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Effects of Regular Endurance Exercise or Acute-exercise and Rest on the Levels of Lipids, Carnitines and Carnitine Palmitoyltransferase-I in rats

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The effects of regular endurance exercise, or acute-exercise and rest on the levels of lipids, carnitines and carnitine palmitoyltransferase-I (CPT-I) were investigated in male Sprague-Dawley rats. The rats were exercise trained on a treadmill for 60 min per day for 60 days (long-term trained, LT), or non-trained for 59 days (NT) and exercised for 60 min on the 60th day. In NT rats, the levels of serum nonesterified carnitine (NEC), acidsoluble acylcarnitine (ASAC), and total carnitine (TCNE) increased significantly during the post-exercise recovery period (PERP). In LT rats, ASAC, and TCNE, which increased right after the 60 min running session decreased to the levels of pre-exercise during the PERP. The levels of skeletal muscle ASAC in NT rats, which increased significantly by the acute-exercise, decreased to the pre-exercise levels during the PERP. However, the ASAC level in LT rats reached its peak at 4 h after running for 60 min. Liver triglyceride (TG) and total lipids (TL), which increased by the acute-exercise, decreased to the pre-exercise levels during the PERP in both NT and LT rats. CPT-I activity in NT rats increased significantly after 1 h of a 60-min exercise and slowly decreased to pre-exercise levels during the PERP. However, the CPT-I activity in LT rats, which increased significantly by the 60 min exercise, decreased slowly and reached its pre-exercise level within 8 h of the PERP. Northern blot analysis showed that the changes of CPT-I activities during the PERP coincided with changes in CPT-I mRNA levels. This study shows that both regular endurance exercise, and acute-exercise and rest, can influence differently the levels of carnitines, lipids and CPT-I in rats. The results suggest that regular endurance exercise, rather than the acute-exercise, can change effectively the distributions of carnitines, lipids and CPT-I in rats during exercise and rest.

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Introduction

At the start of exercise, fats and carbohydrates are utilized. As the intensity of the exercise increases, fat utilization increases significantly at an intensity of about 50% of VO_2max (Brouns and van der Vusse, 1998). Exercise increases the capacity of the oxidative pathways by increasing mitochondrial density. The increased oxidative capacity is believed to reduce dependence on glycogenolysis and glycolysis, as well as to increase fat catabolism during exercise (Gollnick and Saltin, 1982; Brass and Hiatt, 1998).

Carnitine is a quaternary amine (β-hydroxy-γ-trimethylammonium butyric acid) and can be found in almost all cells of higher animals (Cerretelli and Marconi, 1990). It is an essential co-factor in the transfer of long-chain fatty acyl groups (fatty acids with 10 or more carbon atoms) from the outer mitochondria membrane into the inner mitochondrial matrix for β-oxidation to acetyl coenzyme A (Bremer, 1983). The inner mitochondrial membrane is permeable to acylcarnitine, but not to long chain acyl-CoA; therefore, β-oxidation of fatty acids is critically dependent upon carnitine (Brouns and van derVusse, 1998; Brass and Hiatt, 1998). This shuttle mechanism consists of the enzymes, carnitine palmitoyltransferase I & II, and a carnitine acylcarnitine translocase (Pande and Parvin, 1976). Carnitine palmitoyltransferase I (CPT-I) is located on the outer surface of the inner mitochondrial membrane and converts acyl-CoA to acyl-carnitine, which is then transported to the inner mitochondrial membrane to exchange for free carnitine. CPT-1 is the rate-limiting enzyme for fatty acid oxidation and is the first step specific to fatty acid oxidation (McGarry and Foster, 1980; McGarry and Brown, 1997).

Carnitine is obtained from endogenous biosynthesis and dietary sources. Carnitine is synthesized in the body from two

essential amino acids, lysine and methionine. Ascorbate, niacin, and vitamin B_6, as well as reduced iron, are required for its biosynthesis as cofactors. The final step of carnitine biosynthesis occurs predominantly in the liver of rats (Broquist, 1982). Since muscle tissue is incapable of synthesizing carnitine, carnitine that is lost from muscle during exercise must be replaced from the body pool of carnitine (Long *et al.*, 1982).

