SOCIEDADE BRASILEIRA DE ORTOPEDIA E TRAUMATOLOGIA

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Original Article

Effects of simvastatin associated with exercise on the mechanical resistance of muscle and bone in rats☆

Jéssica Suzuki Yamanaka[a,*], *Kaique Eduardo Carvalho Ribeiro*[b], *Gabriela Rezende Yanagihara*[a], *Antônio Carlos Shimano*[a], *Álvaro César de Oliveira Penoni*[c]

[a] *Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP, Brazil*
[b] *Universidade do Vale do Sapucaí (Univás), Pouso Alegre, MG, Brazil*
[c] *Universidade Federal de São João del Rei, São João del Rei, MG, Brazil*

ARTICLE INFO

Article history:
Received 7 April 2017
Accepted 2 May 2017
Available online 5 April 2018

Keywords:
Hypercholesterolemia
Simvastatin
Exercise
Tibia
Gastrocnemius

ABSTRACT

Objective: The aim of the present study was to evaluate the influence of simvastatin on mechanical properties of muscle and bone in hypercholesterolemic rats submitted to physical exercise.

Methods: Ten male Wistar rats were submitted to a high-fat diet rich in cholesterol for 90 days. The animals were then divided into two groups: animals treated with physical exercise (EG) and animals treated with physical exercise and simvastatin (ESG). Protocols for physical exercise in water and simvastatin administration were performed for eight weeks. After this period, the animals were euthanized; the left tibia and right gastrocnemius muscle were dissected for mechanical analysis, and the right tibia for densitometry. The data were analyzed using Student's *t*-test, considering a level of significance of 5%.

Results: The comparison of maximum load and stiffness revealed no significant differences between the groups for both the tibia ($p = 0.851$ and $p = 0.259$) and the gastrocnemius ($p = 0.911$ and $p = 0.083$). The tibia BMD also showed no significant difference between the groups ($p = 0.803$).

Conclusion: Simvastatin had no negative effects on mechanical properties in tibia and gastrocnemius of hypercholesterolemic rats submitted to physical exercise.

☆ Study conducted at Universidade do Vale do Sapucaí, Pouso Alegre, MG, Brazil.

* *Corresponding author.*
E-mail: jsy_suzuki@hotmail.com (J.S. Yamanaka).

https://doi.org/10.1016/j.rboe.2018.03.017

Efeitos da sinvastatina associada ao exercício físico na resistência mecânica de músculos e ossos de ratos

Palavras-chave:
Hipercolesterolemia
Sinvastatina
Exercício
Tíbia
Gastrocnêmio

RESUMO

Objetivo: Avaliar a influência da sinvastatina nas propriedades mecânicas de ossos e músculos de ratos hipercolesterolêmicos submetidos a exercício físico.
Métodos: Foram usados dez ratos machos da raça Wistar, submetidos a dieta hiperlipídica rica em colesterol por 90 dias. Os animais foram então distribuídos em dois grupos: submetidos a tratamento com exercício físico (GE) e submetidos a tratamento com exercício físico e sinvastatina (GE + S). Foi aplicado um protocolo de exercício físico na água e administração de sinvastatina por oito semanas. Após esse período, os animais foram eutanasiados e dissecados a tíbia esquerda e o músculo gastrocnêmio direito de cada animal para análise mecânica e a tíbia direita para densitometria. Para análise dos dados foi aplicado o teste t de Student, considerou-se nível de significância de 5%.
Resultados: A comparação da força máxima e rigidez não revelou diferença significativa entre os grupos tanto para a tíbia (p = 0,851 e p = 0,259) quanto para o músculo gastrocnêmio (p = 0,911 e p = 0,083). A DMO das tíbias também não apresentou diferença significativa entre os grupos (p = 0,803).
Conclusão: A sinvastatina não teve efeitos deletérios nas propriedades mecânicas da tíbia e do músculo gastrocnêmio de ratos hipercolesterolêmicos submetidos a exercício físico aeróbio.

