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ORIGINAL ARTICLE

Changes in Histological Structures of Soleus Muscle of the Male Rat in Response to Nandrolone Decanoate and Exercise

JAMSHED KHAN[1], NASEEMA AHMAD JAN[2], MUHAMMAD HANIF[3], AJMAL KHAN BAZAI[4], SHAZIA RAMZAN[5], USMANULLAH[6]
[1]*Assistant Professor Anatomy Department Lmc Loralai Medical College Loralai*
[2]*Assistant Professor Physiology Department Lmc Loralai Medical College Loralai*
[3]*Assist Professor Of Paediatrics Lmc Loralai Medical College Balochistan*
[3]*Assist Professor Anatomy Lmc Loralai Medical College Balochistan*
[2]*Imo Gynae Unit3 Bmch Quetta*
[6]*Associate Professor Anatomy Gkmc Gajo Khan Medical College Swabi*
Correspondence to: Usman Ullah, Email: drusmanmahsud74@gmail.com, Cell: 03467850599

ABSTRACT

Objective: Present study was conducted to investigate the histological changes produced by ND alone and in combination with exercise on soleus muscle.
Study Design: Randomized controlled trial
Place and Duration: Conducted at Animal House of Postgraduate Medical Institute (PGMI), Lahore during the year 2010 (From 20th Jan, 2010 to 13th April, 2010) for 12 weeks
Methodology: Forty male rats were divided equally by randomized control trial (RCT)i.eAI (control), AII (ND5mg/kg intramuscular twice weekly) AIII (Exercise/swimming) AIV (ND & Exercise) for 12 weeks at the animal house of PGMI, Lahore during the year 2010. Data was recorded on various microscopic parameters viz; internalization of nuclei, splitting in muscle fibers, rounded or angular muscle fibers and diameter of fibers for control and experimental groups.
Results: We found significant (P≤0.025, 0.037) differences among the groups receiving ND in comparison to control and exercise. Highly significant (P≤0.001) differences were observed in animals subjected to ND and exercise in comparison to control regarding internalization of nuclei. Muscle fiber splitting was observed in group AIII and AIV (P≤0.005) in comparison with control. Significant increase in diameter of muscle fiber was observed in group AIII (P≤0.013) and AIV (P≤0.001) in comparison to control. Similarly, minimum degenerative changes were observed showing angular or rounded fibers in group AIII and AIV in comparison to control.
Conclusion: The instant results suggest that significant hypertrophy was observed in animals subjected to ND and exercise.
Keywords: Soleus, splitting, diameter, internalization of nuclei

INTRODUCTION

The widespread abuse of anabolic androgens, the most powerful ergogenic substance, is a serious public health concern, especially among athletes and certain demographics (high school and college students) (Basaria, 2010). Certain populations, including as sportsmen, bodybuilders, and young people, frequently use AAS to increase muscle mass and strength (Trenton and Currier, 2005). Synthetic forms of testosterone, known as anabolic androgenic steroids (AAS), are used for their anabolic and androgenic effects in men (Kanayama et al., 2003). Males' testosterone is made predominantly in the testis, in cells called Leydig cells. Ovaries and adrenal glands only create a small quantity of testosterone (Kicman, 2008; Burger, 2002; Sun et al., 2009). These synthetic analogues are created to mitigate the drug's androgenic effects and boost its anabolic benefits. Testosterone, the active component, can go through a number of different metabolic pathways. When bound to the androgen receptor in the tissues of interest, it produces androgenic and anabolic actions (AR). (Evans, 2004). It has been established that androgen status interacts with muscle loading to impact muscle mass and biochemical indicators of muscle growth (McClung et al., 2004). (Lee, 2003).

The effects of anabolic androgenic steroids (AAS) on the body are widespread. Muscle growth, increased libido, and the emergence of secondary sexual traits in young men are all androgenic effects of AAS (Hoffmann, 2002).

