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Moderate Physical Training Can Increase Muscle Glycogen Levels but Does Not Alter Protein Levels with Exercise in Rats*

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This study investigated the effect of physical training on the utilization and recuperation of stored fuel with exercise in rats. For physical training, animals were exercised on treadmill for 30 minutes everyday. Forty eight rats were given either a physical training or no training for 4 weeks and were then subdivided into 3 groups: before-exercise (BE); during-exercise (DE); after-exercise (AE). The DE group was exercised on treadmill for 1 hour just before being sacrificed. Animals in the AE group were allowed to take a rest for 2 hours after being exercised like the DE group. Glucose and free fatty acids were compared in plasma. Glycogen and triglycerides were compared in liver and skeletal muscle. Protein were compared in plasma, liver and skeletal muscle of rats.

Plasma glucose levels of trained group were not significantly different from those of non-trained group. Muscle glycogen levels of trained group were significantly higher than those of non-trained group. Liver glycogen level of trained group was also significantly higher than that of non-trained group in DE while was not significantly different from those of non-trained group in BE and AE. Plasma free fatty acid levels of trained group were significantly higher than those of non-trained group in BE and AE. Muscle triglyceride levels of trained group tended to be higher than those of non-trained group in BE and DE and significantly higher than those of non-trained group in AE. Plasma and muscle protein levels of trained group were not significantly different from those of non-trained group. Liver protein levels of trained group were not significantly different from those of non-trained group in BE and DE but were significantly higher than that of non-trained group in AE. Thus, it is suggested that an even moderate physical training may delay the onset of fatigue and improve exercise performance by facilitating the mobilization and oxidation of fat and conserving limited carbohydrate store.

Key words: Moderate physical training, Glycogen, Protein, Triglyceride, Stored fuel

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INTRODUCTION

The fuels that support physical activity are glucose, fatty acids, and to a small extent, amino acids. The body uses different mixtures of fuels depending on the intensity and duration of its activities and depending on its own prior training. Numerous studies have investigated positive effects of physical training, but the exact relation between exercise and substrate mobilization from different endogenous fuel stores has not been fully elucidated.[1] The use of each energetic substrate is related to the moderate physical training and exercise. When the body is involved in physical training, certain metabolic processes occur to assure that adequate energy is provided to the exercising muscles.[2-9]

Carbohydrate and fat are the primary energy substrates used for exercise. The availability of carbohydrate to working muscle becomes a limitation to the ability to perform prolonged high intensity exercise. Because carbohydrate becomes increasingly important as the intensity of the exercise increases and because the amount of carbohydrate stored in the body is limited, the depletion of muscle and liver glycogen can become limiting factors during prolonged exercise.[10-15] Thus, a dietary technique such as carbohydrate loading designed to promote an increase of glycogen has been attempted to delay the onset of fatigue. This technique is primarily suited for those individuals who do the strenuous exercise, and the heart rate response should be increased above the resting level by about 50~85% of the maximal heart rate reserve, for a long period. However, there have been little studies on the utilization of fuel sources with moderate physical training which the scheme of the change in the utilization of fuel sources may be different from that with the strenuous exercise.

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Therefore, the aim of this study was to investigate the effect of moderate physical training, recommended to individuals with poor levels of physical fitness, on the storage and utilization of the stored fuels with exercise *in vivo*.

MATERIALS AND METHODS

1. Experimental Animals and Exercise

Forty eight male Sprague-Dawley rats (Deahanbiolink Co., Korea) weighing 70 g were fed a vitamin-free casein based semisynthetic diet which met AIN-93 recommendation for 4 weeks. Rats were given either a physical training or no training for 4 weeks and were then subdivided into 3 groups: before-exercise (BE); during-exercise (DE); after-exercise (AE). For physical training, animals were exercised on treadmill (10 °, 0.5~0.8 km/h) for 30 minutes everyday. The BE group did not exercise before being sacrificed. The DE group was exercised on treadmill for 1 hour just before being sacrificed. Animals of the AE group were allowed to take a rest for 1 hour after being exercised like the DE group.

2. Sample Collection and Biochemical Analysis

At the respective time points, animals were sacrificed by the decapitation under light anesthesia. Immediately following decapitation, blood was collected in heparinized tubes and centrifuged to separate the plasma. Liver and skeletal muscle from the portion of the gastrocnemius were rapidly removed. The plasma and tissues were stored at -40 ℃ until analyzed. Glycogen was measured by a colorimetric procedure.[16] After tissue samples were homogenized in cold sodium phosphate buffer (0.02 M, pH 7.0), aliquots of the tissue homogenate were analyzed for protein and triglyceride. Total protein was determined using a commercial kit based on the Biuret reaction (Asan Pharmaceutucal Co., Korea). Triglyceride was analyzed with a commercial kit utilizing the glycerol phosphate oxidase-quinoneimine coloring method (Asan Pharmaceutical Co., Korea). Plasma glucose was analyzed with a commercial kit based on enzymatic method (Youndong Pharmaceutical Co., Korea). Free fatty acid (FFA) was analyzed with a commercial kit utilizing acyl CoA synthetase-Acyl CoA oxidase (NEFAZYME-S, Eiken Chemical Co., Japan).