Recently, we evaluated the effect of long-term training, acute-exercise, and the combined action of both on the blood and tissue concentrations of lipids, carnitine fractions, and liver CPT-I activity (Cha *et al.*, 1999; unpublished results). In the present work, we monitored changes in the carnitine contents of the rat liver, muscle, and serum during a post-exercise recovery period. We also measured changes in blood and tissue concentrations of lipids during the recovery period.

Materials and Methods

Materials DL-methionine, corn starch, sucrose, fiber, corn oil, choline bitartrate, and carnitine acetyltransferase were purchased from the Sigma Chemical Co. (St. Louis, USA). AIN-76 vitamin and mineral mix were from Teklard (Madison, USA). Casein was from Cottee (Gordon, Australia). [1-^{14}C]acetyl CoA and L-[methyl-^{3}H] carnitine were from Amersham (Buckinghamshire, UK). Nylon membrane, the Psoralen-biotin labeling kit, and the BrightStar™ BioDetect™ kit were from Ambion (Austin, USA). All of the other chemicals were of the highest commercial grade available.

Animal and diets Forty 7-wk-old male Sprague-Dawley rats, weighing about 200 g, were supplied from Daehan Biolink Inc. (Eumsung, Chungbuk, Korea). They were divided into two groups of twenty each, one was exercised daily (long-term trained, LT) and the other was not exercised (non-trained, NT). Each rat was housed individually in stainless steel cages in a temperature (23±1°C), humidity (53±2%), and light controlled room with a 12-h light-dark cycle (Oh and Cha, 2001). The rats were allowed free access to feed, the AIN-76 diet (Table 1), and water. Before the rats were killed, the diet was removed from the cages so they had 12 h of fasting to minimize the effect of food.

Table 1. Composition of AIN-76 purified diet for rats

Ingredients	Percents (%)
Casein	20.0
DL-Methionine	0.3
Corn starch	15.0
Sucrose	50.0
Fiber	5.0
Corn oil	5.0
AIN Mineral mix	3.5
AIN Vitamin mix	1.0
Choline bitartrate	0.2
Total	100.0

Exercise training and sample preparation The rats were run 60 min per day on a treadmill (a 10° incline) at 25 m/min during the 60 days. Exercise was done each morning between 9 : 00~12 : 00. The samples were collected from each rat at each of the following time points: immediately following the 60-min exercise (0 h), 1, 4, and 8 h after the 60-min exercise (Fig. 1). Blood was immediately collected and centrifuged at $1,100 \times g$ for 15 min at 4°C. Serum aliquots were stored at −20°C until analysis. Liver and skeletal muscle were collected and frozen at −80°C until analyzed. To evaluate the liver CPT-I activity and mRNA levels, the liver was immediately used for extraction.

Analysis of lipids Serum total cholesterol levels were measured by using commercial kits from the Asan Pharm. Co. (Seoul, Korea), based on the cholesterol oxidase method (Allain *et al.*, 1974). Serum triglyceride levels were measured by the lipase-glycerol phosphate method (McGowan *et al.*, 1983) using commercial kits (Asan Pharm. Co., Seoul, Korea). Liver lipids were extracted from liver tissues according to the method of Folch *et al.* (1983). The total lipid levels were measured by the sulfo-phospho-vanillin method (Frings and Dunn, 1970) using commercial kits (Kokusai Pharm. Co., Kobe, Japan). The triglyceride levels were measured by the lipase-glycerol phosphate method, described previously.

Carnitine Assay Nonesterified carnitine (NEC)acid-soluble acylcarnitines (ASAC), and acid-insoluble acylcarnitines (AIAC) in serum and tissues, were determined by the radio-enzymatic procedure of Cederblad and Lindstedt (1972), as modified by Sachan *et al.* (1984). In this method, AIAC was precipitated with perchloric acid and centrifugation leaving the ASAC and NEC in the supernatant. An aliquot of the supernatant was assayed to determine the NEC and another aliquot hydrolyzed with 0.5 M KOH to assay all acid-soluble carnitines (ASAC + NEC). ASAC was calculated as the difference between the NEC and the total acid-soluble carnitines. The pellets containing the AIAC were drained, washed, and hydrolyzed in 0.5 M KOH for 60 min in a hot water, both at 60°C. In each case carnitine was assayed by using carnitine acetyltransferase (Sigma, USA) to esterify the carnitine to a [^{14}C]acetate from [1-^{14}C]acetyl CoA (Amersham, UK). Radioactivity of the samples was determined in a Beckman LS3801 liquid scintillation counter (Beckman Instruments, Palo Alto, USA).

