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Effects of physical training on fatty acid metabolism in liver and skeletal muscle of rats fed four different high-carbohydrate diets

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The adaptation of regulatory enzymes of fatty acid metabolism to endurance training and to the administration of four different diets containing fructose, sucrose, maltodextrins, or starch as carbohydrate (CH) was studied in rat liver and skeletal muscle (soleus). (i) In the soleus, physical training induced carnitine palmitoyltransferase I (CPT-I) activity upon complex (but not simple) CH feeding, whereas citrate synthase activity was enhanced by endurance exercise in the four different diets. No effect of diet or training was evident on glycerol 3-phosphate acyltransferase (G3PAT) activity or intramuscular triacylglycerol content. Glycogen levels were notably diminished in the soleus of trained rats fed simple-CH diets compared with their corresponding sedentary controls. (ii) In liver, CPT-I activity was depressed in trained animals fed the fructose diet, whereas citrate synthase was induced by endurance exercise in the four different diets. The activity of key lipogenic enzymes (acetyl-CoA carboxylase, fatty acid synthase, G3PAT) was induced by simple-CH feeding, and this increase was blunted by physical exercise. However, triacylglycerol accumulation and glycogen depletion ensued in liver of trained rats fed simple-CH diets. In these animals this was accompanied by a remarkable decrease of serum triacylglycerol levels and a strong increase of circulating non-esterified fatty acids (NEFA) and glycerol. Results thus suggest that in trained rats fed simple-CH diets (i) the preferential mobilization of adipose tissue triacylglycerols, by increasing the availability of circulating NEFA, may be responsible for liver triacylglycerol accumulation as well as for liver and muscle glycogen depletion, and (ii) decreased triacylglycerol secretion by the liver—but not changes in the activity of regulatory lipogenic enzymes—may be involved in hepatic triacylglycerol accumulation. (J. Nutr. Biochem. 7:348–355, 1996.)

Keywords: endurance exercise; fatty acid synthesis; fatty acid oxidation; liver; skeletal muscle

Introduction

During intense exercise carbohydrate is the predominant fuel utilized by skeletal muscle for contraction.[1–3] Thus, a great deal of work has been performed to elucidate the mechanisms that control glucose and glycogen metabolism during exercise.[1–3] However, during prolonged exercise of moderate intensity, and especially in well-trained subjects or laboratory animals, non-esterified fatty acids (NEFA) contribute a significant portion of substrate for oxidation by skeletal muscle.[4–5] In addition, increased hepatic fatty acid β-oxidation in these conditions supplies acetyl-CoA for ketone body production and enables the liver to increase its gluconeogenic capacity.[4–6] NEFA for muscle and liver metabolism are derived primarily from the hydrolysis of adipose tissue triacylglycerols, and secondarily from the hy-

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drolysis of triacylglycerols in the circulating chylomicrons and very-low-density lipoproteins.[4–6] In addition, triacylglycerols stored within the muscle cell have been implicated as important in supplying NEFA to working skeletal muscle.[7]

It is currently well-established that the use of NEFA during exercise depends on the diet consumed by the athlete or the laboratory animal.[5,8] To ensure a full replenishment of muscle glycogen stores, which in turn may help to sustain prolonged exercise, diets aimed to improve physical performance are usually rich in carbohydrates (CH). Several reports have focused on the relation between the type of dietary CH and the regeneration of glycogen stores in rat and human skeletal muscle.[2,3,8] In contrast, much less is known about the relation between the type of dietary CH and the adaptation of fatty acid metabolism to endurance exercise. Hepatic fatty acid metabolism is markedly affected by the type of CH in the diet, i.e., simple CH (especially fructose) induce lipogenesis much more efficiently than complex CH.[9] However, data on the effects of physical training on activities of liver lipogenic enzymes are not conclusive, and this may be ascribed mainly to differences in training protocols.[10,11] Moreover, as far as we know no study has been published to date on the adaptation of liver and muscle fatty acid metabolism to endurance exercise in rats fed different types of dietary CH.

The present study was undertaken to analyze the adaptation of fatty acid metabolism to endurance training and to the prolonged administration of four different high-CH diets (containing fructose, sucrose, maltodextrins, or starch as CH) in rat liver and slow-twitch skeletal muscle (soleus). Since the four diets were identical except for the type of CH, this study tests whether the adaptations of fatty acid metabolism to physical training depend on the type of CH that is present in the diet. Fatty acid metabolism was studied at the level of key regulatory enzymes involved in fatty acid synthesis, oxidation (translocation into mitochondria, tricarboxylic acid cycle), and esterification.

