Can the Intermittent Training Generate Alterations on the Liver Tissue of Rats Submitted to a Hyperlipidic Diet?

¿El Entrenamiento Intermitente Puede Generar Alteraciones sobre el Tejido Hepático de Ratas Sometidas a una Dieta Alta en Grasa?

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SUMMARY Studies indicate that increasing physical activity and decreasing levels of fat in the liver help to decrease the risk of morbidity from liver conditions. This study aimed to evaluate the effects of an eight-week intermittent training program on the liver tissue of rats subjected to a hyperlipidic diet. The study consisted of 30 male Wistar rats, divided into the following groups: Sedentary Control (SC) Exercise Control (EC) (fed on standard feed and water) Sedentary Obese (SO) and Exercise Obese (EO) (fed on bacon, ham, sausage, biscuits, soda and standard feed), which performed intermittent training through electrically stimulated jumps, with three sets of 12 repetitions, three times per week for eight weeks. At the end of the training period, the animals were euthanized, and their livers were removed for histological processing and hematoxylin and eosin staining (HE). Soon afterwards, caryometric analysis of the hepatocyte nuclei was performed. From the presented results it can be seen that the hepatocyte nuclei of the obese animals were smaller in relation to those of the control animals, therefore, exercise combined with an appropriate diet proved to be efficient in not causing alterations in the hepatocyte nuclei, conserving normal cell function and reducing the chances of the appearance of tissue damage. Furthermore, exercise in isolation cannot be considered a protective factor against the alteration of the liver cells.

KEY WORDS: High fat diet; Exercise; Hepatocytes.

INTRODUCTION

Obesity is characterized by a disproportionate consumption of calories in relation to its expenditure for a long period of time, in which the excess of energy is stored as triglycerides in white adipose tissue cells (Speakman, 2013).

Exercise is a stress factor for the liver, due to its central role in maintaining glucose and lipid homeostasis and its function as an energy supplier for muscular work (Fritsche et al., 2008). An increase in physical activity together with a decrease in levels of fat in the liver can help reduce the risk of morbidity from liver disease (Spassiani & Kuk, 2008).

The pattern of mobilization of energy substrates during exercise can be characterized as a sequence of three phases whose predominant energy substrates are: blood glucose, muscle glycogen and circulating free fatty acids (FFA) (Silveira et al., 2011). In exercises of low to moderate intensity and long duration, an increase in liver and heart glycogen depletion and an increase in FFA oxidation occur (Silveira et al.).

High intensity training diminishes visceral adipose tissue and acts on weight loss by increasing total energy expenditure and excess post-exercise oxygen consumption (EPOC), increasing food-induced thermogenesis and leptin activity and decreasing systolic and diastolic blood pressure whilst inducing the secretion of vasodilator substances such as nitric oxide (Gutierres & Martins, 2008). Thus, the liver plays an important role in providing energy substrates to working muscles during physical exercise, acting synergistically with the energy reserves of the skeletal muscle. However, no studies were found emphasizing the effects of intermittent exercise and how it correlates to morphological changes in the liver tissue.

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The hypothesis of this study was that intermittent training would exercise a protective factor on the liver tissue of animals because, as stated above, physical exercise induces energy expenditure thereby preventing the accumulation of lipids in hepatic tissue, thus avoiding an increase in intrahepatic triglycerides and the subsequent formation of inflammatory foci (steatosis). Therefore, the present study aimed to evaluate the effect of an eight week intermittent training program on the liver tissue of rats with a hyperlipidic diet.

MATERIAL AND METHOD

Animals. Thirty male Wistar rats of 90 days of age were obtained. They were kept collectively in plastic cages (30 x 16 x 19 cm). The animals were housed in groups of three to five animals per cage, at an average temperature of 22±2 °C and light/dark cycles of 12 h with the light cycle starting at 07:00 am. The 30 animals were separated randomly into the following groups (Table I):

<table>
<thead>
<tr>
<th>Group</th>
<th>Initial n</th>
<th>Final n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sedentary Control - SC</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Exercise Control - EC</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>Sedentary Obese - SO</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Exercise Obese - EO</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>27</td>
</tr>
</tbody>
</table>

The animals in the SC and EC groups were fed on standard rodent feed (Supra Lab® brand - Alisul Ind. Alimentos Ltda) with tap water provided ad libitum. The SO and EO groups, from the second month of life, followed a hyperlipidic diet (cafeteria diet), as used by Panveloski-Costa et al. (2011), adapted from Lamas et al. (2004).