It is immoral to provide excessive doses of AAS to humans, yet pharmacological research into the pathological effects of drug misuse requires dosing orders of magnitude above the norm (Takahashi et al., 2004). Abuse of AAS results in gynecomastia and testicular atrophy as well as hepatotoxicity, hypertension, cardiac enlargement, masculinization of children, behavioural abnormalities, and variations of blood lipid levels and coagulation factors. Unpredictable outbursts of violence and cardiac arrest (Evans, 2004; Fineschi et al., 2005; Klotz, 2006). Oral administration, intravenous injection, and transdermal application (through gel or patch) are all viable administration routes for AAS formulations (Evans, 2004).

One of the most often abused anabolic steroid (AAS) compounds is nandrolonedecanoate (ND) (Wouter et al., 2004). Available anabolic steroid drugs, ND included, are not exclusively anabolic but also have androgenic effects (Evans, 2004). Powdered ND is typically a very fine white to creamy white crystalline powder. Chloroform, ethanol, acetone, and vegetable oils are all solvents that work with it. For more information on nandrolone, see (www.drugs.com/pro/ nandrolone.html). It's injected straight into the muscle tissue. This was documented by Ramirez et al. in 2000.

The origins of AAS are steeped in ancient endocrinology and make for an intriguing tale. Castration was found to increase animal domestication by humans around 6,000 years ago (Dotson and Brown, 2007). When administered intravenously (i.v.), an extract from guinea pig and dog testicles boosted the French biologist Charles Edouard Brown-strength, Sequard's stamina, mental acuity, and urine arc length in 1889. (Basaria et al., 2001). The anabolic androgens were first isolated in the late 1930s. Scientists confirmed that androgens, particularly testosterone, may promote muscle growth in the 1940s, when AAS was used in supraphysiological levels in eugonadal persons for anabolic effect (Basaria et al., 2001; Kuhn, 2002; Yesalis and Bahrke, 2005; Handelsman, 2006). Although the use of anabolic agents in sports was outlawed in 1964 by the International Olympic Committee (IOC). Athletes in Germany began using anabolic steroids widely in the 1970s, and their use likely peaked around 1970. (Kuhn, 2002).

Before a big game, sportsmen often take anabolic steroids to give themselves an edge in training. When it comes to doping, the anti-doping agencies are doing everything they can to cut down on and, ideally, eliminate the use of AAS for unethical purposes (Fitch, 2008). In 1990, the Anabolic Steroid Control Act made it illegal to distribute or possess AAS for any purpose other than medicinal. Roughly one million Americans annually spend more than $100 million on illegal anabolic steroid purchases (Hall and Hall, 2005). To combat doping, the Canadian athlete Ben Johnson was disqualified from the Seoul Olympics for using the AAS. There have been other instances of similar incidents since this high-profile incident (Takahashi et al., 2004). We live in a culture that

condones doping in sports. Many different factors contribute to an atmosphere where cheating is seen as a necessary evil in the pursuit of victory. To wit: (Yesalis and Bahrke, 2005). The use of suprapharmacologic doses is suggested in a growing body of literature; nevertheless, the mechanism by which anabolic effects occur is unclear, and neither are the circumstances under which these effects occur. Muscle biopsies taken from ND-using weightlifters show an increase in both the total number of muscle fibres and the average fibre size compared to those of non-ND users. Both of these musculoskeletal events necessitate the activation of satellite cells (Kuhn, 2002). Protein levels of androgen receptors rise in elderly animals with ND when their skeletal muscles are functionally overworked (Lee, 2003). Slow, fatigue-resistant (type-I) fibres in the soleus respond better to ND therapy than rapid (type-II) fibres in the sedentary muscle, extensor digitorum longus (EDL) (Joumaaand Léoty, 2001). Another study, however, finds the opposite effect: that ND administration does not increase soleus muscle growth, even when combined with exercise. Therefore, there is conflicting evidence in the scientific literature on the link between ND use and increased muscle mass (Cunha et al., 2006). Possible causes include species-specific and intraspecific variation in the number of androgen receptors in skeletal muscle (Antonio, 1999). It was found in a study done by Johansen et al. (2006) that heamodialysis patients who engaged in both ND and resistance exercise saw increases in muscle mass. Another study found that there was no increase in the diameter of existing muscle lesions in the sedentary group after ND treatment (Filho et al., 2006). Broeder, et al (2000)

The current study was done to examine the histological alterations generated by ND alone and in combination with exercise on the soleus muscle of the male rat, in light of the importance of ND as the most frequently utilised as performance boosting drug.