Methods and materials

Animals

Male Wistar rats were fed *ad libitum* four different CH-rich diets, with fructose, sucrose, maltodextrins, or corn starch as CH. Diets were provided by "Usine d'Alimentation Rationelle" (Villemoisson, France; ref. 119141708). The terms "simple CH" and "complex CH" (see below) refer to fructose/sucrose and maltodextrins/starch, respectively. The four diets were isocaloric, and they contained 65% of total calories as CH, 20% as fat and 15% as protein. Fat (corn oil), protein (casein supplemented with methionine), vitamins (Vitamine 200), and minerals (CM 205b) were identical in the four diets. Animals were housed individually under an inverted 12-hr light-dark cycle (light, 6 p.m. to 6 a.m.; dark, 6 a.m. to 6 p.m.).

Animals fed the four different diets were ("trained animals") or were not ("sedentary animals") subjected to physical training on a motor-driven treadmill (Columbus Instruments, Columbus, Ohio, USA). The eight resulting animal groups were selected at random and not on the basis of specific characteristics of the animals. Animals were trained at 10 a.m. to 11 a.m., i.e., during their active period. After 2 weeks of adaptation, animals ran continuously for 60 min at 30 m/min during 8 weeks, 6 days per week. These conditions correspond to an intensity of 60 to 65% of VO_{2max} (e.g.,[12–14]). Animals were not exercised for 48 hr before sacrifice to ensure that the potential effects observed are due to the long-term adaptation to physical training and not to acute effects of the last exercise session. Animals were killed by decapitation, and their livers were cold-clamped and frozen in liquid nitrogen. Hind limbs were also rapidly removed, and the soleus was excised and frozen in liquid nitrogen. Blood was collected in centrifuge tubes for serum isolation. Samples were kept at −80°C until they were used.

Carnitine palmitoyltransferase activity

Samples of the liver were homogenized (1:4, wt/vol) in a medium containing 10 mM Tris-HCl (pH 7.4), 0.25 M sucrose and 1 mM EDTA; the homogenization was performed on ice with a tight-fitting Potter-Elvehjem (10 strokes). Samples of the soleus were homogenized (1:9, wt/vol) in a medium containing 10 mM Tris-HCl (pH 7.4), 0.15 M KCl and 1 mM EDTA; the homogenization was performed on ice with a Polytron PT-10 tissue disintegrator (2 × 10 s). Mitochondria were isolated[15] and finally resuspended in 0.3 M sucrose, 10 mM Tris-HCl (pH 7.4), and 1 mM EDTA. Preparations of mitochondria were practically devoid of peroxisomes, as judged by the recovery of catalase activity (always lower than 5%).

Carnitine palmitoyltransferase I (CPT-I) activity was assayed within 15 min of the isolation of mitochondria as the malonyl-CoA-sensitive incorporation of L-[^{3}H]carnitine and palmitoyl-CoA into palmitoylcarnitine as described.[15] Reactions were carried out for 4 min and stopped with 1.0 ml of ice-cold 1 M HCl. [^{3}H]palmitoylcarnitine product was extracted with n-butanol.[15] Carnitine palmitoyltransferase (CPT) activity, which was insensitive to 100 μM malonyl-CoA, representing carnitine palmitoyltransferase II (CPT-II) activity, was always substracted from the enzyme activity experimentally measured. In these preparations of fresh mitochondria, CPT-II activity routinely accounted for less than 10% of the total enzyme activity experimentally determined.

For the determination of (total) CPT-II activity, mitochondria were subjected to three cycles of freezing-thawing and further homogenized on ice with a Polytron PT-10 tissue disintegrator (10 s). This procedure releases more than 95% of glutamate dehydrogenase activity from mitochondria. CPT-II activity was determined in these preparations of broken mitochondria as the malonyl-CoA-insensitive CPT activity using the assay described.

Acetyl-CoA carboxylase activity

Samples of the liver were homogenized (1:4, wt/vol) in a medium containing 50 mM Hepes-NaOH (pH 7.5), 0.25 M mannitol, 4.0 mM citrate, 6.2 mM EDTA, and 5.0 mM 2-mercaptoethanol; the homogenization was performed on ice with a loose-fitting Potter-Elvehjem (10 strokes). The crude homogenate was centrifuged at 12,000g for 5 min and the resulting supernatant was used for enzyme assay.

