Medical Molecular Morphology (2019) 52:15–22
https://doi.org/10.1007/s00795-018-0198-7

ORIGINAL PAPER

Physical exercise alters hepatic morphology of low-density lipoprotein receptor knockout ovariectomized mice

Franciele Mercúrio Rodrigues[1] · Jenifer Inácio Adélio[1] · Vanessa Oliveira Santana[1] · Elisabeth De Marco Ornelas[1] · Romeu Rodrigues de Souza[1] · Clever Gomes Cardoso[2] · Glaucia Luciano da Veiga[3] · Fernando Luiz Affonso Fonseca[3,4] · Laura Beatriz Mesiano Maifrino[1]

Received: 31 January 2018 / Accepted: 18 June 2018 / Published online: 22 June 2018

Abstract
To explore the effects of physical exercise on the liver of animals in menopause, we analyzed the histomorphometric parameters of the hepatic tissue in ovariectomized and dyslipidemic female mice. The animals were distributed in six groups ($n=5$): sedentary control (SC), sedentary ovariectomized control (SOC), trained ovariectomized control (TOC), sedentary LDL knockout (LDL-S), sedentary ovariectomized LDL knockout (LDL-SO), and trained ovariectomized LDL knockout (LDL-TO). At the end of the experiment, the liver and the visceral adipose tissue (VAT) of animals were removed for morphometric and stereological studies. In the LDL-S and LDL-SO animals, both sedentary, results showed reduction in the area (μm^2) and major and minor diameters (μm) of hepatocytes and reduction in the portions of large hepatocytes, and increase in the percentage of Kupffer cells. The trained group showed a tendency of increase in the area and diameter and in the percentage of hepatocytes, as well significant reduction in the percentage of Kupffer cells and interstitial tissue. We suggested that training can prevent cell and tissue damage caused by the process of increase in hepatic fat, lipoperoxidation, and tissue inflammation in animals with privation of estrogen and dyslipidemia, apparently reflecting a better metabolic response of the hepatic tissue in organisms undergoing training.

Keywords Physical exercise · Menopause · Morphology · Dyslipidemia

Introduction

Dyslipidemia, characterized by an increase in triglyceride LDL cholesterol (LDL-c) and of lipoproteins (Lp) and a decrease in the level of HDL-c, is a disease that affects different systems in the organism, especially the renal, cardiac, and hepatic. In the hepatic system, dyslipidemia induces changes in metabolism of carbohydrates, lipids, and proteins, leading to non-alcoholic fatty liver disease (NAFLD), also called hepatic steatosis [1]. NAFLD is characterized by increased concentration of intrahepatic triglycerides (IHTG), which may be associated with inflammation or fibrosis, lobular inflammation, hydropic degeneration, cirrhosis, and hepatocellular carcinoma [2–4]. Elevation in the quantity of fatty acids and other lipids in the hepatocytes can give rise to plasma lipid peroxidation that results in rupture of the cell membrane and induction of inflammatory processes [5].

Menopause, has been associated with dyslipidemia due to privation of the estrogen hormone and loss of its essential functions in the organism, such as cardiovascular protection, control of cholesterol levels, vasodilation, and reduction in oxidative stress [6, 7].

These metabolic impairments, associated with physical and psychological modifications, sedentary lifestyle, and poor eating habits, result in greater susceptibility to obesity and accumulation of fat in the abdominal region,

✉ Fernando Luiz Affonso Fonseca
profferfonseca@gmail.com

1 Stricto Sensu Postgraduate Program in Physical Education and Aging, Universidade São Judas Tadeu, São Paulo, Brazil

2 Department of Histology, Embryology and Molecular Biology, Universidade Federal de Goiás (UFG), Goiania, Brazil

3 Clinical Analysis Laboratory, Faculdade de Medicina do ABC-FMABC2000, Lauro Gomes Avenue, Santo André, SP CEP 09060-650, Brazil

4 Pharmaceutical Sciences Department of Universidade Federal de São Paulo-Unifesp, Diadema, SP, Brazil

dyslipidemia, increasing the risk of development of NAFLD, type 2 diabetes and cardiovascular disease (CVD) [8, 9].

Physical exercise directly assists in treatment and prevention of these metabolic changes. It promotes reduction in visceral adipose tissue and intramuscular lipids, acts in loss of body weight, and promotes reduction in body fat and improvements in the lipid and glycemic profile. It is thus directly associated with reduction in the amount of hepatic fat in patients with NAFLD [10–12].

In addition, the practice of physical exercise attenuates the development of hepatic fibrosis and inflammation and infiltration of macrophages caused by ingestion of a hyperlipidic diet [13].

