The Role of Anabolic Steroids on Hypertrophy and Muscular Strength in Aerobic Resistance and Strength Training

EXERCISE AND SPORTS SCIENCES

ORIGINAL ARTICLE

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ABSTRACT

Introduction: The effects of the anabolic steroids (AS) on muscle mass and strength are controversial and dependent on the training protocol performed and the muscle fibers recruited. Thus, the aim of this study was to evaluate the AS effects combined with strength training or aerobic exercise training on muscle hypertrophy and strength. Methods: Wistar rats (42) were divided into six groups: sedentary control (SC, n = 7), steroid sedentary (SS, n = 7), swimming training control (STC, n = 7), swimming training steroid (STS, n = 7), strength training control (SRC, n = 7) and strength training steroid (SRS, n = 7). AS was administered twice a week (10mg/kg/week). The training protocols were performed for 10 weeks, 5 sessions per week. Soleus, gastrocnemius and plantar hypertrophy (muscle mass corrected for tibia length), total muscle protein (Bradford) and muscle strength in hind limb (resistance to twist) were assessed. Results: No significant differences were observed in soleus muscle hypertrophy. SRC and SRS groups showed hypertrophy of 18% and 31% in plantar muscles compared to the SC group. Hypertrophy was 13% higher in SRS than SRC group. Similar results were found in gastrocnemius muscle. SRC and SRS groups showed significant increases in the protein total amount in the plantar muscles, it was more pronounced in SRS group and positively correlated to muscle hypertrophy. The strength was increase in SRC and SRS groups. Conclusion: AS administration or its association to aerobic training does not increase muscle mass and strength. However, its association to strength training leads to muscle hypertrophy in glycolytic fibers. Therefore, the physical training protocol, muscle recruitment and muscle fibers characteristics, appear to have significant impact on anabolic responses induced by AS.

Keywords: hypertrophy, skeletal muscle, physical training, rats.

INTRODUCTION

Anabolic steroids (AS) were initially synthesized for therapeutic purposes; however, due to their possible effects on the protein synthesis increase, increment of energy storage and reduction in the recovery time after physical training, they started being used by athletes to improve sports performance(1), currently being among the most used ergogenic substances in doping situations(2).

Research shows the remarkable increase of the use of AS by recreational practitioners of strength training(1), who have as main goal improvement in physical appearance. These individuals generally make use of supraphysiological doses, reaching up to values from 10 to 100 times higher than the recommendation for clinical purposes(3). However, the real effects of AS on muscle mass and strength are still controversial in the literature and seem to be dependent, among other factors, on the training regimen used(4), since they will only promote increase in muscle mass and strength if associated with specific stimuli – physical training and diet(5).

A study showed that AS associated with strength training improved performance of athletes in about 1 to 5%, which may not be statistically significant, but may represent the victory in high level sports(4). Strength increase in about 5 to 20% and increase of body mass between 2 and 5kg are also observed, which was directly related to increase of lean mass and muscle size(6). On the other hand, some studies did not find increase of muscle mass and strength induced by AS(7,8). This divergence in the results may be derived from the different AS used, administered doses or training protocols applied. As can be observed, there are still questions in the literature about the real effects of the AS when associated with strength training.

Although the use of AS is more related to practitioners of exercises which involve muscle power and strength, many athletes of aerobic modalities use AS(9). These individuals have as aim to decrease the catabolism induced by the high training volume(9). According to Zyl et al.(10), who investigated endurance in submaximal race with use of AS, improvement of 41% was verified in aerobic performance. However, another study shows that the AS use does not alter fatigue in rats trained in swimming[11]. Therefore, the AS effects associated with aerobic training are controversial. The studies which investigate the association of AS to aerobic training do not evaluate their effects on muscle mass and strength, which make them until the present moment unknown.