Acetyl-CoA carboxylase activity was determined as the incorporation of [^{14}]acetyl-CoA into fatty acids in a reaction coupled to the fatty acid synthase reaction.[16] The use of this procedure avoids the accumulation of malonyl-CoA in the incubation medium, which inhibits acetyl-CoA carboxylase, and obviates any interference with mitochondrial enzymes.[17] Reactions were stopped after 1 min by the addition of 100 μl 10 M NaOH. After saponification, fatty acids were extracted with light petroleum ether.[17]

Fatty acid synthase activity

Samples for determination of fatty acid synthase activity were obtained as described above for the acetyl-CoA carboxylase assay.

Fatty acid synthase activity was determined as the incorporation of [^{14}C]acetyl-CoA into fatty acids in the presence of malonyl-CoA[16]. Reactions were stopped after 5 min by the addition of 100 μl 10 M NaOH. After saponification, fatty acids were extracted with light petroleum ether.[17]

Glycerol 3-phosphate acyltransferase activity

Glycerol 3-phosphate acyltransferase (G3PAT) activity was determined in mitochondria and microsomes from liver and soleus. Mitochondria were isolated as described for the assay of CPT-I activity, whereas microsomes were obtained by spinning the 12,000g supernatant at 105,000g for 60 min exactly.[16] G3PAT activity was measured as the incorporation of [^{14}C]glycerol 3-phosphate and palmitoyl-CoA into lysophosphatidate.[16] Reactions were carried out at 30°C for 2 min (microsomes) or 4 min (mitochondria), and they were stopped with 1 ml methanol supplemented with 1 μmol glycerol 3-phosphate as carrier. Lysophosphatidic acid was subsequently extracted.[16]

Citrate synthase activity

Samples of the liver (ca. 100 mg) were homogenized in 5 ml of a medium containing 10 mM Hepes (pH 7.4), 0.3 M sucrose, 1 mM EGTA, 0.3 mM phenylmethylsulfonyl fluoride, and 25 mg bovine serum albumin (defatted and dialyzed); the homogenization was performed on ice with a tight-fitting Potter-Elvehjem (15 strokes). Samples of the soleus (ca. 50 mg) were homogenized in 2 ml of a medium containing 20 mM Hepes (pH 7.2), 120 mM KCl, 1 mM EGTA, 0.3 mM phenylmethylsulfonyl fluoride and 25 mg bovine serum albumin (defatted and dialyzed); the homogenization was performed on ice with a Polytron PT-10 tissue disintegrator (2 × 10 s). Crude homogenates were filtered through nylon mesh. Citrate synthase activity was determined spectrophotometrically at 412 nm as described by Srere.[18]

Other analytical methods

Serum concentrations of NEFA, glycerol, and triacylglycerols were determined with kits from Boehringer (Mannheim, Germany). Glycogen and triacylglycerols were quantified by standard spectrophotometric methods.[19] Insulin was quantified in serum samples by immunoassay.[20] Protein was determined by the method of Lowry et al.,[21] with bovine serum albumin as a standard.

Statistical analysis

Results shown represent the means ± S.D. of the number of animals of each experimental group (*Table 1*). Assays were always carried out in triplicate. Statistical comparison was performed using a two-way analysis of variance. A *post hoc* analysis was made by the Student-Neuman-Keuls test. Statistical significance was fixed at the 0.05 or the 0.01 level.

Results

The present study tests whether the adaptations of fatty acid metabolism to physical training depend on the type of CH (fructose, sucrose, maltodextrins or starch) that is present in the diet. Determinations were performed in rat liver and slow-twitch skeletal muscle (soleus). The soleus was chosen as a representative type of slow-twitch skeletal muscle because its recruitment during the training protocol performed by our animals is ensured. *Table 1* shows some general characteristics of the eight animal groups after the 10-week experimental period. At the end of the experimental period, trained rats showed a significant 10 to 20% decrease in body weight compared with the corresponding sedentary controls. Nevertheless, the average body weight gain was similar within the four sedentary and the four trained groups irrespective of the type of diet administered. The average food intake was not significantly different among the eight animal groups throughout the 10-week experimental period (data not shown). Neither liver weight nor liver protein content were significantly affected by physical training or the administration of the four different diets. The ratio liver weight:body weight was slightly (though not significantly) increased in the animals subjected to physical training. Therefore, interpretation of data on enzyme activities and substrate levels presented in the present report per mg of protein may be extended to the whole body.

