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Abstract

C57BL/6 mice develop signs and symptoms comparable, in part, to the human metabolic syndrome. The objective of the present study was to evaluate the effects of exercise training on carbohydrate metabolism, lipid profile, visceral adiposity, pancreatic islet alterations, and nonalcoholic fatty liver disease in C57BL/6 mice. Animals were fed one of two diets during an 8-week period: standard (SC, N = 12) or very high-fat (HF, N = 24) chow. An exercise training protocol (treadmill) was then established and mice were divided into SC and HF sedentary (SC-Sed, HF-Sed), exercised groups (SC-Ex, HF-Ex), or switched from HF to SC (HF/SC-Sed and HF/SC-Ex). HF/HF-Sed mice had the greatest body mass (65% more than SC/SC-Sed; $P < 0.0001$), and exercise reduced it by 23% ($P < 0.0001$). Hepatic enzymes ALP (+80%), ALT (+100%) and AST (+70%) were higher in HF/HF mice than in matched SC/SC. Plasma insulin was higher in both the HF/HF-Sed and HF/SC-Sed groups than in the matched exercised groups (+85%; $P < 0.001$). Pancreatic islets, adipocytes and liver structure were greatly affected by HF, ultimately resulting in islet β-cell hypertrophy and severe liver steatosis. The HF group had larger islets than the SC/SC group (+220%; $P < 0.0001$), and exercise significantly reduced liver steatosis and islet size in HF. Exercise attenuated all the changes due to HF, and the effects were more pronounced in exercised mice switched from an HF to an SC diet. Exercise improved the lipid profile by reducing body weight gain, visceral adiposity, insulin resistance, islet alterations, and fatty liver, contributing to obesity and steatohepatitis control.

Key words: Obesity; Lipid metabolism; Metabolic syndrome; Aerobic training; Fatty liver; Stereology

Introduction

The increasing prevalence of obesity in modern society is affected by environmental and behavioral aspects. The percentage of fat energy in diets and lack of physical activity are two important environmental factors influencing obesity (1).

Fat liver infiltration or nonalcoholic fatty liver disease (NAFLD) is a major form of chronic liver disease in children and adults. It is one of the consequences of the current obesity epidemic and can progress to nonalcoholic steatohepatitis. This disease is characterized by steatosis, excessive lipid accumulation, primarily triacylglycerols, within hepatocytes, inflammation, and progressive fibrosis, ultimately leading to cirrhosis and end-stage liver disease (2,3). Fatty liver is macrovesicular or microvesicular steatosis according to the size of the lipid vacuoles. The most common form, which is potentially reversible, is macrovesicular steatosis. Although it is thought to be a benign condition, it may be associated with the development of necroinflammation and fibrosis (steatohepatitis). Microvesicular steatosis is generally the more severe disease (4), whereas macrovesicular fatty liver can be associated with other conditions, including type 2 diabetes, obesity, and metabolic syndrome (5,6). The pathophysiology of fat infiltration leads to an increase of cellular free fatty acids and to a decrease of β-oxidation, which ultimately results in triglyceride accumulation. This accumulation is due to an increase in dietary free fatty acids and lipolysis in peripheral fat tissue (7), with consequently elevated insulin levels generating oxidative stress and organ fibrogenesis (8,9).

Exercise training accompanied by a low-fat diet has long been prescribed as part of the treatment for managing obesity

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and type 2 diabetes. Exercise reduces the adverse effects of dietary fat on insulin levels and improves insulin sensitivity in high-fat fed animals (10). Moreover, during exercise, several adaptive mechanisms are established to supply fuel to skeletal muscle and accelerate glucose and fatty acid metabolism (11). Despite this, there is no firm consensus as to whether exercise training can prevent the induction of hepatic steatosis. Exercise training can prevent the accumulation of fat in the liver of rats receiving a high-fat diet (12). However, recent studies using a similar high-fat diet-induced obesity model in rats have shown that exercise had no effect on liver lipid accumulation (13,14). The reasons for this discrepancy are not clear, although differences in diet-induced fatty liver, in the exercise training program and techniques used for assessing liver lipid infiltration should all be considered.

The objective of the present study was to evaluate the effects of exercise training on carbohydrate metabolism, lipid profile, visceral adiposity, pancreatic islet alterations, and NAFLD in C57BL/6 mice chronically fed a very high-fat diet.

Material and Methods

Animals and treatments

All procedures were carried out in accordance with the conventional guidelines of the Care and Use of Laboratory Animals (US National Institutes of Health 85-23, revised in 1996). The experimental protocols were approved by the local Committee for the Use and Care of Experimental Animals of the State University of Rio de Janeiro, Rio de Janeiro, Brazil (#CEA/195/2007).

Three-month-old male C57BL/6 mice were kept under standard conditions (12-h light/dark cycles, 21 ± 2°C, 60 ± 10% humidity) with free access to food and water. Animals were fed one of two diets for 8 weeks: standard chow (SC, 76% calories from carbohydrates, 10% from fat, and 14% from protein; N = 12) or very high-fat chow (HF, 26% calories from carbohydrates, 60% from fat, and 14% from protein; N = 24). The mineral and vitamin content of the two diets was identical. Diets were elaborated with purified nutrients according to the recommendations of the American Institute of Nutrition - AIN93 (15). After eight weeks of diet, the animals were submitted to the exercise training protocol. SC mice were divided into two groups (N = 6 per group): a) exercised (SC/SC-Ex) and b) non-exercised (sedentary, SC/SC-Sed), and HF mice were divided into four groups (N = 6 per group): a) exercised (HF/HF-Ex), b) non-exercised (sedentary; HF/HF-Sed), c) HF/SC-Ex (exercised and switched to standard chow at the beginning of training), and d) HF/SC-Sed (sedentary and switched to standard chow at the beginning of training). Animals had free access to food and water and their intakes were monitored daily. Body mass (BM) was monitored weekly in conscious animals during the experiment.

