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J Appl Physiol 94:2127-2134, 2003. First published Jan 24, 2003; doi:10.1152/jappphysiol.01164.2002

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A. Charbonneau, C. G. Unson and J.-M. Lavoie

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Lipid Metabolism and Liver Inflammation. I. Hepatic fatty acid uptake: possible role in steatosis

M. W. Bradbury

Am J Physiol Gastrointest Liver Physiol, February 1, 2006; 290 (2): G194-G198.

[Abstract] [Full Text] [PDF]

Alterations in hepatic glucagon receptor density and in Gs{alpha} and Gi{alpha}2 protein content with diet-induced hepatic steatosis: effects of acute exercise

A. Charbonneau, A. Melancon, C. Lavoie and J.-M. Lavoie

Am J Physiol Endocrinol Metab, July 1, 2005; 289 (1): E8-E14.

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Concurrent exercise prevents high-fat-diet-induced macrovesicular hepatic steatosis

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Submitted 18 December 2002; accepted in final form 22 January 2003

Gauthier, Marie-Soleil, Karine Couturier, Jean-Gilles Latour, and Jean-Marc Lavoie. Concurrent exercise prevents high-fat-diet-induced macrovesicular hepatic steatosis. *J Appl Physiol* 94: 2127–2134, 2003. First published January 24, 2003; 10.1152/jappphysiol.01164.2002.—The purpose of the present study was to assess the effect of an exercise training program conducted concurrently with a high-fat (HF)-diet regimen on the induction of hepatic steatosis. Two groups of rats were fed either a standard (SD) or a HF (40% kcal) diet for 8 wk and were additionally assigned either to a sedentary (Sed) or a treadmill-trained (TR) group. Training (5 days/wk) was initiated at the same time as the HF diet and was progressively increased, reaching 60 min at 26 m/min, 10% grade, for the last 4 wk. At the end of the 8-wk period, HF-Sed rats exhibited ~72% higher liver triacylglycerol concentration than SD-Sed rats (means \pm SE: 17.15 \pm 1.5 vs. 9.98 \pm 1.0 mg/g; $P < 0.01$). Histological quantification of lipid infiltration, with the use of an image analysis computing system, revealed that, although fat was mainly stored as microvesicles ($< 1 \mu\text{m}^2$), the HF-diet-induced hepatic steatosis occurred via the accumulation of macrovesicles ($> 1 \mu\text{m}^2$). Concurrent exercise training completely prevented the HF-diet-induced hepatic steatosis. The surface area of liver parenchyma infiltrated by lipid vacuoles was similar in HF-TR as in SD-Sed rats (26.4 \pm 1.8 vs. 29.3 \pm 5.9 $\times 10^3 \mu\text{m}^2/200,000 \mu\text{m}^2$ of liver parenchyma, respectively; $P > 0.05$). The different states of liver lipid infiltration after the HF diet in Sed and TR rats were associated with similar changes in plasma free fatty acids and glycerol, as well as with similar changes in fat pad weights, but not with plasma triacylglycerol levels. It is concluded that, after a HF-diet regimen of 8 wk in rats, hepatic steatosis occurs primarily via the accumulation of lipid as macrovesicles. Exercise training pursued at the same time completely prevents the HF-diet-induced macrovesicular hepatic steatosis.

liver; fatty liver; lipid infiltration; lipid vacuoles; insulin resistance; adiposity

FATTY LIVER, OR STEATOSIS, refers to a histopathological condition characterized by an excess accumulation of lipids, primarily triacylglycerols (TAG), within hepatocytes (6). Although simple steatosis is not strictly a form of hepatitis, it is included as part of the broad spectrum of nonalcoholic fatty liver diseases (1). The clinical significance of fatty liver is generally thought

to be a benign process. A subset of patients, however, go on to develop steatohepatitis, which then progresses to fibrosis, cirrhosis, and liver failure (2, 20, 30). Fatty liver can be classified as macrovesicular or microvesicular steatosis, depending on the size of the lipid vacuoles. Besides chronic ingestion of alcohol, macrovesicular fatty liver is also seen in other conditions, including Type 2 diabetes, obesity, and the metabolic syndrome (18, 30).

Increasing interest in the causes and the consequences of liver lipid infiltration has been recently spurred by the finding that an association exists between the accumulation of TAG in tissues other than adipocytes and the development of a state of insulin resistance (23). Studies in fatless mice have demonstrated that fat accumulation in skeletal muscle and liver is associated with insulin resistance and insulin signaling defects (12, 21). Studies in humans have also indicated that hepatic fat content is closely associated with causes of insulin resistance, such as obesity (18), and with defects in insulin suppression of glucose production, independent of obesity (25). These data provide significant support to the emerging concept that fat accumulation in insulin-sensitive tissues is deleterious for insulin action. Consequently, reduction of hepatic fat accumulation might be considered as a new therapeutic target in the overall problem of insulin resistance.

Exercise training accompanied by a low-fat diet has long been prescribed as part of the treatment in the management of obesity and Type 2 diabetes. Despite this, there is no firm conclusion as to whether or not exercise training can prevent the induction of hepatic steatosis. Two studies conducted in the 1970s have indicated that exercise training can prevent the accumulation of fat in the liver of rats receiving a high-fat (HF) diet (19, 22). In more recent studies, conducted with the use of a similar model of HF-induced obesity in rats, an absence of effects of exercise on liver lipid accumulation was reported (27, 28). The reasons for this discrepancy are not clear, although differences in the diet-induced fatty liver, in the exercise training program, and in the techniques used to assess liver lipid infiltration may all be candidates. The purpose of

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the present study was first to reexamine the effects of an exercise training program pursued at the same time as a HF-diet-induced obesity (40% in kcal) on the accumulation of lipids in the liver and the associated metabolic disturbances in plasma lipid profile, visceral and subcutaneous fat accumulation, and the development of insulin resistance. In addition, with the use of a novel image analysis computing system software program that enables precise morphological quantification of liver lipid infiltration, the present study was intended to precisely quantify the HF-diet-induced macrovesicular and/or microvesicular hepatic steatosis in rats and the extent to which exercise training affects this classification.

METHODS

Animal care. Female Sprague-Dawley strain rats ($n = 37$, Charles River, St-Constant, PQ), weighing 180–200 g (6 wk of age), on their arrival, were housed by pairs and had access to food and tap water ad libitum. Their environment was controlled in terms of light (12:12-h light-dark cycle starting at 6:00 AM), humidity, and room temperature (20–23°C). All experiments described in this report were conducted according to the directives of the Canadian Council on Animal Care.