However, little is known about the effects of the practice of physical exercise on the hepatic tissue of animals with privation of the hormone estrogen and under dyslipidemic conditions. Thus, the aim of our study is to analyze the effects of moderate aerobic exercise on the hepatic tissue, using ovariectomized and LDLr knockout (dyslipidemic) female mice as an animal model.

Materials and methods

Animals and groups

Thirty female mice at 9 months of age (20–30 g) from the Animal House of the Universidade São Judas Tadeu were used. The mice were housed in cages in a location with controlled temperature from 22 to 24 °C and a light/dark cycle of 12/12 h. All the mice were fed water and standard feed "ad libitum". The animals were divided into two groups: 15 genetically modified female mice, with knockout of the low-density lipoprotein receptor (LDLr Knockout), and 15 wild female mice (C57BL/6J). The groups were divided at random into six groups ($n = 5$): sedentary non-ovariectomized control (SC), sedentary ovariectomized control (SOC), trained ovariectomized control (TOC), sedentary non-ovariectomized LDLr knockout (LDLr-S), sedentary ovariectomized LDLr knockout (LDLr-SO), and trained ovariectomized LDLr knockout (LDLr-TO). This study was approved by the research ethics committee of the Universidade São Judas Tadeu (COEP-USJT) in accordance with the following protocol: 058/2007.

Ovariectomy

The ovariectomy was performed at 9 months of age. The animals were anesthetized with a ketamine and xylazine solution (120:20 mg/kg, im) and placed in supine position and a small incision in the lower third of the abdominal region, parallel to the line of the body, was made. The ovaries, the uterine horns, and the blood vessels were located, sectioned, and removed. After that, the musculature and the skin were sutured.

Confirmation of the efficacy of the ovariectomy was determined through colpocytology of the vaginal secretion performed over 4 consecutive days. On the last day of analysis, euthanasia was performed on these animals [14].

Maximal training test

A maximal training test was performed on all the groups at the beginning and at the end of the exercise training program. The test consists of placing the animal to run in an ergometric treadmill at 0.3 km/h for 3 min, and this workload was increased by 0.3 km/h every 3 min until the animal reached exhaustion. The time of the test and the speed of the last workload were noted and served to determine the mean value of aerobic capacity of each group.

Exercise training

Exercise training began 7 days after the ovariectomy surgery; the trained groups were subjected to a physical training protocol on an ergometric treadmill at low–moderate intensity (≈50–70% maximal running speed) for 1 h a day, 5 days a week, for 4 weeks, with a gradual increase in speed from 0.3 to 1.2 km/h. The animals were adapted to the treadmill for 10 min on 3 days prior to beginning the training.

Tissue preparation

After the end of the experimental protocol, the animals were sacrificed by decapitation. The liver and the visceral adipose tissue were revealed by a median incision in the abdominal cavity, removed, and weighed. Tissue samples were washed in phosphate buffered saline solution (PBS) at 0.1 M and pH 7.4 and fixed in 10% buffered formaldehyde solution for 72 h. After that, they were dehydrated in increasing series of alcohols, cleared with xylene, embedded in paraffin, sectioned at 5 microns thickness, collected on glass slides, and stained with hematoxylin and eosin for analysis in an optical microscope.

Morphometric and stereological analyses

The images of the histological sections were captured through a computerized digital image processing and analysis system of the Morphological and Immunohistochemical Studies Laboratory of the Universidade São Judas Tadeu. The system consists of a Sony video microcamera coupled to a Zeiss microscope, which captures images of the histological slides and transmits them to a computer equipped with specific software for quantitative analyses. The image analysis software program Axio Vision 4.8, Zeiss, was used

to perform morphometric analyses and to determine the hepatocyte area; the results were expressed by percentage.

The Image J software (version 1.47—National Institutes of Health) was used to count through a grid the volume density of the structures studied, the results were expressed by percentage [15–19].

For analyses, 100 images/group were captured at 400× magnification. The following morphometric parameters were analyzed: area (μm^2) and major and minor diameters (μm) of the hepatocytes, major and minor diameters (μm) of their nuclei, and the mean nuclear volume (MNV). The mean nuclear volume was calculated by: $MNV = a^2 \times b/1.91$, in which MNV = nuclear volume, a minor nuclear diameter, b major nuclear diameter, and 1.91 is a constant that is used.

The stereological parameters analyzed were density of the nucleus and hepatocyte volume, Kupffer cells, interstitial tissues, and vessels through a 366-point test system [15]. The volume density has the aim of determining the relative occupation of the structures in the test area, and may quantify the main normal and pathological adaptive modifications of the cell structures.