Exercise training protocol

Exercise training was performed on a motor treadmill at moderate-to-low intensity (1.0 km/h maximal running speed) for 8 weeks, 5 days/week for 1 h/day, as previously described (16). The animals were adapted to this procedure for 1 week before beginning the exercise by training at 0.5/0.6/0.7/0.8/1.0 km/h for 12/24/36/48/60 min, respectively, on consecutive days. Sedentary animals were placed on the stationary treadmill five times a week to provide a similar environment.

Euthanasia

At week 16, 24 h after the last exercise, animals were not fed, but had free access to water for 6 h and then were deeply anesthetized (sodium pentobarbital, *ip*, 150 mg/kg), and blood samples were obtained by cardiac puncture for biochemical analyses and radioimmunoassay and centrifuged at 120 *g* for 15 min at room temperature, and plasma was stored at -80°C until assay. Epididymal fat pads were removed from both sides of the animal and weighed. Epididymal fat pads included adipose tissue surrounding the ureters, bladder, and epididymis. The epididymal fat pad/tibia length ratio was used for comparisons of groups. This procedure is particularly important in obesity studies to avoid bias due to BM alteration (17). Both pancreas and liver were also removed and weighed, and then fragments of all portions of the pancreas and all hepatic lobes and epididymal fat pads were quickly collected and fixed in freshly prepared fixative (1.27 M formaldehyde in 0.1 M sodium phosphate buffer, pH 7.2) for 48 h at room temperature.

Metabolic measurements

Both the oral glucose tolerance test (OGTT) and the intraperitoneal insulin tolerance test (IPITT) were performed at weeks 8 and 16 (before and after the exercise training protocol, respectively) in order to evaluate glucose tolerance and insulin resistance, respectively. The OGTT and IPITT were applied 24 h after the last exercise training. For the OGTT, 25% glucose in sterile saline (0.9% NaCl) at a dose of 1 g/kg was administered by orogastric gavage after a 6-h fasting period. IPITT was applied after a 4-h fasting period at a dose of 1 unit/kg (Humalog insulin lispro, Eli Lilly and Company, USA). Blood was taken from a small incision of the tail tip and plasma glucose concentration was measured prior to the incision and at 15, 30, 60, and 120 min after glucose and insulin administration. The curves were analyzed and the area under the curve was calculated using the trapezoid rule to assess glucose intolerance (GraphPad Prism version 5.02 for Windows, GraphPad Software, USA).

Total cholesterol (TC), triglycerides, alanine aminotransferase (ALT), aspartic aminotransferase (AST), and alkaline phosphatase (ALP) were measured by a colorimetric assay (Bioclin, Brazil).

Radioimmunoassay for insulin

Plasma insulin concentrations were measured using an insulin RIA kit, Cat. 07-260121 (Immuchem Coated Tube, USA). All samples were analyzed in a double assay, with an

intra-assay coefficient of variation of 1.4%.

Immunohistochemistry for islet β-cells

Antigen retrieval was performed with citrate buffer, pH 6.0, endogenous peroxidase was quenched with 3% hydrogen peroxide, and finally nonspecific binding was inhibited with phosphate-buffered saline/5% bovine serum albumin. Sections were incubated with anti-insulin antibody (A0564, DAKO, Denmark) and the reaction was amplified with a biotin-streptavidin system (K0679; Universal DakoCytomation LSAB + Kit, Peroxidase, Glostrup, Denmark). The reaction was visualized after incubation with 3,3' diaminobenzidine tetrachloride (K3466, DakoCytomation), and sections were counterstained with Mayer hematoxylin.

Morphometry of pancreatic islets and adipocytes

Fragments of the pancreas and fat pad were embedded in Paraplast plus (Sigma-Aldrich Co., USA), cut into 5-µm thick sections and stained with hematoxylin and eosin. Digital images were acquired at random (TIFF format, 36-bit color, 1280 x 1024 pixels) with an LC Evolution camera and Olympus BX51 microscope. At least 12 islets and 50 adipocytes, representing all regions of the pancreas and epididymal fat pad, were analyzed per mouse, for a total of 60 islets or 250 adipocytes per group. The mean diameters of islet and adipocytes were measured from the digital images of pancreatic tissue and adipose tissue using the Image-Pro Plus software (version 7.0, Media Cybernetics, USA).

Liver stereology

Liver fragments were also embedded in Paraplast plus, cut into 3-µm thick sections and stained with hematoxylin and eosin. Five microscopic fields per animal were analyzed at random using videomicroscopy (Leica DMRBE microscope, Germany) and a test system containing 36 test-points (P_T) (18). The volume density of hepatic steatosis (Vv[steat]) was estimated by point counting: Vv[steat]: = P_P[steat] / P_T (P_P is the number of points that hit the fat store vesicles inside the hepatocyte) (19). This method proved to be more advantageous than image analysis for the evaluation of hepatic steatosis (20).

Image analysis

Digital images from pancreatic islets labeled with anti-insulin antibody were acquired at random (TIFF format, 36-bit color, 1280 x 1024 pixels, LC Evolution camera and Olympus BX51 microscope). A selection tool of the Image-Pro Plus software (version 7.0, Media Cybernetics, USA) was used to identify the islet areas with positive immunoreactions, and this selection was segmented in a black-and-white image, where white shows the immunostained areas and black the remaining tissue. The islet boundary was delimited using an area-over-image tool, and inside this encirclement, the islet area occupied by white color was quantified using the image histogram tool as shown in Figure 1. It was reported as density stain per islet (%) (21).