MATERIAL AND METHODS

This randomized controlled trial was conducted at Animal House of Postgraduate Medical Institute (PGMI), Lahore during the year 2010 (From 20th Jan, 2010 to 13th April, 2010) for 12 weeks. Forty sexually mature albino rats weighing 180-240gms were utilized. Before starting the experiment, they were given two weeks of acclimatisation. All of their nutritional needs, including food and water, were met on a daily basis. Iron cages were used to maintain a constant temperature of 24 2°C and high standards of cleanliness, and the animals were subjected to strict light and dark schedules. Animals were purchased at the National Institute of Health (NIH) Animal House in Islamabad, where albino rats were also found. From Clinix Pharmacy on Jail road in Lahore, Pakistan, I purchased a vial of Nandrolonedecanoate (Deca-durabolin). The local market was shopped for water storage tanks.

To keep things fair, we used a random number system to split the animals into four groups: AI, AII, AIII, and AIV. There were ten critters in each of the two groups. A (control), A (ND treatment), A (exercise), and A (no treatment) (ND-treated & Exercise).

Animals in the AI group did not undergo the swimming or ND injection procedures. Group AII animals were measured and given ND intramuscularly at a human equivalent dose (HED) of 5mg/kg body weight twice weekly using a formulai. Dosage in humans (mg/kg) equals dose in animals (mg/kg) To convert kilometres travelled by humans to those travelled by animals, multiply by x. The Km factor for humans is 37, while that of rats is only 5.9. (Reagan-Shaw, 2007). The animals' dosages were modified weekly as their body weights changed.

The rats in group AIII swam for sixty sessions over the course of twelve weeks (Monday through Friday of each week) with two days off per week to relax. The water temperature in the tank was maintained at 300C 2oC, and there was enough of it such that the rats' tails never touched the bottom. The rats were selected to swim in a group rather than individually because they have a natural tendency to climb over each other when swimming (Matsakas et al., 2006). The training schedule called for five sessions per week, every morning between 8:00 and 11:00 am; this time frame was chosen to maximise the overload effect of swimming. The first week, you swam for 10, 20, 30, 40, and 50 minutes on Monday through Friday. They swam for 50 minutes each day (Monday and Tuesday) and 60 minutes on Wednesday and Thursday of the second week (Wednesday to Friday). The third week included shorter daily times of 60 minutes (on Monday and Tuesday) and longer times of 70 minutes (on Wednesday and Thursday) (Wednesday to Friday). Up to the sixth week, this schedule was maintained without change. Six weeks in, the daily allotment was raised to 90 minutes (Monday to Friday). From week seven through week twelve, this time was increased to a total of 120 minutes per day (Monday to Friday). Animals were dried off after their swim, given access to heat and food and water, and monitored closely. The animals in group AIV were put through the swimming test described above, and then given intramuscular injections of ND at a human equivalent dose of 5mg/kg body weight twice weekly. Heart perfusion with 0.9% saline was used to drain the blood at the same time a nick was made in the right atrium. This was continued until nearly transparent fluid was being expelled from the atrium (Paul et al., 1997). Carefully removing the left pelvic limbs allowed for the removal of a 2.0 cm x 0.8 cm central piece of muscle, which was subsequently handled with great precision to preserve the integrity of the muscle fibres as they remained longitudinally aligned in the larger axis of the fragment (Filho et al., 2006). Haematoxylin and eosin were used in the processing and staining of the tissues (Bancroft and Gamble, 2008).