Diet and exercise protocol. A few days after their arrival, all animals were randomly assigned to a standard (SD) or a HF diet for 8 wk. One-half of the animals in each dietary condition were concurrently exercise trained (TR) for the whole 8-wk period, whereas the other one-half remained sedentary (Sed). Hence, the four experimental groups consisted of SD-fed rats in the Sed (SD-Sed, $n = 9$) or the TR state (SD-TR, $n = 9$), or HF-fed rats in the Sed (HF-Sed, $n = 9$) or the TR state (HF-TR, $n = 10$). The HF diet consisted of 42% lipid, 36% carbohydrate, and 22% protein (kcal) and was provided in small pellets from ICN Pharmaceuticals (New York, NY). The SD diet (12.5% lipid, 63.2% carbohydrate, and 24.3% protein; kcal) consisted of the usual pellet rat chow (Agribrands Purina Canada, Woodstock, ON). Both diets are described in detail in Table 1.

Exercise training consisted of continuous running on a motor-driven rodent treadmill (Quinton Instruments, Seattle, WA) 5 times/wk for 8 wk. Rats were progressively run from 15 min/day at 15 m/min, 0% slope, up to 60 min/day at 26 m/min, 10% slope, for the last 4 wk.

All rats were weighed two times per week, and their food intake in grams was monitored three times per week.

Intravenous glucose tolerance test. Six weeks after the beginning of the dietary and exercise protocols, all animals

were submitted to an intravenous glucose tolerance test (ivGTT) while in an overnight-fasted state (~18 h). All TR animals were restrained from exercise 48 h before the test. The experiments were run between 9:00 AM and 1:00 PM. The ivGTT was conducted according to a modified technique of Bongbele et al. (4). On the morning of the test, animals were anesthetized with pentobarbital sodium (50 mg/kg ip) and shaved on the right side of their neck. A venous catheter (PE-50) was inserted in the right jugular vein and kept patent for the entire duration of the test with a sterile saline heparin solution (5 U/ml). A period of 15 min was allowed between the completion of the surgery and the beginning of the test to standardize the effects of the surgical stress. Blood samples were drawn at time -5 and 0 min to measure glucagon in the fasting state in addition to plasma glucose and insulin. The ivGTT consisted of an injection of a glucose bolus (0.5 g/kg of 50% dextrose solution) administered over a period of 10 s at time 0 min. The catheter was rinsed four to six times with the animal's blood to avoid any residual glucose in the catheter. Blood samples (0.5 ml) were collected in an EDTA-rinsed syringe (15%) at 2.5, 5, 15, 25, 35, and 60 min after the glucose injection, and the plasma was used for subsequent glucose and insulin analyses. Red blood cells from each sampling were resuspended in a Krebs-Heinseleit solution resembling plasma composition and reinjected into the animal. At the end of the ivGTT, the jugular catheter was removed, and the vein was closed, as well as the opening on the neck of the animal. Penicillin (penicillin G procain, 40,000 U/kg im) was injected in the right leg of the rat to prevent risks of infection. Animals were returned to their cages and housed individually for the remaining experimental period (2 wk). TR rats were allowed 1 day of recovery before the resumption of their training program.

Blood and tissue sampling. Two weeks after the ivGTT, all animals were killed between 9:00 AM and 12:00 PM. All TR animals were restrained from training 48 h before death. Food was removed from the animals' cage at least 2 h before death. After complete anesthesia (pentobarbital sodium, 50 mg/kg ip), the abdominal cavity was rapidly opened following the median line of the abdomen. Blood was rapidly (<45 s) and simultaneously drawn from the abdominal vena cava (~4 ml) and the hepatic portal vein (~1.5 ml) into syringes pretreated with EDTA (15%). Blood was centrifuged (3,000 rpm for 8 min, 4°C), and the plasma kept for further analyses. Several organs, muscles, and fat deposits were excised and weighed in the following order: liver, mesenteric fat, right and left limb muscles (plantaris, soleus, medial and lateral gastrocnemius), urogenital fat, retroperitoneal fat, and subcutaneous fat. All tissue samples were frozen in liquid nitrogen immediately after being weighed. The liver median lobe was freeze-clamped and used for glycogen and TAG determinations. For histological analysis of liver lipid infiltration, two cross sections of the large lobe were cut, embedded in histological compound (OCT), and prefrozen in 2-methylbutane brought to liquid nitrogen temperature. Mesenteric fat pad consisted of adipose tissue surrounding the gastrointestinal tract from the gastroesophageal sphincter to the end of the rectum, with special care taken in distinguishing and removing pancreatic cells. Urogenital fat pad included adipose tissue surrounding the kidneys, ureters, and bladder, as well as ovaries, oviducts, and uterus. Retroperitoneal fat pad was taken as that distinct deposit behind each kidney along the lumbar muscles. For subcutaneous deposit measurement, a rectangular piece of skin was taken on the right side of each animal, from the median line of the abdomen to the spine and the right hip to the first rib, as described by Krotkiewski and Bjorntorp (13). All plasma

Table 1. Composition of the high-fat and standard diets

High-Fat Diet	kg	Standard Diet	kg
Casein purified high Nitrogen	20	Crude protein	18.1
Corn starch	39	Crude fat	4.5
Corn oil	3.4	Crude fiber	3.4
Lard	14.6	Ash	6.7
AIN 76 mineral mix	5	Nitrogen-free extract	57.3
Alpha cell nonnutritive Bulk	16	Humidity	10
VDFM (sheet dextrose)	2		
Total	100		100

VDFM, vitamin diet fortification mixture.

and tissue samples were stored at -78°C until analyses, except for the liver cross sections, which were stored at -25°C until histochemical treatment and analysis.

Analytic procedures. Plasma glucose concentration was determined with the use of a glucose analyzer (Yellow Springs Instruments 2300, Yellow Springs, OH). Insulin and glucagon concentrations were measured with commercially available radioimmunoassay kits (Medicorp, Montréal, PQ, and ICN Pharmaceuticals). Plasma nonesterified fatty acid (NEFA) levels were measured with a commercially available kit from Roche Diagnostics (Mannheim, Germany), and glycerol, TAG, and β -hydroxybutyrate were measured with kits from Sigma Diagnostics (St. Louis, MO).