Data analysis

Data are reported as means ± SEM. The differences between groups were tested by one-way ANOVA and the Tukey *post hoc* test, or the Student *t*-test, by comparing the groups during the first 8 weeks of the study. The interaction between diet and exercise was analyzed by two-way ANOVA. For all analyses, a P value of 0.05 was considered to be statistically significant (GraphPad Prism version 5.02 for Windows, GraphPad Software, USA).

Results

Biometry

Figure 2 shows the BM evolution before and during exercise training. The HF chow intake was directly related to BM gain, which led to overweight HF mice in the second week of the diet compared to SC mice. At the eighth week (before exercise training), BM was more than 15% greater in HF than in SC ($P < 0.0001$, one-way ANOVA). At the 16th week, HF/HF-Ex had a BM reduction of 20% compared to HF/HF-Sed ($P < 0.0001$, one-way ANOVA). When the diet was switched from HF to SC and combined with exercise training, there was an additional 24% reduction in BM (HF/SC-Ex compared to HF/HF-Ex; $P < 0.0001$, one-way ANOVA). Both diet and exercise interacted with BM ($P < 0.0001$, two-way ANOVA, Table 1).

The epididymal fat pad/tibia length ratio was smaller in exercised groups than in their sedentary counterparts (data not shown). Moreover, when the diet was switched from HF to SC, this difference was still greater (more than

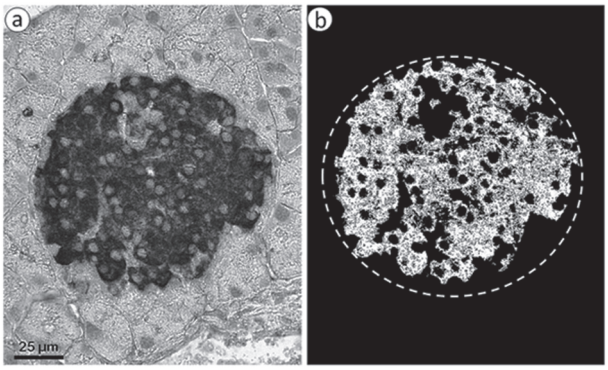

Figure 1. Photomicrograph of a pancreatic islet stained with hematoxylin-eosin (a) and its segmentation to a black and white image (b). The islet was stained with hematoxylin-eosin and selected with the Image-Pro Plus software, and the image was segmented to originate a new image in black and white (b) where the white color represents the expression of insulin and the black color represents the remaining tissue. The pancreatic islet was delimited by the AOI tool (thin outer line), and insulin expression inside it was quantified (see Material and Methods).

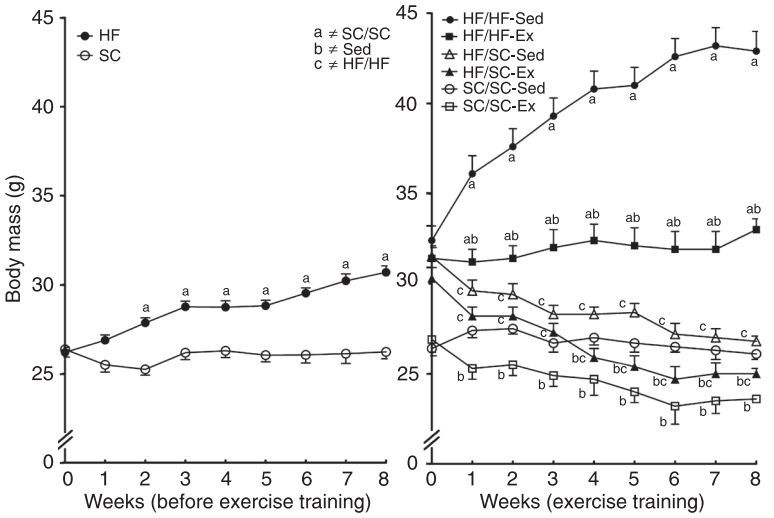

Figure 2. Body mass before and during exercise training. Before exercise training, the HF group had a significant increase of body mass compared to the SC group. During exercise training, both exercise and change of diet reduced body mass. Data are reported as means ± SEM. HF = high-fat diet; SC = standard chow diet; SC/SC-Ex = standard chow/standard chow-exercised; SC/SC-Sed = standard chow/standard chow-sedentary; HF/HF-Ex = high-fat/high-fat-exercised; HF/HF-Sed = high-fat/high-fat-sedentary; HF/SC-Ex = high-fat/standard chow-exercised, and HF/SC-Sed = high-fat/standard chow-sedentary. P < 0.05 when: (a) compared to matched SC/SC, (b) compared to matched sedentary group, (c) compared to matched HF/HF group (one-way ANOVA followed by the Tukey *post hoc* test).

Table 1. Two-way ANOVA of the effect of high-fat diet and exercise training on mice.

	Percent variation* and test significance					
	Interaction		Exercise		Diet	
	%	P	%	P	%	P
Body mass	13%	<0.0001	12%	<0.0001	68%	<0.0001
Epididymal fat	9%	<0.001	10%	<0.001	73%	<0.0001
Cholesterol	6%	<0.05	0.6%	NS	83%	<0.0001
Triglycerides	27%	<0.0001	10%	<0.0001	60%	<0.0001
Insulin	0.5%	<0.001	77%	<0.0001	5%	<0.05
ALP	32%	<0.0001	0.3%	NS	62%	<0.0001
AST	20%	<0.0001	44%	<0.0001	25%	<0.0001
ALT	3%	NS	66%	<0.0001	4%	NS
Adipocyte	14%	<0.0001	9%	<0.0001	75%	<0.0001
Vv steatosis	51%	<0.0001	8%	<0.0001	37%	<0.0001

ALP = alkaline phosphatase; AST = aspartate aminotransferase; ALT = alanine aminotransferase; NS = not statistically significant; Vv = volume density. *Percent variation quantifies the power of each factor (exercise and diet) on the result, and the power of the interaction between the factor as well.

