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Nandrolone decanoate and physical activity affect quadriceps in peripubertal rats

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ABSTRACT

Anabolic androgenic steroids (AASs) are synthetic analogs of testosterone often used by athletes to increase the skeletal muscle mass. Our goal was to examine the effects of physical activity and physical activity combined with supraphysiological doses of nandrolone on functional morphology of the quadriceps muscle. The study included 32 peripubertal Wistar rats, divided into 4 groups: control (T-N-), nandrolone (T-N+), physical activity (T+N-) and physical activity plus nandrolone (T+N+) groups. The T+N- and T+N+ group swam for 4 weeks, 1 h/day, 5 days/week. The T-N+ and T+N+ groups received nandolone decanoate (20 mg/kg b.w.) once *per* week, subcutaneously. Subsequently, the rats were sacrificed and muscle specimens were prepared for the processing. Tissue sections were histochemically and immunohistochemically stained, while the image analysis was used for quantification. Longitudinal diameter of quadriceps muscle cells was increased for 21% in T-N+, for 57% in T+N- and for 64% in T+N+ group while cross section muscle cell area was increased in T-N+ for 19%, in T+N- for 47% and in T+N+ group for 59%, compared to the control. Collagen fibers covered area was increased in T-N+ group for 36%, in T+N- for 109% and in T+N+ group for 159%, compared to the control. Erythrocyte depots were decreased in T-N+ group and increased in T+N- and T+N+ group, in comparison with T-N-. VEGF depots were increased in all treated groups. Chronic administration of supraphysiological doses of AASs alone or in combination with physical activity induces hypertrophy and significant changes in the quadriceps muscle tissue structure.

1. Introduction

The anabolic androgenic steroids (AASs) include testosterone and its numerous synthetic analogs and they are frequently misused by athletes with intention of enhancing their physical performance (Riezzo et al., 2011; Hassan and Kamal, 2013; Nikolic et al., 2015; Piacentino et al., 2015). Nandrolone decanoate (ND) is an anabolic steroid and like the other AASs, was developed in order to maximize anabolic effects (such as muscle growth, protein synthesis and erythropoiesis) and minimize the androgenic ones (Tylicki et al., 2007; Frankenfeld et al., 2014; Piacentino et al., 2015; Frati et al., 2015). These substances can be administered either orally, parenterally, transdermally (by topical gels or patches) or subcutaneously (by implantable pellets) (Evans, 2004; Frati et al., 2015). Most often, athletes use nandrolone in oral or injectable form (Kohler and Lambert, 2002).

Administered androgens that bind to the nuclear androgen receptors (AR), are translocated into the nucleus and regulate the transcription of the group of genes which ultimately leads to increased muscle protein synthesis and muscle growth (Fragkaki et al., 2009; Frati et al., 2015). Studies show that nuclear ARs can be up-regulated when exposed to AASs, while the number and density of ARs is increased by strength training (Evans, 2004). The androgens and training combined complement each other in the mechanisms of the ARs up-regulation, and the strength of these effects is largely determined by the exercise program, sex and age of the athlete as well as the type of AASs misuse (short- or long-term) (Vingren et al., 2010).

Despite the fact that the International Olympic Committee (IOC) prohibited the use of nandrolone in 1976 (Kohler and Lambert, 2002; Evans, 2004), AASs are being abused by competitive and recreational athletes. This is especially related to the bodybuilders and power lifters

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who aim to improve physical appearance or enhance performance by increasing muscle mass and strength (Bhasin et al., 1996; Nieschlag and Vorona, 2015; Sretenovic et al., 2016). In praxis, the doses that they use are up to 10 and even 100 times higher than the therapeutic dose (TD) (Yesalis and Bahrke, 1995; Frankenfeld et al., 2014). Consequences of such AASs abuse are various and depend on dosage, type, frequency and model of use (Riezzo et al., 2011), but extremely high doses can cause acute or chronic adverse side effects in almost all major tissues and organs (Fragkaki et al., 2009; Karila et al., 2004).

It is reported that administration of AASs in puberty leads to early epiphyseal closure and deficit of growth, testicular atrophy, infertility, impotence, prostate hypertrophy, as well as prostate and liver tumors (Rodrigues et al., 2017). In adults, usage of supraphysiological doses of testosterone in combination with strength training leads to marked growth in triceps and quadriceps diameter (Bhasin et al., 1996), but long-term abuse of the AASs can lead to structural and functional alteration of the liver and sometimes even hepatocellular adenoma can occur (Ferrari et al., 2013). Hypertrophy of the left ventricle with disproportional accumulation of extracellular collagen and interstitial fibrosis (Takahashi et al., 2004; Tanno et al., 2011; Hassan and Kamal, 2013; Franquni et al., 2013; Sretenovic et al., 2016) is also detected, as well as higher risk of prostate cancer, impotence and morphological changes in testis (Ferrari et al., 2013). Specific mechanisms of all of these adverse effects are not yet clearly understood, but certain studies suggest that misuse of the AASs is followed by premature death rate (due to suicide, acute myocardial infarction or hepatic coma) in power lifters, that is almost 4 times higher than in normal population (Pärssinen and Seppälä, 2002).

