Exercise training and caloric restriction reduce adiposity index and hepatic lipids in obese rats

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Objective: Obesity is a multifactorial disorder associated with dyslipidemia and hepatic insulin resistance as the consequence of fatty liver accumulation. In the present study, we tested the hypothesis that exercise training or caloric restriction would reduce hepatic steatosis in obese rats. In addition, we investigated whether exercise training associated with caloric restriction would cause a more intense reduction in hepatic steatose than exercise training or caloric restriction alone. Subjects: Male Wistar rats were fed with cafeteria diet with high-fat content for 25 weeks. Then, they were randomly divided into four groups: high-fat-diet (n=13), high-fat-diet submitted to running exercise training (60% peak VO2, n=13), caloric restriction (-20 % of Kcal/day, n=14), and caloric restriction plus training (n=14). This period lasted 10 weeks. Results: (1) Exercise training significantly decreased adiposity index (-23%) and leptin level (-31%) and significantly increased peak oxygen uptake (23%), but caused no changes in body weight and hepatic triglycerides and cholesterol contents. (2) Caloric restriction decreased body weight (-18%), adiposity index (-50%), hepatic triglycerides content (-53%) and leptin (-61%) levels. (3) Exercise training associated with caloric restriction caused decrease in body weight (-22%), adiposity index (-61%), hepatic triglycerides (-54%) and leptin (-75%) levels and, in addition, hepatic cholesterol content (-26%). (4) Exercise associated with caloric restriction caused no significant changes serum lipids, glucose and insulin levels and HOMA-IR. Conclusion: Exercise training associated with caloric restriction is an important nonpharmacological strategy to reduce adiposity index and hepatic lipids in obese rats.

Keywords: obesity; hepatic steatosis; insulin resistance; exercise training; caloric restriction

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Introduction

Obesity has been considered one of the worrisome health problems in occidental countries. This epidemic disease is associated with the development and progression of dyslipidemia, type 2 diabetes and metabolic syndrome. These metabolic alterations are all involved in the non-alcoholic fatty liver disease (NAFLD) [1, 2] which is characterized by large amounts of fat in the liver cells that affects 10 - 24% of the general global population. This prevalence substantially
increases in obese individuals in whom NAFLD reaches more than 50% [3, 4, 5]. Patients with NAFLD have increased risk of developing liver disease, whose end-stage is hepatocellular carcinoma [1]. The primary metabolic cause of hepatic lipid accumulation is not well understood. However, growing evidences suggest that this disorder is a result of insulin resistance and lipid alterations, including uptake, synthesis, degradation or secretion [5].

Exercise training and caloric restriction has been recommended as the initial choice for obesity treatment [6, 7]. This nonpharmacological strategy improves not only insulin-stimulated glucose disposal, but also whole body lipid oxidation [8, 9, 10]. In addition, exercise has been shown to elevate basal lipolysis rate and lipid turnover in healthy individuals [11, 12]. However, the effects of body weight reduction by means exercise training in hepatic lipids content and insulin resistance remain controversy. Some investigators reported that exercise training did not reduce hepatocellular lipids storage in obese individuals [13]. In contrast, in a more recent study, Johnson et al [14] found that exercise training decreased hepatic triglycerides content in obese individuals. This effect of exercise training seems to be time dependent, since the reduction in hepatic triglycerides content time was achieved after 12 weeks of intervention, but not after 6 weeks of intervention [15].

In the present study, we tested the hypothesis that: 1) Body weight loss by exercise training or caloric restriction would cause decrease in fatty liver concentration in obese rats; 2) Exercise training associated with caloric restriction would cause a more intense decrease in fatty liver concentration in obese rats than caloric restriction or exercise training alone.

Methods

Sampling

After 25 weeks on cafeteria diet, male Wistar rats were randomly assigned into four groups: High-fat-diet (n=13), high-fat-diet submitted to running exercise (n=13), caloric restriction (n=14) and caloric restriction and training (n=14). The rats were housed in individual cage in an environmentally controlled clean-air room (23 ± 1°C; 60 ± 5% relative humidity) with a 12 h light/dark cycle (lights on at 7:00 pm). The study was approved by Scientific Committee from the Heart Institute and Human Subject Protection Committee of the Clinical Hospital, Medical School, University of Sao Paulo (#993/05).

Measures and Procedures

Hypercaldoric Diet Protocol. Cafeteria diet with high-fat-content consisted of five different diet (CD1, CD2, CD3, CD4, and CD5) given for seven days each diet as previously described [16, 17]. Briefly, CD1, CD2, CD4, and CD5 were calorically rich (4.4 Kcal/g). In the CD3, the increased caloric intake was achieved by sucrose added in the water (1.2 Kcal/mL). Water and food were provided ad libitum. This experimental protocol lasted for 25 weeks.