60% when HF/SC-Sed was compared to HF/SC-Ex; P < 0.0001, one-way ANOVA). Diet and exercise interacted to establish the epididymal fat pad/tibia length ratio (Table 1; P < 0.001, two-way ANOVA).

Large adipocytes were seen in HF/HF, but exercise training had beneficial effects by reducing adipocyte size (Figure 3). When SC/SC-Sed is compared to HF/HF-Sed, the adipocyte size grew more than 350% (effect of the HF diet; P < 0.0001, one-way ANOVA), while the comparison of HF/HF-Sed to HF/HF-Ex, indicate that the adipocyte size shrank more than 45% (beneficial effect of exercise training; P < 0.0001, one-way ANOVA). With the diet switched from HF to SC in association with exercise, marked beneficial effects were observed with a reduction of adipocyte size. Both diet and exercise interacted with adipocyte size (P < 0.0001, two-way ANOVA; Table 1).

Carbohydrate and fat metabolism

TC and triglyceride levels were higher in both HF/HF-Sed and HF/HF-Ex than their SC/SC counterparts and higher in sedentary mice than in the exercised HF/HF and HF/SC (Table 2; P < 0.001; one-way ANOVA). Diet and exercise interacted with TC and triglyceride levels (Table 1; two-way ANOVA).

ALP was higher in HF/HF and HF/SC than SC/SC. AST was higher in HF/HF-Sed and HF/SC-Sed than in their SC/SC counterparts. ALT was higher in sedentary mice than in the exercised counterparts for all diets (Table 2; one-way ANOVA). Both diet and exercise interacted with AST levels. However, only diet influenced ALP levels, and only exercise influenced ALT levels (Table 1; two-way ANOVA).

Both HF/HF-Sed and HF/SC-Sed showed higher plasma insulin levels than their exercised counterparts (Table 2; P < 0.001, one-way ANOVA). Both diet and exercise interacted with plasma insulin levels (Table 1; two-way ANOVA). OGTT and IPITT values before the beginning of the exercise training protocol (at the eighth week) were higher in HF than in SC mice (P < 0.0001, *t*-test; data not shown). At week 16, HF/HF-Sed had the highest OGTT value, and exercise training reduced it in HF/HF-Ex. When diet was switched from HF to SC, OGTT decreased in both HF/SC-Ex and SC-Sed when compared with their HF/HF

counterparts (Table 3). Likewise, HF/HF-Sed had the highest IPITT value, significantly different from both the HF/HF-Ex and HF/SC-Sed (P < 0.001, one-way ANOVA; Table 3).

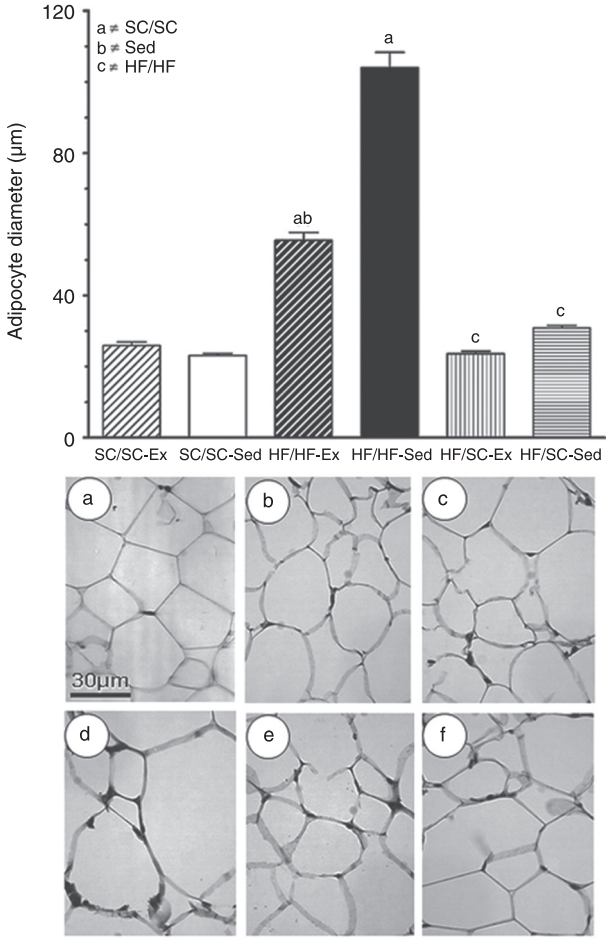

Figure 3. Adipocyte diameter (see abbreviations in Figure 2) and photomicrographs (a-f) of adipose tissue in C57BL/6. Histogram: The letters over the bars indicate a statistically significant difference. P < 0.05 when: (a) compared to matched SC/SC, (b) compared to matched sedentary group, (c) compared to matched HF/HF group (one-way ANOVA followed by the Tukey *post hoc* test). Photomicrographs: *a* and *b*, Usual adipocyte aspect in the SC/SC-Ex and SC/SC-Sed groups, respectively; *c*, HF/HF-Ex group with intermediate adipocyte size; *d*, HF/HF-Sed group with adipocyte hypertrophy; *e* and *f*, adipocytes similar to control in the HF/SC-Ex and HF/SC-Sed groups, respectively.

Pancreas

Islets were more than 120% larger in HF/HF-Sed than in SC/SC-Sed (P < 0.0001, one-way ANOVA; Table 3), while HF/HF-Ex showed an islet size as much as 40% smaller

Table 2. Effect of high-fat diet and exercise training on mouse blood biochemistry.