Liver TAG concentration was estimated from glycerol released after ethanolic KOH hydrolysis by using a commercial kit (Sigma Diagnostics). Although this method does not discriminate between glycerol from phospholipids or TAG, Frayn and Maycock (8) have shown that omitting removal of phospholipids leads to only a $\pm 2\%$ error in the determination of tissue TAG. Liver glycogen content was determined by use of the phenol-sulfuric acid reaction (15). For histological analysis, OCT-embedded liver sections were cut in a cryostat at $5\ \mu\text{m}$ and mounted on microscope slides at -25°C . The slides were then fixed with formaldehyde (10%) and stained with oil red O. Lipid infiltration was assessed with light microscopy by using an image analysis computing system (Northern Eclipse, Empix Imaging). This system enabled a morphological quantification of liver lipid infiltration in terms of lipid vacuole size and number, as well as the overall surface area of liver parenchyma occupied by lipid vacuoles. Lipid vacuoles with a surface area $< 1\ \mu\text{m}^2$ were considered as microvesicles, whereas lipid vacuoles with a surface area $> 1\ \mu\text{m}^2$ were considered as macrovesicles. To account for discrepancies in sinusoid size between animals, the number and the surface area of the lipid vacuoles are expressed for a standardized surface of liver parenchyma of $200,000\ \mu\text{m}^2$.

Statistical analysis. Values are expressed as means \pm SE. Statistical analyses were performed by a two-way ANOVA for nonrepeated-measures design, with diet and exercise training as main effects. Fisher's post hoc test was used in the event of a significant ($P < 0.05$) F ratio. The area under the curve for plasma glucose and insulin levels during the ivGTT was computed by using a trapezoidal model.

RESULTS

The HF diet resulted in a significant ($P < 0.01$) increase in body weight in HF-Sed compared with SD-Sed rats (Table 2). Exercise training in rats on the HF diet resulted in a smaller body weight gain ($P < 0.06$) compared with that in HF-Sed rats, so that, after training, body weight of HF-TR rats was similar to

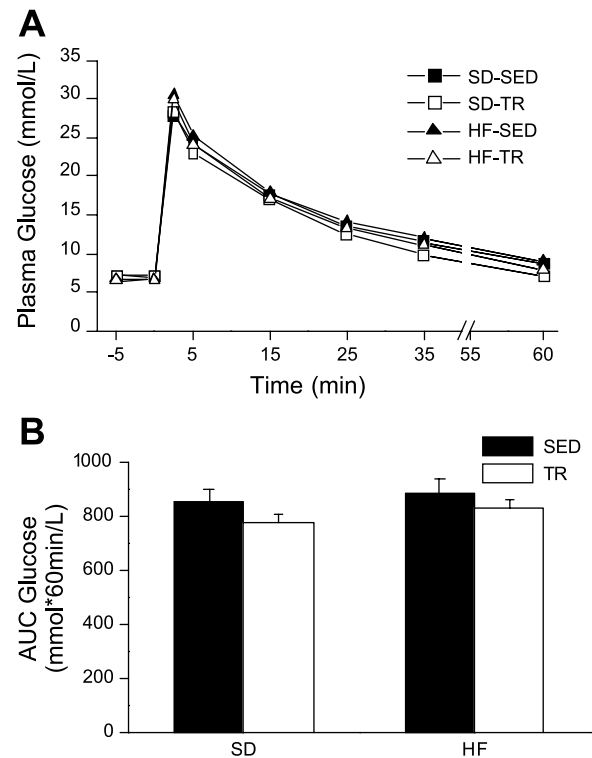


Fig. 1. Plasma glucose response during the intravenous glucose tolerance test (ivGTT; A; means) and total area under the curve (AUC) for glucose concentration (B; means \pm SE) in standard (SD)-fed rats in the sedentary (Sed) and exercise-trained (TR) state and in high-fat (HF)-fed rats in the Sed and TR state. $n = 9$ – 10 Rats/group.

body weight of SD-TR rats. The HF diet in the Sed state was associated with a significantly ($P < 0.05$) higher mean daily energy intake compared with the SD diet (Table 2). Exercise training increased ($P < 0.01$) energy intake only in animals fed the SD diet. A lower ($P < 0.01$) total relative muscle weight was measured after the HF diet in the Sed state, whereas it was only increased ($P < 0.01$) after exercise training in the HF group (Table 2).

There were no significant ($P > 0.05$) effects of either the HF diet or exercise training on plasma glucose response during the ivGTT (Fig. 1). However, the glucose-stimulated insulin response was significantly ($P < 0.05$) higher in HF-fed rats than rats fed the SD diet in both the Sed and the TR state. There was no significant

Table 2. Initial and final body weight, energy intake, and total relative muscle weight in standard- and high-fat-fed rats kept sedentary or exercise trained for 8 wk

	SD-Sed	SD-TR	HF-Sed	HF-TR
n	9	9	9	10
Initial body weight, g	183 ± 2.1	186 ± 1.0	187 ± 1.9	184 ± 1.1
Final body weight, g	284 ± 9.0	306 ± 4.3	$330 \pm 12.7^*$	306 ± 7.0
Total relative muscle weight, g/100 g body wt	0.732 ± 0.01	0.705 ± 0.01	$0.646 \pm 0.02^\ddagger$	$0.721 \pm 0.02§$
Energy intake, kcal/day	70.9 ± 1.4	$75.8 \pm 1.2^\ddagger$	$77.0 \pm 2.3^*$	75.1 ± 1.5

Values are means \pm SE; n , no. of animals. SD, standard diet; HF, high-fat diet; Sed, sedentary state; TR, trained state. Total relative muscle weight represents the sum of soleus, plantaris, and gastrocnemius muscle relative weight of the right leg. Energy intake represents the daily mean over the 8-wk protocol. Significantly different from SD counterpart: $*P < 0.05$, $^\ddagger P < 0.01$. Significantly different from Sed counterpart: $^\ddagger P < 0.05$, $§ P < 0.01$.