Introduction

Hypercholesterolemia is characterized by abnormal elevation of blood cholesterol levels, being considered a significant risk factor for cardiovascular disease. Its treatment and the prevention of cardiovascular diseases involve nutritional therapy, physical exercise practice, and drug therapy.[1]

Simvastatin is a drug used to treat and prevent hypercholesterolemia. It acts as an inhibitor of the enzyme 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, a key enzyme in the biosynthetic pathway of cholesterol in the liver, inhibiting cholesterol synthesis.[2] Due to their pleiotropic effects, other applications of statins are being studied. There are reports of adverse effects of statins use on muscle tissue, including pain, myopathy and, in more severe cases, rhabdomyolysis.[3] Evidence also indicates that statins may have effects on bone tissue,[4] but there are many controversies.

Physical exercise can also act on the musculoskeletal system and may be indicated as a complementary therapy to simvastatin for reducing cholesterol levels. Morphological changes in bones and muscles derived from these therapies have been studied. It is necessary to assess the relationship of simvastatin to exercise in order to demonstrate the effect of these two therapeutic modalities on the mechanical resistance of bone and muscle tissue. Therefore, this study is aimed at evaluating the influence of simvastatin on the mechanical properties of bones and muscles of hypercholesterolemic rats submitted to physical exercise.

Material and methods

This was an experimental pilot study in an animal model, following the rules of the Brazilian College of Animal Experimentation and the International Guiding Principles for Biomedical Research Involving Animals. It was previously approved by the Ethics in the Use of Animals Committee of the University of Vale do Sapucaí (Univás), under protocol 202/13.

Ten male Wistar rats were used, with body mass between 270 and 290 g. The animals were purchased through the Univás Vivarium, and kept under controlled conditions of 21 ± 2 °C, 55–60% humidity, and 12 h light/dark cycle, with water and food *ad libitum*.

The animals were randomly divided into two groups ($n = 5$) according to the proposed treatments: exercise group (EG) – animals were treated with physical exercise; and simvastatin + exercise group (SEG) – animals were treated simultaneously with exercise and simvastatin.

Induction of hypercholesterolemia

The animals received a hypercholesterolemic diet based on the AIN-93 from the American Institute of Nutrition, composed of 18% lipids per kg (1.25% of cholesterol) for 90 days, for hypercholesterolemia induction.[5] After 90 days, blood samples were drawn through cardiac puncture and laboratory tests were performed to confirm the elevation of the animals' cholesterol level.

Simvastatin administration

Simvastatin was administered at a dose of 20 mg/kg. The powdered drug was mixed with grade I purified water in Milli-Q system, and the suspension was supplied through gavage. Due to the growth and the modification of the body weight of the animals, the drug dose was recalculated daily, and administered for eight weeks.

Physical exercise protocol

The exercise protocol consisted in 60 min of water training five times a week for eight weeks, in a swimming system adapted for rats.[6] A rectangular-shaped, smooth-surface swimming tank was used, measuring 120 cm in length, 60 cm in width, and 60 cm in height.[7] The water was maintained at 40 cm depth, with the temperature at $31 \pm 1\,^{\circ}C$.[8] Prior to the start of the experiments, all animals were adapted to the water in the previously described tank under the same conditions. The adaptation lasted five days, in which the animals were placed in the water for an increasing time of 5, 15, 25, 35, and 45 min, respectively, in order to avoid stress of the animal without promoting physiological adjustments during that period. The animals were supervised during all of the exercise period, to avoid floating or being supported.

Morphometry

The animals were weighed weekly to check body mass. After the treatment period, the animals were euthanized using excessive dose of anesthetic (xylazine and ketamine). The right and left tibiae and the right gastrocnemius of each animal were dissected.

The tibiae were weighed on a scale with 0.01 g precision (AC-2000, Marte®). The tibial length was measured with a digital caliper with 0.01 mm precision (Mitutoyo®).