3. Statistical Analysis

All data were subjected to an analysis of variance and tested for significant differences by Duncan's multiple range test (SAS Institute, Cary, NC). p value < 0.05 was considered to be significant. The significance of difference between non-trained group and trained group was tested using student t-test at $p < 0.05$.

RESULTS

As shown in Table 1. there were no differences between non-trained group and trained group in body weight, FER and organ weight.

Table 2 shows the effects of physical training on the levels of plasma glucose, liver glycogen and muscle glycogen. Plasma glucose levels of trained group were not significantly different from those of non-trained group in BE, DE and AE. However, liver glycogen level of trained group was also significantly higher than that of non-trained group in DE while was not significantly different from those of non-trained group in BE and AE. Muscle glycogen levels of trained group were significantly higher than those of non-trained group in BE, DE and AE.

Table 1. The effect of training on body weight and organ weight

	Group		*t*-test
	Non-training	Training	
Initial BW[1] (g)	70.14± 2.66	69.9± 2.81	NS[2]
Final BW (g)	299.79±43	300.93±19	NS
Food intake (g/day)	22.62± 4.85	21.82± 1.78	NS
FER	0.349± 0.09	0.353± 0.03	NS
Liver (g)	13.63± 2.98	14.34± 1.29	NS
Heart (g)	1.18± 0.17	1.23± 0.26	NS
Kidney (g)	1.15± 0.16	1.11± 0.14	NS
Spleen (g)	0.717± 0.13	0.768± 0.31	NS

1) BW: body weight, FER: feed efficiency ratio
2) No significant difference between non-trained group and trained group by t-test $p < 0.05$

Table 2. The effect of physical training on the levels of plasma glucose, liver glycogen and muscle glycogen

		BE[1]	DE	AE
Plasma glucose (mg/dl)	Non-training	154.38±10.63[b2]	183 ±30.28[a]	165 ±12.36[ab]
	Training	159.75± 8.08[a]	162.75±18.49[a]	62 ±17.74[a]
	t-test	NS[3]	NS	NS
Liver glycogen (mg/g)	Non-training	56.64±14.62[a]	29.07±10.68[b]	50.59± 9.30[a]
	Training	49.06±15.31[a]	43.44± 7.72[a]	48.7±11.68[a]
	t-test	NS	*	NS
Muscle glycogen (mg/g)	Non-training	0.11± 0.06[a]	0.051± 0.03[a]	0.095± 0.04[a]
	Training	0.33± 0.23[a]	0.29± 0.08[a]	0.38± 0.12[a]
	t-test	*	*	*

1) BE: before-exercise, DE: during-exercise, AE: after-exercise
2) Values with different superscripts within a column are significantly different at $p < 0.05$.
3) No significant difference between non-trained group and trained group at $p < 0.05$

Table 3 shows the effects of physical training on the level of plasma free fatty acids and triglyceride levels of liver and muscle. Plasma free fatty acids levels of trained group were significantly higher than those of non-trained group in BE and AE and there was a significant increase in plasma free fatty acids of non trained group during exercise and there was no significant difference between trained group and non-trained group in DE. Liver triglyceride levels of trained group tended to be lower than those of non-trained group in BE, DE and AE although these differences were not statistically significant due to the large standard deviation. However, muscle triglyceride levels of trained group tended to be higher than those of non-trained group in BE and DE and significantly higher than those of non-trained group in AE.

Table 4 shows the effects of physical training on the protein levels of plasma, liver and muscle. Plasma and muscle protein levels of trained group were not significantly different from those of non-trained group in BE, DE and AE. Liver protein level of trained group were not significantly different from those of non-trained group in BE and DE but were significantly higher than that of non-trained group in AE because liver protein level was increased in trained group while decreased in non-trained group after exercise.

Table 3. The effect of physical training on the free fatty acid (FFA) and triglyceride (TG) levels of liver and muscle.

		BE[1)]	DE	AE
Plasma glucose (mg/dl)	Non-training	364.49±24.49[a2)]	443.72±97[a]	432.29±34[a]
	Training	428.74± 5.80[b]	449.17±16.85[b]	487.13±54.75[a]
	t-test	*	NS[3)]	*
Liver glycogen (mg/g)	Non-training	314.71±74.13[a]	428.25±55.11[a]	358.00±38.70[a]
	Training	285.86±82.22[a]	317.33±76.07[a]	251.75±75.49[a]
	t-test	NS	NS	NS
Muscle glycogen (mg/g)	Non-training	47.5± 8.69[a]	40.86±11.18[ab]	34.86± 7.55[b]
	Training	54.11±11.11[a]	47.43± 9.91[a]	46.8 ±11.18[a]
	t-test	NS	NS	*

1) BE: before-exercise, DE: during-exercise, AE: after-exercise
2) Values with different superscripts within a column are significantly different at $p < 0.05$.
3) No significant difference between non-trained group and trained group at $p < 0.05$

Table 4. The effect of physical training on the protein levels of plasma, liver and muscle.

