12 - EFFECT OF PHYSICAL EXERCISE ON HDL/LDL RATIO, PROTEIN METABOLISM AND BONES IN RATS SUBMITTED TO HYPERCHOLESTEROLEMIC DIET

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INTRODUCTION

The present work is a continuation of a former one entitled *Effect of Physical Exercise on Lipid Metabolism in Rats Submitted to Hypercholesterolemic Diet*[1], that discussed the effect of physical training on the lipid metabolism as well as muscle and oxidative stress in animals submitted to a hypercholesterolemic diet. We now complete the evaluation of the effect of the exercise protocol on lipid metabolism by verifying the effect of training on the HDL/LDL ratio. More information is supplied in order to improve the understanding about the effect of exercise on other parameters, besides those already presented.

The characterization of the effect of different protocols of hypercholesterolemia on various parameters, amongst which is protein metabolism, has been constantly present in this type of study in order to verify whether these protocols affect the development of the animals[3]. Another relevant factor is the fact that various studies have demonstrated that high lipid ingestion may damage the renal function by fluid concentration and cystic formation, as evidenced by increase in organ size in cases of injury and some of these works focus on the beneficial effects of training in relation to renal stress produced by the hypercholesterolemic diet.

Of equal importance in studies related to physical training using experimental animals, the comprehension of the effect of different protocols of exercise on their organism facilitates the recognition of the effect of training on those parameters that one decide to investigate, beside guiding the choice of researchers about what protocol to use in future experiments.

In the first work it was verified the magnitude of the stress imposed by training on the locomotion active system as well as its adaptative response, when assessing the weight of soleo and *extensor digitorius longus* muscles. It is well accepted that exercise can contribute positively to the maintenance of bone mass and strength due to the tension imposed to bone system by physical activity. Nevertheless the mechanisms by which exercise acts are not well understood and various types of protocols (swimming, running and voluntary exercise in wheels) are utilized in order to try to clarify them. The present study aims at observing the occurrence of exercise-born effects on the HDL/LDL ratio, protein metabolism renal stress parameters and its relation with the hypercholesterolemic diet allied to physical training, as well as evaluating the adaptation of the passive locomotion passive apparatus to the exercise protocol.

METHODS

Animals and diets

Fourty eight adult female Fisher rats were divided into four homogeneous groups according to initial body weight and to the applied treatment: Exercise with hypercholesterolemic diet (EH), exercise with control diet (EC), sedentary with hypercholesterolemic diet (SH) and sedentary with control diet (SC). Each group had 12 animals. These received control diet AIN-93 or hypercholesterolemic diet AIN-93M with fat content modified from 4 to 25% and cholesterol added equivalent to 1% of the diet reducing corn starch content from 72.25% to 50.25% in order to induce hypercholesterolemia. The composition of the diets is indicated in table 1. Animals were given filtered water and food *ad libitum* and were kept in individual cages with light/dark cicle of 12 hours.

position	Control	Hypercholesterolemic
ne	2.5	2.5
mixture[1]	35	35
min mixture[2]	10	10
	50	50
in	140	140
ean oil	40	250
esterol	0	10
Starch	722.5	502.5

[1]Salt mixture (g/kg of mixture): NaCl - 139.3 / KI - 0.79 / MgSO4.7H2O - 57.3 / CaCO3 - 381.4 / MnSO4.H2O - 4.01 / FeSO4.7H2O - 27.0 / ZnSO4.7H2O - 0.548 / CuSO4.5H2O - 0.477 / CoCl2.6H2O - 0.023 / KH2PO4 - 389.0. [2]Vitamin mixture (g/Kg de mistura): Retinol acetate - 2.000.000IU / Cholecalciferol - 200.000IU / p-aminobenzoic acid - 10.00 / I-Inositol - 10,00 / Niacin - 4.00 / Calcium Pantotenate - 4.00 / Riboflavine - 0.80/ Tiamine HCL - 0.50 / Piridoxine HCL - 0.50 / Folic acid - 0.20 / Biotin - 0.04 / Vitamin B12 - 0.003 / Sucrose - q.s.p. 1000. / Choline - 200.0 / -Tocopherol - 10.000IU. Cholesterol Isofar.

Training

Animals that were exercised were adapted to the water medium (water at 31ºC ± 1ºC) in the following way: First and second days, 30 min in a shallow pool; third and fourth days two series of 15 min with 5 min interval in a pool 50 cm deep and in the fifth day they swung 30 min strait in the same depth as in the former day. From the second to the tenth week the exercised animals repeated the session of the fifth adaptation, five days/week. Sedentary animals were submitted to contact with water during 30 min in shallow pool during the hole experiment in order to go through the same stress of handing.