Densitometry

The right tibiae were submitted to densitometry on an X-ray dual-density densitometer (DPX-Alpha, Lunar®, USA) from the Bioengineering Laboratory of FMRP/USP. The images were acquired with the tibiae in the same position, immersed in a 2 cm deep sterile physiological solution in a plastic container. Using the DPX software (version 4.7 E, Lunar®, USA), the bones were manually demarcated in a 9 mm^2 region of interest just below the epiphyseal disk; bone mineral density (BMD) information was collected.

Mechanical analysis

To assess mechanical properties, the left tibia of the animals were submitted to a three-point bending test with a Universal Testing Machine (DL10.000, EMIC®, Brazil) from the Bioengineering Laboratory of FMRP/USP. The bones were placed on two support points, 25 mm apart from each other. A 500N load cell was used, and force was applied at a speed of 1 mm/min until sample rupture. The properties analyzed were maximum strength and relative stiffness.

The right gastrocnemius muscle was subjected to a tensile test with a Universal Testing Machine (DL10.000, EMIC®, Brazil) coupled to a 500-N loading cell to measure the maximum strength and relative stiffness of the muscle fibers. The following parameters were used: speed of 10 mm/min, preload of 5 N, and accommodation time of 30 s.

Statistical analysis

Student's *t*-test was used to compare the groups. Statistical analyses were performed using SPSS (version 20.0), and a significance level of 5% was adopted.

Results

Body mass and feed intake

The body mass of the animals at the beginning of the experiment was similar ($p = 0.183$). The groups maintained similar body mass after hypercholesterolemia induction ($p = 0.132$) and at the end of the experiment ($p = 0.210$). The weight gain was increased until the eighth week (the end of the hypercholesterolemic diet; $p < 0.001$); from the eighth week onwards, with the beginning of the treatment, weight loss was gradual, and the animals lost weight significantly at the end of the experiment ($p = 0.033$).

The mean daily dietary intake was measured until the end of the hypercholesterolemia induction, and no statistically significant differences were observed between the groups during this period ($p = 0.120$). Subsequently, the mean daily feed intake during the treatment period was also measured, and no statistically significant differences were observed between the groups ($p = 0.784$). Feed intake before and after physical training also did not present differences ($p = 0.281$). Fig. 1 presents the body mass and feed consumption values for both groups.

Tibia assessment

The morphometric measurements showed no significant differences in tibial length ($p = 0.834$) and weight ($p = 0.302$) between the groups.

When comparing the maximum strength ($p = 0.851$) and relative rigidity ($p = 0.259$) of the left tibia, no statistically significant differences were observed between the groups. Furthermore, no statistically significant differences were observed in BMD between both groups ($p = 0.803$). The results are shown in Table 1.

Gastrocnemius assessment

The resistance of the gastrocnemius muscle was not affected by the association of simvastatin and exercise. When comparing the maximum strength, no statistically significant differences were observed between the groups ($p = 0.911$). In the traction test, gastrocnemius stiffness also did not present a significant difference ($p = 0.083$). The results are shown in Fig. 2.