	Groups					
	SC/SC-Ex	SC/SC-Sed	HF/HF-Ex	HF/HF-Sed	HF/SC-Ex	HF/SC-Sed
Cholesterol (mg/dL)	121.0 ± 3.6	121.3 ± 1.5	169.3 ± 7.7^{a,b}	190.6 ± 3.6^{a}	135.0 ± 2.5^{b,c}	135.0 ± 2.5^{c}
Triglycerides (mg/dL)	43.4 ± 2.6	49.3 ± 1.4	64.8 ± 3.9^{a,b}	109.8 ± 0.3^{a}	63.8 ± 3.1^{a,b}	55.0 ± 0.3^{c}
ALP (mM)	42.5 ± 1.8	46.7 ± 1.7	59.3 ± 2.0^{a}	75.7 ± 0.7^{a}	80.5 ± 2.4^{a,b,c}	58.3 ± 1.7^{a,c}
AST (mM)	137.0 ± 9.4	141.0 ± 2.5	121.0 ± 6.4^{b}	237.0 ± 14.6^{a}	155.0 ± 10.3^{b}	258.0 ± 7.7^{a}
ALT (mM)	30.3 ± 1.2^{b}	49.3 ± 3.9	31.4 ± 2.1^{b}	61.3 ± 4.0	32.8 ± 4.4^{b}	54.0 ± 5.4
Insulin (mg/dL)	11.0 ± 0.2	17.7 ± 0.2	12.2 ± 1.0^{b}	20.4 ± 0.9^{a}	12.6 ± 1.0^{b}	20.4 ± 1.4

Data are reported as means ± SEM. SC = standard chow; HF = high-fat chow; Ex = exercise training; Sed = sedentary; ALP = alkaline phosphatase; AST = aspartate aminotransferase; ALT = alanine aminotransferase. P < 0.05 when: (a) compared to matched SC/SC, (b) compared to matched sedentary group, (c) compared to matched HF/HF group (one-way ANOVA followed by the Tukey *post hoc* test).

compared to HF/HF-Sed (P < 0.0001, one-way ANOVA). When the diet was switched from HF to SC, the islet size shrank accordingly by 46% from HF/SC-Sed to HF/HF-Sed (P < 0.0001, one-way ANOVA). The islet insulin immunolocalization was 60% greater in HF/HF-Sed than in SC/SC-Sed (P < 0.0001, one-way ANOVA), while it was reduced in both HF/HF-Ex and HF/SC-Ex compared to their sedentary counterparts (P < 0.0001, one-way ANOVA) (Table 3).

Liver

The HF diet and sedentary lifestyle altered the liver structure. HF/HF-Sed had abundant micro- and macrovesicular steatosis (Figure 4) with areas of inflammatory infiltrate characterizing steatohepatitis (Figure 5). Liver steatosis had a relative increase of more than 550% from SC/SC-Sed to HF/HF-Sed (P < 0.0001, one-way ANOVA). Exercise reduced it in HF/HF-Ex by more than 75% compared to HF/HF-Sed (P < 0.0001, one-way ANOVA). When the diet

Table 3. Effect of high-fat diet and exercise training on mouse glucose metabolism.

	Groups					
	SC/SC-Ex	SC/SC-Sed	HF/HF-Ex	HF/HF-Sed	HF/SC-Ex	HF/SC-Sed
AUC of OGTT	19,313 ± 706.5	18,234 ± 461.4	27,660 ± 1628[a,b]	22,673 ± 823.2[a]	18,923 ± 607.1[c]	17,141 ± 660.1[c]
AUC of IPITT	9461 ± 1134	9019 ± 494.5	15,191 ± 694.8[b]	10,877 ± 244.5[a]	10,067 ± 623.2	10,074 ± 243.4[c]
Insulin (mg/dL)	11.0 ± 0.2	17.7 ± 0.2	12.2 ± 1.0[b]	20.4 ± 0.9[a]	12.6 ± 1.0[b]	20.4 ± 1.4
Islet cross-sectional area (μm²)	7101.4 ± 513.6	9992.5 ± 855.9	12,067 ± 882.6[a,b]	22,600 ± 1172.1[a]	10,827 ± 937.9[a]	12,234 ± 599.8[c]
Insulin density by immunoreaction (%)	20.5 ± 1.8[b]	39.6 ± 2.6	30.5 ± 1.6[a,b]	50.3 ± 3.1[a]	30.1 ± 1.4	35.2 ± 1.3[c]

Data are reported as means ± SEM. SC = standard chow; HF = high-fat chow; Ex = exercise training; Sed = sedentary; AUC to OGTT = area under the curve to oral glucose tolerance test (at week 16); AUC of IPITT = area under the curve of intraperitoneal insulin tolerance test (at week 16). P < 0.05 when: (a) compared to matched SC/SC group, (b) compared to matched sedentary group, (c) compared to matched HF/HF group (one-way ANOVA followed by the Tukey *post hoc* test).

