

LIPOPROTEIN(A) AND BODY MASS IN MICE WHICH WERE SUBMITTED TO HYPERCHOLESTEROLEMIA AND STRENGTH AND AEROBIC PHYSICAL TRAININGS

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ABSTRACT

Introduction: The objective of the study was to verify the effects provoked by strength and aerobic exercises on the lipoproteic plasmatic concentrations of lipoprotein(a) [Lp(a)] and body mass in mice which were submitted to a hypercholesterolemic diet. **Materials and Methods**: 43 8-week-old male mice from Wistar family and weight between 230g and 250g were used in the sample. They were divided into six groups: strength training with hypercholesterolemic diet (FD, n=7); aerobic training with hypercholesterolemic diet (AD, n=6); strength training with conventional diet (FN, n=6); aerobic training with conventional diet (AN, n=9); hypercholesterolemic diet (CD, n=7); and conventional diet (CN, n=8). The strength and the aerobic exercises were accomplished during 12 weeks. At the end of the experimental period, the plasmatic concentrations of Lp(a) were dosed. **Results**: No differences have been verified in the plasmatic concentrations of Lp(a) with the different types of physical training. The prolonged treatments with hypercholesterolemic diet did not alter the levels of Lp(a). **Discussion**: The strength and aerobic trainings in animals with hypercholesterolemic and conventional diet did not provoke changes in the plasmatic concentrations of Lp(a).

KEYWORDS

Lipoprotein(a), Diet, Cholesterol, Exercise.

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LIPOPROTEÍNA(A) Y MASA CORPORAL DE RATONES SOMETIDOS A LA HIPERCOLESTEROLEMIA Y ENTRENAMIENTOS FÍSICOS DE FUERZA Y AERÓBICO

RESUMEN

Introducción: El objetivo del estudio fue a verificar los efectos provocados por los ejercicios de fuerza y aeróbico sobre las concentraciones plasmáticas lipoproteicas de la lipoproteína(a) [Lp(a)] y masa corporal, en ratones sometidos a una dieta hipercolesterolémica. **Materiales y Métodos**: Habían sido utilizados 43 ratones machos del linaje Wistar, con ocho semanas, pesando entre 230g y 250g, divididos en seis grupos: entrenamiento de fuerza con dieta hipercolesterolémica (FD, n=7); entrenamiento aeróbico con dieta hipercolesterolémica (AD, n=6); entrenamiento de fuerza con dieta convencional (FN, n=6); entrenamiento aeróbico con dieta convencional (AN, n=9); dieta hipercolesterolémica (CD, n=7); y dieta convencional (CN, n=8). Los ejercicios de fuerza y aeróbico habían sido realizados durante 12 semanas. Al final del periodo experimental fueron dosificadas las concentraciones plasmáticas de la LPa. **Resultados**: No fueron verificadas diferencias en las concentraciones plasmáticas de la LPa, mediante los diferentes tipos de entrenamiento físico. Los tratamientos prolongados con dieta hipercolesterolémica no alteraron los niveles de la Lp(a). **Discusión**: Los entrenamientos de fuerza y aeróbico en animales con dieta hipercolesterolémica y convencional, no proporcionaron modificaciones en las concentraciones plasmáticas de la Lp(a).

PALABRAS CLAVE

Lipoproteína(a), Dieta, Colesterol, Ejercicio.