Effects of high doses of AASs on the skeletal muscle hypertrophy, as mentioned above, have been known for a long time, but there are only a few reports in the available literature with comprehensive histomorphometric results concerning the side-effects of AASs and/or training on the skeletal muscle cells morphology, connective tissue composition and the changes of vascularization of the muscle. Most of the reports suggest that chronic use of AASs increases muscle diameter and muscle cell diameter (Venken et al., 2007), but the collagen dynamics in striated muscle is quite poorly described in the literature. In fact, couple of studies in which this matter was addressed had opposite results. Karpakka et al. (1992) reported that AASs significantly decreased the concentration of hydroxyproline *e.g.* collagen synthesis, while Pärssinen et al. (2000) reported the decrease of degradation with increase of production of collagen type I, in this context. On the other hand, investigators agree that training alone increases collagen type I production in skeletal muscle tissue (Hjorth et al., 2015; Carroll et al., 2015; Martinez-Huenchullan et al., 2017). When it comes to the changes of the vascularization in striated muscle after chronic AASs misuse, it should be noted that Paschoal et al. (2009) reported that AASs inhibit the vascular endothelial growth factor (VEGF) mRNA expression and impair the angiogenesis. VEGF is a potent mitogen of endothelial cells and it has been shown that endurance training induces capillary growth within angiogenic response to exercise (Prior et al., 2003; Waters et al., 2004). Also, Shikatani et al. (2012) suggested that corticosterone inhibits migration and proliferation of endothelial cells thus limiting the angiogenesis.

Although the amount of data regarding the effects of AASs on muscle tissue rapidly increases, there are still controversies and some unknown aspects in this field. Moreover, the synergistic impact of AASs and the physical load has been poorly investigated. Having in mind that the abuse of AASs has become more frequent among young sportsmen in recent decades, the aim of our study was to identify the effects of supraphysiologycal doses of nandrolone decanoate (DECA DURABOLIN®, Organon, Holland) alone, physical activity alone or their combination on the functional morphology of the quadriceps muscle in peripubertal rats.

2. Material and methods

2.1. Experimental animals, study design and organ extraction

Study included 32 peripubertal (5 weeks old) male Wistar albino rats, weighing 150–200 g, that were bred at the Faculty of Medical Sciences, University of Kragujevac, Serbia. Rats were housed in collective cages (four rats *per* cage). The room temperature was kept at 23 ± 1 °C with 12:12h light and dark cycles. Food and water are provided *ad libitum*.

The rats were randomly divided into four groups:

1 T-N-, sedentary rats with no administration of nandrolone decanoate and physical activity (control group),
2 T-N+, sedentary rats with *s.c.* administration of nandrolone decanoate depot (DECA DURABOLIN®, Organon, Holland; 20 mg/kg b.w.) during a period of 4 weeks (nandrolone group),
3 T+N-, physically active rats (swimming 1 h/day, 5 days *per* week, for 4 weeks) with no administration of nandrolone decanoate (the group that had physical activity),
4 T+N+, physically active rats (swimming 1 h/day, 5 days *per* week) with *s.c.* administration of nandrolone decanoate depot (DECA DURABOLIN®, Organon, Holland; 20 mg/kg b.w.) during a period of 4 weeks (the group that had physical activity and was treated with nandrolone).

The initial and final body weights (BW) were measured. During the experiment, the swimming was performed in a glass pool, measuring 120 × 50 × 80 cm (length/width/height), in which the depth of the water was 60 cm. The first week represented the period of adaptation to swimming in which the rats started with 10 min of continuous swimming. Afterwards, swimming time was increased for 10 min every day, until 60 min mark was reached at the end of the fifth day (Nakao et al., 2000). After a period of adaptation, the expermiental period started, during which rats were swimming 1 h *per* day, 5 days *per* week, for four weeks. The swimming was performed every day at 9 a.m. Water temperature was 37 °C. Upon expiry of the experimental period, the rats were sacrificed. Precisely, in order to avoid the effect of acute swimming, the rats were sacrificed 48 h after the last swimming exercise. After short-term ketamine (Ketamin 10%, CP-PHARMA, Burgdof, Germany; 100 mg/kg b.w.) and xilazid (Xyla, Interchemie, Holland; 10 mg/kg b.w.) anesthesia, the animals were premedicated with heparin as an anticoagulant and sacrificed by cervical dislocation (Schedule 1 of the Animals/Scientific Procedures, Act 1986 UK). Their quadriceps muscles were surgically removed for the further examination. All research procedures were carried out in accordance with the European Council Directive (86/609/EEC) as well as the principles of Good Laboratory Practice (2004/9/EC, 2004/10/EC), and were approved by the Ethics Committee for the Welfare of Experimental Animals, Faculty of Medical Sciences University of Kragujevac, Serbia (No. 01-14606, from 7. XII 2016).

2.2. Tissue processing, histochemistry, immunohistochemistry and image analysis

The samples of rat quadriceps muscles were fixed in 4% formalin for 24 h, dehydrated in a series of increasing concentrations of ethanol (50%–100%), enlightened in xylol and embedded in Histowax® (Histolab Product AB, Göteborg, Sweden). Molded blocks of skeletal muscle were cut on a rotational microtome (RM 2125RT Leica Microsystems, Wetzlar, Germany) and 5 µm thick sections were prepared for further quantitative and qualitative histomorphological analyses. The sections were stained with standard H&E (enabling visualization of the tissue structures and some rougher orientation), Masson-Trichrome dye that enables collagen detection, as well as with Novelli staining and VEGF immunostaining, appropriate for the vascularization