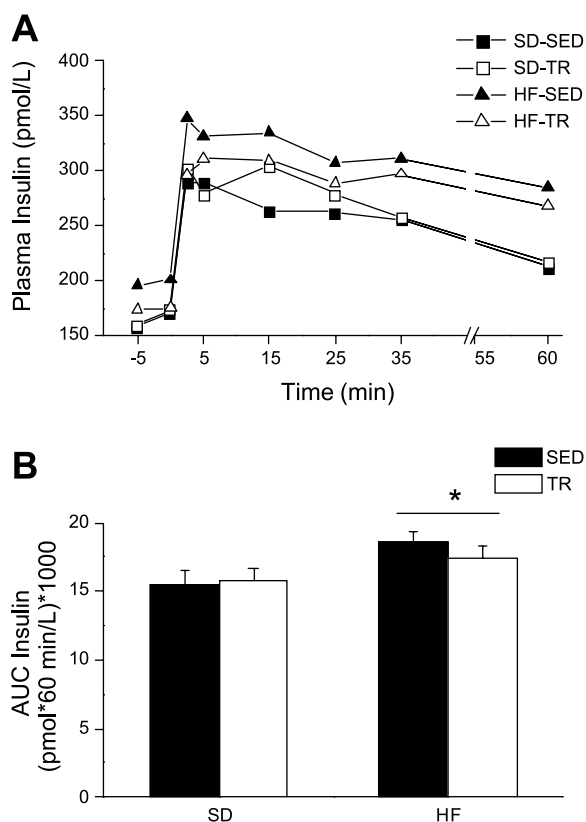


Fig. 2. Plasma insulin response during the ivGTT test (A; means) and total AUC for insulin concentration (B; means \pm SE) in SD-fed rats in the Sed and TR state and in HF-fed rats in the Sed and TR state. $n = 9-10$ Rats/group. *Significantly different from SD-fed animals, $P < 0.05$.

($P < 0.05$) effect of training on glucose-stimulated insulin response, although a tendency for a decrease (7.5%) can be observed in the HF rats (Fig. 2).

The HF diet in the Sed rats resulted in a significant ($P < 0.01$) increase in plasma NEFA and glycerol concentrations, whereas plasma TAG and β -hydroxybutyrate concentrations were not affected by the dietary manipulation (Table 3). Exercise training significantly ($P < 0.05$) reduced plasma NEFA and glycerol concentrations in both dietary conditions, although the values remained higher in rats fed the HF than the SD

diet. There was also a tendency ($P < 0.07$) for plasma TAG to be decreased by training in both dietary groups, whereas β -hydroxybutyrate values were not changed after training (Table 3). Plasma glucose and insulin levels measured in peripheral blood were not affected by either the HF diet or exercise training (Table 3). On the other hand, insulin measured in the portal blood and glucagon measured in the fasting state were all significantly ($P < 0.05$) increased after the HF diet. Although a substantial reduction in fasting glucagon level of $\sim 30\%$ can be observed after training in the HF rats, there were no statistically significant effects of training on the hormones measured in the present study (Table 3). As expected, the HF diet in the Sed rats resulted in a significant ($P < 0.05$) increase (from 133 to 228%) in the relative weight of all visceral and subcutaneous fat pads (Fig. 3A). Exercise training resulted in a significant ($P < 0.05$) decrease in mesenteric and subcutaneous fat deposits, and in the sum of the three visceral and the four measured (including subcutaneous) fat pads in both rats on the SD diet, and even more in rats on the HF diet (Fig. 3B). On the whole, it appears that the HF diet altered most of the variables related to lipid metabolism and that physical training was of significant value in counteracting these deleterious effects.

There were no significant ($P > 0.05$) effects of either HF diet or exercise training on relative liver weight (Fig. 4A). Liver glycogen content was significantly ($P < 0.01$) lower after the HF diet in both Sed and TR rats (Fig. 4B). Training resulted in an increase ($P < 0.05$) in liver glycogen content in both dietary groups (Fig. 4B). The HF diet in the Sed group resulted in a significant ($P < 0.01$) increase in liver TAG concentrations (Fig. 4C). Exercise training in rats on the HF diet prevented the increase in liver TAG ($P < 0.01$), so that, after training, liver TAG concentrations were similar in HF-TR and SD-Sed groups (Fig. 4C). The histological assessment of liver lipid infiltration revealed that the total surface area occupied by lipid vacuoles was largely increased ($\sim 48\%$) after the HF diet in the Sed state, although the statistical significance level of this comparison was at $P < 0.07$ (Fig. 5A). As for the liver TAG levels, the surface area occupied by the lipid

Table 3. Plasma metabolites and hormone concentrations in standard- and high-fat-fed rats kept sedentary or exercise trained for 8 wk

	SD-Sed	SD-TR	HF-Sed	HF-TR
<i>n</i>	9	9	9	10
NEFA, mM	0.09 \pm 0.01	0.05 \pm 0.01 ^d	0.21 \pm 0.04 ^c	0.13 \pm 0.02 ^{c,d}
Glycerol, mM	0.12 \pm 0.01	0.08 \pm 0.01 ^e	0.18 \pm 0.03 ^b	0.11 \pm 0.01 ^{b,e}
TAG, mM	0.75 \pm 0.10	0.60 \pm 0.06	0.92 \pm 0.22	0.59 \pm 0.07
β -OH, mM	0.10 \pm 0.01	0.11 \pm 0.01	0.09 \pm 0.01	0.08 \pm 0.01
Glucose, mM	7.66 \pm 0.21	8.06 \pm 0.21	7.76 \pm 0.23	7.63 \pm 0.22
Peripheral insulin, pM	148.7 \pm 10.5	170.9 \pm 15.7	168.7 \pm 16.5	163.1 \pm 10.7
Portal insulin, pM	225.6 \pm 17.2	219.3 \pm 30.1	265.8 \pm 23.5 ^a	277.0 \pm 26.8 ^a
Fasting glucagon, pg/l	191.7 \pm 9.9	207.3 \pm 30.3	345.6 \pm 70.6 ^a	247.7 \pm 18.2 ^a

Values are means \pm SE; *n*, no. of animals. NEFA, nonesterified fatty acids; TAG, triacylglycerol; β -OH, beta-hydroxybutyrate. Note: all variables were measured in a postprandial state (>2 h) except for fasting glucagon. Significantly different from SD-fed counterpart animals: ^a $P < 0.05$, ^b $P < 0.01$, ^c $P < 0.001$. Significantly different from Sed counterpart animals. ^d $P < 0.05$, ^e $P < 0.01$.