RESULTS

Internalization of nuclei: The mean numbers of muscle fibers with internalized nuclei in group AI were 0.95±0.64, AII 2.1±1.08, AIII 1.1±0.66 and AIV 2.60±0.81. One-way ANOVA test showed statistically significant difference in mean number of muscle fibers with internalization of nuclei of groups AI, AII, AIII and AIV ($p<0.001$ Table 1). Post-Hoc Tukey·s test showed statistically significant difference in number of muscle fibers with internalization of nuclei between groups AI and AII ($p=0.025$), AI and AIV ($p<0.001$), AII and AIII ($p=0.037$) and AIII and AIV ($p<0.001$) showing that number of muscle fibers with internalization of nuclei increased in group AII and AIV. Statistically insignificant difference in mean number of muscle fibers with internalization of nuclei was observed between groups AI and AIII ($p=0.638$) and between groups AII and AIV ($p=0.244$) (Table 2).

Table 1: Showing the mean and standard deviation of number of muscle fibers with internalized nuclei among the control and experimental subgroups in group A (males)

Variable	Group A1 Mean±SD n=10	Group AII Mean±SD n=10	Group AIII Mean±SD n=10	Group AIV Mean±SD n=10	P-value
	Control	ND Treated	Exercise	ND treated + Exercise	
Muscle fibers with Internalized nuclei/200 fiber	0.95±0.64	2.1±1.07	1.1±0.66	2.60±0.81	0.001***

Muscle fibers with splitting: The mean numbers of muscle fibers with splitting in group AI were 1.15±0.47, AII 1.20±0.59, AIII 2.15±0.63 and AIV 2.55±0.93. One-way ANOVA test showed statistically significant difference in mean number of muscle fibers with splitting, in groups AI, AII, AIII and AIV ($p<0.001$, table 3). Post-Hoc Tukey·s test showed statistically significant difference in number of muscle fibers with splitting between groups AI and AIII ($p=0.005$), AI and AIV ($p=0.005$), AII and AIII ($p=0.018$) and AII

and AIV (p=0.006) showing that number of muscle fibers with splitting were increased in group AIII and AIV. Statistically insignificant difference in mean number of muscle fibers with splitting was observed between groups AI and AII (p=0.798) and between groups AIII and AIV (p=0.269) (Table 4).

Table 2: Multiple comparison of number of muscle fibers with internalized nuclei among the control and experimental groups.

(I) Group	(J) Group	Mean difference (I-J)	Std. Error	P-value
AI	AII	1.15	0.428	0.025*
	AIII	0.15	0.308	0.638
	AIV	1.65	0.259	0.001***
AII	AIII	1.00	0.408	0.037*
	AIV	0.50	0.401	0.244
AIII	AIV	1.50	0.307	0.001***

Table 3: Showing the mean and standard deviation of number of splitting muscle fibers and rounded or angular muscle fibers among the control and experimental subgroups in group A (males)

Variable	Group A1 Mean±SD n=10	Group AII Mean±SD n=10	Group AIII Mean±SD n=10	Group AIV Mean±SD n=10	P-value
	Control	ND Treated	Exercise	ND treated + Exercise	
Splitting	1.15±0.47	1.20±0.59	2.15±0.63	2.55±0.93	0.001
Rounded/ Angular	1.10±0.74	1.10±0.46	1.95±0.64	1.70±0.35	0.003

Table 4: Multiple comparison of number of splitting muscle fibers and rounded or angular muscle fibers among the control and experimental groups

(I) Group	(J) Group	Splitting muscle fibers			Rounded/angular muscle fibers		
		Mean difference (I-J)	Std. Error	P-value	Mean difference (I-J)	Std. Error	P-value
AI	AII	0.05	0.189	0.798	0.00	0.236	1.000
	AIII	1.00	0.269	0.005	0.85	0.269	0.012
	AIV	1.40	0.379	0.005	0.60	0.245	0.037
AII	AIII	0.95	0.329	0.018	0.85	0.279	0.014
	AIV	1.35	0.380	0.006	0.60	0.233	0.030
AIII	AIV	0.40	0.340	0.269	0.25	0.201	0.244

