The Egyptian Journal of Hospital Medicine (2009) Vol., 34: 26– 35

Changes Produced by "Nandrolone" in the General Structure and Ultrastructure of Skeletal Muscle Fibers

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Abstract

Anabolic androgenic steroids (AAS) are compounds formed from testosterone or one of its derivatives, which are largely used by amateur and professional athletes to improve the athletic performance. Nandrolone is an anabolic steroid widely used in some sports in which muscle mass and strength are important factors.

The aim of this work: was to evaluate the effects of nandrolone on skeletal muscle general structure and ultrastructure.

Materials and methods: male albino rats were used in this study. They were divided into two groups, control and treated groups. The treated group received i.m. injections of Deca-Durabolin for 10 weeks. At the end of the experiment, the animals were anesthetized, and blood samples were obtained for determination of both lactate dehydrogenase (LD) and creatine kinase (CK) activity then soleus muscles were removed for light and electron microscopic studies.

Resuls: nandrolone treatment caused variation in size and shape of muscle fibers. Small atrophic angular fibers next to hypertrophic ones were seen. Muscle fibers were separated with increased connective tissue. Cross sectional area (CSA) of muscle fibers were slightly increased. EM study showed a dividing satellite cell.

The mean LD activity was increased and the mean CK activity was markedly increased.

In conclusion nandrolone produced hypertrophy of the skeletal muscles.

Keywords: AAS, Nandrolone, Skeletal Muscle

Introduction

Anabolic androgenic steroids are a class of steroid hormones related to the hormone testosterone. The illegal use of these drugs to increase muscle size and strength is widespread. They increase protein synthesis within cells, which results in the build up of cellular tissue (anabolism), especially in muscles (Evans, 2004).

Anabolic steroids were first isolated, identified and synthesized in the 1930s, and are now used therapeutically in medicine to stimulate bone growth and appetite, induce male puberty, and treat chronic wasting conditions, such as cancer and AIDS. Anabolic steroids also produce an increase in muscle mass and physical strength, and are consequently used in sport and bodybuilding to enhance strength or physique (Collins, 2002).

Serious health risks can be produced by long-term use or excessive doses of anabolic steroids. These effects include harmful changes in cholesterol levels (increased low-density lipoprotein and decreased high-density lipoprotein), acne, high blood pressure, liver damage, and dangerous changes in the structure of the left ventricle of the heart (Yesalis, 2000; Collins, 2002).

Men are increasingly concerned about the way they look. A moderately or extremely muscular body is widely accepted as an ideal body shape for young men (Mc Cabe and Ricciardelli, 2004). Young men who are dissatisfied with their body shape and musculature may be more likely to turn to bodybuilding dietary supplements and anabolic steroids to shape their bodies (Field *et al.*, 2005).

AAS have positive anabolic actions on the musculoskeletal system, influencing lean body mass, muscle size, strength, protein metabolism, bone metabolism, and collagen synthesis (Bhasin *et al.*, 2001 and Evans, 2004). Skeletal muscle is a primary target tissue for the anabolic effects of

AAS. Supraphysiological doses of testosterone administered to healthy young men over periods lasting 10-20 wk increase lean body mass, muscle size, and strength, with or without exercise (Bhasin *et al.*, 2001).

AAS have been used by athletes for decades to increase lean body mass, strength, and overall athletic performance. They are widely used by athletes involved in such sports as track and field and weight lifting. Nandrolone is the most abused anabolic steroids, and its use in doping is increasing (Fahey, 1998; Yesalis, 2000 and Baume *et al.*, 2004).

Nandrolone, or 19-nortestosterone, is an anabolic steroid initially introduced for the treatment of anemia, osteoporosis, and breast carcinoma (Basaria *et al.*, 2001). It is available in several pharmaceutical formulations as a 17_-hydroxy ester in an oily matrix or as a nandrolone salt (decanoate or sodium sulfate) in an aqueous solution. The pharmaceutical formulation most widely used is Deca-Durabolin (Kutscher *et al.*, 2002). Because of its anabolic properties, nandrolone accelerates muscle growth (Baume *et al,* 2004), increase lean body mass, strength, gaining weight, power, speed, endurance, aggressiveness and tolerance to stress and allows faster recovery between athletic performances (Mottram and George,2000).