To classify the measurements of the hepatocytes we used as control the sedentary control group. Firstly we identified the hepatocyte measurements in this group, then we verified the smaller and larger sizes and divided by 3 to classify in small, médium and large. In addition, we consider the following measures: small 8833–13,938 μm; medium 13,939–27,876 μm and large 27,876–41,814 μm.

Statistical analysis

The results were presented as mean and standard error of the mean. The one-way analysis of variance (ANOVA) and Tukey post-hoc tests were duly applied for analysis of the data. The level of significance adopted in all the tests was $p < 0.05$.

Results

Biometric and biochemical analysis

The initial body weight (IBW) and final body weight (FBW) and the difference between the weights (FBW − IBW) of the mice did not exhibit a significant difference among the groups.

Menopause (SOC), as well as menopause associated with dyslipidemia (LDLr-SO), both in sedentary organisms, induced an increase in the visceral adipose tissue (VAT%) compared to their respective controls (SC and LDLr-S). The trained groups exhibited consistent reduction in VAT%.

Although not statistically significant, ovariectomy (SOC) induced an increase in liver weight (LIV%) with a tendency toward reduction in the trained group (TOC). In the dyslipidemic animals, changes in LIV% were not found.

The animals of the control group (SC, SOC, and TOC) did not exhibit a significant difference in the levels of triglycerides; nevertheless, ovariectomy induced a significant increase in triglycerides in the dyslipidemic animals (LDLr-SO) compared to the control animals. This process was not reversed in the trained group (LDLr-TO). Although similar results were found in the very low-density lipoprotein (VLDL) measurements in the animals, a reduction was observed in the levels after training (LDLr-TO). In the cholesterol measurements, the animals of the sedentary ovariectomized control group (SOC) showed a significant increase compared to the SC group and a significant reduction in the trained group (TOC) compared to the SOC, LDLr-S, and LDLr-SO groups. In addition, a significant increase in cholesterol was observed in the LDL-S group compared to the LDLr-TO group, as described by Veloso et al. [18].

Morphometry

None of the hepatocytes in the experimental groups exhibited morphological distinction in regard to the presence of fat vacuoles within (Fig. 1), indicating that the animals did not develop non-alcoholic hepatic steatosis.

Morphometric analysis showed that in the ovariectomized groups (SOC and TOC) there was no significant change in the total area and in the major diameter of the hepatocytes compared to the control group (SC). However, the sedentary dyslipidemic groups (LDLr-S and LDLr-SO) showed a significant reduction in the total area of the hepatocytes and in their major and minor diameters in comparison to the control groups. In addition, a reversal of this process was observed in the trained animals (LDLr-TO), with a tendency toward an increase in the total area and a significant increase in the major diameter compared to the LDLr-S group (Table 1). The training normalized the area of hepatocytes in LDLr-TO mice, compared to those in SC mice (Table 1), showing no statistically significant difference, but it supported the good effect of physical exercise.

The ovariectomy and the dyslipidemia associated with the training did not significantly change the area, major and minor diameter of the nucleus of hepatocytes, or the mean nuclear volume (MNV) parameters (Table 2). The training normalized the area of hepatocytes in LDLr-TO mice, compared to those in SC mice (Table 1), showing no statistically significant difference, but it can suggested the good effect of physical exercise.

Ovariectomy (SOC) induced reduction in the frequency of large hepatocytes and reduction in the mean diameter of the hepatocytes. Exercise (TOC) reversed this process compared to the control (SC) (Fig. 1). In the dyslipidemic animals (LDLr-S and LDLr-SO), ovariectomy led to an increase