Intermittent Training. The model of intermittent strength training "Jump Squat" used in the project was adapted from that proposed by Tamaki et al. (1992). The apparatus was designed so that the animal was immobilized over a metal platform by means of an adapted vest.

Electrical stimulation was performed using a metal clip attached to the end of the tail of the animal and to an electrical stimulator (Dualpex 961, from Quarker®), calibrated by Inmetro®. The parameters used were: frequency 1 Hertz (Hz), duration of 0.3 s with an interval of 2 s between each electrical stimulant and the intensity adjusted so that the animal executed the movement, ranging from 3 to 6 milliamperes (mA).

These parameters were adopted as being bidirectional pulses of zero mean, presenting no electrolytic effects and allowing long-term use without risk of tissue damage. At the stimulation, the animals performed full leg extension movements (knee and ankle), lifting a load which had been placed on the back of the vest.

The load was adjusted weekly according to the weekly BW variations (de Lima et al., 2008). This training protocol, proposed by Panveloski-Costa et al., began together with a hyperlipidic diet when the animals reached a young adult age; two months old.

Histological procedures. Twenty-four hours after the last training session, the animals were anesthetized and the euthanasia process was performed.

A paramedian incision was made in the abdomen, and the epithelial tissue folded until the organs were exposed. Shortly after the incision, the liver was removed from the animal, for the extraction of the right upper lobe, the material used for analysis, which was immediately fixed in a 10 % formalin solution.

After 24 hours, the organ was transferred to vials containing 70 % alcohol. This solution was renewed daily for three days, to eliminate any residues of the fixer (Camargo Filho et al., 2011). After fixation and storage in 70 % alcohol, the procedure for inclusion of the material in paraffin was performed and it was subsequently cut in a rotary microtome Microm HM 325®, with transverse cuts of 6 µm thickness. Staining was performed using Hematoxylin and Eosin (HE) according to the methods of Dal Pai Silva (1995).

The caryometric analysis was performed using the methods of Martins et al. (2010), an evaluation was conducted of the hepatocyte nuclei images obtained using an optical microscope Nikon® H550, with a 100x magnification lens, coupled with a Nikon® Infinity 1 digital camera. The images were captured using the NIS-Elements D 3.0 - SP7 – build 547, Nikon® for Windows® software for computerized image analysis. A caryometric analysis was performed using Image-Pro Plus 4.5.0.29 for Windows® 98-NT-2000 software for computerized image analysis from Media Cybernetics Inc.

Statistical Analysis. For statistical comparison of the morphometric results obtained from both groups (Control and Obese), the Levene’s Test was used to verify the normality of the data, and the ANOVA One-Way Test along with the Turkey’s Post-Test (Kruskall-Wallis Test and Dunn’s Post-Test for non-parametric data) for comparison of the results. The significance value was set at (p<0.05) for all procedures. Calculations were performed using the SPSS 17.0 for Windows® application.
RESULTS

The initial and final weight of the animals was measured and there was a significant difference comparing the weight gain values between: SC-EC (p < 0.01), SC-SO (p < 0.02) EC-SO (p < 0.001) EC-EO (p < 0.005) and the SO-EO (p < 0.05).

After analyzing the data, changes obtained by caryometry were observed. The mean values demonstrated that in the comparison made between groups it could be observed that the caryometric variables largest diameter (p = 0.019), smallest diameter (p = 0.005), average diameter (p = 0.010), perimeter (p = 0.003), volume (p = 0.002), area (p = 0.007), and the volume/area ratio (p = 0.006) showed statistically significant differences between the SC and EO groups.

Table II. Mean and Standard Deviation of the values of the largest, smallest and average diameter (µm), D/d, perimeter (µm), volume (µm³), area (µm²), volume/area, shape coefficient, contour index and eccentricity of the liver tissue of the rats between groups.