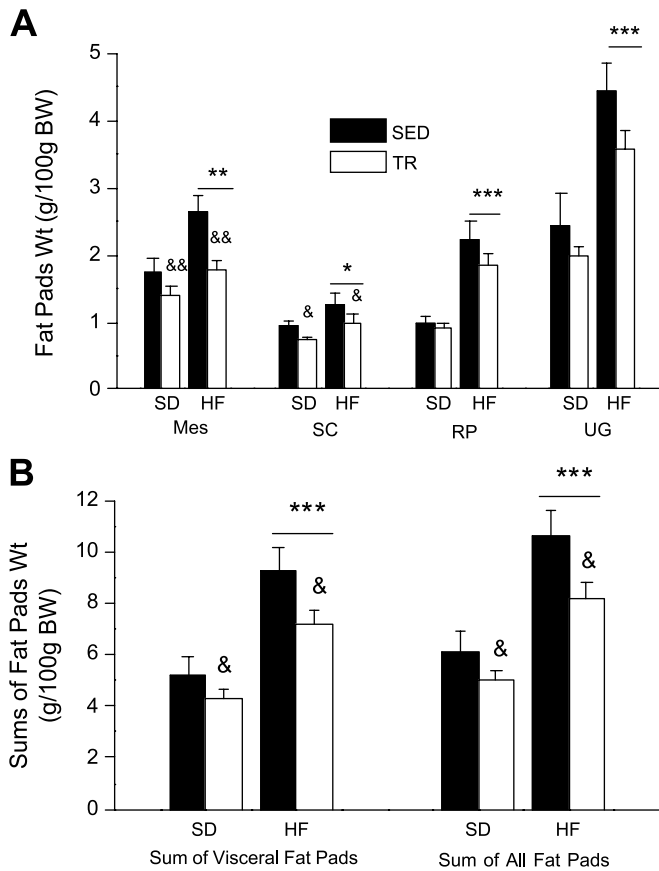


Fig. 3. Mesenteric (Mes), subcutaneous (SC), retroperitoneal (RP), and urogenital (UG) fat weight (Wt) relative to 100 g body weight (BW; A) and the sum of the relative weights of the 3 visceral fat pads (Mes, RP, and UG) and the 4 fat pads (including SC; B) in SD-fed rats in the Sed and TR state and in HF-fed rats in the Sed and TR state. Values are means \pm SE; $n = 9$ –10 rats/group. Significantly different from corresponding SD-fed group: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Significantly different from corresponding Sed group: & $P < 0.05$, && $P < 0.01$.

vacuoles was significantly ($P < 0.01$) reduced by exercise training (Fig. 5A). No statistical differences were found between groups in the total number of lipid vacuoles in the liver (Fig. 5B). There was no statistical difference between all groups in the number of liver lipid vacuoles of surface area $< 1 \mu\text{m}^2$ (Fig. 6). HF-Sed rats had, however, a significantly ($P < 0.05$) higher number of lipid vacuoles with surface area between 1 and $10 \mu\text{m}^2$ than did SD-Sed rats. Exercise training significantly ($P < 0.05$) lowered the number of liver lipid vacuoles in all sizes between 1 and $10 \mu\text{m}^2$ (Fig. 6).

DISCUSSION

The present HF diet regimen in rats resulted in a 72% higher fat accumulation in the liver compared with that of SD-fed animals (Fig. 4C). Histological analyses confirmed these results, with the surface area of liver parenchyma occupied by the lipid vacuoles being $\sim 48\%$ greater in HF- than in SD-fed animals (Fig. 5A). These results are in concordance with those of previous studies in which hepatic steatosis in ro-

dents was induced by HF diets (9, 19, 27). Hepatic steatosis can also be induced by other types of diets, such as high-cholesterol (28), ethanol-containing (29), choline-methionine-deficient (17), and high-sucrose diets (3). In the present study, we used a 40% HF diet in rats (compared with $\sim 12\%$ fat in standard chow, in kcal) over a relatively long period of time (8 wk) for this species, in an attempt to reproduce a common HF diet in humans ($\sim 60\%$ kcal as fat in a HF diet vs. 30% in a recommended standard diet). Our results, using biochemical and histological measurements, clearly show that hepatic steatosis can be induced by such a fat-rich diet without any toxin ingestion or alimentary deficiency.

The major finding of the present study is that, when an exercise training program is pursued at the same time as the HF diet, the induction of hepatic steatosis is completely suppressed. At the end of the 8-wk experimental period, HF-TR animals had similar liver fat content as SD-Sed animals. This was observed by using biochemical as well as histological analyses (Figs. 4C and 5A). There is some evidence that ethanol-induced fatty liver can be attenuated by repeated running exercise in rats (29). The evidence that exercise training

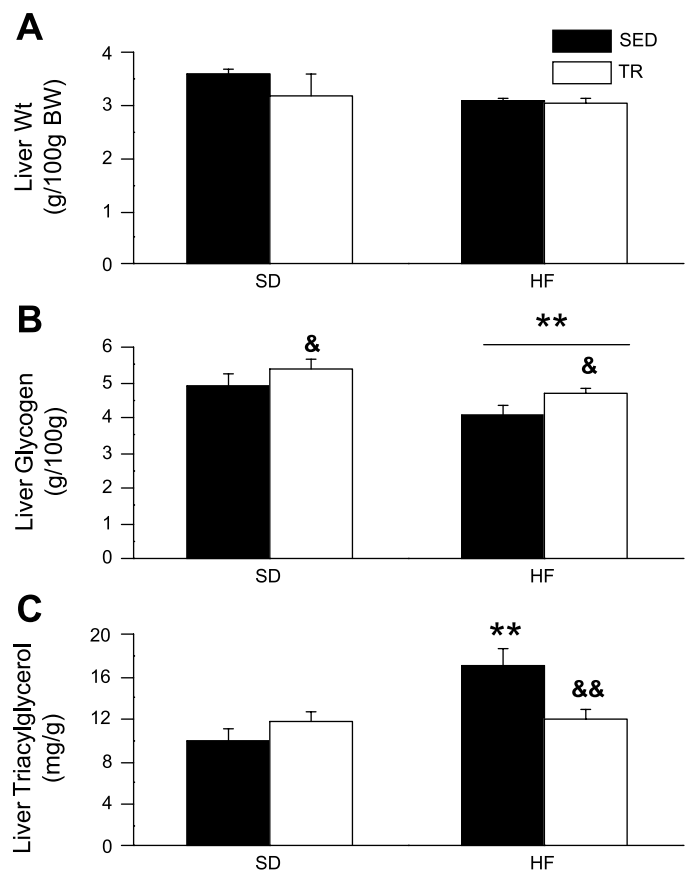


Fig. 4. Liver weight relative to 100 g BW (A), liver glycogen (B), and liver triacylglycerol (C) concentrations in SD-fed rats in the Sed and TR state and in HF-fed rats in the Sed and TR state. Values are means \pm SE; $n = 7$ –10 rats/group. **Significantly different from corresponding SD-fed group, $P < 0.01$. Significantly different from corresponding Sed group: & $P < 0.05$, && $P < 0.01$.