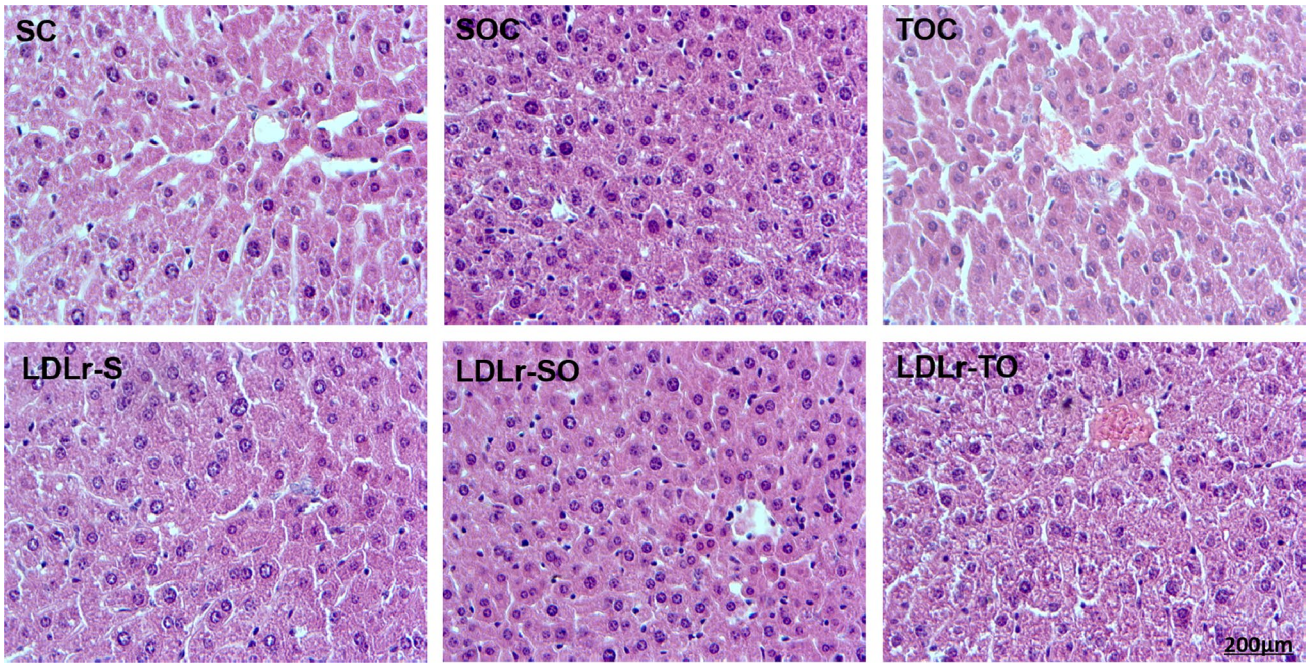

Fig. 1 Photomicrographs of the overall appearance of the hepatic structures revealed by the hematoxylin and eosin (HE) technique. 20× magnification. Groups: sedentary non-ovariectomized control (SC), sedentary ovariectomized control (SOC), trained ovariectomized control (TOC), sedentary non-ovariectomized LDLr knockout (LDLr-S), sedentary ovariectomized LDLr knockout (LDLr-SO), and trained ovariectomized LDLr knockout (LDLr-TO)

Table 1 Area, major and minor diameter of hepatocytes

	Area (μm^2)	Diam hep > (µm)	Diam hep < (µm)
SC	21,238 ± 1069	208.9 ± 8.2	151.6 ± 3.4
SOC	19,947 ± 790	194.9 ± 5.2	155.7 ± 3.9
TOC	22,178 ± 938.3	203.4 ± 5.1	168.3 ± 3.8*
LDLr-S	17,209 ± 928*,+	181.05 ± 6.5*	145.1 ± 4.2+
LDLr-SO	16,457 ± 652*,#,+	174.73 ± 4.2*,+	142. 2 ± 3.6+
LDLr-TO	19076 ± 787.8	190.1 ± 5*,#,+,a	152.6 ± 4.1+

Representative data from area major and minor diameter of hepatocytes of sedentary non-ovariectomized control (SC), sedentary ovariectomized control (SOC), trained ovariectomized control (TOC), sedentary non-ovariectomized LDLr knockout (LDL-S), sedentary ovariectomized LDLr knockout (LDL-SO), and trained ovariectomized LDLr knockout (LDL-TO) groups. Diam hep > (lower diameter of hepatocyte), Diam hep < (larger diameter of hepatocyte). Values expressed by mean ± SEM

*$p < 0.05$ vs SC

#$p < 0.05$ vs SOC

+$p < 0.05$ vs TOC

a$p < 0.05$ vs LDLr-S

b$p < 0.05$ vs LDLr-SO

in the frequency of small hepatocytes and reduction in the frequency of large ones, and exercise (LDLr-TO) reversed this tendency, nearing the values of the non-dyslipidemic control group (TOC) (Fig. 2).

Stereological analysis

In the sedentary groups, ovariectomy (SOC) and ovariectomy associated with dyslipidemia (LDLr-SO) significantly increased the volume density of the Kupffer cells (Vv[kup]) in the hepatic tissue, especially in the LDLr-SO group compared to the controls (SC and LDLr-S). In contrast, training reversed this process, both in the ovariectomized group (TOC) and in the ovariectomized dyslipidemic group (LDLr-TO) (Table 3). The volume of hepatocytes (Vv[hep]) did not show significant change in the animals of the ovariectomized group (SOC and TOC). However, the mice of the trained dyslipidemic group (LDLr-TO) had a significant increase in the percentage of hepatocytes. The blood vessel volume (Vv[ves]) did not change among the groups evaluated. Ovariectomy did not change the volume of interstitial tissue (Vv[int]). However, in the dyslipidemic groups (LDLr-SO and LDLr-TO), an expressive reduction in the quantity of interstitial tissue was found compared to the control group (Table 3).

