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Effect of Physical Exercise on the Renal Structures of Ovariectomized Female LDL Knockout Mice

Efecto del Ejercicio Físico sobre las Estructuras Renales de Ratones Hembras con LDL Ovariectomizadas

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SUMMARY: The reduction of ovarian function and a decrease in estrogen levels marked by menopause are related to increased susceptibility to develop dyslipidemia. These alterations in the lipid profile can have consequences in renal tissue and generate injuries that may progress to renal failure. The practice of physical activity is an important factor for the treatment and prevention of dyslipidemia and its consequences. The objective of this study is to observe the effects of physical exercise on the right kidney of ovariectomized female LDL knockout mice. Animals were submitted to moderate physical exercise, sacrificed, and the right kidney was removed for morphometric and stereological analysis. The results showed that dyslipidemia promoted a decrease in the areas of the corpuscle and renal glomerulus, in the volume density of light in both the proximal and distal convoluted tubules, and an increase in capsular space, particularly more marked in the proximal tubules. We also observed that physical exercise decreased the analyzed parameters. Our results suggest the association of physical training and dyslipidemia presents a tendency to reduce the dimensions of morphometric and stereological parameters of the kidney. These changes may be related to metabolic and physiological adaptation of renal tissue during physical exercise.

KEY WORDS: Kidney; Dyslipidemia; Physical exercise; Menopause.

INTRODUCTION

Dyslipidemia is a disorder in which there is an increase or abnormality in serum cholesterol and triglyceride levels. This change in lipid profile is related to total cholesterol above normal, high levels of LDL-c and low levels of HDL-c in the blood (Caldeira & Garcia, 2011).

Reduced ovarian function and decreased estrogen levels marked by menopause may increase the levels of serum total cholesterol, LDL cholesterol, and triglycerides by 7-19 % on average, comparing pre-menopausal and postmenopausal periods, and decrease HDL cholesterol levels (Phan & Toth, 2014).

In conditions of alterations in lipid metabolism, there is an imbalance between lipogenesis and lipolysis in the kidney and other tissues. Such changes contribute to an accumulation of lipids in the renal tissue, which may lead to thickening of the vascular endothelium, causing lower renal blood flow and greater pumping force of blood in the tissue. This can generate injuries in renal arteries walls, which, chronically, produce renal insufficiency (Wainstein & Lemos, 2007; Batista Peres & Bettin, 2015).

The practice of physical activity can raise HDL cholesterol levels, and reduce triglycerides and LDL

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cholesterol levels. Also, it increases lipase activity by acting efficiently in the prevention and non-pharmacological treatment of dyslipidemia and its consequences. In this sense, exercises are well described in the literature, but studies relating the morphoquantitative aspects of the renal tissue of dyslipidemic ovariectomized animals that perform moderate physical exercise are scarce.

MATERIAL AND METHOD

Animals and groups. The present study was approved by the Research Ethics Committee of the Universidade São Judas Tadeu (COEP-USJT) under protocol number 058/2007. Fifteen female LDL knockout mice with increased plasma LDL levels and 15 female C57BL/6 wild-type mice, 5-week old from the Central Hospital of the Medical School, Universidade de São Paulo (FMUSP) were used. Both groups started the experiment at nine months of age and initial weight ranging from 20 to 30 grams. Mice were housed in cages at controlled room temperature between 22–24 ºC and 12/12 hours light/dark cycle in the USJT Vivarium. All mice were fed with water and standard feed ad libitum. Animals were randomly divided into six groups (n=5 in each group): sedentary non-ovariectomized control (SC); sedentary ovariectomized control (OSC); trained ovariectomized control (OTC); sedentary non-ovariectomized LDL knockout (S LDL); sedentary ovariectomized LDL knockout (OS LDL); trained ovariectomized LDL knockout (OT LDL).

Ovariectomy. Ovariectomy was performed at nine months of age. Animals were anesthetized with ketamine and xylazine solution (120:20 mg/kg) and placed in dorsal decubitus. A small medial incision was made in the lower third of the abdominal region. The ovaries were located and were performed ligation of the uterine horns, including the blood vessels. After sectioning and removal of the structures, the musculature and skin were sutured.

Confirmation of ovariectomy efficacy was determined by two-stage vaginal cytology, before and 20 days after ovariectomy. The state of menopause was characterized in the absence of epithelial cells (diestrus phase) (Vilela *et al*., 2007).