LIPOPROTEÍNA(A) E MASSA CORPORAL DE RATOS SUBMETIDOS À HIPERCOLESTEROLEMIA E TREINAMENTOS FÍSICOS DE FORCA E AERÓBICO

RESUMO

Introdução: O objetivo do estudo foi verificar os efeitos provocados pelos exercícios de força e aeróbico sobre as concentrações plasmáticas lipoprotéicas da lipoproteína(a) [Lp(a)] e massa corporal, em ratos submetidos a uma dieta hipercolesterolêmica. **Materiais e Métodos**: Foram utilizados 43 ratos machos da linhagem Wistar, com oito semanas, pesando entre 230g e 250g, divididos em seis grupos: treinamento de força com dieta hipercolesterolêmica (FD, n=7); treinamento aeróbico com dieta hipercolesterolêmica (AD, n=6); treinamento de força com dieta convencional (FN, n=6); treinamento aeróbico com dieta convencional (AN, n=9); dieta hipercolesterolêmica (CD, n=7); e dieta convencional (CN, n=8). Os exercícios de força e aeróbico foram realizados durante 12 semanas. Ao final do período experimental foram dosadas as concentrações plasmáticas da Lp(a). **Resultados**: Não foram verificadas diferenças nas concentrações plasmáticas da Lp(a), perante os diferentes tipos de treinamento físico. Os tratamentos prolongados com dieta hipercolesterolêmica não alteraram os níveis da Lp(a). **Discussão**: Os treinamentos de força e aeróbico em animais com dieta hipercolesterolêmica e convencional, não proporcionaram modificações nas concentrações plasmáticas da Lp(a).

PALAVRAS-CHAVE

Lipoproteína(a), Dieta, Colesterol, Exercício.

INTRODUCTION

The Coronary Artery Disease (CAD) is resultant from risk factors, among which the abnormal levels of the lipoproteins, called dislipidemy, and the sedentary lifestile¹ are highlited.

The dislipidemies are defined as disturbances of the lipidic metabolism, with repercussions on the levels of the lipoproteins (LP) in the blood circulation, as well as the concentrations of its different components. The high concentration of low density lipoproteins (LDL) and lipoprotein(a) [Lp(a)], as well as the low plasmatic concentration of high density lipoproteins (HDL), have been con-

sidered as independent risk factors for the development of the atherosclerosis. Lp(a) is synthesized in the liver and its lipid composition is similar to LDL, differing because of the presence of an apoprotein(a). Its concentrations are genetically determined, ignoring its function and catabolism. It is known that concentrations of 30mg.dl⁻¹ are associated to the largest risk of developing DAC^{2,3,4}.

On the other hand, the battle against sedentarism through the practice of physical exercises is stimulated for the prevention and treatment of some risk factors, offering a protection against DAC⁵. The decrease of the fat percentage, associated to the diet and to the practice of physical

exercises, can contribute to favorable modifications of lipoprotein concentrations in the blood^{6,7,8}. Studies make the benefit of the aerobic exercise very clear in the reduction of the lipoproteic plasmatic concentrations^{9,10,11}.

According to Prado & Dantas¹², the relation between the alterations of HDL, LDL and the aerobic training seems to be very defined, especially when the diet and the loss of body mass are associated to the obtainment of a good lipidic profile. The benefit exists, as much for the low exercise as for the high one, and either in normolipidemic or dyslipidemic situations. However, the benefit does not seem to happen in the levels of Lp(a), in which most of the studies indicates that there are no alterations in this lipoprotein with aerobic exercises, even when a diet was associated^{13,14,15,16,17,18}. On the other hand, the existence of few and controversial researches¹² involving the strength training does not allow us to affirm (or, at least, to suggest), if beneficial alterations in the lipoproteic plasmatic concentrations, mainly when related to Lp(a) may exist or not.

Therefore, the real effects that a program of aerobic training and of force - not yet well shown in the in the scientific literature - can cause in the lipoproteic concentrations of Lp(a) and in the body mass, especially in normolipidemic and dyslipidemic dietary conditions. This way, the objective of that study was to verify the levels of Lp(a) and body mass of mice submitted to the hypercholesterolemic diet and to physical aerobic and strength training.

MATERIALS AND METHODS

Approval of the study

The study was approved by the Commission of Ethics in Research of the Tiradentes University, under the number. 031005. All the procedures were in agreement with the guidelines of the Manual of Principles in the Care and Use of Animals from the American College of Sports Medicine and it followed the Law no.6638 of May 8, 1979 and the Act no. 24645 of July 10th, 1934.