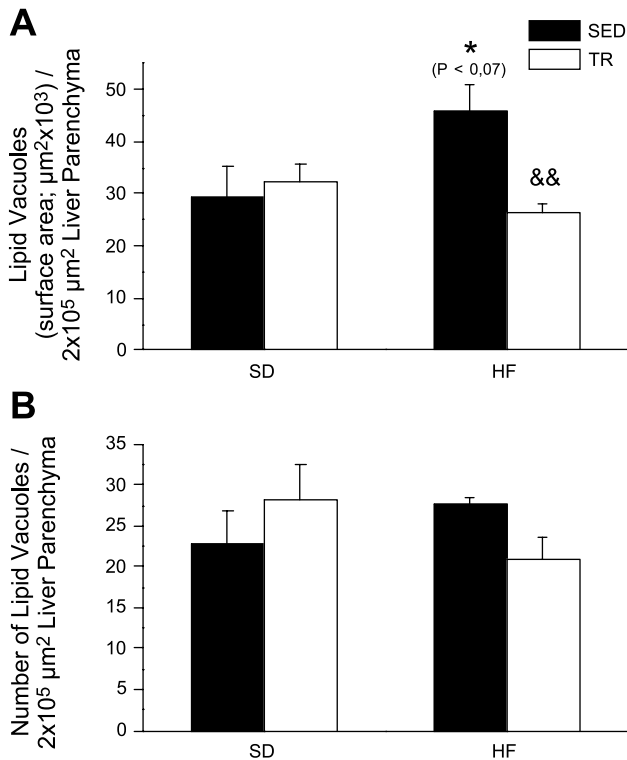


Fig. 5. Total surface area occupied by the lipid vacuoles (A) and total number of lipid vacuoles (B), both expressed for a standardized surface area ($2 \times 10^5 \mu\text{m}^2$) of liver parenchyma in SD-fed rats in the Sed and TR state and in HF-fed rats in the Sed and TR state. Values were obtained from histochemical quantification by using an image analysis computing system (Northern Eclipse, Empix Imaging). Values are means \pm SE; $n = 4-8$ rats/group. *Significantly different from corresponding SD-fed group, $P < 0.07$. &&Significantly different from corresponding Sed group, $P < 0.01$.

reduces liver lipid accumulation induced by fat-rich diets is, however, controversial. In previous studies, an effect (19) and the absence of an effect (28) of exercise training on hepatic steatosis have been reported. Comparison between these two studies reveals that, in the latter study (28), exercise training was started after fatty liver had been induced. It is, therefore, possible that, to be most effective, exercise must be concurrent with the induction of fatty liver. In a recent study, however, Straczkowski et al. (27) failed to show an effect of concurrent exercise training on HF diet-induced hepatic steatosis in rats. In that study, rats were sustained on a 59% fat diet (as kcal), resulting in an $\sim 190\%$ increase in liver TAG and an $\sim 65\%$ decrease in liver glycogen over a period of 3 wk. Exercise training, over a 4-wk period, consisted of running six times per week from 1 to 3 h at 20 m/min, 10% slope. In the present study, liver TAG were increased by $\sim 72\%$, whereas liver glycogen content was decreased by $\sim 20\%$. It is thus possible that, in the study by Straczkowski et al. (27), the metabolic perturbations induced by the HF diet might have been too intense to be compensated by the exercise training program. Although the modalities of application of the exercise regimen seem to play a role, the present results clearly indicate that a 40% HF diet-induced hepatic steatosis

can be totally prevented by a concurrent exercise training program.

Hepatic steatosis can be distinguished as macro- or microvesicular, according to the size of the lipid vacuoles (>1 or $<1 \mu\text{m}^2$ of surface area, respectively). Macrovesicular steatosis has been reported to be more common (26), whereas microvesicular steatosis is usually described in association with more severe clinical cases (7, 10). It has been recently suggested, however, that microvesicular steatosis is more prevalent than was originally believed and that it can be present without liver dysfunction (7) or hepatotoxicity (24). Thus it seems that the clinical implications of microvesicular vs. macrovesicular steatosis require better characterization. In a first step toward this goal, we, for the first time, used a novel image analysis computing system to quantitatively assess the number and the size of lipid vacuoles accumulated within liver parenchyma. The results obtained with the use of this tool indicate that, although the number of microvesicles in the liver is far greater than the number of macrovesicles, HF-diet-induced hepatic steatosis occurs mainly via an increased number of macrovesicles (Fig.

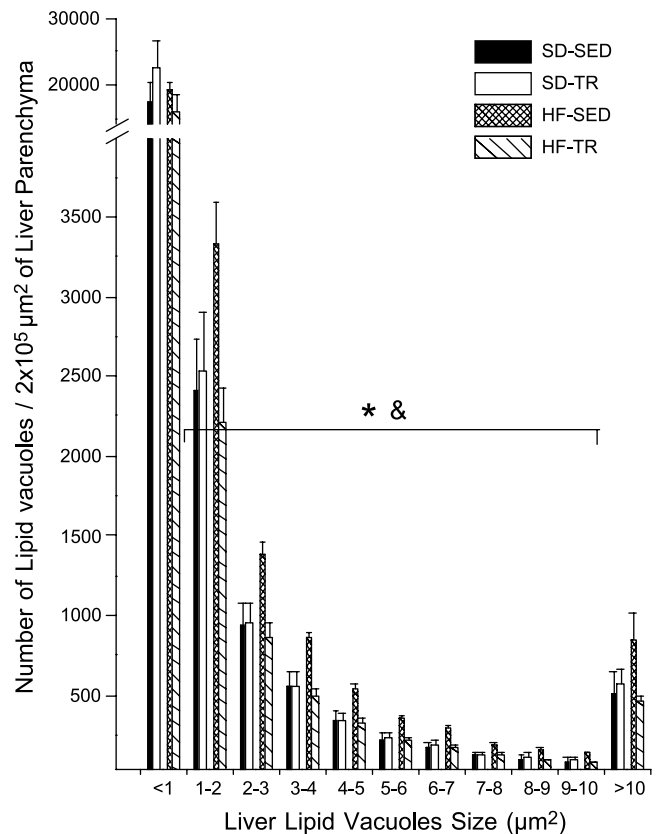


Fig. 6. Number of lipid vacuoles per unit of surface area expressed for a standardized surface area ($2 \times 10^5 \mu\text{m}^2$) of liver parenchyma in SD-fed rats in the Sed and TR state and in HF-fed rats in the Sed and TR state. Values were obtained via histochemical quantification by using an image analysis computing system (Northern Eclipse, Empix Imaging). Values are means \pm SE; $n = 4-8$ rats/group. Significantly different between *HF-Sed and SD-Sed groups and &HF-TR and HF-Sed groups, $P < 0.05$. Statistical significance was for all vacuole sizes between 1 and $10 \mu\text{m}^2$ (shown within brackets).