The analysis of the liver weight, at the end of the experiment, verified that the LDLr-S group presented a lower liver weight statistically different from the animals of the group SOC (Fig. 3).

Table 2 Area, major, minor and medium diameter of the hepatocyte nucleus and medium nuclear volume (MNV)

	Area (μm²)	Diam > nu (μm)	Diam < nu (μm)	MNV
SC	2745.8 ± 103.6	61.8 ± 1.2	56.86 ± 1.4	109,747 ± 6430
SOC	2842.1 ± 118.6	62.3 ± 1.3	57.5 ± 1.3	114,787 ± 7547
TOC	2947.8 ± 114.6	64.2 ± 1.2	58.4 ± 1.2	121,247 ± 7479
LDLr-S	2836.9 ± 122.3	63.6 ± 1.5	57.3 ± 1.8	93,114 ± 6911
LDLr-SO	2741.8 ± 120	62.0 ± 1.3	56. 5 ± 1.3	111,344 ± 7521
LDLr-TO	2832.6 ± 95.2	65.0 ± 1.3	59.6 ± 1.2	127,390 ± 7465

Values of area; Diam hep < nu (lower diameter of nuclei hepatocyte), Diam hep > nu (larger diameter of nuclei hepatocyte) and medium nuclear volume (MNV) of sedentary non-ovariectomized control (SC), sedentary ovariectomized control (SOC), trained ovariectomized control (TOC), sedentary non-ovariectomized LDLr knockout (LDLr-S), sedentary ovariectomized LDLr knockout (LDLr-SO), and trained ovariectomized LDLr knockout (LDLr-TO) groups

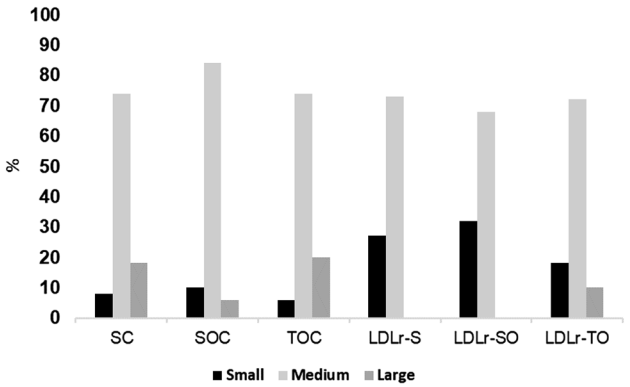

Fig. 2 Histogram of the hepatocyte area. Groups: sedentary non-ovariectomized control (SC), sedentary ovariectomized control (COS), trained ovariectomized control (TOC), sedentary non-ovariectomized LDLr knockout (LDLr-S), sedentary ovariectomized LDLr knockout (LDLr-SO), and trained ovariectomized LDLr knockout (LDLr-TO). Small %, Med %, Large % among the groups

Discussion

In this study, we evaluated the histomorphometric effects of physical exercise on the hepatic tissue of mice with privation of estrogens from ovariectomy and with dyslipidemia due to knockout in the LDL lipoprotein receptor. In addition, the dyslipidemia caused morphological changes and training was able to reverse these effects.

In physiological conditions, estrogen plays pivotal biological roles including hepatic homeostasis. However, when occurs an alteration of metabolic function, either in adipocytes or during menopause, there are a lipid overflow, accumulation of fat in major organs, suppression of glucose disposal, increased lipogenesis and decreased lipolysis [20].

The state of deficiency in estrogens and a sedentary lifestyle have repeatedly been related to accumulation of fat in the liver, which is one more indication that metabolism of fat is modified by the absence of estrogens [21]. Increased fat in the liver can give rise to non-alcoholic fatty liver disease (NAFLD), characterized by various histopathological changes that range from benign steatosis, with accumulation

Table 3 Volume density of Kupffer cells (Vv [kup]), hepatocytes (Vv [hep]), vessels (Vv [vas]), and interstitium (Vv [int])

	SC	SOC	TOC	LDLr-S	LDLr-SO	LDLr-TO
Vv[kup]%	1.2 ± 0.09	2.1 ± 0.1*	1.3 ± 0.1$^{\#}$	0.9 ± 0.2$^{\#}$	3.5 ± 0.2$^{*,\#,+,a}$	1.9 ± 0.1$^{*,+,a,b}$
Vv[hep]%	73 ± 1.4	75.6 ± 1.2	73.1 ± 1.2	74.8 ± 2	78.6 ± 0.9	81.5 ± 0.8$^{*,\#,+,a,b}$
Vv[ves]%	4.4 ± 0.8	3.2 ± 0.5	2.8 ± 0.4	4.1 ± 0.9	3.4 ± 0.5	3.8 ± 0.6
Vv[int]%	20.9 ± 1.1	18.5 ± 0.9	22.4 ± 1.2$^{\#}$	20.2 ± 1.3	14.6 ± 0.8$^{*,\#,+}$	12.9 ± 0.6$^{*,\#,+,a}$