Exercise Training Protocol. Exercise training was performed on a motor treadmill for 10 weeks, 5 days/wk. The running speed and duration of exercise were progressively increased towards 60% maximal oxygen consumption, for 60 min which was achieved at the fourth week of training. The untrained rats were exposed to treadmill exercise (5 min) every week to become accustomed to exercise protocol and handling.

Functional Capacity Protocol. Oxygen uptake (VO2) was measured by means of a rapid-flow, open-circuit, indirect calorimeter as described by others [18]. The rats were submitted to a maximal exercise test to attain the peak VO2. VO2 was continually measured by means of expired gas analysis (Sable Systems Subsample Version 3, SS-3, USA) during a progressive exercise protocol (5 m/min increments every 3 min and no grade) performed on a motor treadmill. Peak VO2 was defined as the highest VO2 attained at the end of the exercise period when the rats could no longer maintain the running speed.

Caloric Restriction Protocol. The caloric restriction consisted of a decrease of 20% of the caloric intake per day determinate at the end of 25th week. This caloric restriction lasted for 10 weeks.

Plasma Hormones and Lipids Measurements. At the end of the protocol, the animals were sacrificed by decapitation after 12-15 h fast. Blood was collected in tubes and centrifuged at 3,000 g for 15 min at 4°C, and stored at -80°C. Serum leptin and insulin level were measured by radioimmunoassay (Linco Research Inc., USA). Serum glucose, triglycerides, cholesterol and fraction of cholesterol level were measured by enzymatic colorimetric assay (CELM®, Brazil).

Insulin Resistance. Liver insulin resistance was estimated by homeostasis model assessment (HOMA-IR) and calculated as following: fasting serum insulin (µU/mL) x fasting serum glucose (mmol/L)/22.5 [19].

Hepatic Lipid Levels. Hepatic triglycerides and cholesterol contents were analyzed by enzymatic assay. Liver samples (100 mg) were added to 2.5 ml of Dole’s reagent
(isopropanol, n-heptane, and H2SO4 – 8N, 4:1:0.25l) and homogenized in Polytron (PT-K Brinkman Instruments) for lipid extraction. The tubes were vortexed three times during the next 30 min and 1.5 mL of n-heptane and 1.5 mL of distilled water were added. The tubes were vortexed again and the mixture decanted for 5 min. One mL of the upper phase was collected and dried by nitrogen gassing. After this step the samples were resuspended in 50 uL de SDS 1%. The triglycerides and cholesterol liver contents were assessed by specifics kits (Laborlab, BR) using a spectrophotometer with green filter (490 – 530 nm).

**Organ Weight.** Liver and visceral fat was harvested and weighted. The visceral fat mass (retroperitoneal, epididymal and mesenteric) was calculated as following: Fat tissue weight/end weight body x 100. Liver weight was corrected by tibia length.

**Statistical Analysis.** Data are reported as mean±SEM. Statistical significance was determined by one-way ANOVA. When significant differences were found, the post hoc Bonferroni multiple comparisons test were carried out. P<0.05 was considered statistically significant.

## Results

Physical characteristics and metabolic measures are shown in Table 1. Caloric restriction alone and caloric restriction plus exercise training caused a significant reduction in body weight in obese rats (Table 1). Both caloric restriction alone and caloric restriction plus exercise training significantly decreased adiposity index in obese rats (Figure 1). Exercise training associated or not with caloric restriction significantly increased oxygen uptake peak in obese rats. Caloric restriction or exercise training caused no significant changes

### Table 1. Physical and metabolic characteristics in obese rats submitted to exercise and caloric restriction.

<table>
<thead>
<tr>
<th>Physical Characteristics</th>
<th>High-fat-diet</th>
<th>Caloric restriction</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Body Weight (g)</strong></td>
<td>463.6±18.1</td>
<td>417.5±13.1</td>
</tr>
<tr>
<td><strong>Body Weight/tibia (g/mm)</strong></td>
<td>12.3±0.5</td>
<td>11.12±0.3</td>
</tr>
<tr>
<td><strong>Peak Oxygen Uptake (mL/kg/min)</strong></td>
<td>58.1±2.8</td>
<td>71.7±3.5</td>
</tr>
</tbody>
</table>

**Metabolic Measures**

| Cholesterol (mg/dL) | 75.9±4.0   | 71.1±3.5   |
| LDL Cholesterol (mg/dL) | 19.8±2.0  | 16.5±2.6   |
| HDL Cholesterol (mg/dL) | 50.7±3.7  | 49.6±2.7   |
| Triglycerides (mg/dL)  | 60.7±4.1   | 58.4±5.5   |
| Glucose (mg/dL)       | 113.9±3.3  | 113.9±2.3  |
| Insulin (ng/dL)       | 0.7±0.1    | 0.7±0.1    |
| HOMA-IR               | 8.6±1.1    | 8.4±1.4    |
| Leptin (ng/dL)        | 6.4±0.7    | 4.4±0.5    |

* Table 1. LDL cholesterol, low-density lipoprotein cholesterol; HDL cholesterol, high-density lipoprotein cholesterol; HOMA-IR- homeostatic Metabolic Assessment-Insulin resistance. † p<0.05 vs high fat diet; † p<0.05 vs high fat diet and high fat diet plus training; ‡ p<0.05 vs high fat diet plus training and caloric restriction plus training.
in LDL-cholesterol, HDL-cholesterol, triglycerides, glucose and insulin levels, and HOMA-IR in obese rats. Caloric restriction or exercise training significantly decreased plasma leptin levels. Caloric restriction associated with exercise training significantly reduced plasma leptin levels and HDL-cholesterol levels in obese rats (Table 1).