Animals

43 male mice of the Wistar lineage were used, originating from the central biotery of the Tiradentes University, with 8 weeks of age, weighing between 230g and 250g. All the animals were maintained in a room with constant temperature ($22\pm2^{\circ}$ C) and clear/dark cycle of 12 hours each.

Diets

Previous to the program of physical training, the animals were divided in two groups, defined by the diet form: normalipidemic animals, which only ingested conventional animal food balanced for rodents, of the NUVILAB® CR1

brand (Nuvital Nutrientes, LTDA Colombo/PR), containing, per weight, 19% of protein, 56% of carbohydrate, 3.5% of lipids, 4.5% of cellulose and 5% of vitamins and minerals, with 3.78 kcal.g⁻¹, and lasted in this way during the whole experiment; and dyslipidemic animals, which only received hypercholesterolemic diet, manufactured by RHOSTER, of the AIN-76 type, aiming at the dyslipidemic induction. The diets and the water were daily offered to the animals, in an amount that was enough to guarantee the *ad libitum* consumption during one month.

Groups and program of physical training

After a month of diet application, the program of physical training was initiated in the normalipidemic and dyslipidemic animals, that continued with their respective diets, being subdivided in 3 subgroups each, defined by the type of executed physical exercise, with a total of 6 groups, described like this: only conventional diet, without physical training (CN, n=8); only hypercholesterolemic diet, without physical training (CD, n=7); aerobic training with conventional diet (AN, n=9); aerobic training with hypercholesterolemic diet(AD, n=6); strength training with conventional diet (SN, n=6); and strength training with hypercholesterolemic diet (SD, n=7). The numbers presented in the sample were different due to the death of some animals during the training.

Types of physical aerobic and of force exercises

Strength Exercise

The program of strength physical exercises was accomplished for 12 weeks, after familiarization, and starting from the established loads in the 1RM test. Three series of 10 repetitions in the crouch apparel, according to the model of Tamaki et al. 19, were accomplished three times a week, with the intensity defined in 75% of the maximum load established in the 1RM test. The load and the intensity were readapted periodically regarding the prescription, due to the improvement of the animals' strength levels, starting from the new maximum loads established by the 1RM test which were weekly accomplished.

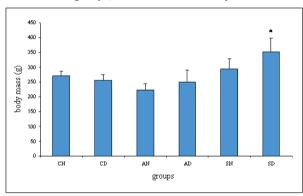
The animals were stimulated to execute the series, through autoadhesives electrodes of ValuTrode brand, model CF3200, size 3.2cm, which were put on the tail and linked to an electroestimulator Quarker, model Dualpex 961, year of production 2000, gauged by INMETRO. The used parameters were: frequency of 1Hz; duration of 1 ms; active cycle 2:4s; and the current intensity, adjusted in such a way that the animal executed the movement, varying from 4mA to 15mA. Those parameters were adopted due

to the bi-directional pulses of null mean, not presenting electrolytic effects and allowing long duration application without lesion risk for the tissues. The short pulse duration is able to promote an effective and comfortable stimulation in the animals.

Aerobic Exercise

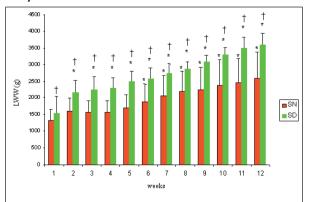
The program of aerobic exercises was started after the familiarization with the technique, that consisted in running in an ergometric treadmill, AVS brand, with capacity for eight animals, variable speed from 0 to 30m. min⁻¹, inclination from 0% to 10%, source of electric stimulation with variations of 0.2, 0.4, 0.6, 0.8, 1.0 and 1.2mA of current intensity, with four independent exits and command in the own display of the treadmill. This program was accomplished three times a week, also for 12 weeks. The time and the speed varied from 10min to 45min and from 25m.min⁻¹ to 30m.min⁻¹, respectively.

