses. Briefly, on the day of an experiment, four animals swam together in a plastic barrel measuring 50 cm in diameter and filled to a depth of about 60 cm. The water temperature was maintained at 35±1 ℃. The animals swam for 30 min×6 cycle bouts (total exercise time was three hours) separated by 5 min rest periods during which time they were dried and placed in their cages. The exercise program chosen for this study was used in our previous study.[19]

3. Blood Biochemical Analyses

Whole blood (~2 mL) was placed into tubes containing EDTA and spun in a centrifuge at 12,000 rpm for 5 min at 4 ℃. Plasma was subsequently analyzed for plasma glucose and free fatty acid (FFA). Plasma glucose was analyzed using Yellow Springs Instruments 2300 (Yellow Springs, OH) and FFA was measured by enzymatic reaction (NEFA C test kit, Wako, Richmond, VA) using a spectrophotometer (@550 nm).

4. Enzyme Activity Measurement

Citrate synthase (CS) activity was determined in a portion of the muscle sample (5~10 mg). The muscle was homogenized in a 1:50 dilution (w/v) of a 175 mM potassium buffer solution and CS activity was assayed spectrophotometrically at 25℃ as previously described.[20] β-HAD activity was assayed spectrophotometrically at 25℃ measuring the disappearance of NADH using the same homogenate as for CS.[21]

5. Muscle and Liver Triacylglycerol Determination

Muscle triacylglycerol (IMTG) concentration was determined as glycerol residue. Briefly, total lipids in muscle fragments (30~60 mg) were extracted for 16 hours at 4 ℃ in 3 mL chloroform-methanol (2:1 v/v) after which 1.5 mL of 4 mM $MgCl_2$ was added.[22] The organic portion was then evaporated under a stream of N_2 and reconstituted in chloroform. Silicic acid was then added to the organic portion and the phospholipids removed by centrifugation. The resultant supernatant was evaporated and saponified in ethanolic KOH for 1 h at 60 ℃ and then centrifuged with 0.15 mM $MgSO_4$. The final supernatant was analyzed fluorometrically for glycerol concentration according to the methods of Wieland.[23]

6. Proglycogen and Macroglycogen Concentration Determination

PG and MG concentration were determined as previously described.[14] Briefly, PG and MG were separated through the addition of 200 µL of ice-cooled 1.5 M perchloric acid (PCA) to a 10~20 mg sample of frozen muscle. The samples were centrifuged at 3000 rpm for 15 min, after which 100 µL of the PCA supernatant were separated for the determination of macroglycogen. The remaining PCA was discarded and the pellet was kept for the determination of proglycogen. The samples were boiled for two hours in 1 N HCl and incubated at 98 ℃ for two hours. Then, all samples were neutralized with 2M Tris base, vortexed, centrifuged at 3000 rpm for 10 minutes and the supernatants were assayed using a fluorometer. Both PG and MG content were expressed as micromoles of glucosyl units liberated per gram wet muscle weight ($\mu mol \cdot g^{-1}$ wet wt). Total glycogen concentration was calculated by adding the totals for PG and MG.

7. Statistical Analysis

Statistical evaluation of the data was undertaken using a two-way analysis of variance (2×2 factorial analysis of variance) with diet (CHOW vs. HF) and exercise (REST vs. EXE) conditions. Interaction between the two factors was first considered and the main effect for each of the two factors was then observed if no interaction was shown. Where any significant main effect was expressed, a *Tukey's post hoc* test was administered to locate the difference. Significance was accepted when $p<.05$. All data are presented as means±S.E.M.

RESULTS

1. Body Weight Changes

At the end of the eight weeks of dietary manipulation, body weight was measured at rest. There was no

Table 2. Body weight changes of CHOW and HF after 8 weeks of experimental diets at rest

	CHOW[1]	HFD[2]	Diet×Exercise
REST	385.8±12.8	407.8±5.0	$p>.05$
EXE	413.6±7.9	417.1±13.0	

1) standard chow diet group; 2) High fat diet group.
All values were means±S.E.M., and expressed in g.

significant difference in body weight among the subjects in the experimental groups (p>.05; Table 2).

