

# L-ARGININE AND PHYSICAL EXERCISE INTERFERENCE ON THE MORPHOLOGY OF THE SKELETAL MUSCLE IN YOUNG RATS

EXERCISE AND  
SPORTS SCIENCES



ORIGINAL ARTICLE

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## ABSTRACT

**Introduction:** Exercise and the amino acid L-Arginine can promote anatomical and physiological changes in the skeletal muscle. **Objective:** The objective of this work was to analyze the influence of L-arginine associated with exercise in the skeletal muscle fibers. **Methods:** To carry out our research, 24 male Wistar rats divided into 4 groups according to the administration of Arginine and physical exercise were used. The experimental groups were distributed as follows: Arginine-Exercise (AE, n = 6), Arginine-Not Exercised (AN, n = 6), Water-Exercise (WE, n = 6) and Water-Not Exercised (WN, n = 6). The amino acid L-Arginine was administered via orogastric intubation, at dose of 300mg/kg, daily from the 7th to 35th days of life of the animal. The exercise was performed on motorized treadmill for 30 minutes/day, 5 times a week, from 15th to 35th days of life of the animal. At the age of 35-45 days, the animals were weighed and sacrificed in order to collect the gastrocnemius muscle. The gastrocnemius muscle was measured, weighed and processed for histological analysis. The muscle's images were taken in order to calculate the mean diameter of the muscle fiber. Data were expressed as mean  $\pm$  standard deviation and analyzed using SPSS. The Shapiro-Wilk, one-way ANOVA and Tukey's tests ( $p < 0.05$ ) were applied. **Results:** Concerning body weight and gastrocnemius weight, there was no significant difference when all the experimental groups were compared. However, the AN group presented the highest mean diameter when compared to the other groups. **Conclusion:** This fact suggests that orogastric administration of Arginine offered to the animals that were not exercised, promotes muscle hypertrophy. On the other hand, exercise by itself did not lead to increase in mean diameter of the skeletal muscle.

**Keywords:** arginine, exercise, skeletal muscle fibers.

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## INTRODUCTION

The body responses to physical exercise may lead to anatomophysiological alterations of many tissues in the body, among which, the muscle tissue<sup>1</sup>. The alterations of the skeletal muscle derive from the functional overload during exercise, promoting hence increase of muscle tension and energy production<sup>2</sup>. In addition to that, increase of muscle work during physical exercise is able to promote chemical reactions which are responsible for the muscle hypertrophy<sup>3</sup>.

Studies have shown that during aerobic exercise, rupture of the body's proteins takes place, which leads to release of amino acids<sup>4</sup>. Thus, athletes may need to increase ingestion of proteins or any other specific amino acid<sup>5</sup>. The amino acids arrest by the skeletal muscle is an early event so that hypertrophy can be initiated<sup>3</sup>.

L-arginine has been widely studied and is part of the composition of many food supplements<sup>6</sup>. This amino acid is considered conditionally essential, since it is not produced by the body in some phases of life and under certain pathological conditions, it needs to be acquired through diet<sup>7,8</sup>.

The oral administration of this amino acid has been related to improvement in physical performance due to reduction in muscular fatigue. This effect is associated with the vasodilation activity promoted by the nitric oxide which is made from the L-arginine. Vasodilation results in increase of muscular perfusion and greater glucose availability by the muscles in activity<sup>9</sup>. It is believed that

the perfusion of the skeletal musculature effect itself contributes to the better training quality, resulting in increase of muscle mass and contractile strength<sup>10</sup>.

Besides the L-arginine effects related to vasodilation, there is also association of this amino acid with improvement of contractile strength through better synthesis of muscle proteins<sup>11</sup>.

In order to study the effects of physical exercise in laboratory animals, the motorized treadmill has been widely used, since this kind of exercise has been also widely applied in rehabilitation programs and physical training<sup>1</sup>. Additionally, with the aim to investigate alterations in the muscle fiber, such as hypertrophy, derived from exercise practice, geometric dimensions of the transversal sections of the fibers, such as diameter, have been used<sup>2</sup>.

Thus, the aim of the present study was to investigate the possible influences of the ingestion of the L-arginine amino acid when associated with physical exercise practice on the body weight and skeletal muscle of young rats.