Representative data from Volume density of Kupffer cells (Vv [kup]), hepatocytes (Vv [hep]), vessels (Vv [ves]), and interstitium (Vv [int]) of sedentary non-ovariectomized control (SC), sedentary ovariectomized control (SOC), trained ovariectomized control (TOC), sedentary non-ovariectomized LDLr knockout (LDLr-S), sedentary ovariectomized LDLr knockout (LDLr-SO), and trained ovariectomized LDLr knockout (LDLr-TO). Values expressed by mean ± SEM

$^{*}p < 0.05$ vs SC

$^{\#}p < 0.05$ vs SOC

$^{+}p < 0.05$ vs TOC

$^{a}p<0.05$ vs LDLr-S

$^{b}p<0.05$ vs LDLr-SO

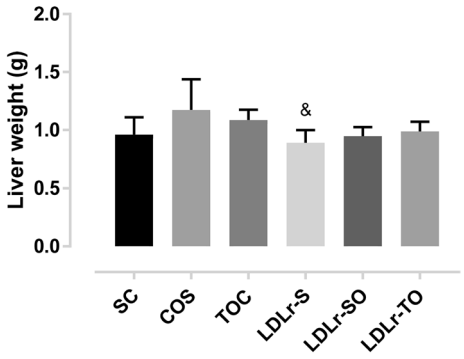

Fig. 3 Values of liver weight at the end of the experiment of the groups, sedentary non-ovariectomized control (SC), sedentary ovariectomized control (COS), trained ovariectomized control (TOC), sedentary non-ovariectomized LDLr knockout (LDLr-S), sedentary ovariectomized LDLr knockout (LDLr-SO), and trained ovariectomized LDLr knockout (LDLr-TO). Data expressed by mean ± SD, &$p < 0.05$, LDLr-S vs COS, ANOVA test

of triglycerides in the hepatic tissue, to severe changes called non-alcoholic steatohepatitis (NASH) [22].

The trained group showed a tendency toward reduction; it may be possible to confirm this from an increase in the training time of the animals. The other groups did not exhibit a significant change in liver weight.

Our findings showed that dyslipidemia (LDL-S) and ovariectomy associated with dyslipidemia (LDLr-SO) in sedentary animals lead to reduction in the area and in the major and minor diameters of the hepatocytes. The non-dyslipidemic animals showed less variation in these parameters, indicating that dyslipidemia may be an important factor related to reduction in the size of hepatocytes. We suggested that these findings may be related to lipid peroxidation and oxidative stress of the hepatic cells in these ovariectomized, dyslipidemic, and sedentary animals, in which there is cell death and replication of mature hepatocytes, leading to expansion of progenitor cells called oval cells [23]. We know that we had a limitation in this study without data about oxidative or inflammatory profile, it is possible that this data could respond more deeply to our hypotheses.

We believe that these factors are related to reduction in the hepatocytes, with reduction in the total area of young cells. The expansion of oval cells is strongly related to the degree of fibrosis and to hepatocellular carcinogenesis, considered a malignant evolution of hepatic steatosis. In contrast, physical exercise resulted in a tendency to reverse the process of reduction of the hepatocytes, with an expressive increase in the area and diameters in the groups that performed physical exercise (TOC and LDLr-TO), indicating that training may be associated with improvement in the physiology and adaptation of the hepatocytes to the effects of lipid peroxidation.

In addition, a reduction was observed in the percentage of large hepatocytes in the animals of the sedentary ovariectomized and dyslipidemic groups (SOC and LDLr-SO), resulting in lower mean diameter, especially in the LDLr-SO group, in which a clear increase in the number of small hepatocytes was observed (Fig. 1). These results are in agreement with what was observed in analysis of the area and diameters (major and minor) of the hepatocytes in the animals of these two groups. In the two trained groups (TOC and LDLr-TO), there was an increase in the frequency of the large hepatocytes, with an expressive increase in the mean diameter of the TOC group compared to the control group.

The increase in hepatic fat in the organism can result in the generation of intracellular lipid droplets with macrovesicular or microvesicular appearance and increase in cell volume and area, in a process characteristic of hepatic steatosis. The presence of fat vesicles was not observed in any group in histological analysis, indicating that ovariectomy alone or associated with dyslipidemia are not able to induce hepatic steatosis. Studies have shown that, in addition to physical activity, diet plays an important role in regulation of hepatic fat [24]. Thus, the increase in area in the hepatocytes of the animals that underwent training is not related to the presence of fat vesicles and to the development of hepatic steatosis (Fig. 1).