2. Blood Glucose and Free Fatty Acid

While 8 weeks of feeding with a high-fat diet induced a significant increase in plasma glucose concentration, three hours of exercise caused a significant decrease in glucose concentration (p<.05, Table 3). Plasma FFA increased significantly as a result of both exercise and feeding with a high-fat diet. Interestingly, plasma FFA concentration increased significantly following prolonged exercise (p<.05) in the CHOW animals, but this was not the case with the HF animals (p>.05, Table 3).

Table 3. The changes of plasma glucose and free fatty acid concentration

	CHOW	HFD	Diet×Exercise
Glucose ($mg \cdot dL^{-1}$)			
REST	71.7±4.5	83.6±2.7†	*P<.05*
EXE	53.4±4.7*	71.8±2.7*#	
FFA ($mmol \cdot L^{-1}$)			
REST	0.34±0.04	0.47±0.23†	*P<.01*
EXE	1.03±0.14*	0.51±0.04#	

* p<.05 compared to REST at the same diet condition.
# p<.05 compared to CHOW EXE.
† p<.05 compared to CHOW REST.
All values were means±S.E.M.

3. Muscle Citrate Synthase and β-hydroxyacyl CoA Dehydrogenase Activities

The muscles of animals that were fed a high-fat diet showed significantly higher CS activity than the muscles of animals on the CHOW diet (p<.05, Table 4). Regardless of dietary conditions, the highest CS activity was observed in red gastrocnemius muscle, followed by soleus and white gastrocnemius muscle (Table 4). The changes in β-HAD activity revealed a similar trend, as shown in CS activity. Thus, the muscles of animals that were fed a high-fat diet showed significantly higher β-HAD activity than the muscles of animals on the CHOW diet (p<.01, Table 5). Regardless of dietary conditions, the highest β-HAD activity was observed in red gastrocnemius muscle followed by soleus and white gastrocnemius muscle (Table 5).

Table 4. Citrate synthase and β-hydroxyacyl CoA dehydrogenase activities in different muscles

	CHOW-REST	HFD-REST	Diet×Exercise
Citrate synthase			
Soleus	27.3±3.5	38.4±2.1*	
Red gastrocnemius	35.2±4.2†	50.5±1.7*†	*P<.05*
White gastrocnemius	13.2±2.1†	18.9±0.9*†	
β-hydroxyacyl CoA dehydrogenase			
Soleus	10.3±1.1	19.3±2.1*	
Red gastrocnemius	18.4±2.0†	29.1±2.0*†	*P<.01*
White gastrocnemius	5.1±0.7†	9.4±0.9*†	

* p<.05 compared to CHOW at the same muscle.
† p<.05 compared to soleus at the same dietary condition.
All values were means±S.E.M, expressed in $mmol \cdot kg^{-1}$ wet wt.

4. Changes in Intramuscular and Liver Triacyglycerol

After long high-fat diet ingestion, triacylglycerol stored in skeletal muscle (p<.01) and liver (p<.05) increased significantly. Interestingly, IMTG concentration only decreased in soleus muscle, but not in red and white gastrocnemius muscles in CHOW animals after three hours of exercise. There were no changes in the muscles of HF animals after three hours of exercise (Table 5).

Table 5. The changes of triacylglycerol in tissues

	CHOW	HFD	Diet×Exercise
Soleus			
REST	16.3±1.5	28.3±2.0†	***P<.05***
EXE	10.2±1.7*	27.4±1.4#	
Red gastrocnemius			
REST	25.4±2.0	38.2±3.1†	***P<.05***
EXE	17.3±1.5*	30.1±2.5*†	
White gastrocnemisu			
REST	2.3±0.2	5.3±0.9†	***P>.05***
EXE	2.0±0.4	4.9±0.7†	
Liver			
REST	4.2±0.3	9.4±1.1†	***P<.01***
EXE	2.9±0.5	4.0±0.7*	

* p<.05 compared to REST at the same diet condition.
# p<.05 compared to CHOW at the same exercise condition.
All values were means±S.E.M, expressed in $\mu moles \cdot g^{-1}$ wet weight.