Figure 1 - Mean and standard deviation of the body mass values of the groups, at the end of the study



^{*} p < 0.05 when compared to the CN group

Figure 2 - Mean and standard deviation of the LWW values of the groups SN and SD, during the 12 weeks of study



^{*} significant in relation to the first week

The body mass control was weekly accomplished, before the realization of the physical trainings, in all groups. With this objective, a precision scale of the brand Marte, model AS2000c, gauged by INMETRO and year of production 1999 was used.

Collection, preparation of the blood and Lp(a) dosage

After the last session of physical exercises, the habitual diet of the groups was maintained and, in the following day, after 12h to 14h of fasting, the blood was collected. With this objective, the animals were anesthetized with thiopental with a concentration of 40mg.kg⁻¹, through intraperitoneal via, removing 2ml of blood for heart puncture, in disposable heparinized syringe. The blood was, soon after, centrifuged to 2500 rotations per minute during 10min, and the obtained serum was stored in a freezer to -20°C, for subsequent determination of Lp(a), that was accomplished by turbidimetry, using a LABTEST dosage kit.

Statistical analysis

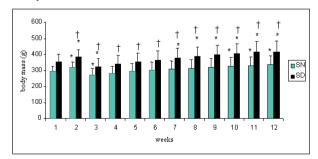
The values were expressed as mean and standard deviation. Variance analysis was accomplished (ANOVA) for two repeated via, verifying the differences between the means of the groups, combined with the *post-hoc* test of Newman-Keuls for multiple comparisons, adopting a 5% significance level.

RESULTS

Body Mass

The results of the *body mass* variable were obtained from the comparison of all groups (CN, CD, AN, AD, SN and SD) in the beginning (1st week) and at the end of the study (12th week). The obtained results indicate that the group SD was the only one to present a body mass

Figure 3 - Mean and standard deviation of body mass values of the groups SN and SD, during the 12 weeks of study

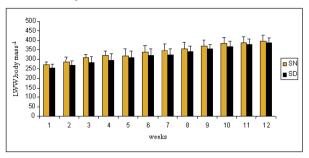


^{*} significant in relation to the first week

† significant in the comparison between SN and SD during the weeks

[†] significant in the comparison between SN and SD during the weeks

Figure 4 - Mean and standard deviation of the relation LWW.body mass⁻¹ of the groups SN and SD during the 12 weeks of study



which was larger than the SN's at the end of the study (Figure 1).

Load of weekly work (LWW)

It was verified that, both groups SN and SD, presented a significant increase of LWW along the 12 weeks of the program, in comparison with the first week. However, SD presented this increase earlier, starting from the 2^{nd} week of training, while SN only started from the 6^{th} week (Figure 2).

There was no difference in the comparison of LWW between SN and SD in the first week, that is to say, in the beginning of the training program both groups, that only differed for the diet variable, had the same muscular strength. However, this same comparison, realized from the 2nd week of training on, showed a difference between the groups, where SD presented larger strength, during the 12 weeks. Maybe, the explanation for those results was related to the body mass gains superior to SD in relation to SN, promoted by the hypercholesterolemic diet (Figure 3). However, when the LWW of each animal was calculated in relation to the body mass (LWW.body mass⁻¹), the results indicated that, when corrected in relation to the body mass, the gains of LWW presented by both groups were equivalent (Figure 4).

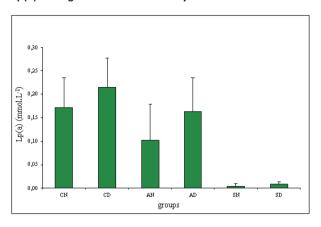
Blood values of Lp(a)

The found results did not demonstrate representatives changes of Lp(a) in this study, in other words, no type of physical training, not even the hypercholesterolemic diet, contributed to change the levels of the lipoprotein (Figure 5).

DISCUSSION

The accomplishment of physical exercises has been the object of study in numerous researches and the less onerous therapeutics is considered in the treatment of several diseases. However, the best form of exercising, until then, is not clear. In this study the two more common ways of physical exercises among the population, the aerobic

Figure 5 - Mean and standard deviation of the variable Lp(a) during the 12 weeks of study



and strength exercise, which were already used in previous studies, in animals and in human beings^{20,21,22,23,24,25,26}.