In regard to the effects of caloric restriction and exercise training on fat liver, we found that caloric restriction alone significantly decreased hepatic triglycerides levels, but did not change hepatic cholesterol levels (Figure 2). Caloric restriction plus exercise training caused decrease in both hepatic cholesterol and triglycerides levels (Figure 2). Exercise training did not change hepatic cholesterol and triglycerides levels.

**Discussion**

The main findings of the present study are: 1) Caloric restriction reduces liver fat contents in obese rats; 2) This effect is greater whether caloric restriction is associated with exercise training.

Previous observations show that obesity is associated with dyslipidemia, insulin resistance, hypertension and metabolic syndrome. In addition, accumulated evidence show that obesity increases liver fat mass (1). On the other, some investigators reported that exercise training decreases hepatic triglyceride content in obese individuals, which was attributed to increase in hepatocyte fat oxidation. The present study extends the knowledge that body weight loss by exercise training associated with caloric restriction potentiates the reduction in hepatic triglyceride and cholesterol contents in experimental model of obesity. The association of caloric restriction and exercise training caused a more intense reduction in liver fatness than exercise training alone and caloric restriction alone.

Despite some investigators have suggested an association between hepatic triglyceride levels and insulin resistance, our study shows dissociation between hepatic triglyceride levels and insulin sensitivity after caloric restriction or caloric restriction and exercise training. These interventions reduced hepatic triglyceride contents, but unchanged HOMA-IR. One definitive explanation for these responses is out of the scope of our study. However, we can speculate that the caloric restriction alone or associated with exercise training increases lipolysis in the adipose tissue and, in consequence, the influx of fatty acid to the liver. These alterations may cause a failure of insulin to stimulate glycogen synthesis. Alternatively, the reduction in leptin levels caused by caloric restriction and exercise training was so dramatic that precluded the improvement in insulin sensitivity in obese rats. It has been reported that leptin plays an important role in insulin sensitivity. It is also possible that HOMA-IR is a limited method to evaluate insulin resistance.

Exercise training alone reduced visceral fat and leptin levels, but failed to change hepatic triglyceride and cholesterol levels. Despite the fact that the exercise regimen used in our study significantly increased peak oxygen uptake which shows its effectiveness, a more intense exercise regimen may be necessary to reduce hepatic steatosis. In a recent study, some investigators showed that aerobic interval training in which obese patients were submitted to moderate and high intensity exercise training was more effective to improve insulin signaling in fat and skeletal muscle. Moreover, a more intense exercise may be necessary to change plasma lipid contents, since our exercise strategy did not change plasma total cholesterol, LDL-cholesterol and HDL-cholesterol levels. We cannot rule out that our experimental model of obesity is inadequate to investigate the effects of exercise training in plasma lipid contents, since in a previous study in humans we observed that the exercise and hypocaloric paradigm significantly reduced total-cholesterol levels and significantly increased HDL-cholesterol levels.

Our study confirms that exercise training and caloric restriction is a powerful strategy for the treatment of obesity.
This non-pharmacological intervention significantly reduced body weight, visceral fat mass and leptin levels in obese rats. Similar results have been previously reported by us in humans. Hypocaloric diet and exercise training decrease body weight, body fatness and leptin levels in adult and children with obesity [23, 24].

Perspectives

Recent statistics point to the fact that the prevalence and incidence of obesity is out of control in the worldwide [7]. In addition, accumulated evidences show that this syndrome dramatically increases the risk of cardiovascular diseases [20], especially whether the body fat is associated with visceral fat mass [25, 26]. Thus, the treatment of obesity is one of the major challenges in nowadays. Our study supports the concept that non-pharmacological intervention based on caloric restriction and exercise training is an unique strategy to reduce body weight and visceral fat mass, especially liver fat mass. Of course, we cannot simply extrapolate these findings to humans. On the other hand, the present study suggests that exercise training and caloric restriction may represent an alternative for the treatment of obesity-induced hepatic steatose in humans. Besides, this nonpharmacological therapy is cheap and causes no side effects.

In conclusion, caloric restriction associated with exercise training reduces liver lipids in obese rats. These findings suggest that this nonpharmacological strategy may represent an alternative in the treatment of human obesity.

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Conflict of interest

The authors declare no conflict of interest.

References