Van Breda *et al.* (1993) reported that, the use of AAS is associated with improvement in physical performance by increasing the muscle energy reserves, such as the concentration of glycogen. Silva *et al.* (2002) reported that the administration of AAS is related to the improvement of athletic performance by increasing the muscle mass and resistance to the training of high intensity. Participation in sports may encourage the use of AAS.

Young adolescence sharing in school sports use AAS more frequently than their non athlete peers to enhance muscle mass and strength (Goldberg *et al.*, 2000), with a total of 28% of all athletes using AAS at some point in their lives (Yesalis and Bahrke, 1995).

Nandrolone decanuate has long extensive attraction from the scientific community, regulatory authorities and media. As a consequence, nandrolone decanuate has been implicated in relation to doping especially in sports as it is used to increase muscle mass as well as muscle strength and was banned by the international Olympic Committee Medical commotion in 1974 (Bagchus *et al.*, 2005).

Because of the wide, and irregular use of AAS in high doses by professional athletes and amateurs, with the objective of increasing muscle mass, improve performance and physical aesthetics body, so this work is designed to study the effect of the most widely used AAS nandrolone decanuate on the skeletal muscle structure.

Material and Methods

Deca-Durabolin (Organon ,Holand) 50mg/ml ampoules containing the active AAS nandrolone decanuate was used in the present study. This drug is a long –acting steroid ester that is hydrolyzed slowly to give a constant tissue level of steroid for >4wk.

Twelve adult male albino rats of 180 gm average weight were used. They were housed in plastic cages, at room temperature, fed commercial animal chew and given water ad libitum. They were divided to 2 equal groups: control group (C) and AAS-treated group (T), deep intramuscularly injected with 1mg/rat nandrolone decanoate every week for 10 weeks (Carson *et al.*, 2002).

At the end of the experiment, rats were anaesthetized by diethyl ether. Blood samples were obtained from the orbital sinuses for the estimation of both lactate dehydrogenase (LD) and creatine kinase (CK) activity. The soleus muscles of both legs were taken. Longitudinal and transverse sections from the midbelly of right soleus muscle were processed, serially cut and stained with H & E for light microscopic examination (Bancroft and Stevens, 1996).

The left soleus muscles were used for the electron microscopic examination. They were initially kept in a slightly stretched state, via pins passed through their tendons into dental wax, while they were fixed for 1 hr in 5% glutaraldehyde in 0.5 M sodium cacodylate buffer, pH 7.4. Very small pieces of muscle, measuring 2×1 mm, were then excised and left overnight in fresh fixative. They were then postfixed in 1%

osmiun tetroxide for 1 hr, dehydrated in graded alcohols, cleared in propylene oxide, and embedded in epoxy resin. During embedding the tissue blocks were oriented longitudinally (Hajibaghori ,1999).Blocks were sectioned and stained with uranyl acetate and examined with Jeol 100 S transmission electron microscope in Regional Mycology and Biotechnology center Al-Azhar University.

Muscle fiber cross sectional area

Skeletal muscle mass may be quantified by determining cross sectional area of the muscle.

For cross sectional area (CSA) analysis, 3 digital images of sections stained with H & E at X 20 magnification were taken and analyzed for soleus fiber CSA (μm^2). All fibers in the images were quantified unless the sarcolemma was not intact (Lee *et al.*, 2003).

Determination of lactate dehydrogenase activity

Lactate dehydrogenase (LD) is a hydrogen transfere enzyme that catalyzes the oxidation of L-lactate to pyruvate. The assay was done using spectrophotometer. The activity was expressed in international units (U/min/L).

Measurement of serum creatine kinase activity

Serum creatin kinase (CK) activity was used as a measure of muscle damage (Tamaki et al., 2001). CK activity was measured using automated analyzer (Roche/Hitachi system 747) and a standard CK NAC kit no. 1360108/ 1360116 (Boehrringer Mannheim, Germany). The activity was expressed in international units (IU/L).

