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Effect of Exercise on Response of Liver Lipogenic Enzymes to a High Fructose Diet[1] (38706)

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Endogenous hypertriglyceridemia is one of the factors predisposing to the development of coronary heart disease (1, 2). Physical exercise has been reported to cause a reduction in serum triglycerides in both humans and in experimental animals (3–7). The mechanism of the exercise-induced reduction in serum triglycerides is not known; both a reduction in the rate of hepatic lipogenesis and an increase in triglyceride clearance from the blood have been suggested as possible mechanisms (3, 4). One of the long-term means of regulating the rate of hepatic lipogenesis may be by alteration of levels of the lipogenic enzymes (8). When animals are fed diets containing substantial amounts of fructose or glucose (up to 70% of dietary calories), levels of the liver lipogenic enzymes increase markedly over a 2- to 3-day period (9). Fasting on the other hand, causes a decrease in the levels of the lipogenic enzymes in the liver (8). In this study we have examined the effect of exercise on the response of liver lipogenic enzymes to a high fructose diet.

Materials and Methods. Male Long-Evans strain rats (Blue Spruce Farms, Altamont, NY) weighing 125–150 g were divided into two groups. An exercising group was run on a motor-driven small animal treadmill (Quinton, Seattle) 5 days per wk. Speed and duration of the daily running bouts was gradually increased so that at the end of 6 wk, rats were running continuously for 1 hr at 1.2 mph up an 8° incline. They were maintained at this daily exercise level for an additional 4–7 wk before sacrifice. All rats ate Purina laboratory chow *ad libitum* during the first 4 wk of the study. Following this time all rats were given access to powdered Purina chow only between 9:00 AM and 12:00 noon. They were allowed free access to water, but were given no food during the remainder of the day. Food intake of the nonexercising sedentary group was restricted so that they gained weight at a rate similar to the runners.

Beginning two days following the last regular one-hr run, both groups were given on three successive days, a high fructose meal over a 3 hr period each morning. The diet contained 48.5% corn starch, 20% fructose, 20% casein, 5% corn oil, 5% Hegsted salt mix, and 1.5% vitamin fortification mix (General Biochemicals, Chagrin Falls, OH). As closely as could be estimated, the total carbohydrate content of this diet was similar to that of Purina Laboratory Chow; however, most of the carbohydrates in Purina chow are complex polysaccharides whereas the monosaccharide fructose makes up 29% of the carbohydrate component of the lipogenic diet used in this study. Each runner was paired with a sedentary animal of similar body weight. Each sedentary control animal was fed the same amount of food as was eaten spontaneously by the runner with which it was paired. The meal-feeding schedule was used to avoid possible differences in time of eating which could result in artifactual differences in hepatic lipogenic enzyme levels between the two groups (10). Beginning approximately 2 hr following the end of the meal on each of the 3 days, the runners were subjected to a 2 hr bout of treadmill running at 1.2 mph

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up an 8° incline. All rats were sacrificed by exsanguination under ether anesthesia 24 hr following the beginning of the last high fructose meal. A homogenate of liver (1:1) was prepared in 100 m*M* KPO_4 containing 10 m*M* glutathione, pH 7.4 using a glass Potter-Elvehjem homogenizer immersed in ice water. Following centrifugation at 28,000 *g* at 4°, an aliquot of the supernatant was passed through a sephadex G-25 column and was used immediately to determine acetyl CoA carboxylase activity. The remainder of the crude supernatant was frozen and saved for all other liver enzyme assays.

Acetyl CoA carboxylase activity was determined as described by Majerus *et al.* (11).

Fatty acid synthetase activity was determined by the method of Hsu *et al.* (12).

Glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase activities were determined by the methods described by Bottomley *et al.* (13).

Malic enzyme was determined by the method of Hsu and Lardy (14).

Citrate cleavage enzyme was determined by the method of Srere (15).

All assays were performed at 30°.

Protein was determined by the biuret method (16).

To determine whether or not differences in liver lipogenic enzyme activities modified triglyceride production by the liver, another group of rats was given 10 g fat-free high fructose diet (starch replacing corn oil in the diet described above) the morning following the 3-day treatment schedule described above. Three hr following the beginning of the meal, rats were anesthetized lightly with ether and a 0.5 ml blood sample was obtained. The rats were then given a saline solution containing Triton WR-1339 (Ruger Chem. Co., Irvington, NJ) at a dosage of 50 mg/100 g body weight by intracardiac injection. Blood samples were collected by cardiac puncture 60 and 120 min later and plasma was frozen for triglyceride assays. Plasma triglycerides were determined by the micro-method of Kaplan and Lee (17).