Considering the controversy found in he literature on the AS effects associated with different physical training protocols on the muscle mass and strength, the aim of this study was to evaluate the effects of the AS association with strength or aerobic training (swimming) on the hypertrophy of the soleus, plantar and gastrocnemius muscles as well as muscular strength.

METHODS

Sample: 42 Wistar male rats, initial body mass of 260 ± 6g were used. The animals were kept in collective cages, three to five animals per cage, separated per groups, in the animal facility of the Laboratory of Biochemistry of Motor Activity of the EEFE/USP with temperature kept between 22 and 24ºC and luminosity control in inverted light-dark cycles of 12 hours. Water and food were administered ad libitum and the animals were weekly weighed. All procedures were approved by the Ethics Committee of the Physical Education and Sports College at USP and in agreement with the guidelines of the Brazilian College of Animal Experimentation.

Treatment: The animals were randomly divided in six experimental groups (each group, n = 7): sedentary control (SC), sedentary anabolic (SA), trained control swimming (TCS), trained anabolic swimming (TAS), strength trained control (STC) and strength trained anabolic (STA). Subcutaneous injections of vehicle (controls) or AS were administered, Nandrolone Decanoate (Decadurabolin; Organon, Roseland, NJ), two times per week, in the dose of 5mg/kg per session, totalizing 10mg/kg/week. This dose equals to the one generally used by athletes (600mg/week)[12].

Swimming training: it was performed according to protocol adapted by Medeiros et al. (2004)[13], in swimming system with water warmed between 30-32ºC, during 10 weeks, with Five weekly sessions being performed, with gradual increase of the session time, until reaching 60 minutes, and of the work overload (weight tied to the animal's tail) until 5% of body mass is reached. This protocol is characterized as aerobic training of low intensity and long duration[13].

Strength training: it was performed according to the model proposed by Tamaki et al. (1992)[14]. The animals were wrapped around a canvas cape inhibiting thus their trunk twist and flexion and were placed on the squat apparatus, where they were sustained on their hinder legs. An electric stimulus (20V, 0.3 second of duration by three seconds of interval) was applied on the rat's tail through an electrode. As a result, the rats extended their hinder legs lifting the apparatus lever with the set load (figure 1).

The animals performed four sets of 12 repetitions with 90 seconds of interval between sets, five times per week for 10 weeks. Two weeks of adaptation to the apparatus were performed before the beginning of the training. After one repetition maximum test (1RM) performance on the squat apparatus, the intensity of 75% of 1RM was set for the training load. The maximum load of each animal was established as being the heaviest weight it could lift after the electrical stimulus. It was performed with two-week periodicity until the last week, an important action to the adjustments at the training intensity.

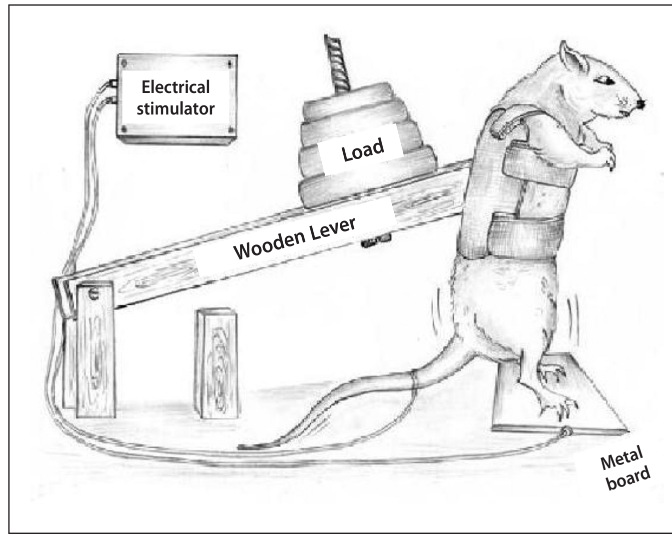

Figure 1. Strength training apparatus adapted from Tamaki et al. (1992).