## METHODS

This experimental study was submitted to the Ethics Committee in Animal Experimentation of the Center of Biological Studies of the Federal University of (CEUA-UFPE), and was approved under protocol nº 23076.005924/2008-80, following the recommendations by the Brazilian Committee of Animal Experimentation (COBEA).

24 male Wistar rats from the colony of the Nutrition Department of the Federal University of Pernambuco, kept in four collective cages with six pups in each were used. These animals were kept under standard conditions of the animal facility, in a room with temperature between  $22 \pm 2^\circ\text{C}$ , submitted to artificial dark/light cycle of 12/12 hours (the dark cycle starting at 7 p.m.), water and food Labina (Purina®, Paulínia, SP, Brasil) ad libitum.

When the rats completed seven days of life, they were separated in two groups according to the L-arginine administration: animals treated with L-arginine (Ar group; 300 mg/kg/day) and animals treated with equivalent volume in distilled water (Ag group). The L-arginine or water were daily administered through gavage from the seventh to the 35<sup>th</sup> day of life, between six and eight o'clock. The total volume administered in each gavage ranged from 0.5 ml/day (seventh to 14th day of life of the animal) to 1.0 ml/day (15th to 35th day of life of the animal)<sup>12</sup>.

When the animals were 15-day old (Ar and Ag) they were subdivided in two groups according to their physical exercise practice: Exercised (AgE and ArE) and Not exercised (AgN and ArN). Each experimental group has seven animals. The physical exercise were performed on motorized treadmill (ET 2000, Insight Equipamentos Científicos, Ribeirão Preto, Brazil) in five weekly sessions, with 30 minutes of duration at six to eight o'clock. The exercise was performed from the 15th to the 35th day of life of the animal with treadmill velocity of 5 m/min, 10 m/min and 15 m/min, on the first, second and third weeks, respectively<sup>12</sup>.

All animals were weighed on the seventh, 14th, 21st and 28th day of life, as well as on the sacrifice day (35-45 days of life). The animals were submitted to anesthesia with solution containing mixture of urethane 10% + chloralose 0.4% at 1,000 mg/kg dose of urethane + 40 mg/kg of chloralose, via intraperitoneal for sacrifice. Afterwards, the gastrocnemius muscle was removed for weighing and length measurement using a line along the muscle from the origin tendon to the insertion tendon. This distance was transferred to a ruler in millimeters with precision measurement of 0.05 cm. After measurement, the muscle was stained in formaldehyde 10% for a minimum period of 48 h, dehydrated and conventionally diaphanized for light microscopy and fixed in paraffin. Transversal sections of the muscle with approximately 4  $\mu\text{m}$  of thickness were obtained through a microtome (Leica). All obtained cuts were stained by hematoxylin-eosin and mounted in Entellan (Merck).

The images of the histological cuts of the muscles (magnification 1,000 x) were selected in microscope, picked and digitalized in a digital camera and attached to a microcomputer. Hypertrophy of the muscle fibers was performed through measurement of the mean diameter in samples of 50 fibers per animal in the ImageJ software<sup>6</sup>.

Statistical analysis was performed with Shapiro-Wilk test for sample normality as well as analysis of variance (one-way ANOVA). In the samples which presented significant difference, Tukey post-test was used. Significance level was considered with  $p < 0.05$ . This statistical analysis was performed with the SPSS 15.0 program (Statistical Package for the Social Sciences, Chicago, USA) for Windows®.

