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ORIGINAL ARTICLE

Effects of Nandrolone Decanoate and Exercise on Histological Structures of Soleus Muscle of the Female Rat

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ABSTRACT

Nandrolone Decanoate (ND) as most frequently used as performance enhancing drug, the present study was conducted to investigate the histological changes produced by ND alone and in combination with exercise on soleus muscle. Forty female rats were divided equally by randomized control trial (RCT)i.e BI (control), BII (ND5mg/kg intramuscular twice weekly) BIII (Exercise/swimming) BIV (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 regarding internalization of nuclei revealed significant (P≤0.006, 0.013) differences among the groups receiving ND in comparison to control and exercise and highly significant (P≤0.001)differences were observed in animals subjected to ND and exercise in comparison to control. Splitting of muscle fiber was observed in group BIII and BIV (P≤0.001) in comparison with control. Significant increase in diameter of muscle fiber was observed in group BIII (P≤0.005) and BIV (P≤0.001) in comparison to control.Similarly, minimum degenerative changes were observed showing angular or rounded fibers in group BIII andBIV in comparison to control. The instant results suggest that significant hypertrophy was observed in animals subjected to ND and exercise.

Keywords: Soleus, splitting, diameter, internalization of nuclei, female

INTRODUCTION

Anabolic androgens remain the most effective and a widely abused ergogenic drug in sports and specific subsets of the population (high school and college students) continues to be a major public health issue (Basaria, 2010). The gain in muscle mass and strength by using AAS is relatively common among certain subpopulations, including athletes, bodybuilders, and young adults (Trenton and Currier, 2005). Anabolic androgenic steroids (AAS) are synthetic derivatives of the male sex hormone testosterone (Kanayama et al., 2003). Testosterone in males is produced primarily by Leydig cells of the testis. Very little amount of testosterone is produced by ovaries and the adrenal glands (Kicman, 2008; Burger, 2002; Sun et al., 2009). These derivatives are manufactured to increase its anabolic effects and reduce androgenic effects. The active ingredient, testosterone, has several possible metabolic fates. To exert its androgenic and anabolic effects, it binds in the target tissues to its androgen receptor (AR).(Evans, 2004). Skeletal muscle is a biological target of anabolic steroid action (McClung et al., 2004) and androgen status has been shown to interact with muscle loading to affect muscle mass and biological markers of muscle growth (Lee, 2003).

Anabolic androgenic steroids (AAS) administration has considerable effects on various organs. The androgenic effects of AAS are development of primary sexual characteristics in males, secondary sexual characteristics during puberty,muscle growth (Hoffmann, 2002).

In general, hundred times the normal dosing is necessary to pharmacologically investigate pathological effects of drug abuse but it is unethical to administer high doses of AAS in humans (Takahashi et al., 2004). AAS abuse causes hepatotoxicity, hypertension, myocardial hypertrophy, musculinization in children, behavioral changes, alterations of blood lipid levels and coagulation factors, gynecomastia and testicular atrophy. sudden cardiac death and uncontrolled violent behaviour (Evans, 2004; Fineschi et al., 2005; Klotz, 2006). AAS formulations may be used via oral and parenteral route as well as transdermally by topical gel or patch (Evans, 2004).

Nandrolonedecanoate (ND) is one of the most popular misused AAS (Wouter et al., 2004). Currently available AAS including ND is not purely anabolic but also possess androgenic properties (Evans, 2004). ND occurs as a fine, white to creamy white, crystalline powder. It is soluble in chloroform, in alcohol, in acetone, and in vegetable oils.(www.drugs.com/pro/ nandrolone .html). It is administered by deep intramuscular injection.(Ramirez et al., 2000).