Endurance tests to inclination: Skeletal muscle strength on hinder legs was assessed with an endurance test to inclination, previously standardized in the literature[15]. The animals were placed on a wooden board, which was inclined at the approximate velocity of five degrees per second, with the aid of a stop watch and a protractor. The angle at which the animals slipped from the board was recorded and it was named fall angle[15].

Material collection: the animals were killed by decapitation 24 hours after the last training session. The soleus, plantar and gastrocnemius muscles were collected, weighed and stored in a freezer at –80ºC for the analyses. The animal's tibia was dissected and its length measured with a pachymeter.

Muscle hypertrophy measurement and protein quantification: In order to evaluate skeletal muscle hypertrophy, the weight of the soleus, plantar and gastrocnemius muscles were normalized by the tibia length of the animal and presented as mg/mm.

Regarding the proteins quantification, the soleus and plantar muscles were thawed and a portion of each sample (0.1g) was homogenized through a homogenizer Polytron (PT-K Brinkman Instruments) with hypotonic lysis buffer containing 10mM TrisHCl and 5mM EDTA, pH 7.4 in the presence of a mixture of protease inhibitors. Homogenization was performed three times during 15 seconds with intervals of 20 seconds. The homogenized tissue was centrifuged at 12,500rpm for 20 minutes at 4ºC. The supernatant was transferred to individual tubes. The protein content of the samples was assessed by the colorimetric method in a spectrophotometer (Bradford – Biorad, USA) with albumin being used as standard (BSA, 1m/ml)[16]. The total protein concentrations were presented in mg/g of muscle tissue.

Statistical analysis: The results are presented as mean ± standard error (SE). Two-way analysis of variance was used for data analysis (training and treatment with AS as independent variables). Whenever significant difference was observed, the Duncan post hoc test was performed. P≤ 0.05 was considered statistically significant.

RESULTS

Body mass and tibial length data did not present significant differences between groups after the experimental period. However, tendency to lower body mass gain was observed in the TAS group compared to the STC group (body mass and tibial length data did not present significant differences between groups after the experimental period), (p 18 vs. 397 ± 37 and 398 ± 3mg, respectively).

When muscle mass normalized by the animal's tibia length and used as muscle hypertrophy index was analysed, significant differences in the soleus muscle have not been observed between groups (figure 2A). On the other hand, the STC group presented hypertrophy of the plantar muscle when compared to the control group (18%, $p < 0.05$), and when it was associated with AS administration, the found hypertrophy in the plantar muscle was even more remarkable – group STA presented increase of 31% ($p < 0.05$) compared to the sedentary control group and increase of 13% when compared to the STC group ($p < 0.05$) (figure 2B). Similar results were observed in the gastrocnemius muscle, in which the STA group presented higher muscle hypertrophy compared to the remaining studied groups ($p < 0.05$) (2C). No significant differences have been observed in the groups which performed swimming training.

Figures 3A and 3B present data concerning the quantification of total protein in the soleus and plantar muscles of all studied groups. Significant difference of total protein in the soleus muscle has not been observed in any of the experimental groups. Nevertheless, the groups which performed strength training (STC and STA) presented significant increase in the total amount of protein in the plantar muscle when compared to the other groups ($p < 0.05$). Furthermore, higher protein concentration can be observed in the group which trained strength and received AS compared to the group which only performed strength training (18.81 ± 0.99 vs. 17.54 ± 0.45; $p < 0.05$). The increase in the total protein concentrations was positively correlated with the muscle hypertrophy observed in these groups ($R^2 = 0.76$, $p < 0.05$) (figure 3C).

The endurance test to inclination used for evaluation of muscular strength on hinder legs, did not present significant differences between groups in the pre-experimental period. However, after 10 weeks of experimental treatment, strength increase was observed on the hinder legs of groups STC and STA compared to the other groups ($p < 0.05$, figure 4).