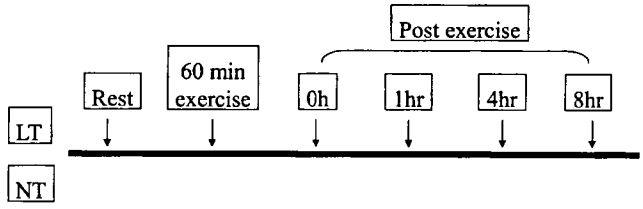

Fig. 1. Experimental design for exercise and post-exercise recovery. Both NT and LT rats were exercised for 60 min on the 60th day on a treadmil at 25 m/min and a 10° incline. Blood, liver and skeletal muscle were collected from each rat right after the 60-min exercise (0 h), 1, 4, and 8 h after the 60-min exercise. Samples were prepared as decribed in Materials and Methods.

Carnitine palmitoyltransferase-I activity Mitochondria isolation: CPT-I activity was assayed in intact mitochondria from liver processed immediately following removal from the animals. Mitochondria were prepared according to the method of Johnson and Lardy (1967). The tissues were first minced by cutting with scissors in cold, Tris-buffered 0.25 M sucrose, then homogenized. The mitochondria were isolated from the homogenate by differential centrifugation, washed, and resuspended three times. After the final wash, the mitochondria were resuspended in the buffer. The mitochondria protein content was determined by the method of Bradford (1976) and the mitochondrial suspension was adjusted to 5 mg protein/ml, as described (Ahn and Kim, 1999).

Table 2. Carnitine concentrations in serum (μmol/L)

	Group	Time course			
		0 h	1 h	4 h	8 h
NEC	NT	28.7 ± 2.3^{b}	42.0 ± 6.0^{ab}	50.5 ± 4.7^{a}	44.3 ± 2.7^{ab}
	LT	17.9 ± 6.5^{b}	17.5 ± 7.3^{b}	12.5 ± 7.3^{b}	11.7 ± 7.9^{a}
ASAC	NT	15.3 ± 5.7^{ab}	19.3 ± 6.6^{a}	22.0 ± 1.5^{a}	17.8 ± 1.5^{a}
	LT	56.2 ± 2.6^{a}	42.8 ± 20.4^{b}	29.3 ± 12.1^{b}	25.9 ± 9.4^{b}
AIAC	NT	2.9 ± 0.1	2.8 ± 0.4	3.6 ± 1.8	3.0 ± 0.5
	LT	4.7 ± 0.6^{a}	2.8 ± 0.4^{ab}	2.6 ± 0.5^{b}	3.4 ± 0.4^{ab}
TCNE	NT	46.9 ± 8.1^{b}	64.1 ± 13.0^{b}	76.1 ± 8.0^{a}	65.1 ± 4.7^{b}
	LT	78.8 ± 9.7^{a}	63.1 ± 28.1^{ab}	44.4 ± 19.9^{b}	41.0 ± 17.7^{b}

All values are mean ± SD (n = 5). Values with different superscripts in the same rows are significantly different ($p<0.05$).
NT, Non-trained; LT, Long-term trained; NEC, Nonesterified carnitine; ASAC, Acidsoluble acylcarnitine; AIAC, Acidinsoluble Acylcarnitine; TCNE, Total carnitine.