Statistical analysis

Data were expressed as means± SD and were statistically analyzed using Student's t- test.

Results

Light microscopic examination of longitudinal sections of control group revealed that, normal muscle was made up of bundles of elongated, multinucleated, and striated myocyte cells (fibers) which were of variable length and diameter. Each fiber was closely invested by delicate connective tissue. The nuclei were elongated and lay along the periphery of the fiber just under the cell membrane. The cytoplasm is filled with numerous longitudinal myofibrils (fig. 1).

Transverse section through muscle fibers showed that they have polygonal outlines. Nuclei were in peripherical position and normal fascicular pattern was observed. The myofibrils appeared as fine dots and are distributed uniformly through the fiber (Fig. 2). The mean fiber CSA was $2403 \pm 167\ \mu m^2$ (histogram 1)

In electron microscopic examination, each muscle fiber is covered by a plasma membrane (the sarcolema) which is covered by basement membrane. The cytoplasm contains the myofibrils which push the elongated nuclei to peripheral position. The myonuclei appeared just beneath the plasma membrane. They were elongated and ovoid in shape, with the chromatin distributed towards the periphery. Cross striation of light and dark bands are present (fig.3).
The mean LD activity was 3161.5 ± 2.138 and the mean CK activity was 1372.13 ± 1.64 (table 1).

Light microscopic examination showed variation in size and shape (polymorphism) of muscle fibers in the AAS-treated group. Some polygonal fibers with peripheral nuclei and fascicular pattern were observed. Small atrophic angular fibers next to hypertrophic ones were seen (fig.4). Increased connective tissue separating the muscle fibers with spacing of muscular fibers was seen (fig.5). Nandrolone treatment was associated with an increase in CSA of muscle fibers. The mean CSA was $2927 \pm 183\ \mu m^2$ (histogram1).

In EM study a dividing satellite cell can be seen. It is a mononuclear myogenic stem cells located between the basal lamina and sarcolemma of the skeletal muscle fiber which is responsible for skeletal muscle growth, repair and regeneration. Once activated, divide and fuse to the muscle fibers where its nuclei become new myonuclei (fig.6).
The mean LD activity was 3586.63 ± 1.99 and the mean CK activity was 2872.0 ± 2.78 (table 1).

Fig. 1: showing longitudinal section of normal muscle. Striated fibers of variable length and diameter were seen. (H&EX200)

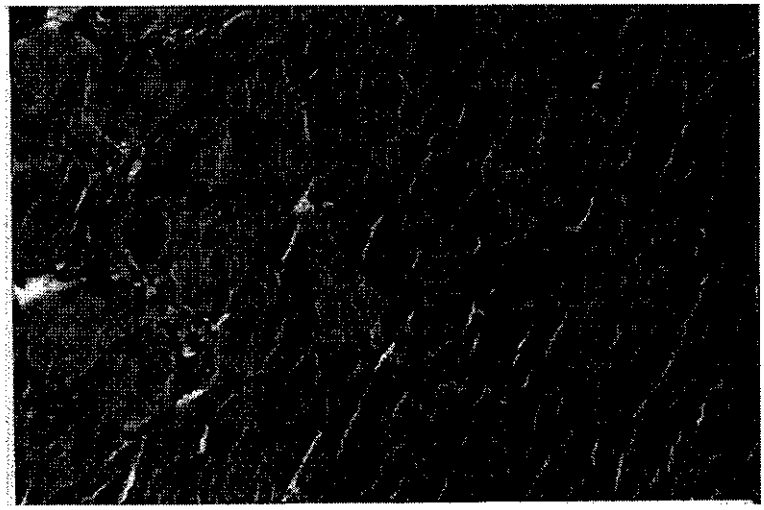


Fig.2: showing transverse section of normal soleus muscle. Notice muscular fibers with polygonal outlines and their nuclei are peripheral in position. (H&EX400).