The history of AAS is an interesting story that has its roots in prehistoric endocrinology. More than 6000 years ago, farmers noted enhanced domestication of animals after castration (Dotson and Brown, 2007).In 1889, French physiologist Charles Edouard Brown-Sequard announced that an extract of guinea pig and dog testicles given intravenous (i.v) results in an increase in physical power, enhancement in intellectual energy, relief of constipation, and lengthening of the arc of his urine (Basaria et al., 2001). In the late 1930s theanabolic androgens wereisolated. In 1940s, the use of supraphysiological doses of AAS in eugonadal individuals for anabolic benefit was practiced, and scientists confirmed that androgens, particularly testosterone, could facilitate muscle growth (Basaria et al., 2001; Kuhn, 2002; Yesalis and Bahrke, 2005; Handelsman, 2006). Although in 1964, the International OlympicCommittee (IOC) banned the use of anabolic agents in sports. In 1970, the use of anabolic steroids spread and probably reached its peak in the athletic competitions in Germany (Kuhn, 2002).The athletes use AAS for greater benefit during training before the competition. The Anti-doping authorities are trying their best to reduce and if possible eradicate cheating by misuse of AAS (Fitch, 2008). Non-medical distribution and possession of AAS was prohibited and declared as a crime in 1990 through the Anabolic Steroid Control Act. In the United States about one million people spent more than $100 million per year to purchase anabolic steroid from the black market (Hall and Hall, 2005). A Canadian athlete, Ben Johnson, who used the AAS, was disqualified from the Seoul Olympics in an effort to control doping. After this notorious incident, reports of such cases have been numerous (Takahashi et al., 2004).In our society there exists an atmosphere that promotes drug use by athletes. Various sources create an environment that promotes doing anything, including doping, to come first.(Yesalis and Bahrke, 2005). A rising literature proposes that use of suprapharmacologic doses can, certainly, be anabolic in certain situations; however, the mechanism by which anabolic effects occurs is unclear, the situations in which these anabolic effects occurs are also not clearly identified. Muscle biopsies of the weightlifters using ND have increase number of muscle fibers and greater average fiber size than those who are not using ND. Satellite cells activation is required for both of these processes

within the muscle (Kuhn, 2002).Androgen receptor protein concentration increases in functionally overloaded skeletal muscle in combination with ND in aged animals (Lee, 2003). ND treatment produces more potent effect in soleus which consists of slow, fatigue resistant (type-I) fibers than extensor digitorum longus (EDL) sedentary muscle consisting of fast (type-II) fibers (Joumaaand Léoty, 2001).Another conflicting report states that administration of ND does not promote hypertrophy of soleus muscle, not even when the use of ND was associated to physical training. So, the scientific information about the relation between the use of ND and muscle hypertrophy is controversial (Cunha et al., 2006). This may be due to variation in the numbers of androgen receptors in muscles within the same species and among different species (Antonio, 1999). A study conducted by Johansen et al. (2006)concluded that ND and resistance exercise both produces an increase in muscle size in patients who were on heamodialysis. Another experimental work suggests that ND treatment presented clear signs of muscular lesions in the sedentary group with no increase in diameter (Filho et al., 2006). Broeder et al. (2000)

Keeping in view the importance of ND as most frequently used as performance enhancing drug, the present study was conducted to investigate the histological changes produced by ND alone and in combination with exercise on soleus muscle of the female rat.

MATERIAL AND METHODS

This randomized controlled trial was conducted in 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. They were acclimatized for two weeks before starting the experiment. They were provided with daily food and water ad libitum during this period.They were kept in iron cages under optimum temperature (24 ±2°C) and hygienic conditions with observation of light and dark cycles.Albino rats were obtained from Animal House of National Institute of Health (NIH), Islamabad. Nandrolonedecanoate (Deca-durabolin) vial was purchased from Clinix Pharmacy, Jail road, Lahore, Pakistan. Water tanks were purchased from local market.

The animals were divided into four groups i.e. BI, BII, BIII and BIV by using random number table. Each group had 10 animals. The groups BI (control), BII (ND-treated), BIII (Exercise) and BIV(ND-treated & Exercise).