A diet rich in fat and the sedentarism are considered the environmental factors that contribute the most for the dislipidemy and for fat mass gains. Lipoproteic elevated levels in the blood are related to larger incidence of cardiovascular diseases, especially coronary diseases ^{27,28}.

In the study accomplished by Pellizzon *et al.*²⁹, reductions of body mass were observed in mice submitted to physical exercises. Their results are not in accordance with the present study, when they affirm that the mice with a diet rich in fat, accomplishing aerobic training, during six weeks, 2h.day⁻¹ and 5 days.week⁻¹, weighed significantly more than the ones of the control group in the beginning of the study and, at the end of the study, the total body mass was reduced.

In this study, it was observed that there were no differences between the groups fed with different diets and submitted to different exercises, except for the group SD, which presented larger body mass during the whole experiment. Maybe an explanation for this was the fact that there was an association of factors, such as the hypercholesterolemic diet (what favored the weight gains), the changes found in their body mass - mainly due to their thin mass (muscles), obtained either by the natural growth of the animals or by the physiologic and biochemical current muscular adaptations of the training program. According to Tamaki et al. 19, there is a direct relation between muscle weight and total body weight. These authors, in their investigations, submitted mice to strength training and they found superior values of body mass for the strength training in relation to the running training in the treadmill for mice, being the muscle hypertrophy the responsible for the increase of the total body weight. In this study, a significantly larger difference in the body weight in SD was found. In agreement with the same author, it is extremely difficult to produce muscle hypertrophy in laboratory animals, especially in mice. This limitation is especially significant when we consider that the mouse is, today, the principal animal for experimental research. Although the running in treadmill has been used to produce muscle hypertrophy, this exercise modality consists mainly in endurance training, and it is not considered to be an ideal type of strength training, so that it becomes difficult to compare this model to those found in human beings. This way, the magnitude of a strength training program which is able to promote some of those alterations in mice, is still broadly unknown.

In this study, it was also observed that the groups treated with diet and submitted to strength exercise presented, besides larger body weight, larger values of LWW. However, when the reason LWW.body mass⁻¹ was analyzed, the values were similar.

These results are partially in accordance with those found by Yaspelkis et al.³⁰, that investigated, in their studies, the improvement of the musculoskeletal glucoses metabolism in mice after application of strength exercises. They demonstrated, among the variables studied, that there was an increase in the animals' body mass, as well as an increase of the muscular mass, mainly of the plantar muscles. However, such data are contrary to the one found by Hornberger & Farrar³¹ which, when studying the hypertrophy of the Hallux's long flexor muscle, in eight weeks of progressive strength training in mice, it was verified that the body mass did not suffer changes during the initial and final period of the training.

In that way, it is evident that the protocol of strength exercise in question, accomplished for 12 weeks, with weekly frequency of three sessions, in male mice, in a squat machine, was able to promote an increase in the muscular force, being this an effective program of training. In the study of Tamaki et al.¹⁹ it was observed that the animals in the squat machine developed maximum force twice larger than the sedentary groups, corroborating the results of the work. Contrarily, in this study, the groups that were submitted to the aerobic training did not present significant difference regarding the body mass.

Chen et al.³² investigated the lipid metabolism in hypercholesterolemic mice, with different amounts of soy protein fraction. The animals were fed during four weeks with a cholesterol-free diet, diets with 2%, 5% and 10% of cholesterol and soy protein fractions. In their results they found that, despite the fact that the animals were fed with different cholesterol fractions, they obtained gains of progressive weight, but they were not significant.

In a previous study, Kibenge & Chan³³ demonstrated that the mice fed with a diet rich in fat and submitted to aerobic swimming exercise, presented a body mass significantly smaller than the sedentary control mice. Those results do not corroborate our study, because there was no difference of body mass among the ani-

mals fed with hypercholesterolemic diet, submitted to the exercises treatment and its control. Those discoveries are in accordance with those found by Steinberg et al.³⁴, in which, when comparing the mice trained in treadmill and fed with the diet rich in fat, sedentary and fed with diet rich in fat mice, mice trained with normal diet and sedentary mice with normal diet, for four weeks, they verified that the body mass did not suffer significant difference among the groups at the end of the study. In the current study it was only found a difference between the group AD and control mice, in the beginning of the study; during the time of training that difference was eliminated.