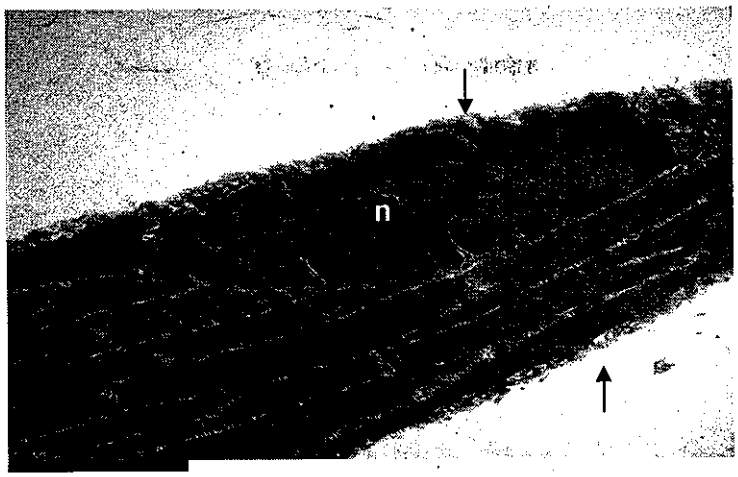


Fig.3: showing a myonucleus (n) located inside the sarcolemma () and not surrounded by an independent cytoplasm.
(EM,Control group, Uranyl acitate & lead citrateX 4000).

Fig. 4:showing spacing of muscular fibers with increased endomysium,
(Treated group H&EX200).

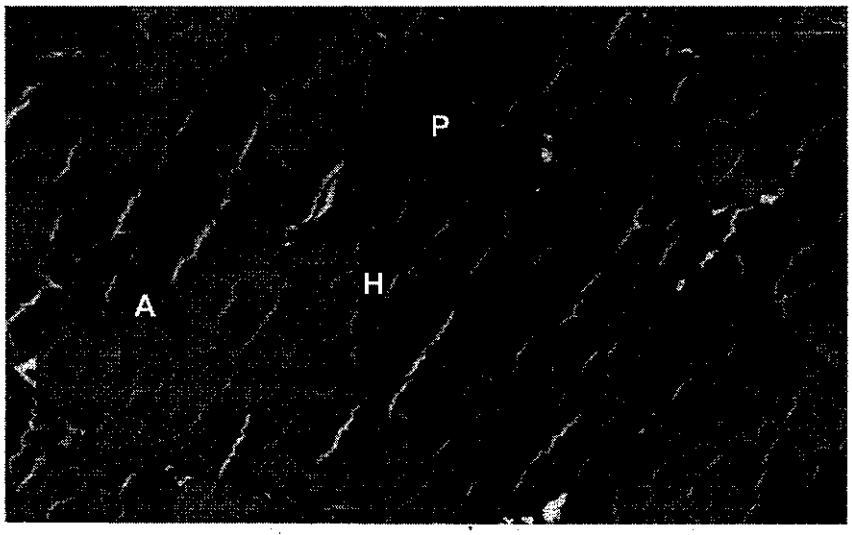


Fig.5: showing : polymorphic fiber (P), small atrophic and angular fiber (A) and hypertrophic (H) one. (Treated group H&EX400).