<table>
<thead>
<tr>
<th>Variables</th>
<th>SC (n= 5)</th>
<th>EC (n= 9)</th>
<th>SO (n= 4)</th>
<th>EO (n= 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. Diameter M ± SD</td>
<td>0.718±0.065*</td>
<td>0.671±0.051</td>
<td>0.642±0.041</td>
<td>0.635±0.026</td>
</tr>
<tr>
<td>S. Diameter Med (IR)</td>
<td>0.410 (0.04)*</td>
<td>0.380 (0.01)*</td>
<td>0.360 (0.04)</td>
<td>0.330 (0.03)</td>
</tr>
<tr>
<td>A. Diameter Med (IR)</td>
<td>0.540 (0.05)*</td>
<td>0.520 (0.10)</td>
<td>0.485 (0.05)</td>
<td>0.460 (0.03)</td>
</tr>
<tr>
<td>Perimeter M ± SD</td>
<td>1.826±0.135*</td>
<td>1.690±0.155</td>
<td>1.605±0.094</td>
<td>1.562±0.063</td>
</tr>
<tr>
<td>D/d</td>
<td>1.844±0.268</td>
<td>1.771±0.273</td>
<td>1.860±0.072</td>
<td>1.990±0.111</td>
</tr>
<tr>
<td>Volume M ± SD</td>
<td>0.092±0.014*</td>
<td>0.076±0.021*</td>
<td>0.067±0.012</td>
<td>0.055±0.010</td>
</tr>
<tr>
<td>Area Med (IR)</td>
<td>0.240 (0.04)*</td>
<td>0.220 (0.07)</td>
<td>0.190 (0.04)</td>
<td>0.170 (0.02)</td>
</tr>
<tr>
<td>Volume/Area Med (IR)</td>
<td>0.360 (0.04)*</td>
<td>0.350 (0.07)</td>
<td>0.320 (0.03)</td>
<td>0.310 (0.02)</td>
</tr>
<tr>
<td>S. Coefficient M ± SD</td>
<td>0.872±0.051</td>
<td>0.888±0.049</td>
<td>0.872±0.012</td>
<td>0.847±0.019</td>
</tr>
<tr>
<td>Outline Index M ± SD</td>
<td>3.810±0.119</td>
<td>3.780±0.125</td>
<td>3.817±0.029</td>
<td>3.875±0.052</td>
</tr>
<tr>
<td>Eccentricity M ± SD</td>
<td>0.806±0.061</td>
<td>0.788±0.049</td>
<td>0.810±0.008</td>
<td>0.831±0.016</td>
</tr>
</tbody>
</table>

M= Mean; SD= Standard Deviation; Med= Median; IR= Interquartile Range. The ANOVA One-Way (Tukey Post-Hoc) or Kruskall-Wallis (Dunn Post-Hoc) for comparison of the results. The significance value was set at (p<0.05) for all procedures. * Statistically significant difference compared to group EO.

Fig. 1. Usage of the software Image-Pro Plus 4.5.0.29 for Windows 98-NT-2000, from Media Cybernetics Inc. for Windows®.
submitted to the same diet who did not perform physical activities.

Eder (2009) corroborates the results of this study, finding that intermittent training showed an increase in depletion of post-exercise VLDL-TAG and decrease in hepatic deposits of TAG, suggesting higher transport of triacylglyceride to the periphery, seeking to replenish the intramuscular deposits in the post exercise.

Garekani et al. (2011) points out that the total fat levels in animals with high and moderate levels of exercise are lower compared to sedentary animals, because such alterations also depend on the adaptations the body realizes in the metabolism to meet the energy expenditure during the practice of the exercise.

In the caryometric analysis, the comparison between groups points out that the caryometric variables largest diameter (p=0.019), smallest diameter (p=0.005), average diameter (p=0.010), perimeter (p=0.003), volume (p=0.002), area (p=0.007), and the volume/area ratio (p=0.006) presented statistically significant differences between the groups SC and EO.

Besides the variables of smallest diameter (p=0.019), volume/area ratio (p=0.044) for EC and EO groups, there were no statistically significant differences comparing the SC group variables with the other groups.

In a study by Menguini et al. (2012) it was observed that obesity in the liver of Wistar rats induced macrovesicular steatosis similar to grade 2, characterized principally by ballooning degeneration. In a study by Syn et al. (2012), significant development in hepatic steatosis and fibrosis was observed in an eight week period, due to the natural death of T cells. Thus, the alterations presented in the liver of the animals belonging to the SO group could be correlated with the diet (responsible factor).

The variation in the nuclei sizes in the SO group corresponds to the anisonucleosis process, also observed by Guzman et al. (2011) in cases of toxic and metabolic steatohepatitis, autoimmune hepatitis and other liver diseases. Jarrar & Taib (2011) also observed a slight anisonucleosis in sub toxic doses of lead poisoning in Wistar rats.

The pathogenesis of anisonucleosis in non-neoplastic liver diseases is not well understood, but is probably consistent with the morphological manifestation of nuclear injury from a variety of mechanisms (hepatitis C virus, metabolic disorders, toxic steatohepatitis, autoimmune hepatitis and other chronic liver diseases in humans) (Guzman et al.).