An additional experiment was conducted for the purpose of determining the effects of exercise on the response of the liver lipogenic enzymes to a more prolonged exposure to the high fructose diet. In this study, rats were given the high fructose meals for 5 days in succession. The running group was exercised daily as described for the 3-day treatment schedule. All animals were sacrificed 24 hr following the beginning of the last meal. Liver lipogenic enzymes were measured as described above.

Results. The results of the lipogenic enzyme assays were qualitatively the same whether expressed on the basis of μmoles/min/mg protein, μmoles/min/g liver wet weight, μmoles/min/liver, or μmoles/min/100 g body wt. We have arbitrarily chosen to express the results in terms of μmoles/min/g liver, wet weight.

As can be seen from Table I, activities of the various liver lipogenic enzymes were two to sevenfold higher in rats given the high fructose diet than in Purina-fed animals.

Prolonged exercise during the period following a high fructose meal appears to retard the increase in liver lipogenic enzymes induced by the high fructose diet. Exercised animals were observed to have significantly lower activities of five of the six liver lipogenic enzymes studied when compared to sedentary animals (Table I). Fatty acid synthetase was not influenced by the exercise.

Although the rate of post-prandial accumulation of serum triglycerides after Triton WR-1339 injection was higher in the sedentary than in the exercised animals on the high fructose diet the differences were not statistically significant (Table II).

When rats were fed the lipogenic diet for a more prolonged period, no difference was observed between exercised and sedentary animals with respect to the lipogenic enzymes studied, with the exception of citrate cleavage enzyme which remained lower in the livers of the runners (Table III).

Discussion. Each of the enzymes examined in this study plays an important role in synthesis of fatty acids in the liver. Acetyl CoA carboxylase and fatty acid synthetase are directly involved in formation of fatty acids from acetyl CoA (8). The hexose monophosphate shunt enzymes, glucose-6-phosphate dehydrogenase and 6-phospho-

TABLE I. EFFECT OF TWO-HR DAILY EXERCISE BOUTS ON RESPONSE OF LIVER LIPOGENIC ENZYMES[a] TO A HIGH FRUCTOSE DIET GIVEN THREE DAYS IN SUCCESSION.

	Purina chow sedentary (I)	High fructose diet sedentary (II)	runners (III)
Acetyl CoA carboxylase	0.19 ±0.01	0.94 ±0.10	0.71 ±0.09[b]
Fatty acid synthetase	0.035 ±0.004	0.22 ±0.02	0.19 ±0.01
Glucose-6-phosphate D. H.	2.3 ±0.4	8.5 ±0.9	5.2 ±0.5[c]
6-Phosphogluconate D. H.	8.1 ±0.4	14.0 ±0.7	10.2 ±0.6[d]
Citrate cleavage enzyme	0.7 ±0.1	4.8 ±0.5	2.6 ±0.3[c]
Malic enzyme	2.0 ±0.4	8.6 ±0.5	5.2 ±0.3[d]

[a] Enzyme activities expressed as μmoles substrate converted to product per g wet wt of liver per min; mean ± SEM; $n = 4$ for Purina-fed animals; $n = 8$ for all other values.
[b] $P < 0.05$, II vs III.
[c] $P < 0.01$, II vs III.
[d] $P < 0.001$, II vs III.

TABLE II. EFFECTS OF TWO-HR DAILY EXERCISE BOUTS ON POST TRITON SERUM TRIGLYCERIDES[a] IN RATS GIVEN HIGH FRUCTOSE DIET FOR THREE DAYS IN SUCCESSION.