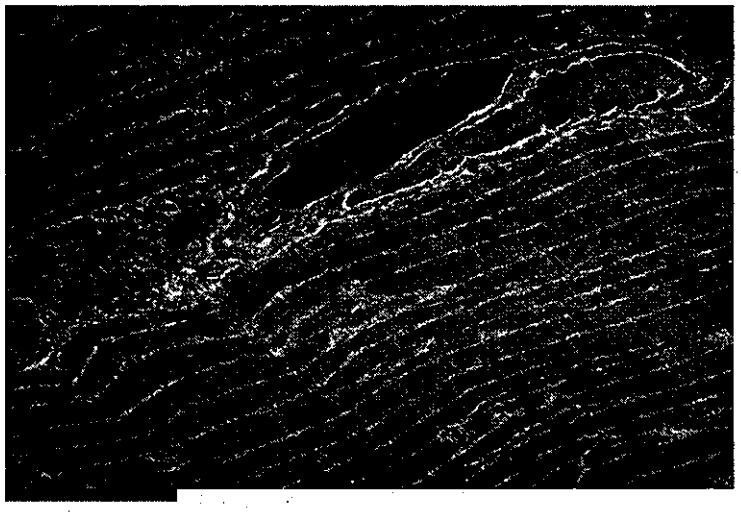
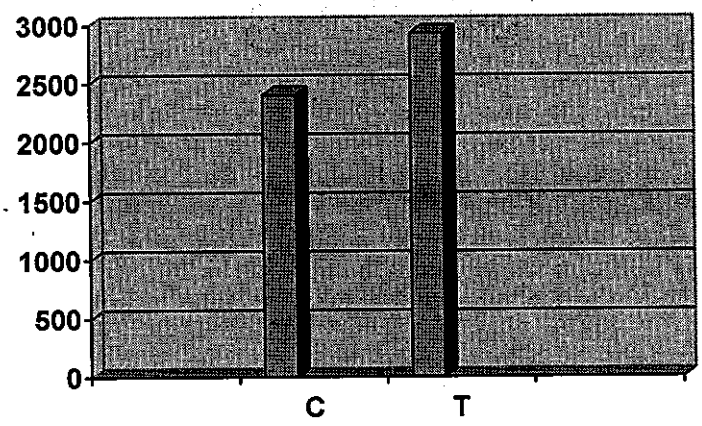


Fig.6: showing a dividing satellite cell separated from the muscle fiber by sarcolemma ().↑ (EM, Treated group, Uranyl acitate & lead citrateX 3000).



Histogram (1): showing mean CSA of soleus muscle in control(C) and treated group (T).

Table (1): showing LD and CK activity.

Groups	LD u/l	CK u/l
Control group	3161.5 ±2.138	1372.13±1.64
Treated group	3586.63 ±1.99	2872.0 ±2.78

Each value represents mean ±SD (P<0.05).

Discussion

AAS are synthetic derivatives of the male hormone testosterone. They can exert strong effects on the human body that may be beneficial for athletic performance (Fred and Harm,2004). Athletes and bodybuilders have recognized for several decades that the use of anabolic steroids can promote muscle growth and strength (Kicman, 2008).

The anabolic steroid nandrolone decanuate (Deca-durabolin: Organon) was used in this study because of its long biological half-life and previous studies demonstrating that, skeletal muscle is a biological target of AAS, and that they exert an anabolic effect in rat skeletal muscle (Griffin,1996 and Carson *et al.*,2002).

The results of the present study showed that nandrolone administration was associated with hypertrophy, polymorphism of skeletal muscle fibers and increased cross sectional area.

Harridge *et al.* (1996) proved that, skeletal muscle can adapt to the variable functional requirements through a quantitative mechanism based on changes in muscle mass and fiber size, and a qualitative mechanism based on a change in fiber type distribution.

Fahey, (1998) and Inoue *et al.* (1994) noticed that, the anabolic effect of AAS is mediated primarily by androgen receptors in skeletal muscle. They added that, androgen receptors regulate the transcription of target genes that may control the accumulation of DNA required for muscle growth. Fahey,(1998) added that, AAS may have an anti-catabolic effect and they may block the effects of hormones such as cortisol involved in tissue breakdown during and after exercise. So AAS may prevent tissue from breaking down following of an intense work-out. Kuhn, (2002) suggested that, AAS exert several complementary anabolic actions, including a psychoactive effect on the brain, glucocorticoid antagonism, and stimulation of the growth hormone (GH)-insulin-like growth factor-1 (IGF-1) axis.

The studies of Urban (1999) and Sinha-Hikim *et al.* (2002) proved that, testosterone has an anabolic effect that

stimulates the growth of muscle by increasing muscle mass, strength and endurance. Sinha-Hikim *et al.*(2002) added that testosterone-induced increase in muscle size and strength was due to a dose-dependent hypertrophy that resulted from an increase in cross-sectional area of muscle fibers and an increase in myonuclear number or increased differentiation of mesenchymal stem cells into the myogenic lineage. They also added that, increased levels of testosterone have been shown to increase protein synthesis, muscle strength, and lean body mass..