Table 3. Carnitine concentrations in tissues

	Group	Time course			
		0 h	1 h	4 h	8 h
Liver (nmol/g)					
NEC	NT	313.6 ± 98.7	331.8 ± 105.1	362.1 ± 80.8	451.6 ± 141.6
	LT	393.8 ± 142.4	386.4 ± 157.5	367.5 ± 15.6	268.8 ± 128.1
ASAC	NT	34.7 ± 12.5	129.6 ± 3.8	117.5 ± 75.7	122.4 ± 114.6
	LT	44.6 ± 66.2	52.5 ± 35.5	45.5 ± 12.3	31.0 ± 15.6
AIAC	NT	1.2 ± 0.7	3.5 ± 4.7	6.5 ± 7.9	3.1 ± 1.6
	LT	7.5 ± 3.2	12.8 ± 9.4	1.2 ± 0.6	14.2 ± 3.8
TCNE	NT	349.5 ± 111.9	464.9 ± 113.6	486.1 ± 164.4	577.1 ± 257.8
	LT	445.9 ± 211.8	451.7 ± 202.4	414.2 ± 28.5	314.0 ± 147.5
Skeletal muscle (nmol/g)					
NEC	NT	934.0 ± 104.0	1101.1 ± 252.4	1160.6 ± 58.3	1014.0 ± 184.8
	LT	1716.4 ± 140.5^{a}	848.0 ± 61.2^{c}	922.5 ± 425.4^{bc}	1542.7 ± 379.4^{ab}
ASAC	NT	956.2 ± 132.7^{a}	358.2 ± 95.4^{b}	484.3 ± 13.8^{b}	397.0 ± 45.8^{b}
	LT	446.9 ± 169.9^{ab}	278.7 ± 78.2^{b}	1053.3 ± 633.4^{a}	525.2 ± 515.0^{ab}
AIAC	NT	204.3 ± 28.6	220.6 ± 11.5	183.1 ± 95.2	177.9 ± 33.9
	LT	222.3 ± 98.3	133.1 ± 18.7	209.6 ± 19.6	217.6 ± 24.6
TCNE	NT	2094.5 ± 265.3	1579.8 ± 276.8	1828.0 ± 167.3	1588.9 ± 264.5
	LT	2385.6 ± 408.7^{a}	1259.9 ± 158.1^{b}	2185.4 ± 1078.4^{a}	2285.5 ± 919^{a}

All values are mean ± SD (n = 5). Values with different superscripts in the same rows are significantly different ($p<0.05$).
NEC, Nonesterified carnitine; ASAC, Acidsoluble acylcarnitine; AIAC, Acidinsoluble acylcarnitine; TCNE, Total carnitine. NT, Non-trained; LT, Long-term trained.

CPT-I activity: A modified procedure of Guzman *et al.* (1987) was used to estimate CPT-I activity in liver mitochondria. Each assay mixture contained a total volume of 1.0 ml : 80 mM sucrose, 1.0 mM EGTA, 70 mM imidazol, 1 μg antimycin A, 2 mg bovine serum albumin, 0.5 mM L-carnitine (0.4 mCi/mmol of L-[methyl-^{3}H] carnitine), and 40 μM palmitoyl CoA. The reactions were initiated by the addition of mitochondria or detergent extracts. The reaction was linear up to 10 min. All of the incubations were performed at 37°C for 5 min. The reactions were stopped by the addition 4 ml of 1.0 M perchloric acid. The reaction mixture was centrifuged at 2,000 × *g* for 10 min, and an aliquot of the butanol phase was transferred to a vial for counting radioactivity.

CPT-I mRNA levels Total RNAs from fresh rat liver were isolated by the guanidine thiocyanate/phenol/chloroform extraction procedure (Chomcznski and Sacchi, 1987), as described by Park (Park *et al.*, 2000). CPT-I mRNA abundance was measured by Northern blot analysis (Mynatt *et al.*, 1994). The RNA was resolved on an agarose gel and transferred to a Nylon membrane (Ambion, Austin, USA). The CPT-I DNA fragment that was obtained from the CPT-I cDNA (Mynatt *et al.*, 1994) was labeled with biotin using a Psoralen-biotin labeling kit (Ambion, Austin, USA). Hybridization of the probe to the membrane-bound mRNA was conducted at 42°C for 20 h. The membrane was washed to remove the non-specifically bound probe and incubated in the blocking, conjugation, blocking and CDP-star solutions of the BrightStar™ BioDetect™ kit (Ambion, Austin, USA), successively. Then, the membrane was exposed to x-ray film (Fuji, Tokyo, Japan) for 45 min.

Statistical analysis All of the values are expressed as group means ± SD. The significance of the differences were determined using a 2-way analysis of the variation (ANOVA) using the Statistical Analysis System version 6 (SAS Institute, Cary, NC, USA). A p value <0.05 was considered significant and determined with the Student's t-test.