Time of blood collection	Serum triglycerides (mg/100 ml) Sedentary	Runners
Before triton	46 ± 11	30 ± 8
60 min post triton	283 ± 40	197 ± 16
120 min post triton	592 ± 60	468 ± 44

[a] Values are means ± SEM; $n = 8$ for each group. Rats were given 10 g fat-free high fructose diet 3 hr before the Triton WR-1339 injection and 24 hr following the beginning of the third high fructose meal.

gluconate dehydrogenase, as well as malic enzyme, are important sources of NADPH for fatty acid synthesis (18, 19). Citrate cleavage enzyme is involved in the generation of extramitochondrial acetyl CoA for lipogenesis (20), which takes place primarily in the cytoplasmic compartment of the hepatocyte (8). All of these enzymes increase several fold in liver in response to a lipogenic diet (containing sucrose, fructose, or glucose in substantial amounts) over the course of a 2- to 3-day period (8, 9, 21). It is thought that this metabolic adaptation in the liver may be a long-term means of regulating the rate of hepatic lipogenesis (8, 20).

TABLE III. EFFECT OF TWO-HR DAILY EXERCISE BOUTS ON RESPONSE OF LIVER LIPOGENIC ENZYMES[a] TO A HIGH FRUCTOSE DIET GIVEN FIVE DAYS IN SUCCESSION.

	Sedentary	Runners
Acetyl CoA Carboxylase	0.49 ±0.06	0.57 ±0.06
Fatty acid synthetase	0.20 ±0.02	0.20 ±0.01
Glucose-6-phosphate D. H.	7.3 ±0.7	7.4 ±0.8
6-Phosphogluconate D. H.	11.7 ±0.8	11.0 ±0.5
Citrate cleavage enzyme	3.5 ±0.3	2.2 ±0.16[b]
Malic enzyme	6.8 ±0.5	6.8 ±0.5

[a] Enzyme activities expressed as μmoles substrate converted to product per g wet wt of liver per min; mean ± SEM; $n = 4$ for all values.
[b] $P < 0.05$.

Several investigators have observed decreases in serum triglyceride concentration in response to exercise (3–7). In the present study, activities of several liver lipogenic enzymes were significantly lower in exercised than in sedentary animals that had eaten equal amounts of the lipogenic diet for 3 days. Insulin has been implicated as being the inducer of lipogenic enzyme synthesis in liver after high carbohydrate feeding (21). The reduction in plasma insulin levels which accompanies exercise (22, 23) could conceivably be responsible for the lower levels of hepatic lipogenic enzymes in our exercised animals.

If any of these enzymes were rate limiting for fatty acid synthesis, a lower rate of triglyceride synthesis in the exercised animals might be expected. The experiment with Triton WR-1339 was undertaken to test this possibility. Triton WR-1339 has been shown to inhibit clearance of triglycerides

from the blood (24, 25). The rate of accumulation of triglycerides following Triton injection shortly after a fat-free meal is thus a crude measure of the rate of triglyceride production by the liver. Although the exercised animals tended to show lower rates of triglyceride accumulation than the controls after Triton, the differences were not statistically significant (Table III). Acetyl CoA carboxylase is generally thought to be the rate limiting step for fatty acid synthesis (26), although definitive experimental evidence is apparently lacking (8). Our results are consistent with the idea that the carboxylase is rate limiting. Only a small and marginally significant difference was found between exercised and sedentary animals with respect to liver acetyl CoA carboxylase activity.

It appears from our second study that with the exception of citrate cleavage enzyme, lipogenic enzymes reach similar levels in both exercised and sedentary animals after 5 days on the lipogenic diet. Sedentary control levels of some of the hepatic lipogenic enzymes were lower after 5 days than after 3 days on the lipogenic diet. This difference is consistent with the time course of the sugar-induced increase in lipogenic enzymes reported by other workers (21) wherein a temporary overshoot is seen prior to establishment of the new steady state. These results provide evidence that exercise does not influence the final steady state levels of the lipogenic enzymes, but only the time course of the approach to a new steady state.

Summary. Meal-fed Long-Evans rats fed a high fructose diet and exercised for 2-hr daily on a treadmill for three days had lower levels of several hepatic lipogenic enzymes (acetyl CoA carboxylase, glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, malic enzyme and citrate cleavage enzyme) than did sedentary rats pair-fed the diet. Accumulation of triglycerides in plasma following ingestion of a fat-free, high fructose meal and injection of Triton WR-1339, an inhibitor of plasma triglyceride clearance, was not significantly different in the two groups of animals.

All of the hepatic lipogenic enzymes measured, with the exception of citrate cleavage enzyme, attained similar levels in the runners as in the controls after 5 days on the high fructose diet. Thus the exercise appeared to affect the time course of the increase in the levels of activity of most of the lipogenic enzymes but not the final steady state levels attained.

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