Fatty acid metabolism in skeletal muscle

Fatty acid oxidation. CPT-I and CPT-II activities were not affected by the type of dietary CH (*Table 2*). Interestingly, physical training increased CPT-I activity in the animals fed complex CH, and especially in the starch group, but not in the animals fed simple CH (*Table 2*). This was accompanied in the starch group by a less remarkable increase of CPT-II activity (*Table 2*).

The differential behavior of CPT-I activity versus the type of dietary CH was not evident in the case of citrate synthase from soleus mitochondria. Thus, citrate synthase activity was not affected by the type of dietary CH, and it

Table 1 General characteristics of the eight animal groups used in the present study

Diet	Training	n	Body wt (g)			Liver wet wt (g)	Liver wet wt/ Body wt (%)	Liver protein content (g/g liver wet wt)
			Initial	Final	Gain			
Fructose	No	5	163 ± 6	380 ± 15	217 ± 11	14.1 ± 0.7	3.7 ± 0.4	0.18 ± 0.02
	Yes	5	160 ± 5	323 ± 34[a]	163 ± 23[a]	13.0 ± 1.4	4.0 ± 0.7	0.18 ± 0.01
Sucrose	No	5	164 ± 2	397 ± 18	233 ± 12	13.7 ± 0.8	3.5 ± 0.2	0.19 ± 0.03
	Yes	4	169 ± 7	346 ± 23[b]	177 ± 21[a]	13.1 ± 0.5	3.8 ± 0.4	0.19 ± 0.02
Maltodextrins	No	5	166 ± 3	409 ± 12	243 ± 19	14.0 ± 1.3	3.4 ± 0.6	0.21 ± 0.02
	Yes	4	170 ± 4	342 ± 16[a]	172 ± 10[a]	13.2 ± 0.8	3.9 ± 0.4	0.20 ± 0.02
Starch	No	5	169 ± 3	383 ± 29	214 ± 17	13.9 ± 1.2	3.6 ± 0.7	0.22 ± 0.01
	Yes	5	161 ± 5	335 ± 28[b]	174 ± 14[a]	13.3 ± 0.5	4.0 ± 0.4	0.21 ± 0.03

[a]$P < 0.01$ vs. the corresponding sedentary controls; [b]$P < 0.05$ vs. the corresponding sedentary controls.

Table 2 Effect of high-CH diets and endurance exercise on CPT-I and CPT-II activities in the soleus and the liver

Diet	Training	Soleus		Liver	
		CPT-I	CPT-II	CPT-I	CPT-II
Fructose	No	2.57 ± 0.33	1.45 ± 0.12	2.18 ± 0.38	2.45 ± 0.29
	Yes	2.24 ± 0.48	1.38 ± 0.08	1.33 ± 0.18[a]	1.70 ± 0.07[a]
Sucrose	No	2.82 ± 0.22	1.58 ± 0.28	2.00 ± 0.31	2.63 ± 0.22
	Yes	2.86 ± 0.44	1.71 ± 0.44	2.09 ± 0.35	2.90 ± 0.46
Maltodextrins	No	2.53 ± 0.33	1.45 ± 0.34	2.01 ± 0.11	2.45 ± 0.45
	Yes	3.47 ± 0.26[bd]	1.63 ± 0.12	1.85 ± 0.26	2.73 ± 0.18
Starch	No	2.50 ± 0.78	1.41 ± 0.23	2.03 ± 0.27	2.59 ± 0.13
	Yes	4.48 ± 0.89[ac]	2.22 ± 0.55[bd]	2.14 ± 0.50	2.47 ± 0.29

Values of CPT activity are expressed in nmol palmitoylcarnitine/min × mg protein.
[a]$P < 0.01$ versus the corresponding sedentary controls; [b]$P < 0.05$ versus the corresponding sedentary controls; [c]$P < 0.01$ versus trained rats fed complex-CH diets; [d]$P < 0.05$ versus trained rats fed complex-CH diets.

was enhanced by endurance exercise in a similar fashion in animals fed simple or complex CH (*Table 3*).

Lipogenesis. G3PAT catalyzes the first commited step of fatty acid esterification and is considered to constitute one of the rate-limiting reactions of this process.[22] Although it has been reported that the liver enzyme exists in two different subcellular locations (namely the mitochondrion and the endoplasmic reticulum),[22] we could not detected G3PAT activity in muscle microsomes (data not shown). Mitochondrial G3PAT in the soleus was not affected by either the type of CH in the diet or the practice of physical exercise (*Table 4*).