Table 5: Showing the mean and standard deviation of diameter of muscle fiber among the control and experimental subgroups in group A (males)

Variable	Group A1 Mean±SD n=10	Group AII Mean±SD n=10	Group AIII Mean±SD n=10	Group AIV Mean±SD n=10	P-value
	Control	ND Treated	Exercise	ND treated + Exercise	
Diameter (µm)	17.74±1.26	18.04±2.24	21.41±3.71	24.61±1.27	0.001

Muscle fibers with rounded or angular shapes: The mean numbers of muscle fibers with rounded or angular shapes in group AI were 1.10±0.74, AII 1.10±0.46, AIII 1.95±0.64 and AIV 1.70±0.35. One-way ANOVA test showed statistically significant difference in mean number of muscle fibers with rounded or angular shape of groups AI, AII, AIII and AIV (p=0.003, table 3). Post-Hoc Tukey's test showed statistically significant difference in number of muscle fibers with rounded or angular shapes between groups AI and AIII (p=0.012), AI and AIV (p=0.037), AII and AIII (p=0.014) and AII and AIV (p=0.030) showing that number of muscle fibers with rounded or angular shapes were increased in group AIII and AIV. Statistically insignificant difference in mean number of muscle fibers with rounded or angular shapes was observed between groups AI and AII (p=1.000) and between groups AIII and AIV (p=0.244) (Table 4).

Muscle fiber diameter: The mean diameter of muscle fibers in group AI were 17.74±1.26, AII 18.04±2.24, AIII 21.41±3.71 and AIV 24.61±1.27. One-way ANOVA test showed statistically significant difference was observed in mean diameter of muscle fibers of groups AI, AII, AIII and AIV (p<0.001, table 5). Post-Hoc Tukey's test showed statistically significant difference in mean diameter of muscle fibers between groups AI and AIII (p=0.013), AI and AIV (p<0.001), AII and AIII (p=0.026), AII and AIV (p<0.001) and between groups AIII and AIV (p=0.014) showing that mean diameter of muscle fibers were increased in group AIII and AIV. Statistically insignificant difference in mean diameter of muscle fibers was observed between groups AI and AII (p=0.727) (Table 6).

Table 6: Multiple comparison of diameter of muscle fiber among the control and experimental groups.

(I) Group	(J) Group	Mean difference (I-J)	Std. Error	P-value
AI	AII	0.300	0.834	0.727
	AIII	3.663	1.190	0.013
	AIV	6.864	0.509	0.001
AII	AIII	3.363	1.263	0.026
	AIV	6.564	0.854	0.001
AIII	AIV	3.201	1.047	0.014

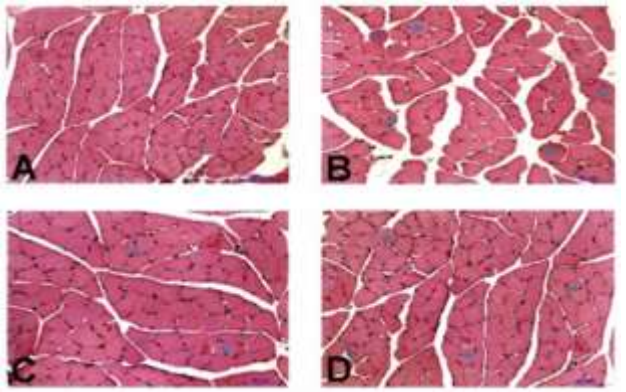

Fig.1: A Group AI showing transverse section of soleus muscle of control group. Fig.B Group AIIsubmitted to Nandrolone decanoate (ND).Fig.C Group AIII submitted to swimming. Fig. D Group AIVsubmitted to exercise and nandrolone decanoate showing internalization of nuclei (blue arrow) splitting (red arrow) angular and rounded with red and blue stars. H&E stain X100.