DISCUSSION

The main results found in the present study show that AS are efficient in increasing hypertrophy as well as muscle protein concentrations only when associated with strength training. These effects seem to be dependent on the characteristics of the muscle fibers and the AS effects are observed in the plantar muscle, characterized thus as being predominantly composed

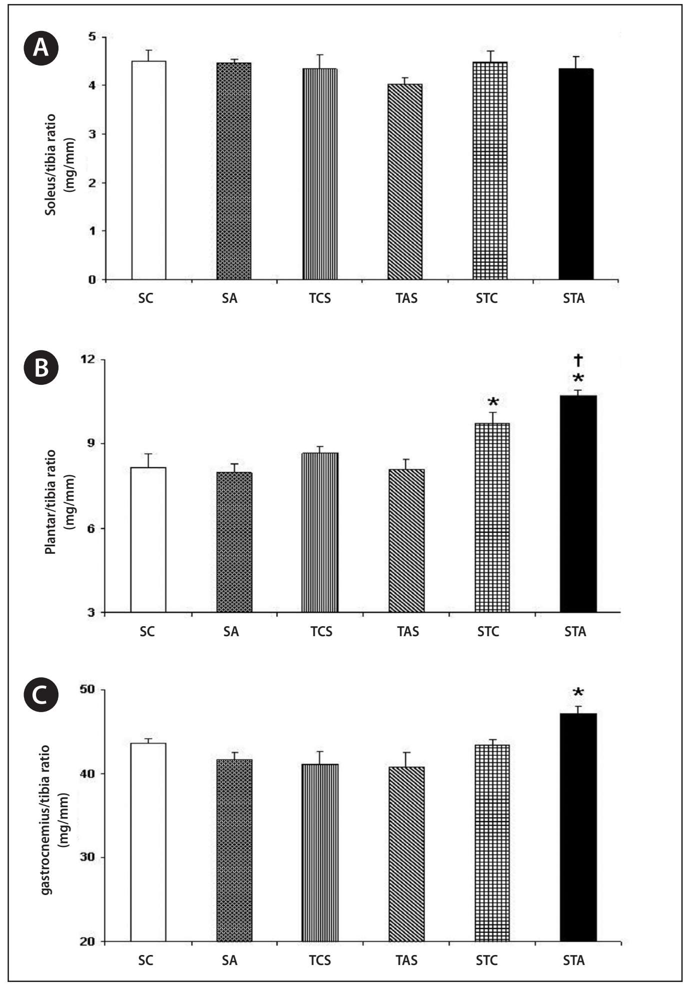

Figure 2. Skeletal muscular hypertrophy index. Mass of the soleus (2A), plantar (2B) and gastrocnemius (2C) muscles corrected by the tibia length in sedentary control (SC), sedentary anabolic (SA), trained control swimming (TCS), trained anabolic swimming (TAS), strength trained control (STC), strength trained anabolic (STA) animals. The data are represented as mean ± SE. *Significant difference concerning the remaining groups. †Significant difference concerning group STC. $p < 0.05$.

of glycolytic fibers. Strength training was efficient in increasing muscle strength on the animals' hinder legs; however, AS do not seem to have influence on this increase.

Although TAS group has presented higher tendency to lower mass gain when compared to the groups which performed strength training, significant difference has not been observed in body mass after the experimental period. The AS influence on body mass is still very argued in the literature. In previous investigations, Yu-Yahiro et al.[17], showed reduction in body mass in rats treated with AS, being it associated with appetite decrease in these animals. On the other hand, other authors have not observed differences in rats treated with AS[18]. In a study previously published by our team, results similar to the ones here were observed, in which the group treated with AS and treated with swimming presented lower body mass gain when compared to the other groups, which was explained by the reduction in intraperitoneal fat found, suggesting thus that the use of AS associated with physical aerobic exercise may favor lipolysis[19].