Experimental protocol of physical activity. All animals underwent a maximal treadmill exercise test until the animal was no longer able to run by increasing treadmill speed. From this maximum exercise test, was prescribed a 4-week training.

Physical training started 15 days after ovariectomy surgery. Trained groups were submitted to a protocol of physical training on the treadmill with speed and progressive load (1 hour per day/5 days per week at 50 to 60 % of the maximum speed of effort) for four weeks, as previously described. Animals were adapted on the treadmill during ten minutes for three days preceding the start of training.

Euthanasia and organ collection. At the end of the experiment, animals were sacrificed by decapitation. Then, the right kidney was removed, sectioned longitudinally and fixed in 10 % formalin solution in phosphate buffer for 48 hours. After this procedure, the pieces were dehydrated, kept in 70 % alcohol for 24 hours, then in 95 % alcohol for 2 hours and soon after, started the process of inclusion in paraffin. After inclusion, the blocks were used to make 6mm thick longitudinal histological sections through a conventional microtome. Ten non-consecutive sections per animal were made, slides were Hematoxylin and Eosin (HE) stained, mounted between slide and coverslip and examined under a light microscope (Zeiss).

Morphometric and stereological analysis. Morphometric analyzes were used to evaluate the following parameters: area (mm^2), renal corpuscle area (mm^2), and capsular space area (mm^2), and renal glomerular volume (V= π/6 x md∏) (µm∏).

Stereological analyzes were also performed by using a grid of 324 points to estimate the volume density of the light of distal and proximal contorted tubules. Results were given in percentage.

For the analyses, we captured 25 images per animal through a light photomicroscope (Zeiss) with a 20X objective. Images were transferred to image analysis programs (Axio Vision Software, Zeiss and Image J) located at the Laboratory of Morphoquantitative and Immunohistochemical Studies of the USJT.

Statistical Analysis. Results were presented as mean and standard error of the mean. The two-way ANOVA test and post-hoc Tukey's test were applied for data analysis. The significance level adopted in all tests was $p < 0.05$.

RESULTS

Biometric analysis. In the studied groups, there was no significant difference between the final and initial masses (FBM-IBM) (Table I). Ovariectomy promoted an increase in VAT (visceral adipose tissue) of 53 % in control groups (OSC), and of 16 % in dyslipidemic groups (OS LDL). Physical exercise promoted mean VAT reduction of 46 % in both groups (OTC and OT LDL) compared to their peers. In

Table I. Initial body mass (IBM), final body mass (FBM), difference between masses (FBM-IBM) and percentages of visceral adipose tissue and kidney in relation to final body mass (VAT% and Kidney%).

Parameters	SC	OSC	OTC	S LDL	OS LDL	OT LDL
IBM(g)	22.65±0.39	22.39±0.33	22.42±0.24	22.89±0.73	22.47±0.53	23.90±0.44
FBM(g)	24.04±0.37	23.76±0.19	23.20±0.46	23.16±0.73	24.88±1.87	25.05±0.47
FBM-IB(g)	1.39±0,20	1.37±0.4	0.88±0.52	0.27±0.13	1.84±0.71	1,14±0.23
VAT (%)	2.36±0,06	3.62±0.03*	1.93±0.06*	2.59±0,20*+	3.01±.0.03*#a	1.62±0.07*#ab
Kidney (%)	0.50±0.02	0.67±0.07*	0.55±0.01	0.48±0.01#	0.48±0.01#	0.49±0.01#

Mean±SEM. *p< 0.05 vs. SC; #p< 0.05 vs. OSC; +p< 0.05 vs. OTC; ap< 0.05 vs. S LDL; bp< 0.05 vs. OS LDL.

the control group (OSC), ovariectomy increased the KIDNEY% by 34 % compared to the CS and induced a tendency to its reduction in dyslipidemic animals (S LDL, OS LDL and OT LDL) (Table I).

Stereological analysis. Ovariectomy (OSC) did not alter the volume density of the light of proximal tubules (PT) and distal tubules (DT) compared to the control group (SC). Training (OTC) promoted an increase in light of PT compared to SC and OSC groups, and decrease in light of DT compared to the OSC group.