The animals in group BI were neither submitted to swimming nor to intramuscular injection of ND. The animals in group BII were weighed before giving an intramuscular injection of ND at a human equivalent dose (HED) of 5mg/kg body weight twice a week by using a formulai.eAnimal equivalent dose (mg/kg) = Human dose (mg/kg) X Human km factor/ Animal km factor. Human km factor is 37 and Rat Km Factor is 5.9 (Reagan-Shaw, 2007). Dose was adjusted on weekly basis according to weights of the animals.The animals in group BIII were submitted to sixty swimming sessions for twelve weeks (from Monday to Friday, each week) with a rest of two days per week in a tank containing water at 30^0C ± 2ºC in quantity enough to avoid that the rats could touch the bottom of the tank with tips of their tails. Group swimming was chosen because rats usually climb over each other, and in this way more vigorous muscle activity is achieved than when animals are allowed to swim alone (Matsakas et al., 2006).The training programme consisted of daily swimming sessions, 5 times per week, between 08:00 and 11:00 am, with overload produced by the increase of the training time. In the first week, the time of swimming was 10, 20, 30, 40, and 50 minutes from Monday to Friday. In the second week, they swam 50 minutes/day (Monday and Tuesday) and 60 minutes/day (Wednesday to Friday). In the third week, this time was of 60 minutes/day (Monday and Tuesday) and 70 minutes/day (Wednesday to Friday). This time was kept same until the sixth week. In the sixth week, the time was increased to 90 minutes per day (Monday to Friday). From the seventh to twelfth week, this time was increased to 120 minutes/day (Monday to Friday). After the swimming session the animals were toweled, kept warm, and given food and water ad libitum. The animals of group BIV were submitted to swimming as mentioned above, and weighed before giving an intramuscular injection of ND at a human equivalent dose of 5mg/kg body weight twice weekly.The blood was removed by heart perfusion with 0.9 % saline at the same time a nick was given in right atrium. This was carried until almost clear fluid was coming out of atrium (Paul et al., 1997). Left pelvic limbs were dissected out, and then a central fragment of 2.0 cm by 0.8 cm of the muscle was removed and carefully handled in order to keep the muscular fibers longitudinally placed in the bigger axis of the fragment(Filho et al., 2006). The tissues were processed and stained using Haematoxylin and Eosin (Bancroft and Gamble, 2008).

RESULTS

internalization of nuclei: The mean number of muscle fibers with internalized nuclei in group BI were 0.95±0.69, BII 2.05±0.83, BIII 1.05±0.60 and BIV 2.60±0.88. One way ANOVA test showed statistically significant difference in mean number of muscle fibers with internalization of nuclei of groups BI, BII, BIII and BIV ($p<0.001$). Post-Hoc Tukey's test showed statistically significant difference in number of muscle fibers with internalization of nuclei between groups BI and BII ($p=0.006$), BI and BIV ($p<0.001$), BII and BIII ($p=0.013$) and BIII and BIV ($p<0.001$) showing that number of muscle fibers with internalization of nuclei were increased in group BII and BIV. Statistically insignificant difference in number of muscle fibers with internalization of nuclei was observed between groups BI and BIII ($p=0.758$) and between groups BII and BIV ($p=0.154$) (Table 1).

Table 1: 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
BI	BII	1.10	0.306	0.006
	BIII	0.10	0.314	0.758
	BIV	1.65	0.366	0.001
BII	BIII	1.00	0.325	0.013
	BIV	0.55	0.353	0.154
BIII	BIV	1.55	0.229	0.001

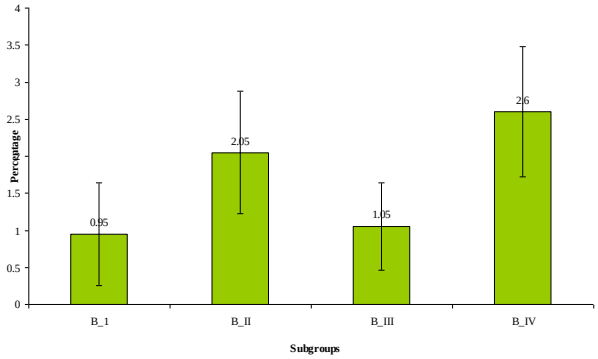

Fig.16: The mean and standard deviation of number of muscle fibers with internalized nuclei among the control and experimental groups (Female)

Muscle fibers with splitting: The mean numbers of muscle fibers with splitting in group BI were 0.90±0.66, BII 1.45±0.73, BIII 2.30±0.26 and BIV 2.50±0.88. One way ANOVA test showed statistically significant difference in mean number of muscle fibers with splitting of groups BI, BII, BIII and BIV ($p<0.001$). Post-Hoc Tukey's test showed statistically significant difference in number of muscle fibers with splitting between groups BI and BIII ($p<0.001$), BI and BIV ($p<0.001$), BII and BIII ($p=0.012$) and BII and BIV ($p=0.012$) showing that number of muscle fibers with splitting were

increased in group BIII and BIV. Statistically insignificant difference in mean number of muscle fibers with splitting was observed between groups BI and BII (p=0.162) and between groups BIII and BIV (p=0.522) (Table 2).