Biochemical evaluation

After nine weeks of training animals were sacrificed 48 hours after the last exercise session and diets were withdrawn 12 hours before sacrifice. Animals were anesthesized with ether and cut in the abdominal region up to the torax in order to expose the right braquial artery which was cuted for blood collection. Blood was collected in eppendorf tubes and immediately centrifuged for serum or plasma separation. Biochemical dosages were carried out according to manufactor's instructions (Labtest Diagnóstica). The kidneys, spleen and the femur bone were also localized, removed and weighted.

Statistical analysis

Comparison between groups was made by two-way ANOVA at p<0.05.

RESULTS

Effect of training on the HDL/LDL ratio:

Training interfered in the HDL/LDL ratio. The animals in the trained groups showed better ratios than sedentary ones. There was a diet effect, with animals receiving the hyper diet having lower values). An interaction between the two treatments was also observed as trained rats in the control diet showed better results than the others (table 2).

Table 2. Values (mean and standard deviation) of HDL/LDL reason and comparison of means in relation to exercise, diet and interaction (p<0,05).

Parâmetro	Razão HDL/LDL
EC	3.116 ± 1.338[a#]
EH	0.058 ± 0.080[c]
SC	1.984 ± 0.870[b]
SH	0.025 ± 0.020[c]
Valor de p	
Exercício	p<0.05
Dieta	p<0.05
Interação	p<0.05

Different letters indicate significant difference p<0.05. NS = non significant.

Effect of training treatment on the protein profile:

In order to assess the effect of moderate endurance training on protein metabolism animals of the four groups were compared at the end of the experiment regarding total protein, albumin and urea concentrations. Total protein values were higher in exercised rats than in sedentary ones. An effect of diet was also observed with exercised animals of the hyper group having higher values as compared to controls. Interaction between the two treatments was also observed; exercised control rats having higher total protein values than sedentary receiving the same diet. Significant results were not observed relating training and albumin and urea concentrations. Albumin concentration of hyper rats was lower than that of the controls but an inverse pattern was shown when urea was measured (table 3)

Table 3. Values (mean and standard deviation) of total proteins, albumin and urea and comparison of means in relation to exercise, diet and interaction (p<0.05).

Parâmetros	Proteínas Totais	Albumina	Uréia
EC	45.45 ± 2.44[b#]	487.89 ± 57.8	4,19 ± 0.79
EH	50.16 ± 4.23[a]	445.52 ± 36.82	6,82 ± 3.94
SC	41.46 ± 3.09[c]	491.54 ± 74.47	4,21 ± 0.84
SH	49.83 ± 2.23[a]	446.28 ± 43.55	6,2 ± 0.95
Valor de p			
Exercício	p<0.05	NS	NS
Dieta	p<0.05	p<0.05	p<0.05
Interação	p<0.05	NS	NS

Different letters indicate significant difference p<0.05. NS = non significant.

Evaluation of renal stress and spleen weight:

In relation to kidneys weight an effect of training was observed: trained animals had heavier organs than sedentary ones. Creatinin values were not significantly different between trained or non-trained animals. On the other hand diet had an effect, with hyper animals showing higher values than control ones. An interaction between the two treatments was also observed: trained/hyper animals had lower creatinin concentration than sedentary receiving the same diet. It was even lower than that of trained/control rats. Spleen weight was not affected significantly by training but only by diet, with hypercholesterolemic animals showing heavier spleens than the control ones (table 4).

Table 4. Values (mean and standard deviation) of kidney weight, creatinine and spleen weight and comparison of means in relation to exercise, diet and interaction (p<0,05).

Parâmetros	Peso do Rim	Creatinina	Peso do Baço
EC	0.58 ± 0.08	32.1 ± 19.59[a]	0.42 ± 0.04
EH	0.61 ± 0.04	26.14± 8.48[a, b]	0.49 ± 0.06
SC	0.65 ± 0.06	15.07 ± 7.07[b]	0.43 ± 0.05
SH	0.62 ± 0.04	36.92± 16.44[a]	0.47 ± 0.03
Valor de p			
Exercício	p<0.05	NS	NS
Dieta	NS	p<0.05	p<0.05
Interação	NS	p<0.05	NS

Different letters indicate significant difference p<0.05. NS = non significant.