There was an influence of dyslipidemia on the volume density of light in PT and DT in dyslipidemic groups (S LDL, OS LDL and OT LDL), with a marked reduction of 43 % in PT and 70 % in DT compared to non-dyslipidemic control groups (SC, OSC and OTC). No significant difference was observed in the volume density of light of the proximal and distal tubules between dyslipidemic groups about ovariectomy (OS LDL) and physical exercise (OT LDL) (Table II).

Morphoquantitative analysis. The results of morphoquantitative analysis (Figs. 1 and 2) show the significant increase in the areas of a corpuscle and renal glomerulus promoted by ovariectomy (OSC). Physical exercise (OTC) reversed the process when compared to the SC group.

In dyslipidemic groups, was observed a tendency to decrease the corpuscle and renal glomerulus areas, compared to control groups, and there was no influence from ovariectomy and exercise (Figs. 2A and 2B).

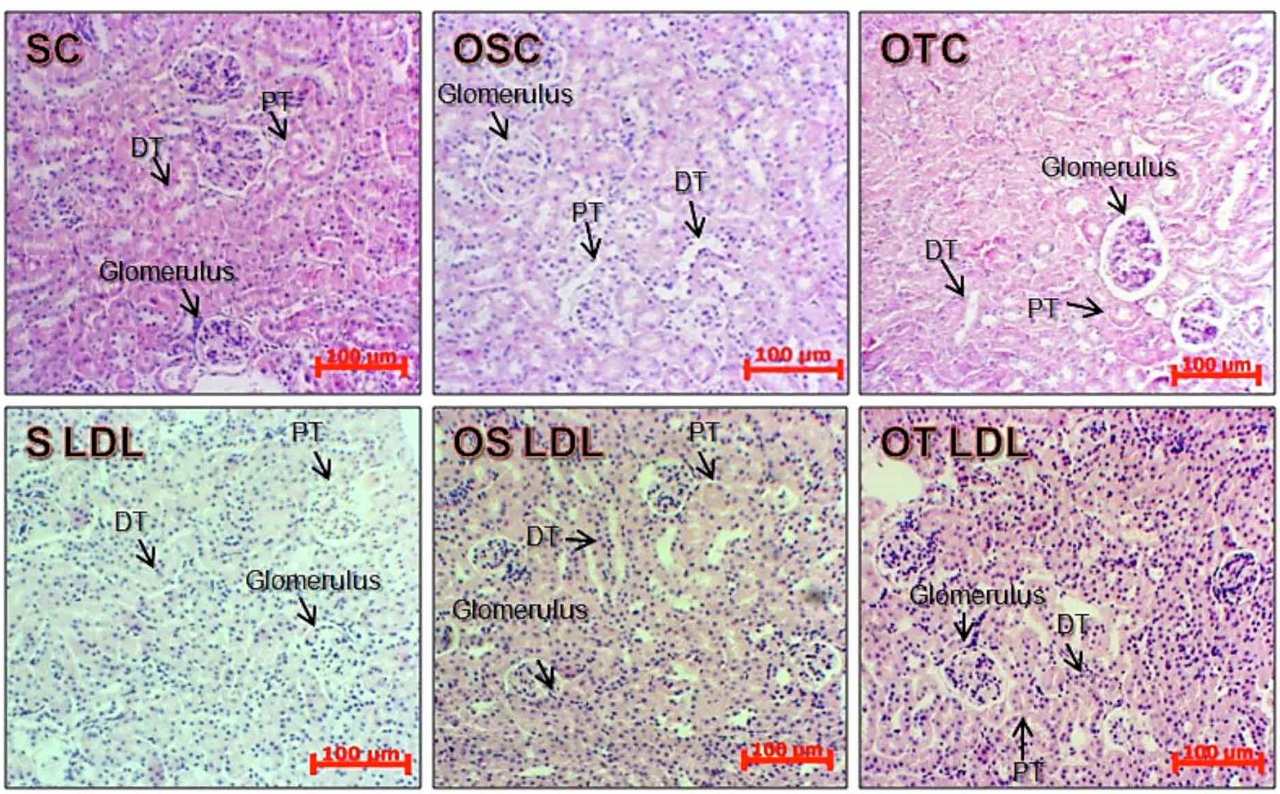

Fig. 1 - Photomicrographs of the general aspect of renal structures: proximal (PT) and distal (DT) tubules; glomerulus: evidenced by the HE technique. X200.Bar 100µm.