Mobilization of intramuscular triacylglycerol stores ensues on prolonged exercise.[5,7] However, no differences were evident in any of the 8 experimental groups with regard to muscle triacylglycerol content (*Table 5*). In addition, the effect of diet on fuel selection by the working muscle is considered to be accomplished through the size of the muscular glycogen stores.[5,6] In the case of the present study, in sedentary animals glycogen content was not significantly affected by the type of dietary CH, but physical training combined with simple-CH feeding induced a marked decrease of glycogen levels in the soleus (*Table 5*). In contrast, compared with sedentary rats, trained animals had similar glycogen content in the soleus when fed the complex-CH diets (*Table 5*).

Related blood parameters. Several blood parameters were determined to test whether any of them could be related to this differential replenishment of muscle glycogen stores upon the type of dietary CH. In sedentary rats, serum insulin concentration was higher upon the ingestion of simple-CH diets. However, no significant effect of physical training on insulinemia was evident in any of the four types of diet (Table 6). Interestingly, muscle glycogen levels were inversely related to the serum concentration of NEFA and glycerol (*Table 6*). Thus, the serum concentration of NEFA and glycerol, the two products of adipose tissue lipolysis, were only significantly increased in rats fed simple-CH diets and subjected to physical training, i.e., in those in which liver glycogen levels were lower (*Table 6*).

Fatty acid metabolism in liver

Fatty acid oxidation. In sedentary rats, CPT-I and CPT-II activities were not significantly different in the 4 types of diet (*Table 2*). However, physical training induced a specific decrease of CPT-I and CPT-II activities in the fructose

Table 3 Effect of high-CH diets and endurance exercise on citrate synthase activity in the soleus and the liver

Diet	Training	Citrate synthase (μmol/min × mg protein)	
		Soleus	Liver
Fructose	No	1.16 ±0.08	0.30 ± 0.03
	Yes	1.64 ± 0.24[a]	0.45 ± 0.04[a]
Sucrose	No	1.16 ± 0.16	0.29 ± 0.05
	Yes	1.64 ± 0.14[a]	0.43 ± 0.03[a]
Maltodextrins	No	1.06 ± 0.08	0.33 ± 0.06
	Yes	1.56 ± 0.15[a]	0.42 ± 0.05[b]
Starch	No	1.04 ± 0.06	0.30 ± 0.06
	Yes	1.82 ± 0.36[a]	0.41 ± 0.04[b]

[a]$P < 0.01$ versus the corresponding sedentary controls; [b]$P < 0.05$ versus the corresponding sedentary controls.

Table 4 Effect of high-CH diets and endurance exercise on G3PAT activity in the soleus and the liver

Diet	Training	Soleus	Liver	
		Mitochondrial G3PAT	Microsomal G3PAT	Mitochondrial G3PAT
Fructose	No	6.89 ± 0.68	29.7 ± 5.8	25.9 ± 6.7
	Yes	7.44 ± 0.97	29.0 ± 3.6	9.7 ± 1.5[a]
Sucrose	No	6.92 ± 0.54	34.8 ± 4.6	27.4 ± 4.9
	Yes	6.61 ± 0.39	35.8 ± 2.1	14.1 ± 1.7[a]
Maltodextrins	No	6.28 ± 0.42	16.8 ± 1.8[b]	23.1 ± 3.0
	Yes	6.42 ± 1.31	15.3 ± 3.7[c]	22.9 ± 2.7[c]
Starch	No	6.56 ± 0.96	18.0 ± 2.4[b]	25.0 ± 5.4
	Yes	6.23 ± 0.49	17.9 ± 5.1[c]	29.1 ± 3.1[c]

Values of G3PAT activity are expressed in nmol glycerol 3-phosphate into product/min × mg protein.
[a]$P < 0.01$ versus the corresponding sedentary controls; [b]$P < 0.01$ vs. sedentary rats fed simple-CH diets; [c]$P < 0.01$ vs. trained rats fed simple-CH diets.

group (*Table 2*). Like in muscle, the behavior of liver citrate synthase did not parallel that of the two CPT enzymes. Thus, physical exercise induced citrate synthase activity in a similar fashion in animals fed simple or complex CH (*Table 3*).

Lipogenesis. The activity of G3PAT from liver microsomes increased upon simple versus complex CH feeding (*Table 4*). However, no significant effect of endurance exercise was evident in any of the four different types of diet (*Table 4*). In contrast, G3PAT activity determined in liver mitochondria was not affected by the diet but by the practice of physical exercise. Thus, a training-induced decrease of mitochondrial G3PAT activity was observed in liver of rats fed simple-though not complex-CH (*Table 4*).