Table 2: Multiple comparison of number of splitting muscle fibers and rounded or angular muscle fibers among the control and experimental groups in B (Females)

(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
BI	BII	0.55	0.361	0.162	0.55	0.404	0.207
	BIII	1.40	0.208	0.001	1.00	0.307	0.010
	BIV	1.60	0.348	0.001	0.80	0.226	0.006
BII	BIII	0.85	0.269	0.012	0.45	0.189	0.041
	BIV	1.05	0.337	0.012	0.25	0.352	0.054
BIII	BIV	0.20	0.300	0.522	0.200	0.249	0.273

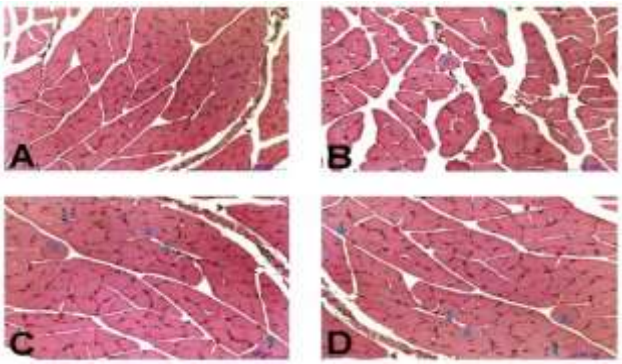

Fig. A Group BI showing transverse section of soleus muscle of control. Fig. B Group BII submitted to Nandrolone Decanoate (ND). Fig. C Group BIII submitted to swimming. Fig. D Group BIV submitted to swimming and nandrolone decanoate showing internalization of nuclei (blue arrow) splitting (red arrow) angular and rounded shape with red and blue stars respectively. H&E stain X 100.

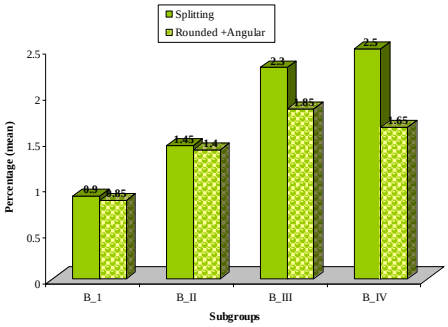

Fig.20: The mean value of splitting and rounded + angular msucle fibers among the control and experimental groups in females

Muscle fibers with rounded or angular shapes: The mean numbers of muscle fibers with rounded or angular shapes in group B (Female) were BI 0.85±0.71, BII 1.40±0.78, BIII 1.85±0.47 and BIV 1.65±0.47. One way ANOVA test showed statistically significant difference in mean number of muscle fibers with rounded or angular shapes of groups BI, BII, BIII and BIV (p=0.006). Post-Hoc Tukey's test showed statistically significant difference in number of muscle fibers with rounded or angular shapes was observed between groups BI and BIII (p=0.010), BI and BIV (p=0.006) and BII and BIII (p=0.041) showing that number of muscle fibers with rounded or angular shapes were increased in group BIII and BIV. Statistically insignificant difference in mean number of muscle fibers with rounded or angular shapes was observed between groups BI and BII (p=0.207), BII and BIV (p=0.495) and between group BIII and BIV (p=0.273) (Table 2).

Muscle fiber diameter: The mean diameters of muscle fibers in group B (Female) were BI 17.27±1.31, BII 17.51±2.67, BIII 20.57±3.18 and BIV 23.84±2.49. One way ANOVA test showed statistically significant difference in mean diameter of muscle fibers of groups BI, BII, BIII and BIV (p<0.001). Post-Hoc Tukey's test showed statistically significant difference in mean diameter of muscle fibers between groups BI and BIII (p=0.005), BI and BIV (p<0.001), BII and BIV (p<0.001) and between groups BIII and BIV (p=0.032) showing that mean diameter of muscle fibers were increased in group BIII and BIV. Statistically insignificant difference in mean diameter of muscle fibers was observed between groups BI and BII (p=0.816) and between groups BII and BIII (p=0.093) (Table 3).