6). The present study also provides the first evidence that prevention of HF-diet-induced hepatic steatosis by exercise training occurs mainly by preventing the accumulation of TAG as macrovesicles, without any apparent changes in the pool of microvesicles. It is not possible, however, to determine from the present data whether lipid macrovesicles are newly synthesized as large vesicles or whether they result from the aggregation of small lipid vesicles into larger vesicles (5), accompanied by an increased synthesis of microvesicles. Nevertheless, the increased number of macrovesicles without any reduction in quantity of microvesicles should have led to an increase in the total number of vesicles after the HF diet. Although a tendency toward an increase in the total number of vesicles after the HF diet and a decrease in the total number of vesicles after exercise training can be observed among HF-fed rats, the differences did not reach statistical significance (Fig. 5B). This could be due to the remarkably greater number of microvesicles compared with macrovesicles and the fact that the number of microvesicles was not affected by the present dietary and exercise manipulations (Fig. 6).

The precise mechanism by which macrovesicular hepatic steatosis can be prevented through exercise training remains speculative. Mechanisms involved in the pathogenesis of HF-diet-induced hepatic steatosis are themselves nebulous. The accumulation of lipids within the liver must be the result of an imbalance among 1) lipid uptake by the liver, 2) lipid oxidation inside the liver, and 3) very-low-density lipoprotein (VLDL)-TAG secretion. In the present experiment, HF diet resulted in more than a twofold increase in plasma NEFA. The increased delivery of fatty acids to the liver should result in a higher uptake by the liver, because NEFA uptake is mostly done in a concentration-dependent manner (5). Although we did not directly measure liver lipid oxidation, plasma β -hydroxybutyrate levels, which can be used as an index of the activation of this metabolic pathway, were not altered by either the HF diet or exercise training. An increase in lipid uptake by the liver should have resulted in an increased output through incorporation of TAG into VLDL. The increased VLDL secretion by the liver should have led to an increased plasma level of TAG (11). In our study, plasma TAG levels were not, however, increased by the HF diet. During increased fat availability, the synthesis and secretion of VLDL have already been reported to be insufficient to ensure the normal lipid cycling between adipocytes and hepatocytes (16, 31). Altogether, the changes in blood lipid profiles after the HF diet suggest that fat accumulation inside the liver was the result of an increased uptake of fatty acids by the organ, leading to an increased TAG synthesis rather than an increased oxidation. Furthermore, it appears that the increased liver TAG synthesis could not be compensated by an increase in VLDL secretion, resulting in fat accumulation within hepatocytes.

Consistent with the above-discussed mechanisms involved in the development of HF-diet-induced hepatic steatosis, there are three possibilities through which

exercise training could have prevented liver fat accumulation: 1) a diminished delivery of lipids to the liver, 2) an increased hepatic oxidation, and/or 3) an increased incorporation of TAG into VLDL. Concurrent training in rats receiving the present HF diet resulted in a large decrease in circulating plasma NEFA, no change in plasma β -hydroxybutyrate levels, and a tendency ($P < 0.07$) for lower plasma TAG levels. Altogether, these results suggest that the preventing effect of exercise training on hepatic fat accumulation is a consequence of a diminished delivery of NEFA to the liver rather than an increased hepatic oxidation or an increased VLDL synthesis.

The reduced NEFA and glycerol plasma concentrations in TR animals are most likely related to the decreased level of adiposity in these rats, most likely due to an increased fat utilization. The interpretation that the decrease in liver lipid accumulation with exercise training is due to a decrease in circulating lipids rather than an increased output from the liver would be consistent with the above-mentioned suggestion that training is more efficient in decreasing hepatic fat accumulation if it is conducted at the same time that fatty liver is induced than if it is conducted after fatty liver has been induced. Another interesting observation is that, similar to the liver, fat accumulation in the mesenteric fat pad, which is drained into the hepatic portal vein, was completely prevented by exercise training. In contrast, HF-diet-induced enlargement of other visceral fat pads (urogenital and retroperitoneal), which are drained into the vena cava, was not completely abolished by exercise training. It has been suggested in a recent review (14) that intracellular TAG storage depot within the liver could act as an internal buffer to temporarily protect the hepatocytes against the potential toxicity of NEFAs when their uptake is greater than their oxidation rate and VLDL secretion. Similarly, it can be postulated that, along with the mesenteric fat pad, the liver could act as a systemic buffer to temporarily lower circulating NEFAs.

A decrease in liver lipid accumulation may have clinical significance. An increase in liver TAG content has been associated with impaired insulin signaling pathway within hepatocytes of fatless mice, resulting in decreased insulin suppression of hepatic glucose production (12). Normal insulin action on hepatic glucose production was reestablished when liver TAG were reduced by redistribution of fat into transplanted adipocytes (12). In our experiment, hepatic insulin sensitivity was not determined. However, the results of the ivGTT revealed that HF-fed rats developed mild systemic insulin resistance, which was only somewhat improved by training (Fig. 2). HF-fed rats also depicted higher portal insulin and fasting glucagon levels than their SD-fed counterpart animals, which was not fully counteracted by training. The rather small effect of training on these insulin-sensitivity-related variables contrasts, however, with the large effect of training observed on hepatic lipid accumulation. Hepatic steatosis also constitutes the first hit in the pathogenesis of nonalcoholic fatty liver, which can progress to ste-

atohepatitis, steatonecrosis, fibrosis, cirrhosis, and even liver failure (2, 20). Exercise training can, therefore, constitute a tool contributing to the protection of the liver against clinical complications of fatty liver.

In summary, the results of the present study indicate that a HF diet administered over a period of 8 wk in rats induced a state of hepatic steatosis, which was primarily attributable to an accumulation of lipids into macrovesicles ($>1 \mu\text{m}^2$ of surface area). Exercise training conducted concurrently with the HF diet completely prevented the accumulation of lipids into the liver. The exercise-induced decrease in hepatic fat accumulation was mainly attributed to a reduction in the number of macrovesicles.

The authors acknowledge Dr. Claudette Léger-Gauthier for assistance in histological analysis of hepatic lipid infiltration.

This work was supported by grants from the Natural Sciences and Engineering Research Council of Canada and Fonds pour la Formation de Chercheurs et d'Aide à la Recherche (government of Quebec).