Unlike skeletal muscle, the liver synthesizes fatty acids *de novo* in a very efficient fashion. Acetyl-CoA carboxylase and fatty acid synthase catalyze the two enzymatic steps commited to hepatic fatty acid synthesis *de novo*.[9] In agreement with previous reports,[9] these two enzyme activities were induced in parallel by simple-CH feeding compared to complex-CH administration (*Table 7*). In addition, physical training decreased hepatic acetyl-CoA carboxylase and fatty acid synthase activities only in animals fed simple-CH diets (*Table 7*). It is noteworthy that these long-term changes were more remarkable in the case of fatty acid synthase than in the case of acetyl-CoA carboxylase (*Table 7*).

In rats fed complex-CH diets, the lack of effect of endurance exercise on the activity of key enzymes of fatty acid synthesis (acetyl-CoA carboxylase and fatty acid synthase, *Table 7*) and esterification (G3PAT, *Table 4*) was also evident on liver triacylglycerol content (*Table 5*). However, the exercise-induced decrease of lipogenic-enzyme activities observed in liver of rats fed simple-CH diets (*Tables 4* and *7*) did not correlate with hepatic triacylglycerol content. Surprisingly, physical training led to hepatic triacylglycerol accumulation in rats fed simple-CH diets (*Table 5*). Like in the soleus, simple-CH feeding did not enable a complete regeneration of liver glycogen stores upon physical training, whereas complex-CH ingestion did (*Table 5*).

Related blood parameters. As indicated above for muscle glycogen levels, this does not seem to be determined by the circulating levels of insulin (*Table 6*). However, a strong

Table 5 Effect of high-CH diets and endurance exercise on triacylglycerol and glycogen levels in the soleus and the liver

Diet	Training	Triacylglycerol content (μmol/g wet weight)		Glycogen content (μmol glucose residues/g wet weight)	
		Soleus	Liver	Soleus	Liver
Fructose	No	1.05 ± 0.08	10.9 ± 2.0	14.8 ± 2.5	391 ± 73
	Yes	1.18 ± 0.29	23.6 ± 1.8[a]	10.3 ± 1.8[b]	130 ± 27[a]
Sucrose	No	1.14 ± 0.29	9.7 ± 2.1	14.2 ± 3.2	353 ± 37
	Yes	1.21 ± 0.06	19.3 ± 2.2[a]	7.7 ± 2.0[a]	79 ± 6[a]
Maltodextrins	No	0.99 ± 0.11	7.1 ± 0.7	10.6 ± 1.0	203 ± 33[c]
	Yes	1.18 ± 0.24	7.4 ± 0.9[d]	13.4 ± 1.4	201 ± 61[d]
Starch	No	1.04 ± 0.11	7.7 ± 1.2	14.5 ± 3.4	224 ± 64[c]
	Yes	0.90 ± 0.18	7.5 ± 1.5[d]	15.3 ± 2.4	190 ± 45[d]

[a]$P < 0.01$ versus the corresponding sedentary controls; [b]$P < 0.05$ versus the corresponding sedentary controls; [c]$P < 0.01$ versus sedentary rats fed simple-CH diets; [d]$P < 0.01$ versus trained rats fed simple-CH diets.

Table 6 Effect of high-CH diets and endurance exercise on serum levels of insulin, NEFA, glycerol, and triacylglycerols

Diet	Training	Insulin (μU/ml)	NEFA (mM)	Glycerol (mM)	Triacyl-glycerols (mM)
Fructose	No	27.2 ± 2.8	0.25 ± 0.08	0.17 ± 0.01	2.07 ± 0.29
	Yes	27.5 ± 2.0	0.52 ± 0.07[a]	0.24 ± 0.03[a]	0.94 ± 0.04[a]
Sucrose	No	30.0 ± 3.4	0.29 ± 0.04	0.13 ± 0.02	2.17 ± 0.34
	Yes	30.7 ± 3.4	0.48 ± 0.12[b]	0.18 ± 0.01[a]	1.01 ± 0.13[a]
Maltodextrins	No	24.2 ± 4.0[d]	0.33 ± 0.09	0.13 ± 0.02	0.94 ± 0.09
	Yes	24.7 ± 3.4	0.29 ± 0.07[e]	0.13 ± 0.02[e]	0.79 ± 0.12
Starch	No	22.7 ± 0.9[c]	0.32 ± 0.02	0.13 ± 0.02	1.07 ± 0.24
	Yes	26.0 ± 3.1	0.32 ± 0.03[e]	0.13 ± 0.01[e]	0.80 ± 0.07