DISCUSSION

When people talk about "anabolic steroids," they're usually referring to testosterone derivatives that are employed for their anabolic effects in the clinic and in the athletic world. Although testosterone and its derivatives have been used for decades to build muscle in healthy men, their anabolic effects have been called into question. Most scientists came to the conclusion that

anabolic steroids do not increase muscle strength or size in adults with normal gonadal function, dismissing any good results as being skewed by the high expectations of athletes, poor study design, or sloppy data analysis. Athletes are firm in their belief that these medicines work, whereas scientists are firm in their belief that they do not (Kuhn, 2002). It is therefore still somewhat debatable whether or not the usage of anabolic androgenic steroids is beneficial in achieving the strength goals and the promotion of the increase in muscle mass. The media and surveys both indicate rising interest in these substances (Evans, 2004). With that in mind, the current research aimed to compare the benefits of ND to those of resistance exercise training (swimming) on the soleus muscle of male rats.

When comparing male animals from group AI to groups AII and AIV, we found a statistically significant increase in the proportion of muscle fibres with internalised nuclei. And when comparing groups AII and AIII, as well as groups AIII and AIV, it was likewise statistically significant.

These findings show that ND administration enhanced the proportion of muscle fibres with internalised nuclei in ND-treated rats. When comparing Group AI to Group AIII, it was clear that exercise alone had no influence on the percentage of muscle fibres that had internalised nuclei. Furthermore, nucleus internalisation in myofibers has been demonstrated in rats given ND at a weekly dose of 6mg/kg body weight by McClung et al. (2005). They prove that finding internalised nuclei in a mature muscle fibre indicates a healthy state for regeneration. Activation of satellite cells, which are responsible for postnatal growth, repair, and maintenance of skeletal muscle and are found in the subbasal lamina of mature skeletal muscle fibres, prompted the internalisation of nuclei in order to facilitate the prompt repair of degenerating muscle fibres (Chen and Goldhamer, 2003). Muscle injury triggers the satellite cells to transform into myoblasts, which then fuse with the broken myofibers to repair the damage and close the gap that has opened up (Baoge et al., 2012). Testosterone and its synthetic derivatives, such as ND, can control satellite cell activity (McClung et al., 2005).

When comparing male animals from group AI to those from groups AIII and AIV, there was a statistically significant increase in the proportion of muscle fibres that had split when looking at the AI group. If we compare group AII to groups AIII and AIV, we find that it is likewise statistically significant.

These findings show that, regardless of ND, exercise causes a rise in the number of muscle fibres that split. Muscle hypertrophy, as described by Goldberg et al. (1975), involves the gradual expansion of muscle fibres and the occasional longitudinal splitting that occurs after six days of intense exercise. Overload during 28 days, according to Jose and William (1994), doubled the percentage of muscle fibres that split from 0.3% to 5.25%. According to research by Nascimento et al. (2008), hypertrophied muscle fibres can split if under too much tension.

When comparing male animals from group AI to those from groups AIII and AIV, there was a statistically significant difference in the proportion of animals having round versus angular muscle fibres. Even when comparing group AII to groups AIII and AIV, there was a substantial difference.

These findings show that, whether or not ND was used, vigorous exercise increased the proportion of muscle fibres with a rounded or angular form in the experimental groups. Increasing either the external overload or the length of exercise increases the intensity of the workout. In the present study, the animals were exercised for longer periods of time, which is indicative of rigorous exercise. Our findings are consistent with those of Edgerton (1970), who used an external overload of 4% of the rats' body weight attached to the tip of their tail during a daily swimming session to induce angular and necrotic fibres in the soleus muscle of sedentary, moderately exercised, and heavily exercised rats. This overload was applied for 30 minutes at a time.