Table 3: 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
BI	BII	1.10	0.306	0.006
	BIII	0.10	0.314	0.758
	BIV	1.65	0.366	0.001
BII	BIII	1.00	0.325	0.013
	BIV	0.55	0.353	0.154
BIII	BIV	1.55	0.229	0.001

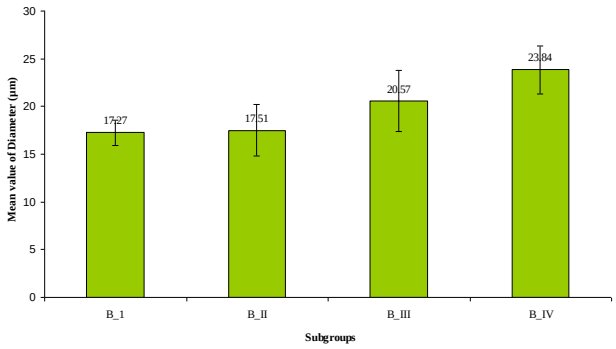

Fig.24: The mean and standard deviation of diameter of muscle fiber among the control and experimental groups (Female)

DISCUSSION

The term "anabolic steroids" refers to testosterone derivatives that are used both clinically and by athletes for their anabolic effects. However, for decades, researchers have queried the anabolic effects of testosterone and its derivatives in healthy men. Most researchers concluded that anabolic steroids do not improve muscle strength or size in people with normal gonadal function and have taken no notice of positive results as unduly influenced by positive expectations of athletes, inferior experimental design, or poor data analysis. There has been a great disagreement between the conviction of athletes that these drugs are effective and the conviction of scientists that they aren't (Kuhn, 2002). Therefore, the effectiveness of the strength achieved and the muscular mass increase promoted by the use of anabolic androgenic steroids is still relatively controversial. According to surveys and media

reports, the legal and illegal use of these drugs is gaining popularity (Evans, 2004). Therefore, the purpose of this study was to see the effects of ND and resistance exercise training (swimming) on soleus muscle of the male rat.

In animals, the numbers of muscle fibers with internalization of nuclei were statistically significant when group BI was compared with group BII and BIV. It was also significant while comparing group BII with BIII and group BIII with BIV.These results demonstrate that animals receiving ND increased the number of muscle fibers with internalized nuclei. Exercise alone had no effect on the number of muscle fibers with internalized nuclei as evident while comparing group BI with group BIII. McClung et al. (2005) has also observed internalization of nuclei in myofibers in animals receiving ND weekly at a dose of 6mg/kg body weight which demonstrate that identification of internalized nuclei in intact mature muscle fiber is associated with regeneration. This internalization of nuclei was due to activation of satellite cells which are responsible for post-natal growth, repair and maintenance of skeletal muscle located beneath the basal lamina of mature skeletal muscle fibers, for timely repair of degenerating muscle fibers (Chen and Goldhamer, 2003). The satellite cells after muscle injury eventually differentiate into myoblasts, fuse with the injured myofibers, the gap formed between the two ends of the injured myofiber is refilled (Baoge et al., 2012). The activity of satellite cells can be regulated by testosterone and its synthetic derivatives including ND (McClung et al., 2005).

In animals, the numbers of muscle fibers with splitting were statistically significant when group BI was compared with group BIII and BIV. It was also significant while comparing group BII with group BIII and BIV.

These results demonstrate that muscle fibers with splitting were increased in animals submitted to exercise, irrespective of ND. Goldberg et al. (1975) reported that overworked muscle involves an enlargement of muscle fibers and occasional longitudinal splitting after six days which is indicative of muscle hypertrophy. Jose and William (1994) were also of the opinion that overload for 28 days increased the percentage of splitting muscle fibers from 0.3% to 5.25%. Nascimento et al. (2008) reported that overload results in splitting due to stress on hypertrophied muscle fibers.

In animals, the numbers of muscle fibers with rounded or angular shapes were statistically significant when group BI was compared with group BIII and BIV. It was also significant while comparing group BII with group BIII and BIV.

These results demonstrate that intense exercise had increased the number of muscle fibers with rounded or angular shape in experimental groups irrespective of the use of ND. The intensity of exercise depends on two factors either by increasing external overload or duration of exercise. In the present study the animals were subjected to intense exercise by increasing the duration of exercise. Edgerton (1970)also observed angular and necrotic fibers in the soleus muscle but not the gastrocnemius and plantaris of sedentary, moderately exercised and heavily exercised rats which may be a characteristics of normal soleus of sedentary and chronically exercised rats, using an external overload 4% of their body weight attached to the tip of the tail during 30 minutes of daily swimming session consistent with our results.