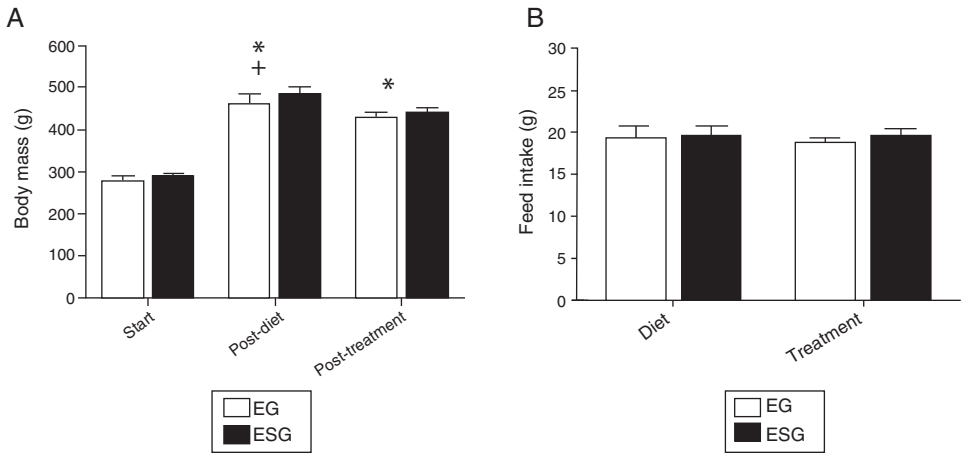

Fig. 1 – (A) Comparison between exercise (EG) and exercise + simvastatin (ESG) groups at three different times. Both groups presented weight gain until the end of the diet and had weight loss with treatment, with no significant difference between groups at any of the times. *Significant difference in relation to the beginning. +Significant difference in relation to post-treatment; (B) feed intake did not change before and after treatment and did not differ between groups.

Table 1 – Results of bone length, weight and bone mineral density (BMD) of the right tibiae and maximal strength and rigidity of the left tibiae in the exercise (EG) and exercise + simvastatin (ESG) groups.

	Right tibia			Left tibia	
	Length (mm)	Weight (g)	BMD (g/cm^2)	Maximum force (N)	Relative stiffness (N/mm)
EG	43.37 ± 0.45	0.96 ± 0.04	0.12 ± 0.01	129.57 ± 18.21	264.91 ± 38.46
ESG	43.32 ± 0.25	1.01 ± 0.09	0.12 ± 0.04	127.91 ± 5.96	233.23 ± 43.76

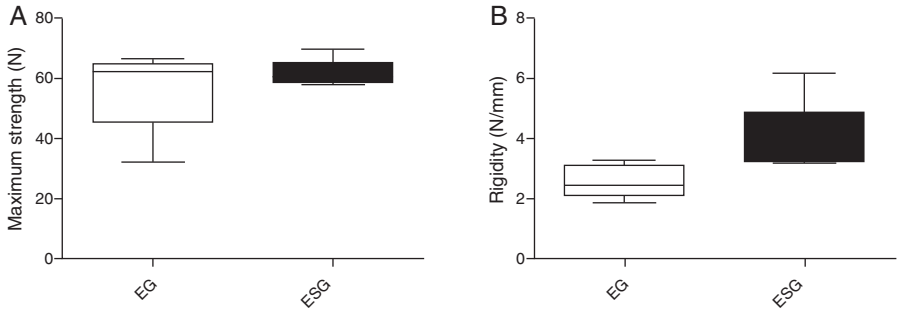

Fig. 2 – (A) Exercise (EG) and exercise and simvastatin (ESG) groups presented similar maximum strength at the mechanical gastrocnemius test; (B) rigidity was similar between groups.

Discussion

The present study evaluated the use of simvastatin associated with exercise on the tibia and gastrocnemius of hypercholesterolemic rats, since both are lipid-lowering therapeutic modalities that affect the musculoskeletal system. The results of the present study did not demonstrate an influence of physical exercise on the morphometric, mechanical, and BMD properties of the tibiae of rats regardless of simvastatin use. When associated with exercise, simvastatin also did not affect the mechanical resistance of the gastrocnemius muscle.

Even a low-impact exercise such as swimming stimulates bone formation. The muscular action in physical exercise promotes a mechanical stimulation to the bones, which, due to their piezoelectric properties, respond positively to this stimulus.[9] In turn, simvastatin did not affect bone stimulation; both groups presented similar BMD and mechanical properties. In the present study, physical exercise, as a strong factor influencing bone tissue, may have suppressed the simvastatin response.