Concerning the effects of the AS use on the muscle hypertrophy, the results will mainly depend on the drugs used, dose and kind of training performed. When we speak about training, it is already well-established in the literature that the

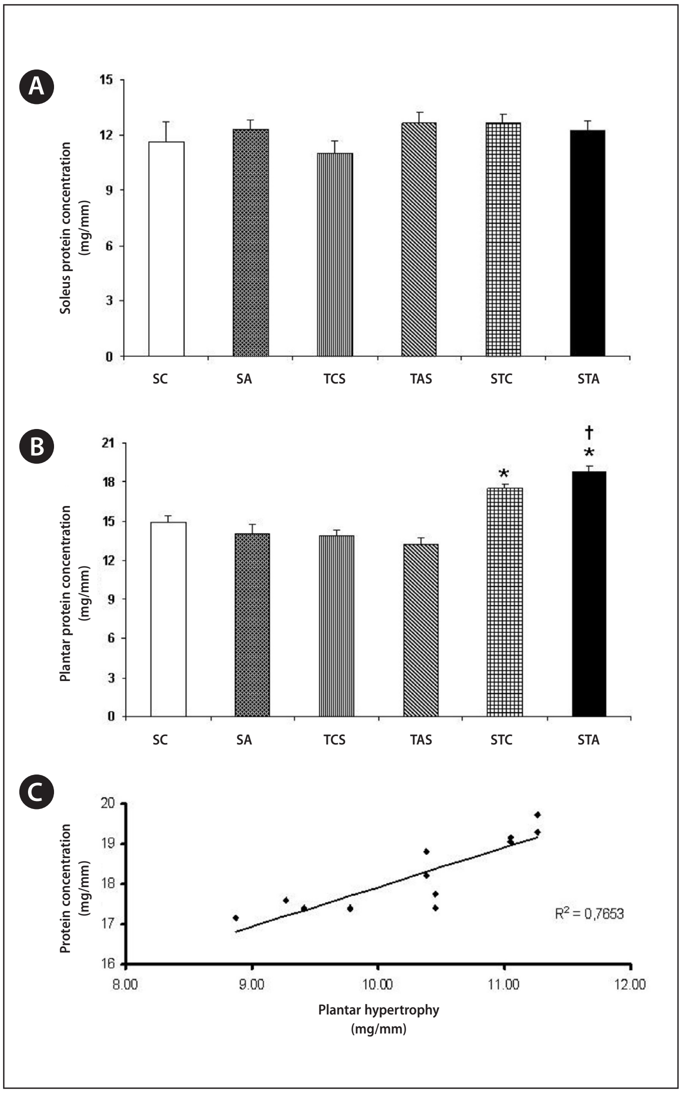

Figure 3. Muscular total protein concentration. Total protein concentration of the soleus (3A) and plantar (3B) muscles in sedentary control (SC), sedentary anabolic (SA), trained control swimming (TCS), trained anabolic swimming (TAS), strength trained control (STC), strength trained anabolic (STA) animals. Chart of correlation between protein concentration and hypertrophy in the plantar muscles in the strength trained groups (3C). Data are represented as mean ± SE. *Significant difference concerning the remaining groups, $p < 0.05$. †Significant difference concerning group STC. $p < 0.05$.