5. Changes in Total Muscle Glycogen

As shown in Fig. 1, there was no significant difference between the CHOW and HF groups (p>.05) in terms of total glycogen content of rested soleus, red gastrocnemius and the liver. Unlike in other tissue, total glycogen content was significantly higher in the white gastrocnemius muscle of the CHOW animals than it was in that of the HF animals. Following three hours of exercise, while total glycogen concentration from both diet conditions dropped significantly in all muscle groups, the glycogen content of the liver decreased significantly only in the tissue of the CHOW animals. Interestingly, total glycogen content differed significantly in soleus and red gastrocnemius muscle as well as in the liver, something that did not occur in white gastrocnemius muscle after three hours of exercise.

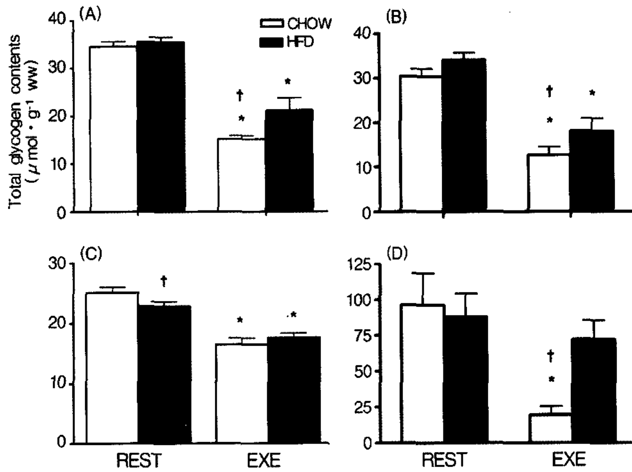

Fig. 1 Effect of prolonged exercise on total glycogen contents in skeletal muscles and liver between CHOW and HFD group.
CHOW, standard chow diet; HFD, high fat diet.
(A) soleus, (B) red gastrocnemius, (C) white gastrocnemius, (D) liver.
*p<.05 vs. REST at the same dietary condition. †p<.05 between CHOW and HFD when the exercise condition was the same. Values are expressed by means±S.E.M.

6. Changes in Proglycogen

At rest, there was no difference between the CHOW and HF groups (Fig. 2; p>.05) in terms of proglycogen content in soleus, red gastrocnemius and the liver (but not in white gastrocnemius). Similar to the changes in total glycogen content after three hours of exercise, the proglycogen concentration of both the CHOW and HF groups dropped significantly in all muscles, whereas proglycogen in the liver only decreased significantly in the tissue of the CHOW group. Proglycogen content after prolonged exercise was significantly lower in soleus and red gastrocnemius muscle and in the liver in the CHOW group than it was in that of the HF group.

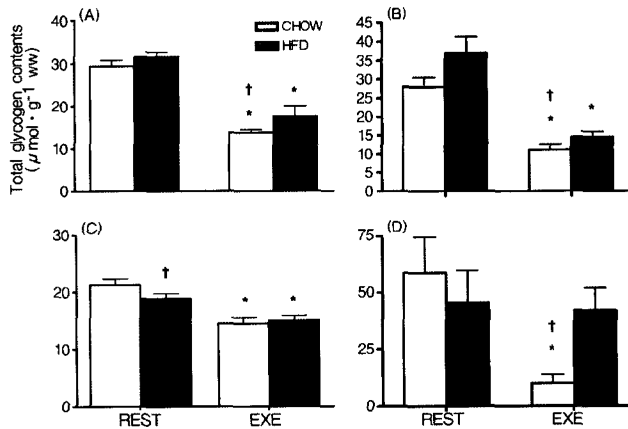

Fig. 2 Effect of prolonged exercise on proglycogen contents in skeletal muscles and liver between CHOW and HF group.
CHOW, standard chow diet; HFD, high fat diet.
(A) soleus, (B) red gastrocnemius, (C) white gastrocnemius, (D) liver.
*p<.05 vs. REST at the same dietary condition. †p<.05 between CHOW and HFD when the exercise condition was the same. Values are expressed by means±S.E.M.

7. Changes in Macroglycogen

Alterations in macroglycogen content during exercise revealed a different pattern than alterations in proglycogen content. Thus, there was almost no change in red gastrocnemius and in the liver in the HF group, whereas major changes in macroglycogen content occurred in the tissue of the CHOW group after three hours of exercise.