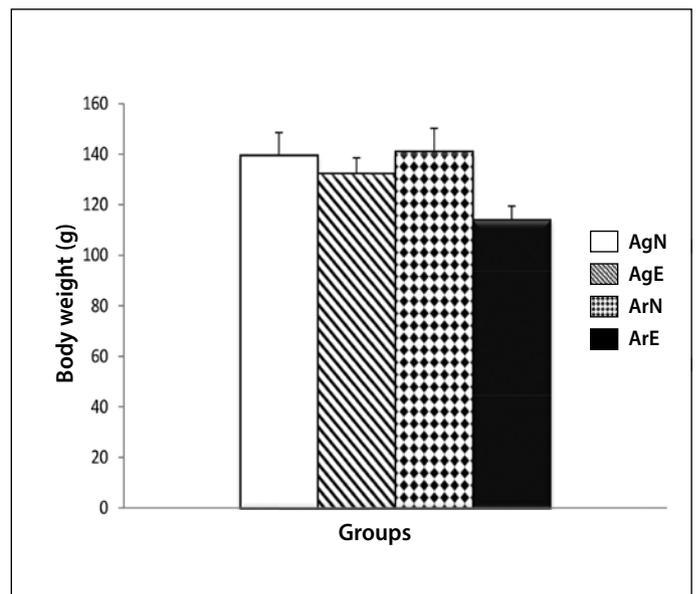
## RESULTS

Regarding the animals' weight on the day of the sacrifice, there was no significant difference between the experimental groups (0.07525) when their means are compared (figure 1). The results were expressed as mean  $\pm$  standard deviation of mean. Body weight (g) of the animals at the sacrifice day was  $139.53 \pm 21.96$  for the AgN group,  $132.41 \pm 15.01$  for the AgE group,  $141.11 \pm 22.37$  for the ArN group and  $114.05 \pm 13.27$  for the ArE group.

Absolute and relative weight of the gastrocnemius muscle of the animals at the pre-set age was, respectively:  $0.6136 \pm 0.1068$  and  $0.004416 \pm 0.1068$  for the AgN group,  $0.60195 \pm 0.13747$  and  $0.004466 \pm 0.00024$  for the AgE,  $0.66488 \pm 0.13873$  and  $0.00468 \pm 0.000365$  for the ArN,  $0.52945 \pm 0.15007$  and  $0.004183 \pm 0.000299$  for the ArE. After statistical analysis, it was observed that these data did not present significant difference (figure 2).

The length of the gastrocnemius muscle was  $2.11 \pm 0.26$  for the AgN group,  $2.18 \pm 0.26$  for the AgE,  $2.21 \pm 0.19$  for the ArN and  $2.08 \pm 0.27$  for the ArE (figure 3). The groups presented similar length values; therefore, there was no statistical difference between them ( $p = 0.164672$ ).

The mean diameter of the muscle fiber ( $\mu\text{m}$ ) of the gastrocnemius (figure 4) was of  $30.855 \pm 1,627.19$  for the AgN group,  $31,671.51 \pm 2,657.79$  for the AgE,  $35,784.9 \pm 3,037.78$  for ArN and  $30,857.03 \pm 2,483.21$  for ArE. The ArN group had diameter increase when compared with AgN ( $p = 0.00031$ ) and ArE groups ( $p = 0.013677$ ).



**Figure 1.** Effect of the physical exercise and/or L-arginine amino acid during the body weight development (g) of young Wistar rats on the sacrifice day. The four experimental groups are represented: AgN (n = 6), AgE (n = 6), ArN (n = 6) and ArE (n = 6). Data presented as mean  $\pm$  standard error (one-way ANOVA,  $p < 0.05$ ).

## DISCUSSION

Our results evidence that neither physical exercise nor oral ingestion of the L-arginine amino acid were able to influence on body weight of the studied animals. There are reports in the literature that the nitric oxide produced by the L-arginine ay reduce food ingestion, resulting in decrease of body weight of rats and