Regarding sub-capsular space, physical exercise promoted a decrease in this parameter in both groups (control and dyslipidemic), while dyslipidemia promoted the increase of sub-capsular space (Fig. 2C).

The frequency distribution histogram of the medium diameter of glomeruli showed that dyslipidemia (S LDL) promoted an increase in medium diameter (46-73 µm) and decrease in large diameter (74-105 mm). Ovariectomy (OS LDL) increased the small diameter (17 to 45 mm) and reduced the medium diameter. Physical exercise (OT LDL) did not reverse the process (Fig. 3).

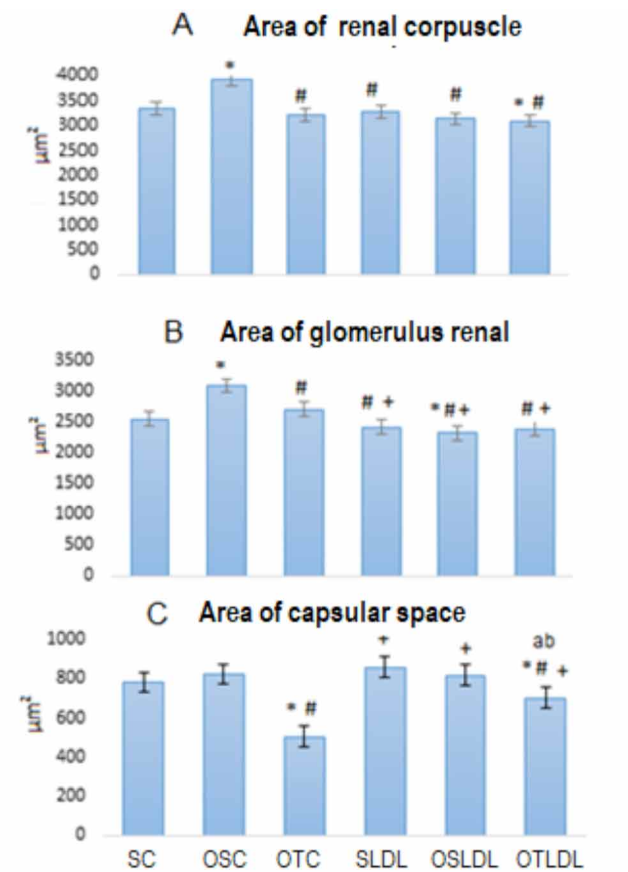

Fig. 2. Mean area of corpuscle (A), glomerulus (B) and capsular space (C) of the kidney in the studied animals. M±SEM. *p< 0.05 vs. SC; #p< 0.05 vs. OSC; +p< 0.05 vs. OTC; ap< 0.05 vs. S LDL; bp< 0.05 vs. OS LDL.

There was a significant decrease in renal glomerular volume of dyslipidemic animals when compared to the control (Fig. 4).

Regarding the number of glomeruli/area, in dyslipidemic animals, ovariectomy promoted an increase in the number of glomeruli, while training reversed the process (Fig. 5).

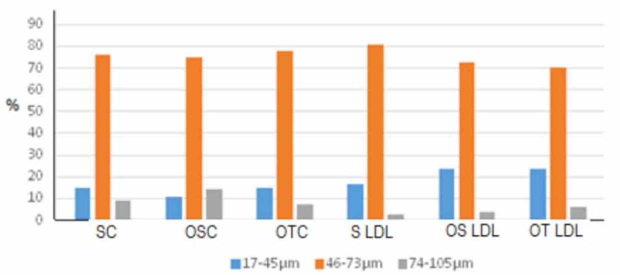

Fig. 3 - Histogram of frequency distribution of the mean diameter of renal glomeruli.

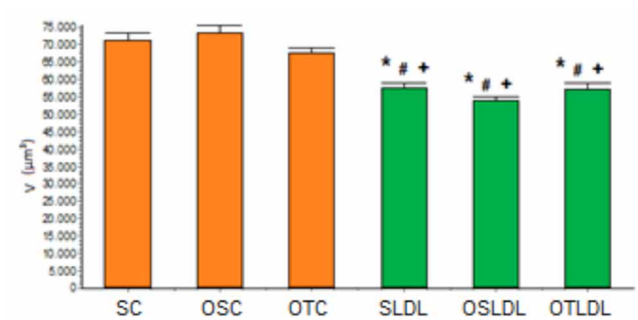

Fig. 4 - Renal glomerular volume (µm∏) in the studied animals. M±SEM *p< 0.05 vs. SC; #p< 0.05 vs. OSC; +p< 0.05 vs. OTC.