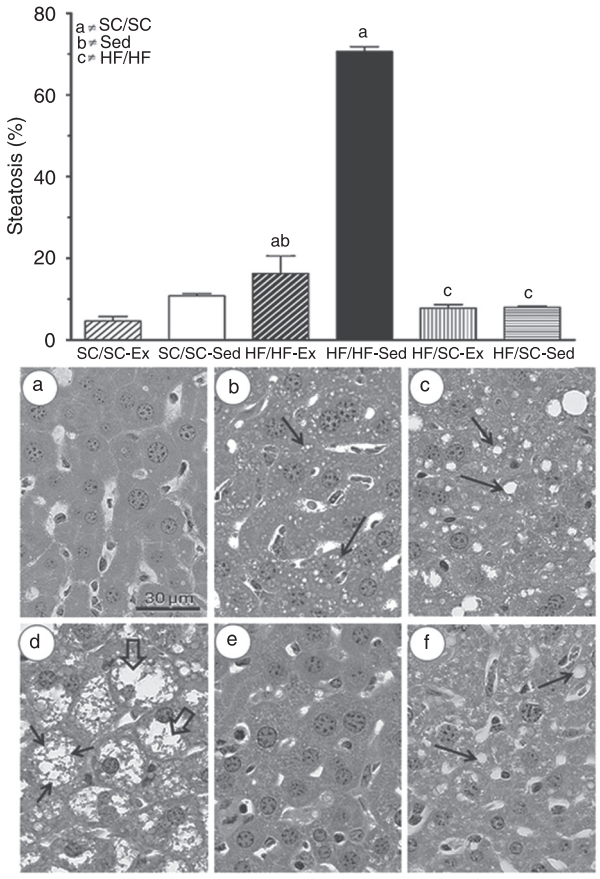

Figure 4. Volume density of hepatic steatosis (see abbreviations in Figure 2) and photomicrographs (a-f) of the liver structure in C57BL/6. Histogram: The letters over the bars indicate statistically significant difference. P < 0.05 when: (a) compared to matched SC/SC, (b) compared to matched sedentary group, (c) compared to matched HF/HF group) (one-way ANOVA followed by the Tukey *post hoc* test). Photomicrographs: *a*, Healthy hepatocyte with the structures preserved; *b*, hepatocytes with dispersed microvesicles (arrows); *c*, hepatocytes with scattered macrovesicles and few microvesicles (arrows); *d*, hepatic structure with macrovesicles (large arrows) and several matrix microvesicles (arrows) characterizing marked liver steatosis; *e*, preserved liver structure similar to that of the SC/SC-Ex group; *f*, hepatocytes exhibiting some macrovesicles (arrows).

was switched from HF to SC, steatosis was significantly lower in both HF/SC-Sed and HF/SC-Ex than in their HF/HF counterparts ($P < 0.0001$, one-way ANOVA). Both diet and exercise acted on liver steatosis ($P < 0.0001$, two-way ANOVA; Table 1).

Discussion

HF diet intake resulted in body mass gain and corresponding adipocyte hypertrophy, fat accumulation within hepatocytes, and changes in carbohydrate metabolism. Animals switched from a long period of HF to SC showed reduction or reversal of the negative features observed, especially when the animals were submitted to an exercise training protocol.

The effects of HF consumption on visceral adiposity and its relation to the development of chronic diseases and obesity are complex, and the effects of exercise training in reducing the risk caused by an HF diet and sedentarism merit study (22). However, in this evaluation it is necessary to consider the intensity, frequency and duration of the exercise and the type of diet, which can lead to different metabolic adaptations (12). It is questionable whether all models of exercise have the same effects on body mass and visceral adiposity, fatty liver and lipid profiles. Aerobic exercise at a moderate level of intensity over a relatively long period of time, as used in the present study, promotes reduction of body mass and adiposity by improving the lipid profile (23,24).

One of the mechanisms underlying the positive effects of exercise training is related to fat utilization by increased lipid oxidation during exercise (25). The combination of exercise and caloric restriction is a common therapeutic strategy for obesity control. In diet-induced obese rats, for instance, exercise plus caloric restriction have additive effects on the reduction of adiposity, which are greater than the effect of either exercise or caloric restriction alone (26,27).

The present findings demonstrated that HF intake led to visceral adiposity with dyslipidemia in sedentary mice, in agreement with the literature (13,14). Thus, treadmill exercise was able to reduce visceral adiposity, serum triglycerides, and serum cholesterol and to improve HDL-cholesterol concentrations. The activity of lipoprotein lipase increases through exercise and is responsible for releasing fatty acids from the lipoproteins by promoting an imbalance between HF diet and the effects of exercise (24). Furthermore, exercise improves insulin action in the liver (28) because, during exercise and after glycogen depletion, fatty acid becomes the major fuel for the exercising metabolism in muscle; during the recovery period, glycerol is necessary for glycogen repletion (23,29).

The importance of applying different protocols of exercise training associated with other therapies to control and reduce the high prevalence of NAFLD in the obese population, including adolescents, is recognized (30). As demonstrated here, the exercise training protocol used in the present study was effective in reducing fatty liver and suppressing HF diet-induced steatosis.

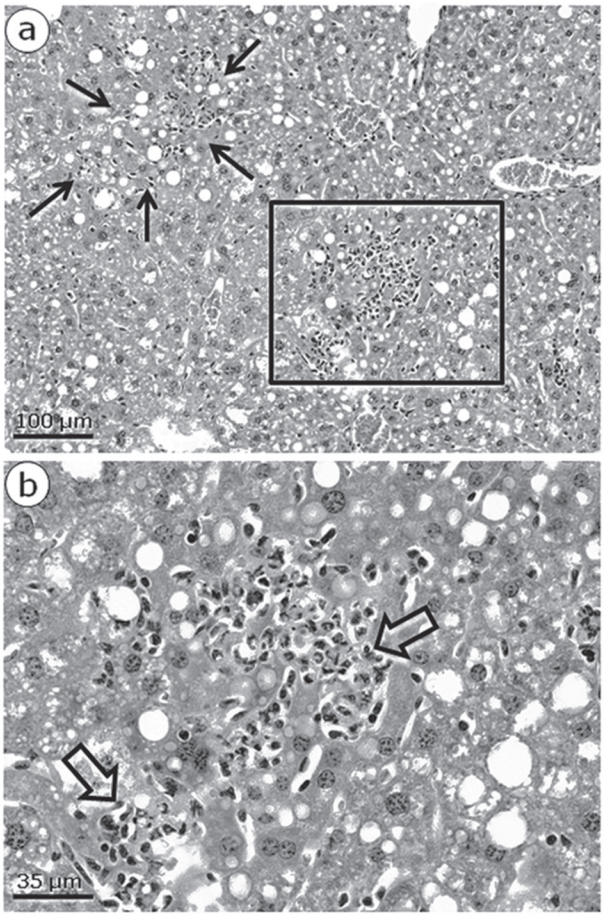

Figure 5. Photomicrographs of steatohepatitis in a C57BL/6 mouse of the HF/HF-Sed group. *a*, Abundant micro- and macrovesicular steatosis with some areas of inflammatory infiltrate (arrows and inset square) and *b*, magnification of the inset of figure *a* showing infiltrate with abundant macrophage cells (open arrows).