		BE[1)]	DE	AE
Plasma glucose (mg/dl)	Non-training	7.6±0.96[a2)]	7.98±0.83[a]	8.17±1.94[a]
	Training	7.6±0.77[a]	7.33±0.84[a]	6.97±0.675[a]
	t-test	NS[3)]	NS	NS
Liver glycogen (mg/g)	Non-training	3.375±1.11[a]	4.7±1.76[a]	3.58±0.97[a]
	Training	4.56±1.45[a]	4.43±2.14[a]	5±1.50[a]
	t-test	NS	NS	*
Muscle glycogen (mg/g)	Non-training	1.45±0.19[a]	1.4±0.11a	1.32±0.21[a]
	Training	1.37±0.13[a]	1.525±0.23[a]	1.48±0.30[a]
	t-test	NS	NS	NS

1) BE: before-exercise, DE: during-exercise, AE: after-exercise
2) Values with different superscripts within a column are significantly different at $p < 0.05$.
3) No significant difference between non-trained group and trained group at $p < 0.05$

DISCUSSION

This study demonstrated that moderate physical training induced a favorable turn in the utilization and recuperation of fuel with exercise, which is most evident in stored fuel. The evidence of an alteration in fuel utilization of stored fuel with training is based on the change of fuel sources with exercise. The biochemical indices might not be influenced by the difference of body weight and organ weight because there were no differences between non-trained group and trained group in body weight, FER and organ weight.

Maintaining adequate stores to meet energy needs helps prevent fatigue during exercise. Carbohydrate stores in muscle and liver are important for sustained energy. It has been reported that a low or depleted glycogen stores limit exercise time and intensity and lead to decrease the time to exhaustion during physical activity.[17] Especially, muscle glycogen becomes important as a fuel for muscular exercise as the intensity of exercise increases. Compared with non-trained group, the higher level of muscle glycogen was shown regardless of exercise. Also, the higher level of liver glycogen was shown during exercise in trained group. Thus, it can be suggested that physical training made the animal adapt either to slow down glycogen depletion or to store more glycogen. It is reported that exercise increases the muscle's sensitivity to insulin, predominately, during the 4 to 6 hours after exercise and muscle glycogen can be resynthesized near pre-exercise levels within 24 hours. After 24 hours muscle glycogen can be increased very gradually succeeding normal levels over the next few days.[18] Also, there were the reports that muscle glycogen synthesis was greater within 2 hours proceeding exercise[15] and greatest 45 minute post workout. In this study muscle glycogen level of trained group was higher than that of non-trained group. Liver glycogen level of trained group was not different from that of non-trained group in before exercise but in during exercise glycogen level of trained group was higher than that of non-trained group. Thus, it is suggested that the storing more glycogen might be the primary adaptive mechanism underlying the greater capacity of trained muscle to keep the glycogen levels.

A depletion of liver glycogen may lead to hypoglycemia during exercise because gluconeogenesis normally cannot keep pace with glucose utilization by the muscle.[19] However, plasma glucose levels of trained group of this study were not significantly different from those of non-trained group regardless of exercise. It has been reported that as endurance athletes deplete the endogenous carbohydrate stores, the body catabolizes some of its protein for energy or eventual conversion to glucose. Protein catabolism has been shown to be increased significantly when muscle glycogen is depleted by only about 33~35 percent.[20,21] Since there was no difference in protein levels between non-trained group and trained group regardless of exercise or recuperation in this study, protein appeared to be a relatively minor source of energy and was not affected by physical training.

When exercise is initiated, energy turnover is increased with rapid mobilization and oxidation of both carbohydrates and lipids stored within contracting muscle; increases in fat oxidation is most important in low intensity exercise.[22,23] A relative increase in the availability of free fatty acids during exercise has been shown to delay the onset of exhaustion.[24] Free fatty acids may be released by adipose tissue triglycerides and travel through the blood to the muscle cells, and may also be derived from muscle triglyceride for oxidation by muscle during exercise.[25] Because plasma free fatty acids levels of trained group were significantly higher than those of non-trained group, trained group might utilize efficiently free fatty acid as a fuel source. Compared with non- trained group, muscle triglyceride was significantly higher after exercise and tended to be higher before and during exercise. This intramuscular fat utilization could also be a good fuel sources in prolonged exercise because it was reported that the increased content and use of muscle triglyceride may be the primary adaptive mechanism underlying the greater capacity of trained muscle to oxidize fatty acids during exercise.[26] Thus, it is suggested that an even moderate physical training may delay the onset of fatigue and improve exercise performance by facilitating the mobilization and oxidation fat and conserving limited carbohydrate store.

Therefore, moderate physical training can increase glycogen levels but does not alter protein levels with exercise in rats. Protein appears to be a relatively minor source of energy and was not affected by physical training. It is suggested that an even moderate physical training may delay the onset of fatigue and improve exercise performance by facilitating the mobilization and oxidation fat and conserving limited carbohydrate store.

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