[a]$P < 0.01$ versus the corresponding sedentary control; [b]$P < 0.05$ versus the corresponding sedentary controls; [c]$P < 0.01$ versus sedentary rats fed simple-CH diets; [d]$P < 0.05$ versus sedentary rats fed simple-CH diets; [e]$P < 0.01$ versus trained rats fed simple-CH diets.

increase in plasma NEFA and glycerol concentrations was evident in trained rats fed simple-CH diets and subjected to physical training (*Table 6*). In these animals this was accompanied by a remarkable decrease of serum triacylglycerol concentration compared with their sedentary controls (*Table 6*).

Discussion

Prolonged exercise of moderate intensity is one of the well-known physiological situations in which NEFA contribute a significant portion of substrate for muscle and liver oxidative metabolism.[1,5] CPT-I is supposed to catalyze the rate-limiting step in the transport of long-chain fatty acids into the mitochondrial matrix in liver, and this reaction in turn constitutes a key step of long-chain fatty acid oxidation overall.[23,24] Thus, in nutritional or hormonal states in which plasma NEFA concentration and hepatic fatty acid oxidation change, the flux through the step catalyzed by hepatic CPT-I changes in parallel.[23,24] These changes are confined to liver CPT-I, whereas the enzyme from extrahepatic tissues (including skeletal muscle) is not affected.[24] Therefore, endurance exercise is a physiological situation of particular interest because CPT-I activity is increased in the soleus but not in the liver. It is worth noting that the induction of CPT-I in the soleus of trained animals was dependent on the type of dietary CH, and it only occurred upon administration of complex CH, especially starch. Previous reports show that rat[25] and human[26] skeletal muscle CPT-I activity, as well as rat skeletal muscle CPT-II mRNA levels,[27] are increased by physical training. Because the type of diet was not specified in these reports, it may be assumed that they were complex-CH diets. The reasons for the lack of effect of endurance training combined with simple-CH feeding on rat muscle CPT-I activity are still unknown. Induction of rat liver CPT-I occurs in pathophysiological situations in which the contribution of NEFA to hepatic oxidative metabolism increases at the expense of CH availability.[24] Extrapolation of data on the induction of liver CPT-I to the behavior of muscle CPT-I may not be accurate because liver and muscle CPT-I are enzyme proteins with very different properties.[28] However, the lack of induction of CPT-I in the soleus of trained rats fed simple-CH diets could be connected with the competition of CH and fatty acids for muscle oxidative metabolism, i.e., the availability of readily-absorbable monosacharides for muscle and/or the mobilization of muscle glycogen stores might be enhanced in that situation. In this respect we have observed (authors' unpublished re-

Table 7 Effect of high-CH diets and endurance exercise on acetyl-CoA carboxylase and fatty acid synthase activities in the liver

Diet	Training	Acetyl-CoA carboxylase	Fatty acid synthase
Fructose	No	1.27 ± 0.08	4.13 ± 0.70
	Yes	0.79 ± 0.07[a]	2.82 ± 0.28[a]
Sucrose	No	1.23 ± 0.08	4.41 ± 0.99
	Yes	0.78 ± 0.05[a]	2.67 ± 0.31[a]
Maltodextrins	No	0.74 ± 0.05[b]	1.59 ± 0.67[b]
	Yes	0.76 ± 0.01	1.69 ± 0.05[c]
Starch	No	0.82 ± 0.08[b]	1.68 ± 0.37[b]
	Yes	0.80 ± 0.05	1.70 ± 0.47[c]

Values of acetyl-CoA carboxylase activity are expressed in nmol malonyl-CoA formed/min x mg protein, whereas those of fatty acid synthase are expressed in nmol malonyl-CoA into fatty acids/min x mg protein.
[a]$P < 0.01$ versus the corresponding sedentary controls; [b]$P < 0.01$ versus sedentary rats fed simple-CH diets; [c]$P < 0.01$ versus trained rats fed simple-CH diets.

sults) that the glycogenic capacity of liver and skeletal muscle of trained rats is notably depressed by simple-CH administration compared with complex-CH administration.