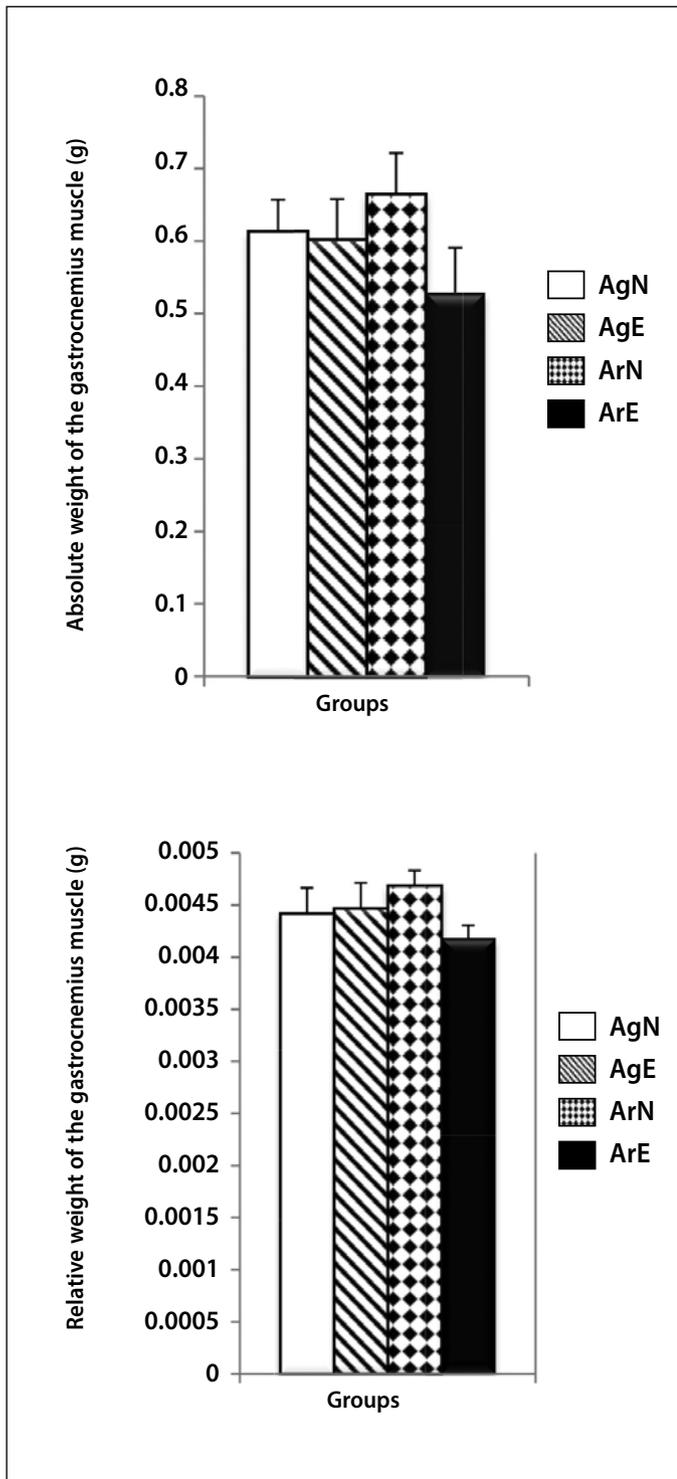


Figure 2. Effect of the physical exercise and/or of the L-arginine amino acid during the absolute and relative weight development (g) of the gastrocnemius muscle of young Wistar rats. Data presented as mean  $\pm$  standard error (one-way ANOVA,  $p < 0.05$ ) of the experimental groups: AgN (n = 6), AgE (n = 6), ArN (n = 6) and ArE (n = 6).

mice<sup>13,14</sup>. However, our results clash with these, corroborating more recent studies. Lin *et al.*<sup>15</sup>, Suzuki<sup>16</sup> and Huang *et al.*<sup>17</sup> analyzed body weight of Wistar rats with different ages, submitted to many different kinds of physical exercises and administration of L-arginine in variable quantities. Such investigations did not prove difference in body weight of the studied animals. Thus, we suggest that regardless of the amino acid dose, there is no relation between its ingestion and physical exercise practice concerning weight of the animals.

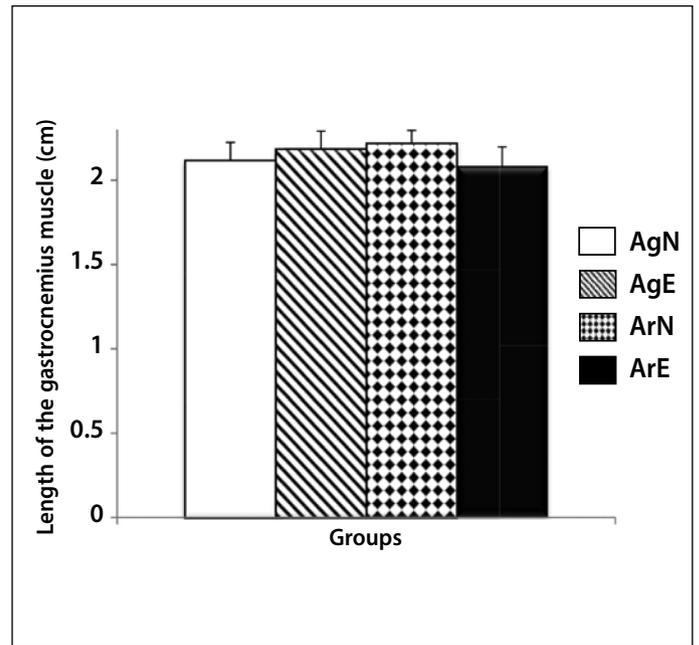


Figure 3. Effect of physical exercise and/or L-arginine amino acid during the development on length of gastrocnemius muscle of young Wistar rats. Data are presented in mean  $\pm$  standard error (one way ANOVA,  $p < 0.05$ ) of the four experimental groups: AgN (n = 6), AgE (n = 6), ArN (n = 6) and ArE (n = 6).