Bhasin, (2003) proved that, androgen-induced increase in muscle mass appeared to arise from muscle fiber hypertrophy rather than hyperplasia.

Sheffield-Moore *et al.* (1999) suggested that these morphometric effects are the result of a testosterone-induced increase in muscle protein synthesis.

In the present study, the muscle fibers were separated by increased connective tissue. Bagatell *et al.* (1996) and Parssinen *et al.* (2000) noticed that, AAS enhance collagen synthesis.

In present EM study of treated group a dividing satellite cell can be seen.

Satellite cells are mononuclear myogenic stem cells located between the basal lamina and plasmalemma of the skeletal muscle fiber. They are responsible for postnatal skeletal muscle growth, repair and regeneration. Once activated, SCs divideand fuse to the muscle fibers where their nuclei become new myonuclei (Gartner and Hiatt, 2007).

Fred, and Harm (2004) found that, AAS-induced increment of muscle tissue can be attributed to hypertrophy and the formation of new muscle fibres, in which key roles are played by satellite cells. Bhasin, (2003) proved that, Androgen induced increases in muscle fiber cross-sectional area were correlated with the increase in myonuclear number and satellite cell number. These findings suggest that androgen increases satellite cell number, resulting in muscle fiber hypertrophy and myonuclear number increase.

Taylor *et al.* (2002) reported that androgen increases satellite cell proliferation and decreases in satellite cell apoptosis.

In the present study LD activity was slightly increased. Lichtenbelt *et al.* (2004) reported that, increased LD activity indicates increase of the cellular metabolic activity in AAS treated group.

In the present study CK activity was markedly increased. Tamaki *et al.* (2001) reported that nandrolone increased creatine and creatinine excretion and creatine kinase.

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التغيرات التي تحدث فى التركيب العام و الدقيق للعضلات نتيجة تعاطى عقار "ناندرولون" (المنشط الإستيرويدى)

منى عبد الرحمن سالم- سمية يوسف مصطفى- إيمان بدوي عبد الفتاح .

أقسام الهستولوجي_ الطب الشرعي والسموم_ التشريح كلية طب بنات الأزهر

يعتبر عقار ناندرولون (ديكاديورابولين) المنشط أحد مركبات التستوستيرون أو أحد مشتقاته التي يستخدمها الرياضيين المحترفين و الهواه لتحسين ادائهم و يستخدم ناندرلون علي نطاق واسع في بعض الالعاب التي تعتمد علي القوة العضلية و الهدف من هذه الدراسة هو دراسة تأثير عقار ناندرلون المنشط علي التركيب الغام و الدقيق للعضلات.

استخدم في هذا البحث عدد إثناعشر من الجرذان البيضاء قسمت إلى مجموعتين: الأولى ضابطه و الثانية حقنت أسبوعيا بعقار ديكاديورابولين لمدة عشر اسابيع. و في نهاية التجربة خدرت الحيوانات وتم الحصول علي عينات الدم لتحديد نشاط كلا من لاكتات دهيدروجينز و كرياتين كيناز و أخذت عينات من عضلة الكورونا لدراسة التركيب العام و الدقيق.

تسبب ناندرولون في اختلاف شكل و حجم الالياف العضلية و زيادة مساحه مقطعها. كما ظهرت الالياف العضلية منفصلة عن بعضها وصاحب ذلك زيادة النسيج الضام بينهما

كما أوضحت دراسة مقاطع العضلات بالميكروسكوب الإلكتروني وجود انقسام في الخلايا الساتلية

كما زاد نشاط كل من لاكتات دهيدروجينز و كرياتين كيناز

و لقد أوضحت هذه الدراسة أن تعاطى عقار ناندرولون يسبب تضخم في الألياف العضلية الهيكلية و قد يكون سبب هذا التضخم ناتج عن إنقسام الخلايا الساتلية.