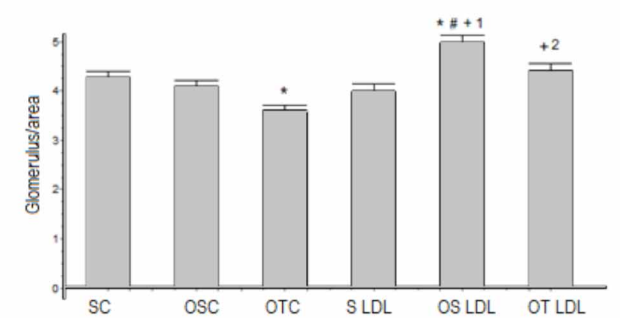

Fig. 5 - Number of glomeruli per area analyzed in the studied animals. M±SEM. *p< 0.05 vs. SC; #p< 0.05 vs. OSC; +p< 0.05 vs. OTC; 1p< 0.05 vs. S LDL; 2p< 0.05 vs. OS LDL.

Table II. Volume density of light of proximal (Vv [light PT]) and distal (Vv [light DT] tubules in the studied groups.

Parameters	SC	OSC	OTC	S LDL	OS LDL	OT LDL
Vv [Light PT] %	4.09 ± 0.37	4.13 ± 0.31	8.24 ±0.76$^{*\#}$	3.85 ± 0.35+	2.65± 0.31+	2.73 ± 0.28+
Vv [Light DT] %	7.0 ±2.71	8.66 ± 0.92	5.52 ±0.35#	2.8 ±0.22$^{*\#+}$	2.22 ± 0.18$^{*\#+}$	1.34 ± 0.27$^{*\#}$

Mean±SEM. *p<0.05 vs SC; #p<0.05 vs OSC; +p<0.05 vs OTC

DISCUSSION

The present study findings demonstrate the increase of visceral adipose tissue promoted by ovariectomy in control and dyslipidemic groups. Training supports a reduction of this parameter.

In fact, ovariectomy accelerates the gain of body mass, more specifically of adipose tissue (Vieira Potter *et al*., 2012). A study with ovariectomized rats indicated a reduction in UCP1 expression in brown adipose tissue and decrease in UCP2 expression in white adipose tissue, which may be associated with reduced energy expenditure and consequent increase in body mass (Pedersen *et al*., 2001). Also, estrogen has a central effect on food intake and energy expenditure, and their balance affects fat deposition, which explains the increase in visceral tissue observed in ovariectomized groups (OSC and OS LDL) (Cooke & Naaz, 2004).

Latour *et al*. (2001) demonstrated that regular treadmill exercise over an eight-week period did not decrease body weight gain or dietary intake of ovariectomized rats treated with estrogen or not. However, this does not mean that body composition has not been altered with training (Latour *et al*.). In the study by Melton *et al*. (2000), swimming training in ovariectomized rats during an eight-week period prevented mass body gain after ovariectomy despite the increased food intake. These results demonstrate the importance of physical exercise in individuals with estrogen deprivation.

Dyslipidemia and obesity have been identified as important causes of kidney diseases, including chronic kidney disease (CKD). Overweight and obesity result in hemodynamic, structural and histological changes in the kidneys (Chen, *et al*., 2017; Silva Junior *et al*., 2017). Fat excess also results in a significant decrease in renal weight that may be related to a progressive loss of nephrons due to benign nephrosclerosis. According to Kovesdy *et al*. (2017), in the development of CKD, many nephrons are lost due to physiological changes caused by hyperfiltration, which causes a reduction in the size of the kidney, and consequently, the remaining nephrons try to compensate for the body hemodynamics.

The proximal tubule is susceptible to a variety of metabolic and hemodynamic factors associated with dyslipidemia, obesity and diabetes (Thomas *et al*., 2005; Mount, *et al*., 2015). In our study, we evidenced the negative influence of dyslipidemia on the volume density of the proximal and distal tubules (Table 2), with a significant reduction in the areas of a corpuscle and renal glomerulus compared to ovariectomized control groups (OTC), and associated with a decrease in the diameter of large glomeruli.