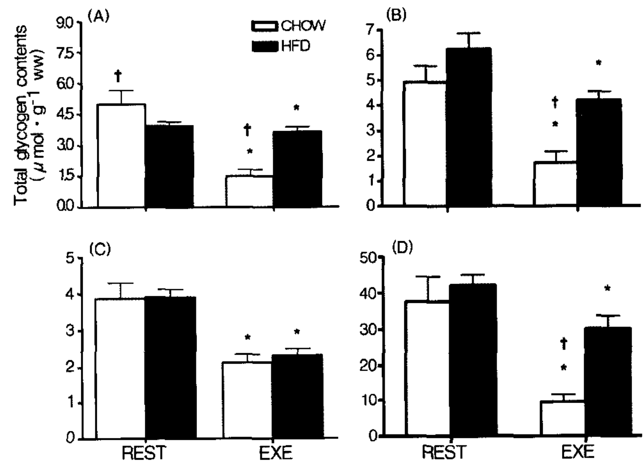

Fig. 3 Effect of prolonged exercise on macroglycogen contents in skeletal muscles and liver between CHOW and HFD group.
CHOW, standard chow diet; HFD, high fat diet.
(A) soleus, (B) red gastrocnemius, (C) white gastrocnemius, (D) liver.
*p<.05 vs. REST at the same dietary condition. †p<.05 between CHOW and HFD when the exercise condition was the same. Values are expressed by means±S.E.M.

DISCUSSION

Biochemical and electron microscopic studies have shown that glycogen exists in two distinguishable forms in skeletal muscle:[13)] PG (acid-insoluble) and MG (acid-soluble). These two forms of glycogen differ in the ratio of protein-to-carbohydrate and are therefore separable via acid solubilization (MG is soluble and PG precipitates.[9-13,24,25)] The functional differences, however, between these glycogen forms during exercise have not been well documented. Specifically, the effect of dietary intervention on PG and MG concentration has never been studied. In the present study, we investigated the effect of a long-term high-fat diet on PG and MG accumulation and mobilization during exercise. The analyses were extended

to three different types of muscle fiber and the liver.

The feeding of a high-fat diet for eight weeks, as opposed to a CHOW diet, did not induce any difference in body weight ($p>.05$). Similar results have been reported in other studies that had designs similar to that of the present study.[26,27] Blood glucose levels increased significantly after eight weeks on a high-fat diet, possibly indicating the development of insulin resistance. In the present study, blood glucose dropped significantly after three hours of exercise. This result implies that glucose was used to supply energy during exercise and that it was used to compensate for muscle and liver glycogen content depletion during prolonged exercise. Such prolonged exercise will rapidly deplete stored glycogen.[28] Ingestion of a high-fat diet significantly increased the concentration of plasma FFA. Similar results have been reported in other studies,[29-31] thus implying that fat may be preferable as an energy source to other energy substrates in the body. Moreover, in the present study plasma FFA increased approximately 3.5-fold in the CHOW group after three hours of exercise, something that did not occur in the HF group. This may have been due to cellular adaptation (i.e., activation of fat metabolism) such that the rate of FA transportation and utilization increased. These results suggest that a high-fat diet alone was sufficient stimulus to induce adaptations which apparently increase the ability of muscle to oxidize FA or spare glycogen.[26,29,31-35]

This study showed that regardless of the type of muscle fiber and tissue, triacylglycerol concentration was significantly higher after eight weeks of ingesting a high-fat diet. Increased tissue triacylglycerol should be considered as an energy source during prolonged exercise to spare muscle and liver glycogen storage.[34] There have been many studies showing that IMTG content is closely associated with glucose oxidation rates and that increased IMTG content decreases the rate (or degree) of glucose oxidation and/or glycolytic utilization.[36-39] In the present study, IMTG concentration decreased significantly in soleus (which represents type I muscle fiber) and red gastrocnemius (which represents type IIa muscle fiber) but did not change significantly in white gastrocinemius (represents for Type IIb muscle fiber) following the three hours of exercise. These results support the notion that IMTG can *only* be mobilized in Type I and Type IIa muscle fiber (but not in Type IIb muscle fiber) to provide energy during prolonged exercise. In addition, it was also postulated that the intensity of the exercise adopted in this study was not sufficient to mobilize the IMTG stored in Type IIb muscle fiber. Interestingly, IMTG selectively decreased in Type I muscle fiber following three hours of exercise. That is, IMTG in soleus muscle decreased significantly in the CHOW group but not in the HF group. This indicates that tissue taken from animals in the HF group was much more dependent on aerobic metabolism for energy during prolonged exercise than that taken from animals in the CHOW. The results were different with the liver. Thus, intratissue triacylglycerol did not change significantly in the tissue of animals in the CHOW group but IMTG decreased significantly in the tissue of animals in the HF group. These metabolic differences in IMTG mobilization during prolonged exercise between animals on different diets must be further examined through research based on a more sophisticated design.