Bone evaluation (femur):

The effect of training on the bones was assessed by measuring femur weight, size, length and density. No significant differences were observed.

Table 5. Values (mean and standard deviation) of femur weight, length, volume and density and comparison of means in relation to exercise, diet and interaction (p<0,05).

Parâmetros (Fêmures)	Peso	Comprimento	Volume	Densidade
EC	0.5740 ± 0.055	33.96 ± 0.65	0.47 ± 0.09	1.26 ± 0.22
EH	0.6011 ± 0.069	33.86 ± 1.04	0.45 ± 0.07	1.36 ± 0.23
SC	0.6003 ± 0.057	33.4 ± 0.67	0.44 ± 0.05	1.37 ± 0.14
SH	0.5738 ± 0.073	33.83 ± 0.61	0.49 ± 0.09	1.21 ± 0.21
Valor de p				
Exercício	NS	NS	NS	NS
Dieta	NS	NS	NS	NS
Interação	NS	NS	NS	NS

NS = non significant.

DISCUSSION

An improvement in the HDL/LDL ratio was seen in trained animals as compared to sedentary ones, what confirms the tendency sown in our previous work[1] of increase in HDL and decrease in LDL. The improvement was even more effective in the trained/control animals. We believe that a significant difference can be found as we change the exercise protocol, altering the volume and/or the intensity of the exercise, making it more tiresome.

The results also show that exercise provokes an increase in total protein concentration, although this effect was more evidenced in the animals that received the hyper diet. Creatinin concentration were not influenced directly by training; nevertheless an interaction between the two treatments (diet and exercise), with animals of the EH group having lower values as compared to SH ones. Increased creatinin concentration may indicate renal damage[6] and exercise seems to have a protective effect against the renal stress produced by the hypercolesterolemic diet. This argument is reinforced by the fact that trained animals had lighter kidneys than sedentary ones.

No interference of the treatments was found in the femur bone of the experimental animals. The swimming protocol does not impose intense overcharge in the bones due to minimization of the gravity force by water[4] and that was not enough to produce alterations in the bones of the studied animals.

CONCLUSION

The exercise protocol improved the ratio HDL/LDL especially in the control animals. Training increased total protein concentration and reduced that of creatinin in the exercised animals receiving the hypercholesterolemic diet. The swimming protocol did not provoke alterations in the structure of the femur bone and we suggest that new protocols be performed altering volume and/or intensity of training.

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EFFECT OF PHYSICAL EXERCISE ON HDL/LDL RATIO, PROTEIN METABOLISM AND BONES IN RATS SUBMITTED TO HYPERCHOLESTEROLEMIC DIET

ABSTRACT

The correct characterization of hypercholesterolemic models involves the knowledge of the effect of diet on parameters the go beyond lipid metabolism and that nevertheless reflect the effect of the hypercholesterolemic diet on the development of the animals. Equally important, the exact notion of the magnitude of the stress provoked by physical training helps the understanding of its effect on the objects of investigation and guide the choice of the better protocol to be utilized in different experiments. In the present study 48 female adult Fisher rats were distributed in 4 groups: Exercise (swimming 30 min/day, 5 days/week) with hypercholesterolemic diet, exercise with control diet, sedentary with hypercholesterolemic diet and sedentary with control diet. After 9 weeks animals were sacrificed. Results show that exercise was efficient in improving the ratio HDL/LDL and there was an effect on total protein values and kidney weight of the animals. There was no alteration in the structure of the femur bone induced by training. The results indicate that low intensity trainning is capable of altering positively the protein profile and suggest that new studies be performed with different volume and intensity of exercise.

KEY WORDS: Physical exercise, hypercholesterolemia, metabolism

L'EFFET DE L'EXERCICE PHYSIQUE SUR LE RAPPORT HDL/LDL, LE METABOLISME PROTEIQUE ET L'OS DES SOURIS SOUMISES A DIETE HYPERCHOLESTEROLEMIQUE.