REFERENCES

- Angulo P. Nonalcoholic fatty liver disease. *N Engl J Med* 346: 1221–1231, 2002.
- Bacon BR, Farahvash MJ, Janney CG, and Neuschwander-Tetri BA. Nonalcoholic steatohepatitis: an expanded clinical entity. *Gastroenterology* 107: 1103–1109, 1994.
- Bacon BR, Park CH, Fowell EM, and McLaren CE. Hepatic steatosis in rats fed diets with varying concentrations of sucrose. *Fundam Appl Toxicol* 4: 819–826, 1984.
- Bongbele J, Gutierrez A, Cardin S, and Lavoie JM. Effects of exercise training on insulin response to intravenous glucose in pubertal rats. *Int J Sports Med* 13: 225–229, 1992.
- Burt AD, MacSween RNM, Peters TJ, and Simpson KJ. Non-alcoholic fatty liver: causes and complications. In: *Oxford Textbook of Clinical Hepatology*, edited by McIntyre N. New York: Oxford University Press, 1991, p. 865–871.
- Burt AD, Mutton A, and Day CP. Diagnosis and interpretation of steatosis and steatohepatitis. *Semin Diagn Pathol* 15: 246–258, 1998.
- Fraser JL, Antonioli DA, Chopra S, and Wang HH. Prevalence and nonspecificity of microvesicular fatty change in the liver. *Mod Pathol* 8: 65–70, 1995.
- Frayn KN and Maycock PF. Skeletal muscle triacylglycerol in the rat: methods for sampling and measurement, and studies of biological variability. *J Lipid Res* 21: 139–144, 1980.
- Han LK, Takaku T, Li J, Kimura Y, and Okuda H. Anti-obesity action of oolong tea. *Int J Obes Relat Metab Disord* 23: 98–105, 1999.
- Hautekeete ML, Degott C, and Benhamou JP. Microvesicular steatosis of the liver. *Acta Clin Belg* 45: 311–326, 1990.
- Hecht Y and Chevrel B. *Les Stéatoses Hépatiques*. Paris: Baillière, 1975.
- Kim JK, Gavrillova O, Chen Y, Reitman ML, and Shulman GI. Mechanism of insulin resistance in A-ZIP/F-1 fatless mice. *J Biol Chem* 275: 8456–8460, 2000.
- Krotkiewski M and Bjorntorp P. The effect of progesterone and of insulin administration on regional adipose tissue cellularity in the rat. *Acta Physiol Scand* 96: 122–127, 1976.
- Lewis GF, Carpentier A, Adeli K, and Giacca A. Disordered fat storage and mobilization in the pathogenesis of insulin resistance and type 2 diabetes. *Endocr Rev* 23: 201–229, 2002.
- Lo S, Russell JC, and Taylor AW. Determination of glycogen in small tissue samples. *J Appl Physiol* 28: 234–236, 1970.
- Lombardi B. Considerations on the pathogenesis of fatty liver. *Lab Invest* 15: 1–20, 1966.
- Lombardi B, Pani P, and Schlunk FF. Choline-deficiency fatty liver: impaired release of hepatic triglycerides. *J Lipid Res* 9: 437–446, 1968.
- Marceau P, Biron S, Hould FS, Marceau S, Simard S, Thung SN, and Kral JG. Liver pathology and the metabolic syndrome X in severe obesity. *J Clin Endocrinol Metab* 84: 1513–1517, 1999.
- Narayan KA, McMullen JJ, Butler DP, Wakefield T, and Calhoun WK. Effect of exercise on tissue lipids and serum lipoproteins of rats fed two levels of fat. *J Nutr* 105: 581–587, 1975.
- Paradis V, Perlemuter G, Bonvoust F, Dargere D, Parfait B, Vidaud M, Conti M, Huet S, Ba N, Buffet C, and Bedossa P. High glucose and hyperinsulinemia stimulate connective tissue growth factor expression: a potential mechanism involved in progression to fibrosis in nonalcoholic steatohepatitis. *Hepatology* 34: 738–744, 2001.
- Reue K, Xu P, Wang XP, and Slavin BG. Adipose tissue deficiency, glucose intolerance, and increased atherosclerosis result from mutation in the mouse fatty liver dystrophy (fld) gene. *J Lipid Res* 41: 1067–1076, 2000.
- Rothfeld B, Levine A, Varady A, Loken S, and Karmen A. The effect of exercise on lipid deposition in the rat. *Biochem Med* 18: 206–209, 1977.
- Saltiel AR and Kahn CR. Insulin signaling and the regulation of glucose and lipid metabolism. *Nature* 414: 799–806, 2001.
- Scott DA, Gholson CF, Netchvolodoff CV, Ray M, Gonzalez E, and Bacon BR. Incidental microvesicular steatosis due to valproic acid anticonvulsant therapy. *Am J Gastroenterol* 86: 500–502, 1991.
- Seppala-Lindroos A, Vehkavaara S, Hakkinen AM, Goto T, Westerbacka J, Sovijarvi A, Halavaara J, and Yki-Jarvinen H. Fat accumulation in the liver is associated with defects in insulin suppression of glucose production and serum free fatty acids independent of obesity in normal men. *J Clin Endocrinol Metab* 87: 3023–3028, 2002.
- Sheth SG, Gordon FD, and Chopra S. Nonalcoholic steatohepatitis. *Ann Intern Med* 126: 137–145, 1997.
- Straczkowski M, Kowalska I, Dzienis-Straczkowska S, Kinalski M, Gorski J, and Kinalska I. The effect of exercise training on glucose tolerance and skeletal muscle triacylglycerol content in rats fed with a high-fat diet. *Diabetes Metab* 27: 19–23, 2001.
- Terao T, Fujise T, and Nakano S. Effects of long-term exercise and high-cholesterol diet on lipid-lipoprotein metabolism in rats. *Tokai J Exp Clin Med* 12: 243–251, 1987.
- Trudell JR, Lin WQ, Chrystof DA, Kirshenbaum G, and Ardies CM. Induction of HSP72 in rat liver by chronic ethanol consumption combined with exercise: association with the prevention of ethanol-induced fatty liver by exercise. *Alcohol Clin Exp Res* 19: 753–758, 1995.
- Van Steenberghe W and Lanckmans S. Liver disturbances in obesity and diabetes mellitus. *Int J Obes Relat Metab Disord* 19: S27–S36, 1995.
- Yao ZM and Vance DE. The active synthesis of phosphatidylcholine is required for very low density lipoprotein secretion from rat hepatocytes. *J Biol Chem* 263: 2998–3004, 1988.