**DISCUSSION**

The present study aimed to investigate the effects of intermittent exercise on the liver tissue of animals subjected to a hyperlipidic diet.

The body weight of the animals was measured at the start and end of the experiment, in which was found with a statistically significant difference that the body weight of the EC group is less than the ones from the EO and SO groups.

Chapados & Lavoie (2010) found that visceral fat mass levels of animals that exercised and were submitted to a hyperlipidic diet were lower at the end of the experimental phase in comparison to the visceral fat mass of animals
Possibly, this phenomenon occurred in the hepatocyte nuclei of the SO group due to the accumulation of lipids (with consequent formation of steatohepatitis). Portela et al. (2007), verified in pregnant rats that the administration of aspartame, diluted in distilled water at room temperature, also presented a decrease in the values of the variables related to the size of the hepatocyte nuclei compared to the control group, due to the toxicity of the substance on the liver.

Batista et al. (2006), in a caryometric study, observed the action of an infusion of Cayaponia tayuya on mice and found that the hepatocyte nuclei were higher than the nuclei of animals which had not been treated with the plant. These latter findings indicate that hepatocyte anisonucleosis occurs due to the exposure of the tissue to agents (toxic or not) or high stress situations which provide greater work demands, with consequent cellular apoptosis and tissue injury.

The results also demonstrate the size of the EO group cell nuclei to be smaller in relation to the EC. However, the EO group had a differentiating factor, in addition to diet; intermittent training. The results of Chapados et al. (2009) corroborate the findings of this study, stating that coupled with a reduction in the content of triacylglycerides in the liver, physical training reduces the synthesis of VLDL (Very Low Density Lipoprotein) in fed rats which have had obesity induced by high fat levels, via microsomal triglyceride transfer protein.

A study by Almeida et al. (2008) showed that an increase in the hepatic lipid content occurs proportionally to the original value. The effects of exercise training in this study are possibly associated with the effects of nutritional intake, i.e., the diet of the EO animals may have mitigated the effects of physical training.

Studies in rats indicate that a diet rich in fatty acids can modulate the damage caused in liver tissue, generating dyslipedemia, excessive weight and liver injury with increased steatosis and fibrosis in the liver tissue, due to the production of large quantities of TNF-α (Tumor Necrosis Factor) generated by the adipose tissue (Neves et al., 2006).

Possibly, the decrease in the nuclear volume showed by caryometric analysis in the animal’s tissues of SO and EO groups in relation to the animal’s tissues of SC and EC groups, reflects the decrease in metabolic activities of these ones. Chapado & Lavoie, state that the excess lipids mobilized from the adipose tissue during exercise in addition to the lipids from the diet that are mobilized through the liver while inhibiting the synthesis of VLDL and MTP (Microsomal Triglyceride Transfer Protein), can aggravate framework of hepatic steatosis even in physical training situation.

Fabbrini et al. (2010) add to this by stating that high levels of intrahepatic triglycerides can cause metabolic abnormalities independent of BMI, percentage body fat or visceral fat mass. Therefore, from the histological findings, it appears that physical exercise acting in isolation was not a protective factor against the alteration of the liver cells; it needs to be combined with other factors such as a low-calorie diet.

This study was limited to analyzing nucleic changes of liver tissue. However, future studies may collaborate with the literature by quantifying the number of cells, in which it can be pointed out in what proportion the liver needs to adapt its number of hepatocytes, from a normal condition to an exercised condition or a condition of obesity, as well as possible changes in extracellular matrix in the liver tissue, for example, quantifying the concentration of fatty acids.

It can be concluded therefore, that the exercise combined with proper diet was efficient in not causing significant changes in the nuclei of hepatocytes, being able to retain its normal functions and reducing the chances of the appearance of lesions in the tissue. In addition to that, the exercise alone cannot be considered a protection factor of liver cells.

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extrajeron sus hígados para el procesamiento histológico y tinción con hematoxilina y eosina (HE). Luego se realizó el análisis cromiometrico de los núcleos de los hepatocitos. Se observó que los núcleos de los hepatocitos fueron menores en los animales obesos en comparación con los núcleos de hepatocitos de los animales de control, pues el ejercicio combinado con una dieta adecuada se mostró eficiente para que causar alteraciones en los núcleos de hepatocitos, y esa combinación puede retener la función normal de las células y disminuir las posibilidades de la aparición de daños en los tejidos. Además, el ejercicio aislado no puede ser considerado como un factor de protección contra la alteración las células del hígado.

PALABRAS CLAVE: Dieta alta en grasa; Ejercicio; Hepatocitos.

REFERENCES


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