RÉSUMÉ

La correcte caractérisation des modèles d'hypercholestérolémie inclut la connaissance de l'effet de la diète sur les paramètres qui vont au-delà du métabolisme lipidique et qui, cependant, reflètent l'effet de la diète hypercholestérolémique sur le développement des animaux. De même, l'exacte notion de la magnitude du stress provoqué par l'entraînement aide la compréhension de son effet sur les objets d'investigation, et orientent le choix du meilleur protocole à d'être utilisé en différentes expérimentations. Dans cette recherche, 48 souris Fischer adultes ont été distribuées en quatre groupes : exercice avec diète hypercholestérolémique (EH), exercice avec diète contrôle (EC) (natation 30 min/jour, 5 jour/semaine), sédentaire avec diète hypercholestérolémique (SH) et sédentaire avec diète contrôle (SC). Après 9 semaines, les animaux ont été sacrifiés. Les résultats montrent que l'exercice a été efficace, en améliorant le rapport HDL/LDL. Il a été constaté l'effet de l'entraînement sur les valeurs totales de protéines et dans le poids des reins des animaux étudiés. Ils n'existent pas des altérations dans les structures de l'os du fémur induites par l'entraînement. Les résultats indiquent que l'entraînement de baisse intensité est capable d'altérer le profil protéique positivement. En plus ces résultats suggèrent la nécessité d'autres études avec « différents volumes et intensités et entraînement ».

MOTS-CLÉS : Exercice physique, hypercholestérolémie, métabolisme.

EFECTO DEL EJERCÍCIO FÍSICO SOBRE LA RELACIÓN HDL/LDL, METABOLISMO PROTEICO Y HUESOS DE RATAS SUBMETIDAS A DIETA HIPERCOLESTEROLÉMICA.

RESUMEN

La caracterización correcta de la hipercolesterolemia envolucra el conocimiento del efecto de la dieta sobre parâmetros que van mas allá del metabolismo de lípidos y que sin embargo reflete el efecto de la dieta hipercolesterolémica sobre el desarollo de los animales. Igualmente importante, la exata noción de la magnitud del stress provocado por el entrenamiento ayuda el endendimiento de su efecto sobre los objectos de investigación y nortean la escoja del mejor protocol a ser utilizado en diferentes experimentos. En el presente trabajo 48 ratas Fisher hembras adultas fueron distribuidas en cuatro grupos: Ejercício (natación 30min/dia, 5 dias/semana) con dieta hiprcolesterolémica (EH), Ejercicio con dieta control (EC), sedentario con dieta hipercolesterolémica y sedentario con dieta control (SC). Después de 9 semanas los animales fueron sacrificados. Los resultados muestran que el ejercicio fué eficaz en amejorar la relación HDL/LDL. Hubo efecto del entrenamiento sobre los valores de proteinas totales y en el peso de los riñones de los animales estudiados. No hubo alteraciones en la estructura del femur inducidas por el entrenamiento. Los resultados indican que el entrenamiento de baja intensidad es capable de alterar el perfil protéico positivamente y sugeren que sean hechos nuevos trabajos con diferentes volumens y intensidades de entrenamiento.

PALABRAS-LLAVE: Ejercício físico, hipercolesterolemia, metabolismo.

EFEITO DO EXERCÍCIO FÍSICO SOBRE A RELAÇÃO HDL/LDL, METABOLISMO PROTEICO E OSSOS DE RATAS SUBMETIDAS À DIETA HIPERCOLESTEROLÊMICA.

RESUMO

A correta caracterização dos modelos de hipercolesterolemia envolve o conhecimento a respeito do efeito da dieta sobre parâmetros que vão além do metabolismo lipídico e que, entretanto, refletem o efeito da dieta hipercolesterolêmica sobre o desenvolvimento dos animais. De igual importância, a exata noção da magnitude do estresse provocado pelo treinamento auxilia o entendimento do seu efeito sobre os objetos de investigação, e norteiam a escolha do melhor protocolo a ser utilizado em diferentes experimentos. Neste estudo, 48 ratas Fisher adultas foram distribuídas em quatro grupos: Exercício com dieta hipercolesterolêmica (EH), exercício com dieta controle (EC) (natação 30 min/dia, 5 dias/semana), sedentário com dieta hipercolesterolêmica (SH) e sedentário com dieta controle (SC). Após 9 semanas os animais foram sacrificados. Os resultados mostram que o exercício foi eficaz em melhorar a relação HDL/LDL. Houve efeito do treinamento sobre os valores totais de proteínas e no peso dos rins dos animais estudados. Não houveram alterações na estrutura do osso fêmur induzidas pelo treinamento. Os resultados indicam que o treinamento de baixa intensidade é capaz e alterar o perfil protéico positivamente, e sugerem que sejam feitos novos trabalhos com diferentes volumes e intensidades e treinamento.

PALAVRAS-CHAVE: Exercício físico, hipercolesterolemia, metabolismo.