Since mechanical stress is one of the key variables that might cause muscular injury during exercise, and since strong physical workouts develop lesions in the skeletal muscles, and since high peaks of contractions are demanded, the animals are placed in a mechanical stress condition (Filho et al., 2005). Exercise-induced muscle injury is a well-documented phenomena that happens after an intense workout the body isn't used to. This was reported by Eston et al. Muscle fibres can undergo dramatic morphological changes during eccentric contractions, including edoema and swelling that can lead to a more rounded appearance of the fibres.

When comparing the male groups AI and AIII and AIV, a statistically significant difference was found in muscle fibre diameter. There was also a statistically significant difference when comparing groups AIII and AIV, and a similar pattern emerged when comparing groups AII and AIII.

These findings show that the diameter of muscle fibres was not raised by ND alone but was considerably increased by exercise alone and in combination with ND.

There was no discernible increase in muscle fibre diameter in ND-only-treated mice. Our findings are in line with those of Bisschop et al. (1997), who found that low dose (1.5mg/kg) ND increases the diameter of type I muscle fibres while high dose (7.5mg/kg) ND increases the diameter of type II muscle fibres. The dose of ND used in our study (5mg/kg) is close to high dose which caused hypertrophy of type II fibres. Type I fibres are primarily found in the soleus muscle (Filho et al., 2006). Significant hypertrophy in type II muscular fibres of the adult female rat diaphragm was also reported after ND administration (6.6mg/kg body weight; Lewis et al., 2002). To some extent, the amount of androgen receptors present in target tissues determines how anabolic and androgenic AAS behave in different species and in different muscle groups within the same species. Some muscles in rats respond more strongly to castration or androgen administration than others. In particular, the muscles involved in reproduction (the bulb cavernous muscle and the levatorani) are affected. In contrast, the plantar muscles, the extensor digitorum longus, and the soleus muscle are only mildly affected. Because of this, the lack of noticeable AAS-induced hypertrophy may be because the soleus muscle is not particularly sensitive (Cunha et al., 2006).

According to research by Filho et al. (2006), rats fed a hyperproteinic and hypercaloric diet show hypertrophy in immobilised muscles after taking anabolic steroids. A high-calorie, high-protein diet was not used in this investigation.

Our findings corroborated those of Goldberg et al. (1975), who found that 6 days of overload led to a considerable increase in muscle fibre diameter thanks to exercise. Rennie and Tipton (2000) found that both humans and rats benefited from an increase in mixed muscle proteins as a result of exercise, which explains the resulting enlargement in circumference. It has been shown in another study by Pikosky et al. (2006) that exercise boosts both protein synthesis and breakdown in the muscles, but that the benefits of the former far outweigh the latter, leading to a positive net muscle protein balance following exercise. Intensity, modality, and training status all play a role in how much and which way exercise effects muscle protein turnover. Our findings contrast those of Filho et al. (2006), who found that animals subjected to a combination of anabolic steroids and physical exercise did not increase their muscle mass relative to those subjected to physical training alone.

Muscle fibre diameter increased in both sexes thanks to the complementary effects of exercise and ND. Muscle biopsies in weightlifters were shown to have a higher number of muscle fibres and larger average fibre size in the trapezius muscle of AAS users compared to nonusers (Kuhn, 2002). Hemodialysis patients who took ND and exercise saw a rise in quadriceps muscle cross sectional area, as shown by Johanson et al. (2006). Since Lee et al. (2003) found that ND and overload for 7 days synergistically enhanced androgen receptors in aged rat soleus but not plantaris muscle, it is possible that changes in androgen receptor levels are responsible for this additive effect of ND and exercise. In contrast

to the effects shown in other muscles after functional overload, plantaris muscle showed no change after ND administration, suggesting that the interaction between anabolic steroids and exercise is phenotypic specific.

CONCLUSION

The results of this study show that both ND and exercise together significantly increase soleus muscle size, while exercise alone also significantly increases soleus muscle size.

Further studies on ND are recommended at receptor levels for better understanding of its anabolic effects in young individuals.

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