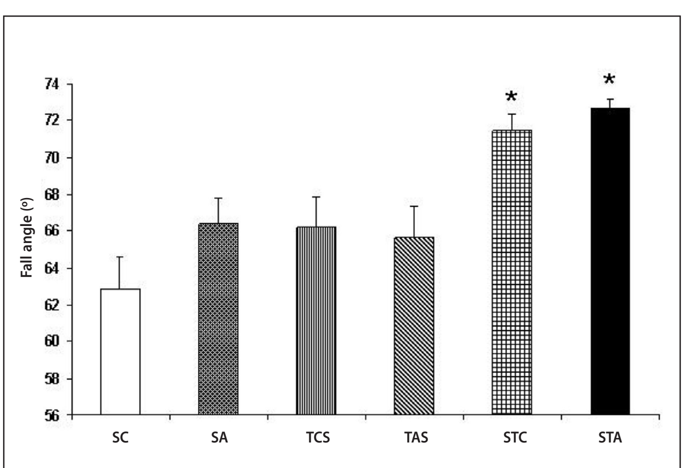

Figure 4. Muscular strength on hinder legs. Muscular strength in hinder legs assessed by the resistance to inclination test (fall angle) in sedentary control (SC), sedentary anabolic (SA), trained control swimming (TCS), trained anabolic swimming (TAS), strength trained control (STC), and strength trained anabolic (STA) animals. Data are presented as mean ± SE. *Significant difference concerning the remaining groups. $p < 0.05$.

most used method for promotion of muscle hypertrophy is the high intensity and short duration one, with progressive overload increase, such as weight lifting[14]. Due to the difficulty in studying muscle hypertrophy in humans with invasive methods and performance of biopsy as well as the impossibility to apply AS supraphysiological doses, we chose to use a strength training model proposed by Tamaki et al.[14], which developed a squatting apparatus which was effective in promoting muscle hypertrophy in rats by mechanisms similar to the ones found in humans.

Since AS are more related to modalities which involve muscle power and strength, the effects of its association with aerobic training on the muscle hypertrophy and strength are not much studied and are therefore little known. However, athletes of aerobic modalities[9], as well as individuals who practice physical activity as leisure[1] and perform aerobic activities during their training programs, make use of AS, which makes the understanding on the real effects of the AS important when associated with this kind of exercise.

In the present study, both groups which performed swimming training, even when treated with AS, did not present hypertrophy in any of the assessed muscles, showing thus that AS when associated with aerobic training is not efficient in inducing muscle hypertrophy, suggesting that the AS effects depend on the characteristics of the performed training.

When we analysed the groups which performed strength training, we did not observe hypertrophy in the soleus and gastrocnemius muscles; however, hypertrophy was found in the plantar muscle of these animals. When the AS was associated with strength training, hypertrophy in the gastrocnemius and plantar muscles was observed, being even more remarkable when compared to the group which only performed physical training, suggesting thus that AS when associated with strength training may lead to greater muscle hypertrophy.

Similar results were observed by Giorgi et al.[20], who showed in their study pronounced increase of the circumference of the rectus femoris muscle in the group which performed strength training associated with AS compared to the group which performed training and received placebo, which corroborates our results.

Interesting results observed in the present study suggest that AS may have greater effect on the muscles predominately composed of glycolytic muscle fibers, as in the case of the plantar muscle, since in the soleus muscle with characteristics predominantly oxidative, hypertrophy was not observed, which may explain hypertrophy in the gastrocnemius muscle observed in group STA, which may have occurred by the effects of the AS on the glycolytic fibers, being the gastrocnemius characterized as a mixed muscle, composed of glycolytic and oxidative fibers. Results found in the literature corroborate our results, in which it has been suggested that AS seem to act more on muscle fibers with glycolytic characteristics of fast contraction than on fibers with oxidative characteristics of slow contraction[21]. Kuipers et al.[21], in their study observed that eight weeks of AS administration were effective in increasing the fibers of the deltoids muscle of athletes who performed strength training, being this increase more evident in glycolytic fibers than in oxidative fibers. However, the same work also highlights that the use of AS can induce hypertrophy in oxidative fibers, especially if the drug administration occurs for a long time period.

The more remarkable hypertrophy observed in glycolytic fibers may be related to the higher concentration of androgenic receptors, which may be altered by many factors, including contractile activity, testosterone concentrations and type of muscle fiber[22]. Investigations have shown increase in the concentrations of the androgenic receptors induced by strength training in the extensor longus muscle of the fingers of rats; conversely, reduction of these receptors was observed in the soleus muscle, demonstrating the importance of the characteristics of the muscle fibers on the training effects[23], which may explain the results observed in the present study.