Studies have reported that statins act in the stimulation of bone tissue.[4,10] This action occurs due to the stimulation of bone morphogenic protein (BMP-2), which causes osteoblastic

proliferation and differentiation, resulting in a greater and better bone tissue formation. According to Anbinder et al.,[11] all statins stimulate BMP-2, except pravastatin. Statins were also associated with a decrease in osteoclast activity and consequent reduction in bone absorption observed by an increased osteoprotegerin (OPG) expression and decreased expression of nuclear kappa-B binding factor (RANKL).[12] Thus, the possible use of statins to potentiate the treatment of bone deficiencies has been investigated. Oxlund and Andreassen[13] assessed the effects of simvastatin on the tibia and vertebra of ovariectomized rats and observed a reduction in trabecular bone loss. Skoglund et al.[14] also reported that rats with femoral fractures treated with 120 mg/kg simvastatin presented better resistance than those not treated with simvastatin.

However, other studies have failed to observe bone stimulation after simvastatin treatment in rats.[15,16] Maritz et al.[16] also stated that high doses of simvastatin increase bone formation, while low doses of simvastatin may decrease it. Different doses, times, and modes of administration may have been the cause of the controversial results. To avoid side effects derived from high doses of simvastatin, local administration in the bones appears to be an attractive solution for the use of this drug in bone therapy.[17]

Simvastatin is also associated with morphological and structural damage in skeletal muscle.[18] It does not act exclusively on the biosynthetic pathway of cholesterol, but also inhibits the synthesis of coenzyme Q_{10}, which affects mitochondrial oxidative phosphorylation and can lead to apoptosis in muscle fibers.[19] Rats in which simvastatin is used present morphological and structural changes that may be aggravated when the use of this drug is associated with exercise.[5,20] Bonfim et al.[21] reported that the association of simvastatin with treadmill exercise led to an increase in the muscle injury in rats. In the present study, no statistically significant differences were observed in the mechanical properties of animals, regardless of the use of simvastatin. The study by Padulla et al.[22] indicated that the cardiac musculature of trained rats underwent beneficial adaptations in response to exercise, becoming more resistant to the apoptotic effects of statins. Physical exercise is a powerful stimulator in mitochondrial biogenesis. Thus, although some studies describe that exercise causes microlesions that can aggravate myopathy due to the use of statins, physical activity promotes muscle strengthening, which may have prevented musculoskeletal damage related to biomechanical resistance.

The animals were fed a hypercholesterolemic diet to cause hypercholesterolemia and better mimic the effects of treatment.[5] The animals gained increasing weight throughout the hypercholesterolemic feeding. This increased weight gain can be explained by the natural growth of the animals combined with a diet rich in lipids. From the end of the diet to the beginning of physical activity practice, the animals lost weight. Although the treatment led to weight loss, the feed consumption did not change. This fact can be explained by the increase in energy expenditure due to the practice of physical exercise, mimicking the dietary and physical reeducation involved in a conservative treatment for cholesterolemic control that, due to new habits, leads to weight loss.

In the present study, swimming was chosen because continuous aerobic exercise is the physical activity of choice for patients with hypercholesterolemia. The low-impact exercise did not decrease the muscular resistance associated with the use of simvastatin and it may even have promoted muscle strengthening, avoiding this damage. Bone tissue was also unaffected by the use of simvastatin. Although simvastatin can be considered a bone formation stimulator, it did not potentiate or suppress the effects of physical exercise. Moreover, the combination of simvastatin with exercise acts as a regulator of metabolic disorders that may be harmful to the musculoskeletal system.[23] Therefore, the practice of low-intensity physical exercise is indicated as an adjunctive treatment for dyslipidemia control, not only because of its lipid-lowering effect, but also because of the accumulation of musculoskeletal benefits that avoid damage associated with medication use.

It is important to know the biomechanical response of bones and muscles to a hypercholesterolemia therapy. The present study complements others that evaluated the morphological and structural effects of simvastatin and exercise in the musculoskeletal system. However, the study included small samples, and may not be possible to extrapolate the results into a larger group. Further studies with larger samples are therefore encouraged. The findings permit the conclusion that simvastatin had no deleterious effects on the mechanical properties of the tibia and gastrocnemius muscle of rats submitted to aerobic exercise.

Conflicts of interest

The authors declare no conflicts of interest.

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