Also, the ratio that measures kidney weight by the final body mass, kidney% (Table I), indicates that ovariectomy promotes kidney enlargement (OSC), and dyslipidemia (LDL knockout groups) induces its reduction. Our studies demonstrate that ovariectomy (OSC) results in increased renal corpuscle size and increased number of glomeruli, and training (OTC) reverses this process. The size of capsular space is also reduced with training.

The fat excess encapsulating in the kidney causes an increase in intra-renal hydrostatic pressure, slows the filtrate flow in tubules, and the blood flow in the vasa recta, increasing sodium reabsorption in the proximal convoluted tubule.

Also contributing to the process is intra-abdominal pressure coming from deposits of visceral fat and modifications in the extracellular matrix of the renal medulla, with deposition of fat, glycosaminoglycans and hyaluronate that lead to an increase in volume of interstitial fluid and greater compression on the loop of Henle and vasa recta (Hall, *et al*., 2010). Histological analysis of glomeruli indicates there is glomerulosclerosis, cell proliferation, basement membrane thickening, and other damage that may lead to loss of glomeruli, and possibly to vascular injury, leading to nephron ischemia, inflammatory infiltrates, tubular dilation and tubular atrophy (Pinhal *et al*., 2013; Amaral *et al*., 2014).

Little is known in the literature about the relationship between dyslipidemia and physical exercise in the renal structures of menopausal individuals. Our results suggest that physical training associated with dyslipidemia also presents a tendency to decrease the dimensions of renal structures.

By receiving 20 % of cardiac output, the kidney plays an important role in systemic circulation, and the decline in this percentage of blood flow occurs during physical exercise and affects alterations in the function of the organ, such as lower vasoconstriction of the renal vascular tree, neoglucogenesis and increased glucose absorption, which contribute to a better homeostasis in kidney (Master Sankar Raj *et al*., 2017). Studies in humans and animals recognize that renal vasoconstriction is less pronounced during intense exercise after a period of training. The mechanisms involved in these adaptations that arise in the first stages of exercise suggest the reduction of stimulation of SNS (sympathetic nervous system) and vasopressin are involved in less renal vasoconstriction (McAllister, 1998). By promoting adaptations in the stimulation and serum concentration of the hormones involved in renal vasoconstriction, planned and structured physical activity allows a more physiological hemodynamic response and better homeostasis (Silva Junior *et al*.).

Recent studies in exercise training in adults with CKD found an association between regular exercise and improved health outcomes in individuals with CKD (Aoike *et al*., 2015). In general, we observed that both dyslipidemia and physical exercise lead to a decrease in parameters of kidney%, volume density of the proximal and distal tubules, renal corpuscle area and renal glomerulus area, glomerular volume, and an increase in capsular space and number of glomeruli. We suggest that the improvement in metabolic and physiological activities promoted by physical exercise is responsible for these changes in volume and area reduction, and increase in the number of glomeruli, which act as the adaptive response of the renal tissue submitted to changes induced by dyslipidemia.

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RESUMEN: La reducción de la función ovárica y una disminución en los niveles de estrógeno, marcados por la menopausia, se relacionan con una mayor susceptibilidad a desarrollar dislipidemia. Estas alteraciones en el perfil lipídico pueden tener consecuencias en el tejido renal y generar lesiones que pueden progresar a insuficiencia renal. La práctica de la actividad física es un factor importante para el tratamiento y la prevención de la dislipidemia y sus consecuencias. El objetivo de este estudio fue observar los efectos del ejercicio físico en el riñón derecho de ratones hembras con LDL ovariectomizados. Los animales fueron sometidos a ejercicio físico moderado, se sacrificaron y se extrajo el riñón derecho para el análisis morfométrico y estereológico. Los resultados mostraron que la dislipidemia promovió una disminución en las áreas del corpúsculo y el glomérulo renal, en la densidad volumétrica de la luz en los túbulos contorneados proximales y distales, y un aumento en el espacio capsular, particularmente más marcado en los túbulos proximales. También observamos que el ejercicio físico disminuyó los parámetros analizados. Nuestros resultados sugieren que la asociación del entrenamiento físico y la dislipidemia presentan una tendencia a reducir las dimensiones de los parámetros morfométricos y estereológicos del riñón. Estos cambios pueden estar relacionados con la adaptación metabólica y fisiológica del tejido renal durante el ejercicio físico.

PALABRAS CLAVE: Riñón; Dislipidemia; Ejercicio físico; Menopausia.

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