In a trial to understand the AS effects on the muscle hypertrophy, we assessed the total amount of protein in the plantar muscle, predominantly glycolytic, and soleus muscle, predominantly oxidative. Muscle hypertrophy may occur by increase of the transversal section area of the fiber or by incorporation of new fibers[24], where the AS have been shown due to their important role in this process, acting in the proliferation and differentiation of satellite cells as well as myonuclei[25], which ends up reflecting on higher protein concentration in these muscles.

Significant differences have not been observed in the total protein concentrations in the soleus muscle in any of the studied groups. Conversely, when the plantar muscles are assessed, increase of the protein concentrations is observed in both groups which performed strength training when compared to the other groups. Likewise the observed results concerning muscle hypertrophy, the increase of total protein in the group which trained strength and received AS was even more remarkable compared to the group which only trained strength. Muscle hypertrophy was positively correlated with increase of total protein concentration in the plantar muscle, which lead us to suggest that the hypertrophy observed in groups STC and STA was induced by the increase of the protein synthesis induced by training, which can be exacerbated by the AS association.

Similarly to the results observed in humans, the strength training method used in this study has been effective in triggering increase of the muscle protein synthesis[14]. Strength training is effective in increasing the protein synthesis and degradation; however, the degradation ends up occurring in a smaller scale than the synthesis, causing a positive protein balance, increasing the total protein concentrations[26]. On the other hand, AS, besides being associated with the increase of protein synthesis, also has an effect on the degradation, inhibiting the action of the glucocorticoid receptors, decreasing hence the protein catabolism, which causes balance even higher than in the strength training alone[24]. These data may explain the more remarkable increase of protein concentrations in the plantar muscle of the group trained in strength associated with the AS use.

Finally, it is important to highlight that muscle hypertrophy is not always followed by increase in muscle strength[15]. In order to evaluate the AS effects and its association to different physical training protocols on muscle strength, we used an endurance test to inclination according to Kennel et al.[15], which assesses strength on hinder legs.

The endurance test to inclination showed significant increase in both groups which performed strength training, not being observed additional effects induced by the AS association. These results show that AS did not influence on the increase of muscle strength induced by training in this study. The strength increase may be caused by neural adaptations, which occur in the beginning of the training program, and by muscular adaptations, muscle hypertrophy, which occurs subsequently[27]. Both groups which performed strength training presented hypertrophy in the plantar muscles, which may partly explain the strength increase in the hider legs observed. However, when the animals were treated with AS, the higher hypertrophy observed does not seem to have had influence on the muscle strength.

In investigations carried out with individuals who performed strength training and received AS, increase of 22% of muscle strength compared to the placebo group at the end of the experimental group I was observed, the authors conclude that the AS use may increase strength up to two times faster than training alone [20]. The muscle strength induced by AS may be related to the higher activation of the neural androgenic receptors, increasing the neurotransmitter concentrations, which may reflect on higher strength production[28].

The AS effects on the strength increase depend on the type of AS, training protocol and study methodology used. In the present study, when we assess the evolution of the repetition maximal tests (data not published), we observe significant increase of the lifted load in group STA compared to group STC. However, for muscle strength analysis, we chose to perform the endurance test to inclination so that the animals which performed swimming and strength training were assessed in the same conditions, excluding possible interference of training adaptations, which would occur if the maximal strength test was performed.

It was concluded by the results of this study that the AS administration alone or its association with aerobic endurance physical training, does not lead to increase of muscle mass and strength. The AS association with strength training resulted in greater muscle hypertrophy and protein concentrations in fibers with glycolytic characteristics. Therefore, the type of physical training, neuromuscular recruiting and characteristics of the muscle fibers seem to be important to the anabolic responses induced by the AS.

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