Hyperinsulinemia is a compensatory response to insulin resistance causing adipogenesis, and this compensatory effect eventually leads to diabetes (31). HF intake is associated with excessive circulating free fatty acids and glucose, aggravating insulin resistance and increasing lipolysis and insulin secretion (32,33). A sedentary lifestyle also contributes to the development of insulin resistance in humans (34). In contrast, insulin sensitivity may increase with exercise, independent of body mass loss or changes in body composition (35). The main effect of exercise on insulin resistance is the increased expression of intracellular insulin signaling pathway components, in particular of glucose transporters (GLUT4) in skeletal muscle (36).

The effects of exercise are of particular interest regarding islet β-cell lipid metabolism since an abnormal accumulation of lipid in β-cells has been implicated in the pathogenesis of islet failure and β-cell death in type 2 diabetes. Whereas fatty acids in chronic excess, particularly in association with hyperglycemia, are toxic to islets (37), free fatty acids are essential for normal islet glucose-stimulated insulin secretion (38). Changes in fatty acid metabolism and signaling, therefore, could also be involved in physiological adaptations of pancreatic islets to exercise (39). Likewise, in the present study, the reduction of both islet size and β-cell insulin immunolocalization in exercised mice was significant compared to sedentary mice.

The composition of adipose tissue cells is the major determinant of metabolic activity and responds to environmental changes of a specific fat deposit (40). The present findings demonstrated abundant small adipocytes in the exercised mice, while the sedentary mice usually showed large adipocytes. An acceptable explanation of this fact is a probable enhanced lipolysis, blunted lipogenesis, or both mechanisms.

We have shown that mice chronically fed an HF diet develop several issues associated with increased risk factors for cardiovascular diseases and metabolic disorders. Exercise training has beneficial effects in reducing these risk factors contributing to the control of obesity and comorbidities, such as NAFLD. Understanding these data from animal experiments can be of help in ongoing studies related to clinical research.

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References

1. Jequier E. Pathways to obesity. *Int J Obes Relat Metab Disord* 2002; 26 (Suppl 2): S12-S17.
2. Gentile CL, Pagliassotti MJ. The role of fatty acids in the development and progression of nonalcoholic fatty liver disease. *J Nutr Biochem* 2008; 19: 567-576.
3. Jiang J, Torok N. Nonalcoholic steatohepatitis and the metabolic syndrome. *Metab Syndr Relat Disord* 2008; 6: 1-7.
4. Burt AD, Mutton A, Day CP. Diagnosis and interpretation of steatosis and steatohepatitis. *Semin Diagn Pathol* 1998; 15: 246-258.
5. Marceau P, Biron S, Hould FS, Marceau S, Simard S, Thung SN, et al. Liver pathology and the metabolic syndrome X in severe obesity. *J Clin Endocrinol Metab* 1999; 84: 1513-1517.
6. Van Steenbergen W, Lanckmans S. Liver disturbances in obesity and diabetes mellitus. *Int J Obes Relat Metab Disord* 1995; 19 (Suppl 3): S27-S36.
7. Pessayre D. Role of mitochondria in non-alcoholic fatty liver disease. *J Gastroenterol Hepatol* 2007; 22 (Suppl 1): S20-S27.
8. Chitturi S, Abeygunasekera S, Farrell GC, Holmes-Walker J, Hui JM, Fung C, et al. NASH and insulin resistance: Insulin hypersecretion and specific association with the insulin resistance syndrome. *Hepatology* 2002; 35: 373-379.
9. Mathur A, Marine M, Lu D, Swartz-Basile DA, Saxena R, Zyromski NJ, et al. Nonalcoholic fatty pancreas disease. *HPB* 2007; 9: 312-318.
10. Ropelle ER, Pauli JR, Prada PO, de Souza CT, Picardi PK, Faria MC, et al. Reversal of diet-induced insulin resistance with a single bout of exercise in the rat: the role of PTP1B and IRS-1 serine phosphorylation. *J Physiol* 2006; 577: 997-1007.
11. Pagano C, Marzolo M, Granzotto M, Ricquier D, Federspil G, Vettor R. Acute effects of exercise on circulating leptin in lean and genetically obese fa/fa rats. *Biochem Biophys Res Commun* 1999; 255: 698-702.
12. Horowitz JF. Fatty acid mobilization from adipose tissue during exercise. *Trends Endocrinol Metab* 2003; 14: 386-392.
13. Estadella D, Oyama LM, Damaso AR, Ribeiro EB, Oller Do Nascimento CM. Effect of palatable hyperlipidic diet on lipid metabolism of sedentary and exercised rats. *Nutrition* 2004; 20: 218-224.
14. Burneiko RC, Diniz YS, Galhardi CM, Rodrigues HG, Ebaid GM, Faine LA, et al. Interaction of hypercaloric diet and physical exercise on lipid profile, oxidative stress and antioxidant defenses. *Food Chem Toxicol* 2006; 44: 1167-1172.
15. Reeves PG, Nielsen FH, Fahey GC Jr. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition *ad hoc* writing committee on the reformulation of the AIN-76A rodent diet. *J Nutr* 1993; 123: 1939-1951.
16. Ferreira JC, Rolim NP, Bartholomeu JB, Gobatto CA, Kokubun E, Brum PC. Maximal lactate steady state in running mice: effect of exercise training. *Clin Exp Pharmacol Physiol* 2007; 34: 760-765.
17. Yin FC, Spurgeon HA, Rakusan K, Weisfeldt ML, Lakatta EG. Use of tibial length to quantify cardiac hypertrophy: application in the aging rat. *Am J Physiol* 1982; 243: H941-H947.
18. Mandarim-De-Lacerda CA. Stereological tools in biomedical research. *An Acad Bras Cienc* 2003; 75: 469-486.
19. Aguila MB, Pinheiro AR, Parente LB, Mandarim-De-Lacerda CA. Dietary effect of different high-fat diet on rat liver stereology. *Liver Int* 2003; 23: 363-370.
20. Franzen LE, Ekstedt M, Kechagias S, Bodin L. Semiquantitative evaluation overestimates the degree of steatosis in liver biopsies: a comparison to stereological point counting. *Mod Pathol* 2005; 18: 912-916.
21. Mandarim-de-Lacerda CA, Fernandes-Santos C, Aguila MB. Image analysis and quantitative morphology. In: Hewitson TD, Darby JA (Editors), *Histology protocols: methods in molecular biology*. New Jersey: Humana Press; 2010. p 211-225.
22. Hida K, Wada J, Eguchi J, Zhang H, Baba M, Seida A, et al. Visceral adipose tissue-derived serine protease inhibitor: a