Overloaded muscle had angular fibers, with a tendency to rounding because intense physical exercises produce lesions in the skeletal muscles, and high peaks of contractions are demanded, the animals are in mechanical stress situation which is one of the main factors that can cause muscular damage during exercise (Filho et al., 2005). Eston et al. (2003) also reported that exercise induced muscle damage is a well-documented phenomenon which results after a bout of unaccustomed exercise. Eccentric contractions can cause severe morphological changes in the muscle fibers, edema and swelling of muscle fibers, resulting in rounded shape of muscle fiber.

As for as the diameter of muscle fiber was concerned the group revealed statistically significant difference when group BI was compared with group BIII and BIV. It was also significant while comparing group BII with group BIII and BIV, furthermore a significant difference was observed while comparing group BIII with BIV.

These results demonstrate that ND alone had not increased the diameter of muscle fibers but exercise alone and in combination with ND had significantly increased the diameter of muscle fibers.

The muscle fiber diameter in animals receiving ND alone did not increase significantly. These results are consistent with the experimental work done by Bisschop et al. (1997) that low dose (1.5mg/kg) increases the diameter of type I muscle fibers where as high dose (7.5mg/kg) increases the diameter of type II muscle fibers, since the dose (5mg/kg) of ND used in our study is near to high dose which caused hypertrophy of type II fibers. The soleus muscle presents predominance of type I fibers (Filho et al., 2006). Lewis et al. (2002) also observed significant hypertrophy in type II muscle fibers of adult female rat's diaphragm when ND was given at a dose of 6.6mg/kg body weight. The actions of anabolic and androgenic AAS vary between species and also between different muscle groups of the same species, depending on the number of androgen receptors present in target tissues. In rats, the muscles related to reproduction (bulb cavernous muscle and levatorani) are more responsive to the administration of AAS, while the plantar muscles, extensor digitorum longus and soleus muscles have minor changes coming to castration or androgen administration. Thus, the absence of significant hypertrophic effects of AAS may be due to small responsiveness of the soleus muscle (Cunha et al., 2006).

Filho et al (2006) reported that anabolic steroids produce hypertrophy even in immobilized muscles of animals on hyperproteinic and hypercaloric diet. In the present study hyperproteinic and hypercaloric diet was not used.

In our study exercise had significantly increased the diameter of muscle fibers which is consistent with the Goldberg et al. (1975) reported that exercise is a well-known fact that resulting in hypertrophy of muscle fibers in soleus muscle after 6 days of overload. This increase in diameter was because of increased protein synthesis as reported by Rennie and Tipton (2000) that exercise increased mixed muscle proteins in both human beings and rats. Another study conducted by Pikosky et al. (2006) stated that exercise increased the rate of mixed muscle protein synthesis and breakdown but the magnitude and duration of the increase in synthesis exceed that of break down, resulting in an improved net muscle protein balance after exercise. The magnitude and direction by which exercise influences muscle protein turnover are affected by many factors including the intensity, mode and the training state of the individual. Filho et al. (2006) observed that there was no significant increase in muscular mass of animals submitted to combination of anabolic steroids and physical training when compared with those submitted to only physical training, which is in contradiction with our results.

Exercise and ND synergistically increased the diameter of muscle fibers regardless of sex. Kuhn (2002) reported that muscle biopsiesin weightlifters had greater number of muscle fibers and greater average fiber size in the trapezius muscle of AAS users than nonusers. Johanson et al. (2006) also observed the additive effects of ND and exercise, resulting in increased cross sectional area of quadriceps muscle in patients who were on hemodialysis. This synergistic effect of ND and exercise may be due to alterations in levels of androgen receptors as Lee et al. (2003) noted that ND and overload for 7 days synergistically increased androgen receptors in aged soleus but not in the plantaris muscle of the rat. This anabolic steroid and exercise interaction appears to be phenotype specific because plantaris muscle had no change after functional overload regardless of ND treatment.This study concludes that ND in combination with exercise results in significant hypertrophy of soleus muscle andexercise alone also induces significant hypertrophy of soleus muscle.

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

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