Results and Discussion

In the present study we monitored the changes of carnitine levels and lipid concentrations during the post-exercise recovery period (PERP) in the blood and tissue of the trained and untrained rats. This study allowed us to evaluate the differential effects of acute exercise and rest on long-term trained (LT) rats and non-trained (NT) rats. In NT rats, the levels of serum NEC, ASAC, and TCNE increased gradually and reached its peak at 4 h after the acute-exercise (Table 2).

Table 4. Lipid concentrations in serum and liver

Lipid	Group	Time course			
		0 h	1 h	4 h	8 h
Serum					
TG	NT	0.99 ± 0.19	0.93 ± 0.16	1.13 ± 0.42	0.72 ± 0.36
(mmol/L)	LT	0.50 ± 0.05	0.51 ± 0.08	0.49 ± 0.11	0.51 ± 0.11
TC	NT	2.17 ± 0.31	1.91 ± 0.54	2.14 ± 0.26	1.80 ± 0.61
(mmol/L)	LT	1.03 ± 0.67	1.72 ± 0.47	1.30 ± 0.29	1.98 ± 1.23
TL	NT	0.018 ± 0.000	0.017 ± 0.001	0.026 ± 0.008	0.020 ± 0.005
(g/L)	LT	0.024 ± 0.007	0.026 ± 0.002	0.026 ± 0.005	0.028 ± 0.007
Liver					
TG	NT	58.9 ± 31.6^a	26.1 ± 4.0^{ab}	20.2 ± 6.8^b	16.9 ± 6.1^b
(μmol/g)	LT	26.5 ± 9.3	23.7 ± 4.2	24.6 ± 0.8	18.1 ± 5.8
TL	NT	0.081 ± 0.036^a	0.035 ± 0.016^b	0.037 ± 0.012^b	0.036 ± 0.024^b
(mg/g)	LT	0.080 ± 0.004	0.040 ± 0.025	0.056 ± 0.023	0.038 ± 0.014

All values are mean ± SD (n = 5). Values with different superscripts in the same rows are significantly different (p<0.05).
TG, Triglyceride; TC, Total cholesterol; TL, Total lipid; NT, Non-trained; LT, Long-term trained.

Table 5. Carnitine palmitoyltransferase-I activity in liver (nmol/min/mg protein)

Group	Time course			
	0 h	1 h	4 h	8 h
NT	0.4 ± 0.2^b	4.5 ± 2.2^a	3.4 ± 1.6^{ab}	3.2 ± 1.9^{ab}
LT	8.6 ± 4.0^a	7.9 ± 4.6^a	5.2 ± 1.8^b	2.8 ± 1.5^b

All values are mean ± SD (n = 5). Values with different superscripts in the same rows are significantly different (p<0.05).
NT, Non-trained; LT, Long-term trained.

On the other hand, in LT rats, the ASAC and TCNE levels increased during the exercise, then decreased and maintained the pre-exercise level (Table 2). In addition, the NEC level, which decreased significantly in the LT rats during exercise (unpublished results), was unchanged during the PERP (Table 2). Interestingly, the changes of carnitine fractions in the rat sera showed an opposite pattern in skeletal muscle. As shown in Table 3, in the skeletal muscle of NT rats, the ASAC level, which increased significantly by the acute-exercise, decreased to the pre-exercise level during the PERP. However, the levels of ASAC in LT rats reached its peak at 4 h after the 60 min running session. The liver carnitines in both NT and LT rats were unchanged by the acute-exercise (unpublished results) and during the PERP (Table 3). Previously, we showed that both exercise and a high fat diet can increase blood carnitine concentration (Cha *et al.*, 1999). We also showed that exercise alone increased carnitine concentrations in rat skeletal muscle independent of a high fat diet (Cha *et al.*, 1999). The current experiments, which provide support for our previous findings, also evaluate the interaction of long-term exercise and one time acute-exercise and rest on carnitine concentrations in rats. The current study shows that the pattern of increase and decrease in the carnitine concentrations in NT rats is different from that in LT rats.