unique insulin-sensitizing adipocytokine in obesity. *Proc Natl Acad Sci U S A* 2005; 102: 10610-10615.
23. Gauthier MS, Couturier K, Latour JG, Lavoie JM. Concurrent exercise prevents high-fat-diet-induced macrovesicular hepatic steatosis. *J Appl Physiol* 2003; 94: 2127-2134.
24. Schrauwen P, Westerterp KR. The role of high-fat diets and physical activity in the regulation of body weight. *Br J Nutr* 2000; 84: 417-427.
25. Helge JW. Long-term fat diet adaptation effects on performance, training capacity, and fat utilization. *Med Sci Sports Exerc* 2002; 34: 1499-1504.
26. Aucott L, Poobalan A, Smith WC, Avenell A, Jung R, Broom J, et al. Weight loss in obese diabetic and non-diabetic individuals and long-term diabetes outcomes - a systematic review. *Diabetes Obes Metab* 2004; 6: 85-94.
27. Poobalan A, Aucott L, Smith WC, Avenell A, Jung R, Broom J, et al. Effects of weight loss in overweight/obese individuals and long-term lipid outcomes - a systematic review. *Obes Rev* 2004; 5: 43-50.
28. Charbonneau A, Couturier K, Gauthier MS, Lavoie JM. Evidence of hepatic glucagon resistance associated with hepatic steatosis: reversal effect of training. *Int J Sports Med* 2005; 26: 432-441.
29. Gauthier MS, Couturier K, Charbonneau A, Lavoie JM. Effects of introducing physical training in the course of a 16-week high-fat diet regimen on hepatic steatosis, adipose tissue fat accumulation, and plasma lipid profile. *Int J Obes Relat Metab Disord* 2004; 28: 1064-1071.
30. Tock L, Prado WL, Caranti DA, Cristofalo DM, Lederman H, Fisberg M, et al. Nonalcoholic fatty liver disease decrease in obese adolescents after multidisciplinary therapy. *Eur J Gastroenterol Hepatol* 2006; 18: 1241-1245.
31. Ferrannini E. Is insulin resistance the cause of the metabolic syndrome? *Ann Med* 2006; 38: 42-51.
32. Gallou-Kabani C, Vige A, Gross MS, Rabes JP, Boileau C, Larue-Achagiotis C, et al. C57BL/6J and A/J mice fed a high-fat diet delineate components of metabolic syndrome. *Obesity* 2007; 15: 1996-2005.
33. Karasawa H, Nagata-Goto S, Takaishi K, Kumagae Y. A novel model of type 2 diabetes mellitus based on obesity induced by high-fat diet in BDF1 mice. *Metabolism* 2009; 58: 296-303.
34. Bassuk SS, Manson JE. Epidemiological evidence for the role of physical activity in reducing risk of type 2 diabetes and cardiovascular disease. *J Appl Physiol* 2005; 99: 1193-1204.
35. O'Donovan G, Kearney EM, Nevill AM, Woolf-May K, Bird SR. The effects of 24 weeks of moderate- or high-intensity exercise on insulin resistance. *Eur J Appl Physiol* 2005; 95: 522-528.
36. Teran-Garcia M, Rankinen T, Koza RA, Rao DC, Bouchard C. Endurance training-induced changes in insulin sensitivity and gene expression. *Am J Physiol Endocrinol Metab* 2005; 288: E1168-E1178.
37. El-Assaad W, Buteau J, Peyot ML, Nolan C, Roduit R, Hardy S, et al. Saturated fatty acids synergize with elevated glucose to cause pancreatic beta-cell death. *Endocrinology* 2003; 144: 4154-4163.
38. Dobbins RL, Chester MW, Daniels MB, McGarry JD, Stein DT. Circulating fatty acids are essential for efficient glucose-stimulated insulin secretion after prolonged fasting in humans. *Diabetes* 1998; 47: 1613-1618.
39. Lamontagne J, Masiello P, Marcil M, Delghingaro-Augusto V, Burelle Y, Prentki M, et al. Circulating lipids are lowered but pancreatic islet lipid metabolism and insulin secretion are unaltered in exercise-trained female rats. *Appl Physiol Nutr Metab* 2007; 32: 241-248.
40. Yang X, Smith U. Adipose tissue distribution and risk of metabolic disease: does thiazolidinedione-induced adipose tissue redistribution provide a clue to the answer? *Diabetologia* 2007; 50: 1127-1139.