According to Hoyos et al.³⁵, in his study, No significant difference of body mass among mice fed with hypercholesterolemic and normolipidemic diet was found at the end of four months of study. The same is applied to the study of Hornberger & Farrar³¹, in which their investigations verified that the mice submitted to strength exercise and controlled diet did not show significant difference of body mass, between the beginning and the end of the training period.

On the other hand, Kin Isler et al.³⁶, in their investigations, affirm that a program of physical aerobic exercises, applied with intensity control, duration and frequency, are able to promote reduction in the body mass, as well as in the plasmatic levels of lipoproteins. Äguila et al.³⁷ also report in his study that mice are animals resistant to the hyperlipidemy and that, when evaluating the lipidic metabolism in mice with different diets, no differences were found.

It is important to point out that the dyslipidemic mice maintained the diet rich in fat during the treatment with exercise, which, comparing with the practice, would constitute therapeutic method with medicine not associated to the change of the alimentary habit. It can be speculated, based on in this fact, that the chronic treatment with exercise, allied to a balanced diet, could cause the decrease in the lipoprotein concentrations.

Although no causal relation has been established between these parameters, authors also suggested that the association between blood lipids and heart frequency can be under the influence of the autonomic nervous system, because the increase of the sympathetic activity, that elevates the heart frequency, also exercises enormous influence in the lipid blood metabolism. In this way, the catecholamines deactivate the lipoprotein lipase, in a way in which the reduction of its activity can proportion the reduction of the removal tax of blood fat, elevating its plasmatic concentration ^{38,39}. This may be a justification for the found results, taking into account that the exercise is a stressful activity for the mice, altering their heart frequency and the metabolic regulation.

The results of the current study, found in Lp(a), were in accordance with most of the literature, but we must, however, take into consideration that no works relating physical exercise and plasmatic concentrations of Lp(a) in mice were found, being the results, therefore, compared with studies obtained with human beings 13,14,15,16,17,18,40,41,42,43,44.

Elevated concentrations of Lp(a) have been considered as independent risk factors for the development of the atherosclerosis 45,46. After a session of physical aerobic exercise (acute effect), those modifications were not verified. Hubinger et al.40 investigated the acute effect of the aerobic exercise in Lp(a), in two groups. One of them, with young men, running 60min in an intensity of 90% of FC_{max} in rolling treadmill, and the other, composed of 6 individuals (3 young women and 3 men of the first group), running during 40min, in an intensity of 75% to 80% of FC_{max} . The researchers didn't verify significant changes in the Lp(a) levels after the aerobic exercise session. That same work concludes, through this study and previous accomplished ones, that neither the aerobic acute exercise nor the chronic change, beneficially, the Lp(a) levels. In this study, no changes were verified in the Lp(a) levels, but there is a clear tendency of improvement in the groups SN and SD. It seems that the diet was the main factor for the elevation of Lp(a) in the serum. This fact reinforces the conclusions of Mackinnon & Hubinger⁴⁷, which states that the relation between the physical exercise and Lp(a) is not clear yet.

A protocol of strength exercise in the squat machine, accomplished for 12 weeks, with frequency of three times a week, in male mice, was able to promote an increase in LWW and, consequently, in the muscular strength and in the body mass, not being, however, able to promote lipoproteic reductions. The aerobic exercise was also not able to promote beneficial changes in the lipoproteic profile.

There were no significant changes in the Lp(a) levels of the male mice which were treated or not with hyperlipidemic diet, submitted to physical aerobic and of strength exercise. In this way, the obtained results suggest that the aerobic and strength exercises in the levels of this lipoprotein are still unknown. This way, new studies should be accomplished so that they can explain such aspects better.

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