We observed an increase in the percentage of Kupffer cells in the hepatic tissue of the ovariectomized control (SOC) and ovariectomized dyslipidemic (LDLr-SO) animals. The trained groups showed clear reduction in these values, even though the LDLr-TO group still exhibited a discrete increase in the percentage of Kupffer cells (Table 3). An increase in free fatty acids in the hepatic tissue triggers inflammation by activation of the NF-κβ pathways, which is associated with a rise in the expression of inflammatory cytokines, such as TNF-α, IL-6, interleukin 1-beta (IL-1β), which results in activation and proliferation of the Kupffer cells [25–27]. These high cytokine serum levels are correlated with histological severity [28], and also promote resistance to insulin [29].

In situations of hepatic injury, there is a repair mechanism through replication of mature hepatocytes [30]. However, when the injury in continuous, there is reduction in the ability of proliferation of these hepatocytes. The increase found in the percentage of hepatocytes in the group of trained dyslipidemic animals (LDLr-TO) may be related to reduce the hepatic injury in the animals that underwent physical training. This indicates improvement in the physiological activity of the hepatic tissue and reduction in the inflammatory process. This change in the number of cells of the hepatic tissue was not reflected in an increase in the fraction of blood vessels in the groups analyzed, once more suggesting tissue stability.

We observed that both ovariectomy alone and associated with dyslipidemia results in an increase in visceral fat and levels of plasma fat, as well as reduction in the area of the hepatocytes and in the number of large hepatocytes. The results clearly showed that physical training is responsible for reversing this process, increasing the percentage of hepatocytes and their area, as well as increasing the percentage of large hepatocytes. Training also led to a reduction the percentage of Kupffer immunological cells and an increase in the portion of interstitial tissue.

Conclusion

Taken together, we suggest that these modifications result from improvement in metabolic and physiological activities promoted by physical exercise in the ovariectomized and dyslipidemic animals. These changes may be related to better adaptation of the organism and of the liver to metabolism of lipids. However, the possible influence of adaptive mechanisms to inflammation, that may be present in this experimental condition, can not be ruled out. In fact, further studies are needed to confirm this hypothesis.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