In contrast to the induction of muscle CPT-I, the well-described exercise-induced increase of citrate synthase activity[1] occurred in the soleus and the liver and it was not dependent on the type of CH present in the diet. Citrate synthase catalyzes a central reaction of cell oxidative metabolism, namely the first step of the tricarboxylic acid cycle. In addition, citrate serves as a carbon source for lipid biosynthesis and is an important feed-forward allosteric activator of acetyl-CoA carboxylase.[9] Hence, it is likely that non-selective induction of citrate synthase occurs when tissue oxidative metabolism increases irrespective of whether NEFA or CH are the main oxidative substrates.

It is well established that triacylglycerols stored within the muscle cell are an important source of NEFA for working skeletal muscle.[5,7] Thus, the concentration of endogenous triacylglycerols in the muscle fiber decreases during exercise, and it becomes normalized in hours or days after an exercise session.[5,7] The mechanisms that control the hydrolysis and replenishment of intramuscular triacylglycerol stores are still poorly understood. Nevertheless, it has been shown that changes in triacylglycerol lipase activity do not correlate with changes in soleus triacylglycerol content in rats recovered from a 2-hr swim.[29] In the present report we show that endurance exercise does not affect G3PAT activity in rat soleus, indicating either that this is not a regulatory enzyme of triacylglycerol repletion or that potential changes affecting this enzyme activity disappear 48 hr after the exercise session (see Methods and materials). The latter possibility seems unlikely because changes in other enzyme activities are still evident after that time of recovery (the present report).

Hepatic lipogenesis is markedly affected by the type of CH in the diet.[9] In particular, simple CH induce acetyl-CoA carboxylase and fatty acid synthase more efficiently than complex CH.[9] Interestingly, physical training blunted the simple CH-mediated induction of acetyl-CoA carboxylase and fatty acid synthase. Liver G3PAT activity overall followed the same pattern than these two enzymes, although induction by simple CH was restricted to microsomal G3PAT and depression by endurance exercise in animals fed simple CH was confined to mitochondrial G3PAT, in line with the notion that mitochondrial and microsomal G3PAT display different properties and may be distinct enzymes.[22,30] Nevertheless, data in the literature on the effects of physical training on activities of liver lipogenic enzymes are not conclusive, and this may be ascribed mainly to differences in training protocols.[10,11]

Surprisingly, depression of lipogenic enzyme activities in trained rats fed simple-CH diets was accompanied by triacylglycerol accumulation, indicating that factors apart from these enzymatic reactions exert control over the synthesis of hepatic triacylglycerol stores in these animals. On one hand, prolonged physical exercise combined with fructose feeding lowered hepatic CPT-I and CPT-II activities. By increasing the availability of fatty acids for esterification at the expense of oxidation, this may contribute to triacylglycerol accumulation in liver of trained rats fed the fructose diet. Likewise, depressed CPT-I activity also seems to contribute to the generation of fatty liver in pathological situations such as chronic ethanol feeding.[15,24] On the other hand, decreased plasma concentration of triacylglycerols in trained rats fed simple-CH diets suggests that the output of hepatic triacylglycerols associated to very-low-density lipoproteins may be depressed, and this could be another factor involved in hepatic triacylglycerol accumulation upon simple-CH administration to trained rats. It is well established that physical training depresses hepatic triacylglycerol output.[5,10,31] Nevertheless, we are aware that enhanced clearance of plasma triacylglycerols might also contribute to lowering plasma triacylglycerol concentration.

Triacylglycerol accumulation in the liver of trained animals fed simple-CH diets was in turn associated with glycogen depletion, i.e., carbon biosynthetic sources are diverted to lipogenesis at the expense of glycogen synthesis. Similarly, glycogen stores were depleted in the soleus of these animals, although triacylglycerol accumulation did not ensue. As in other studies, data in this report show that the relation between the type of dietary CH and the regeneration of liver and skeletal muscle glycogen stores is not only dependent on differences in plasma insulin concentration.[2,32] Diets rich in fructose or sucrose are associated with insulin resistance and reduced glucose tolerance in rats and humans.[33] This may in turn stimulate the release of NEFA from adipose tissue and their use by other tissues.[33,34] In liver[35] and slow-twitch skeletal muscle,[36,37] NEFA inhibit glucose transport, glucose phosphorylation, and glycogen synthesis. This is precisely what was observed in this study, namely an increase in plasma NEFA and glycerol in trained rats fed fructose or sucrose and subjected to physical training. Our results thus suggest that the preferential mobilization of adipose tissue triacylglycerols, by increasing the availability of circulating NEFA, might be a factor involved in liver triacylglycerol accumulation and liver and muscle glycogen depletion upon the administration of diets containing simple CH to trained rats.

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