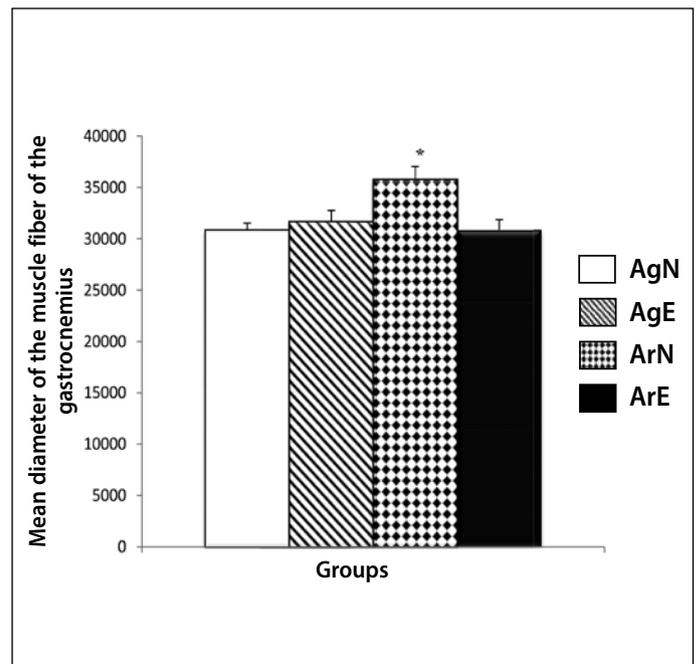


Figure 4. Effects of physical exercise and/or L-arginine amino acid during the development on the mean diameter ( $\mu\text{m}$ ) of muscle fiber of young Wistar rats on the sacrifice day. The four experimental groups are represented: AgN (n = 6), AgE (n = 6), ArN (n = 6) and ArE (n = 6). Data presented in mean  $\pm$  standard error (one-way ANOVA, Tukey post hoc;  $p < 0.05$ ). The symbol \* expresses that the ArN group presented greater mean diameter of the muscle fiber when compared with the other groups.

Concerning the length of the gastrocnemius muscle, there was no significant difference either between the studied groups. However, there is not record in the literature of studies which evaluate such macroscopic parameter, relating it with physical exercise and ingestion of this amino acid. It is believed that muscle contraction derived from physical exercise practice may lead to hypertrophy of the muscle fibers<sup>10</sup>, which reflects on increase of

length of the skeletal muscle venter. Additionally, L-arginine, due to promotion of vasodilation in the skeletal muscle, could boost this hypertrophy due to greater blood and nutrients contribution for this muscle in activity<sup>9,10</sup>.

The results of this study evidence that there was no significant difference between the mean diameter of the fibers of the animals which perform physical exercise when compared with the sedentary group. The mean diameter of the muscle fiber is a datum which reflects on muscle hypertrophy, being hence a measurement fairly used in the study of the effects of physical exercise on the striated musculature in rats<sup>1,2</sup>. Hypertrophy is derived from the functional demand increased due to physical overload and is a result of alterations in the cellular metabolism and proliferation<sup>8</sup>. Studies present increase of mean diameter in rats submitted to physical exercise practice<sup>1,2</sup>. Nevertheless, our results are different from the already mentioned; since we did not obtain increase of mean diameter of the fibers of the animals submitted to physical exercise practice. We believe that it can be explained by the exercise protocol used, being hence, insufficient overload to stimulate hypertrophy. When the protocol of this investigation is compared with the ones by Camargo Filho *et al.*<sup>1</sup> and Brito *et al.*<sup>2</sup>, we observed that exercise duration was the same; however, the duration of the present study is lower and the velocity on the treadmill was not mentioned by these authors. Such situation let us infer that in order to have suitable muscle response, factors which characterize physical exercise, such as: duration, frequency and intensity, should be considered. Exercise intensity is defined according to the maximal oxygen consumption ( $VO_{2max}$ ) and maximal heart rate ( $HR_{max}$ ) during the activity, and can be classified as: light, moderate and intense<sup>19</sup>. The exercise performed in this investigation is based on an alteration in the protocol by Silva *et al.*<sup>20</sup>, who classify it as moderate.