References

1. Lavoie JM, Pighon A (2012) NAFLD, estrogens, and physical exercise: the animal model. J Nutr Metab 2012(914938):10
2. Novelli EL, Diniz YS, Galhardi CM, Ebaid GM, Rodrigues HG, Mani F et al (2007) Anthropometrical parameters and markers of obesity in rats. Lab Anim 41(1):111–119
3. Sjogren K, Hellberg N, Bohlooly YM, Savendahl L, Johansson MS, Berglindh T et al (2001) Body fat content can be predicted in vivo in mice using a modified dual-energy X-ray absorptiometry technique. J Nutr 131(11):2963–2966
4. Shi H, Strader AD, Woods SC, Seeley RJ (2007) The effect of fat removal on glucose tolerance is depot specific in male and female mice. Am J Physiol Endocrinol Metab 293(4):24
5. Koek GH, Liedorp PR, Bast A (2011) The role of oxidative stress in non-alcoholic steatohepatitis. Clin Chim Acta 412(15–16):1297–1305
6. Cardoso JA, Toscano AE, Tashiro T, de Carvalho CA, de Moraes SR (2006) Morphometry of human myocardium in senile individuals. Arq Bras Cardiol 86(5):374–377
7. Coylewright M, Reckelhoff JF, Ouyang P (2008) Menopause and hypertension: an age-old debate. Hypertension 51(4):952–959
8. Lamas O, Martinez JA, Marti A (2004 Jul) Energy restriction restores the impaired immune response in overweight (cafeteria) rats. J Nutr Biochem 15(7):418–425
9. de Lima C, Alves LE, Iagher F, Machado AF, Bonatto SJ, Kuczera D et al (2008) Anaerobic exercise reduces tumor growth, cancer cachexia and increases macrophage and lymphocyte response in Walker 256 tumor-bearing rats. Eur J Appl Physiol 104(6):957–964
10. Jarrar BM, Taib NT (2012) Histological and histochemical alterations in the liver induced by lead chronic toxicity. Saudi J Biol Sci 19(2):203–210
11. Guzman G, Chennuri R, Voros A, Boumendjel R, Locante A, Patel R et al (2011) Nucleometric study of anisonucleosis, diabetes and oxidative damage in liver biopsies of orthotopic liver transplant recipients with chronic hepatitis C virus infection. Pathol Oncol Res 17(2):191–199
12. Leite RD, Prestes J, Bernardes CF, Shiguemoto GE, Pereira GB, Duarte JO et al (2009) Effects of ovariectomy and resistance training on lipid content in skeletal muscle, liver, and heart; fat depots; and lipid profile. Appl Physiol Nutr Metab 34(6):1079–1086
13. Berglund ED, Lustig DG, Baheza RA, Hasenour CM, Lee-Young RS, Donahue EP et al (2011) Hepatic glucagon action is essential for exercise-induced reversal of mouse fatty liver. Diabetes 60(11):2720–2729
14. Marcondes FK, Bianchi FJ, Tanno AP (2002) Determination of the estrous cycle phases of rats: some helpful considerations. Braz J Biol 62(4A):609–614
15. Mandarim-de-Lacerda CA, Pessanha MG (1995) Stereology of the myocardium in embryos, fetuses and neonates of the rat. Acta Anat 154(4):261–266
16. Cui A, Hu Z, Han Y, Yang Y, Li Y (2017) Optimized analysis of in vivo and in vitro hepatic steatosis. J Vis Exp. https://doi.org/10.3791/55178
17. Zhao YY, Yang R, Xiao M, Guan MJ, Zhao N, Zeng T (2017) Kupffer cells activation promoted binge drinking induced fatty liver by activating lipolysis in white adipose tissues. Toxicology 390:53–60
18. Veloso AGB, Lima NEA, de Marco Ornelas E, Cardoso CG, Marques MR, da Costa Aguiar Alves Reis B, Fonseca FLA, Maifrino LBM (2018) Effects of moderate exercise on biochemical, morphological, and physiological parameters of the pancreas of female mice with estrogen deprivation and dyslipidemia. Med Mol Morphol 51:118–127
19. Marchon C, de Marco Ornelas E, da Silva Viegas KA, Lacchini S, de Souza RR, Fonseca FL et al (2015) Effects of moderate exercise on the biochemical, physiological, morphological and functional parameters of the aorta in the presence of estrogen deprivation and dyslipidemia: an experimental model. Cell Physiol Biochem 35(1):397–405
20. Kim MH, Kim EJ, Choi YY, Hong J, Yang WM (2017) Lycium Chinense improves post-menopausal obesity via regulation of PPAR-γ and estrogen receptor-α/β expressions. Am J Chin Med 45(2):269–282
21. Tarantino G, Pizza G, Colao A, Pasanisi F, Conca P, Colicchio P et al (2009) Hepatic steatosis in overweight/obese females: new screening method for those at risk. World J Gastroenterol 15(45):5693–5699
22. 18 Fabbrini E, Sullivan S, Klein S (2010) Obesity and nonalcoholic fatty liver disease: biochemical, metabolic, and clinical implications. Hepatology 51(2):679–689
23. Kolaja KL, Klaunig JE (1997) Vitamin E modulation of hepatic focal lesion growth in mice. Toxicol Appl Pharmacol 143(2):380–387
24. Kenneally S, Sier JH, Moore JB (2017) Efficacy of dietary and physical activity intervention in non-alcoholic fatty liver disease: a systematic review. BMJ Open Gastroenterol 4(1):000139
25. 21 Ryan AS, Pratley RE, Goldberg AP, Elahi D (1996) Resistive training increases insulin action in postmenopausal women. J Gerontol A Biol Sci Med Sci 51(5):M199–M205
26. Suzuki A, McCall S, Choi SS, Sicklick JK, Huang J, Qi Y, Zdanowicz M, Camp T, Li YX, Diehl AM (2006) Interleukin-15 increases hepatic regenerative activity. J Hepatol 45(3):410–418

27. Funke A, Schreurs M, Aparicio-Vergara M, Sheedfar F, Gruben N, Kloosterhuis NJ, Shiri-Sverdlov R, Groen AK, van de Sluis B, Hofker MH, Koonen DP (2014) Cholesterol-induced hepatic inflammation does not contribute to the development of insulin resistance in male LDL receptor knockout mice. Atherosclerosis 232(2):390–396
28. Ryan AS, Pratley RE, Elahi D, Goldberg AP (1985) Resistive training increases fat-free mass and maintains RMR despite weight loss in postmenopausal women. J Appl Physiol 79(3):818–823
29. Brochu M, Malita MF, Messier V, Doucet E, Strychar I, Lavoie JM et al (2009) Resistance training does not contribute to improving the metabolic profile after a 6-month weight loss program in overweight and obese postmenopausal women. J Clin Endocrinol Metab 94(9):3226–3233
30. Hansen D, Dendale P, Berger J, van Loon LJ, Meeusen R (2007) The effects of exercise training on fat-mass loss in obese patients during energy intake restriction. Sports Med 37(1):31–46