There was no significant difference in serum TG, TC, and TL levels in both LT and NT rats during the PERP (Table 4). In the NT rats, the significantly increased levels of liver TG and TL by the 60 min acute-exercise decreased to the pre-exercise level after a one-hour rest and maintained that level during the PERP. The LT animals, which showed relatively small increases in the levels of liver TG and TL by the 60 min exercise (unpublished results), also recovered the level of pre-exercise after the one-hour rest and maintained that level (Table 4). The small increases of the TG and TL levels in LT rats are most likely due to the enhanced utilization for fuel (Hurley *et al.*, 1986) and/or a reduction in the substrate availability for TG synthesis (Froberg, 1971). These results may suggest that regular endurance exercise has a beneficial effect on hepatic lipids. Previously, it was also shown that both exercise and a low fat diet resulted in a lower body fat percentage in humans (Tucker *et al.*, 1997). A high energy expenditure is probably required to do the exercise training. Therefore, exercise could be one of the best ways to maintain body weight balance in humans. Recently, it has been reported that 92% of individuals who were successful at long-term maintenance of substantial weight loss used exercise as a part of their weight loss and maintenance program (Klem *et al.*, 1997).

Liver carnitine palmitoyltransferase-I (CPT-I) activity in NT rats increased significantly after 1 h of 60 min acute-exercise and decreased to the pre-exercise level during the PERP. The CPT-I activity in LT rats, which showed a significant increase in the activity by the 60 min exercise (unpublished results), decreased slowly and reached the pre-exercise level after 8 h of the recovery period (Table 5).

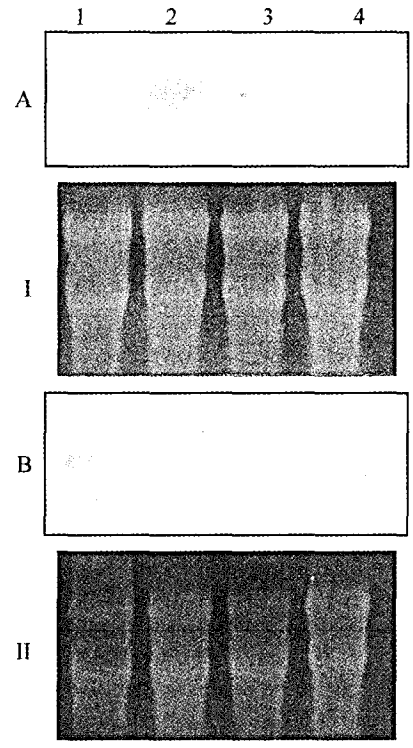

Fig. 2. Northern blot analysis showing changes in hepatic CPT-I mRNA during post-exercise recovery period. Liver samples were collected from each rat at each of the following time points: immediately following 60-min exercise (lane 1), 1 h (lane 2), 4 h (lane 3), and 8 h (lane 4) after the 60-min exercise. Total RNA (30 μg) was separated by 1% agarose gel electrophoresis and transferred to a Nylon membrane. Hybridization and visualization were conducted as described in Materials and Methods. A, Northern blot of NT rat mRNA; B, Northern blot of LT rat mRNA. I and II, ethidium bromide stain of RNAs from NT and LT rats, respectively.

Northern blot analysis showed that the changes of CPT-I activities during the PERP paralleled the alteration changes in the CPT-I mRNA levels (Fig. 2). These data may suggest that the CPT-I is regulated at the transcriptional level by exercise and rest. In a different study, the changes of CPT-I mRNA abundance, produced by hyperthyroidism and hypothyroidism, paralleled the changes in CPT-I activity in the rat liver. This suggests that the CPT-I is regulated at the transcriptional level by thyroid hormones (Mynatt *et al.*, 1994). Transcription of the rat liver CPT-I gene was also elevated by both high fat diets and exercise, suggesting that control of the CPT-I gene expression is a key feature in the regulation of fatty acid oxidation during exercise (Shon *et al.*, 1999). On the other hand, the activity of rat liver CPT-I was not significantly altered by long-term physical training (Guzman and Castro, 1988). In the study, the animals were not exercised for 24 h prior to killing.

Overall, this study shows that both regular endurance exercise, and acute-exercise and rest, can influence differently

the levels of carnitines, lipids, and CPT-I in rats. Further, the results may suggest that regular endurance exercise, rather than acute-exercise, can change effectively the distributions of carnitines, lipids, and CPT-I in rats during exercise and rest. Future studies with more detailed and extended time scales may provide further insights into the different modulation of carnitines, lipids, and CPT-I levels for the one-time and long-term exercises, as well as for the recovery period.

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