The mean diameter of the group of not exercise animals to which L-arginine was administered was significantly higher when compared with the other groups. In adults, L-arginine associated with exercise is related to the increase of muscle mass explained by the vasodilator effect of the nitric oxide produced by this amino acid<sup>10</sup>. Vasodilation increases muscle perfusion and increase of nutrients to the muscle in activity, which favors the increase of muscle mass. This mechanism explains the increase of diameter in individuals who made use of the L-arginine amino acid while they performed physical exercise. Moreover, recent research found out that the L-arginine amino acid stimulates the secretion of the growth hormone (GH). The GH promotes muscle hypertrophy, since it facilitates the transport of amino acids to the cells<sup>21</sup>. The influence of physical exercise on the GH was contradictory, being dependent on the kind and intensity of exercise performed<sup>22,23</sup>. Stokes *et al.*<sup>23</sup> and Collier *et al.*<sup>24</sup> observed that there is increase in the GH levels even at rest in individuals supplemented with L-arginine. Thus, we believe that the L-arginine dose (300 mg/kg) of the present study was able to cause hypertrophy of the skeletal striated musculature, possibly through the increase of the GH levels; since the vasodilator effect is associated with the ingestion of

this amino acid concomitantly to the physical exercise practice. Thus, our results corroborate Chiyoda *et al.*<sup>25</sup>, who evidenced that L-arginine orogastric administration did not interact with physical exercise. When mean diameter of the groups of exercised animals is compared, we observe that there was no significant difference between those which ingested the amino acid and those which did not. Hypertrophy, expressed by the increase of the mean diameter of the muscle fiber, is understood as the positive balance between the protein synthesis and degradation induced by physical exercise<sup>26</sup>. Thus, these physical exercise effects depend on its better definition, as specific intensity. Moreover, L-arginine has proved to be efficient in muscle hypertrophy, since it increases the protein synthesis rate. Matsumoto *et al.*<sup>27</sup> observed that the L-arginine orogastric supplementation (2 g) associated with physical exercise of moderate intensity suppressed muscle proteolysis during this exercise, resulting in positive protein balance and consequently, in hypertrophy. Nevertheless, this effect has not been observed in our studies, which may be justified by the L-arginine dose used in this work.

The oral ingestion of the L-arginine amino acid has been efficient in increasing the angiogenesis induced by physical exercise through increase of expression of the vascular endothelial growth factor (VEGF)<sup>16</sup>. Some authors have reported that the increase of the VEGF levels in patients results in increase of collateral arterial circulation<sup>28</sup>. This angiogenesis increase may be related to the increase of muscle hypertrophy. However, in our results, this probable correlation does not seem to be connected with practice of physical exercise, since hypertrophy only occurred in the animals from the arginine non-exercised group.

It is known that disuse of skeletal musculature due to lack of stimulus by overload, leads to destruction of intramuscular proteins, which results in muscle atrophy<sup>29</sup>. Ingestion of 500 mg/kg of L-arginine was sufficient to avoid atrophy by disuse in muscles of hinder legs of Wistar rats<sup>30</sup>. This result is in agreement with ours; however, it should be considered that the 300 mg/kg dose, used in this study may present the same result.

## CONCLUSION

In short, the ingestion of the L-arginine amino acid in the 300mg/kg dose, without physical exercise interference, was able to promote hypertrophy of the skeletal striated musculature. In our study, a dose able to promote a positive effect on the skeletal striated musculature was used, since there is no record of the specific dose of this amino acid for this aim. However, the L-arginine relation with physical exercise has not been well-established yet. Hence, the need of more detailed systematization of the physical exercise protocol so that it positively influences in the mean diameter of the muscle fiber should be considered. Thus, parameters such as exercise duration, intensity and frequency should be better defined.

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All authors have declared there is not any potential conflict of interests concerning this article.

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