In the present study, we measured CS and β-HAD activity as an index of aerobic capacity. After feeding with a high-fat diet, the experimental animals showed a significant increase in CS and β-HAD activity. This occurred regardless of the type of muscle fiber and tissue. The result supports many previous findings showing that a high-fat diet improved metabolic activity associated with oxidative enzyme activity.[26,27,31,35] Increased oxidative enzyme activity was associated with elevated levels of FFA and IMTG.

Although in animals on the HF diet (as opposed to the CHOW diet), white gastrocnemius showed a significant reduction in muscle glycogen, muscle glycogen content in all other tissue did not differ statistically. Although total glycogen content in all muscle types and in the liver decreased significantly in the animals in both groups following three hours of exercise, the degree of reduction was different. There was a smaller decrease among animals on the high-fat diet and a higher degree of reduction with a high-carbohydrate diet. This may indicate that energy production was heavily reliant on carbohydrate in the animals in the CHOW group. On the other hand, it appears to have been heavily reliant on fat metabolism in the animals in the HF group. Similar results have been reported previously.[26,34] The glycogen content of the liver after different dietary interventions did not differ significantly at rest, but it was markedly different in the livers of the animals in the HF group and decreased by approximately 80% in the animals in the CHOW group. These results strongly suggest that a deterioration of carbohydrate metabolic function in the liver occurs with the ingestion of long-term high-fat diet.

Changes in PG content showed a trend similar to those in total glycogen with different diets and exercise

conditions. The changes in macroglycogen content during exercise were different. Thus, there was almost no change in red gastrocnemius and in the livers of animals in the HF group, whereas major changes in MG content were seen in the tissue of animals in the CHOW group after three hours of exercise.

Although it has been well documented in many previous studies that a long-term high-fat diet results in decreased carbohydrate metabolism, the exact mechanism responsible for this remains unclear. It is also true that deterioration of carbohydrate metabolism is only understood as an aspect of changes in total glycogen. The results of this study, however, suggest that MG, rather than PG, is responsible for the metabolic disorders related to a long-term high-fat diet. Further study is needed to determine what role is played by glycogen in tissue where metabolic disorders (i.e., insulin resistance, obesity) have already occurred. Moreover, the differential regulatory effects of these two forms of glycogen during prolonged exercise must be considered. Recently, Graham *et al.*[40] reported that PG declined markedly during exercise having three different intensities and durations (@70% VO_{2max} for 45 min, 85% VO_{2max} to exhaustion, 100% VO_{2max} at 3 min bouts separated by 6 min rest). This did not occur with MG. The authors reported that the rate of catabolism increased depending on the intensity of the exercise and that this was mainly attributable to PG breakdown.[40] Unlike the PG response, the rate of glycogenolysis decreased as the duration of the exercise increased, the reason being that the MG catabolism rate decreased.[40] Derave *et al.*[41] reported that both PG and MG decreased significantly during two hours of swimming with weights (5% of body mass) but that the degree of reduction was different. Specifically, MG was regulated during and after exercise. Adamo *et al.*[6] investigated whether carbohydrate ingestion plays a different role in the resynthesis of PG and MG after exhaustive exercise and found that the synthesis of PG occurred faster than that of MG, which indicates that PG is more sensitive to carbohydrate supplementation than MG.

In the present study, we examined the effects of diet manipulation on PG and MG accumulation and mobilization in different types of muscle fiber and tissue during exercise. Whereas changes in PG concentration showed a trend similar to that of total glycogen, alterations in MG concentration differed from those seen in these forms of glycogen. Specifically, the degree of reduction of MG following three hours of exercise was substantially greater in the tissue of animals in the CHOW group than it was in the tissue of animals in the HF group. These results suggest that MG rather than PG may be the cause of metabolic impairment induced by a long-term high-fat diet.

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