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The effects of high doses of nandrolone decanoate and exercise on prostate microvasculature of adult and older rats

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ABSTRACT

Aims: The present study aimed to investigate the effects of the interaction between the abusive use of nandrolone decanoate (ND) and physical activity on the prostate structure of adult and older rats. We evaluated whether the use of ND, associated or not with physical exercise during the post-pubertal stage, interferes with the morphophysiology of the prostate.
Main methods: Fifty-six male Sprague–Dawley rats were divided into eight groups. The animals were treated for eight weeks and divided into sedentary and trained groups, with or without ND use. Four groups were sacrificed 48 h after the end of the eight week experiment (adult groups), and four other groups were sacrificed at 300 days of age (older groups). The prostate was collected and processed for stereological and histopathological analysis and for the expression of AQP1 and VEGF by the Western blotting technique.
Key findings: Both ND and physical activity altered the ventral prostate structure of the rats; the AQP1 and VEGF expression increased in young animals subjected to physical exercise.
Significance: Thus, it was concluded that the use of ND, associated or not with exercise during the post-pubertal stage, interferes with the morphophysiology of the prostate.

Introduction

Nandrolone decanoate (ND) is one of the most used anabolic androgenic steroids (AAS) among athletes [31]. AAS are synthetic derivatives of testosterone [6] and when administered, in combination with physical exercise, increase muscle mass and strength. The side effects of AAS can range from psychological effects to morphological changes in testes [25] and prostate [12]. Physical exercise and AAS may induce subclinical changes in the hypothalamus–pituitary–gonad axis [10] which affects the reproductive organs.

Some studies have shown that physical activity may be a protective factor and exert direct effects on a decreased risk of prostate cancer (PCa) [11]. It may benefit PCa [1] patients and be used as a preventive measure for diseases that affect the prostate [33]. In general, the prostatic stroma changes because of pathological alterations in the gland, for example, increased angiogenesis [36].

Angiogenesis is a complex process involving endothelial cell division, selective degradation of the vascular basal membrane and the surrounding extracellular matrix and migration of endothelial cells. VEGF (Vascular Endothelial Growth Factor) has the ability to affect all of these activities and has increased expression at sites where new blood vessels form. Thus, VEGF is recognized as an important factor in angiogenesis [9].

According to a review by Dvorak et al. [9], VEGF overexpression is associated with angiogenesis under different pathological and physiological conditions. In addition to VEGF, studies have shown that AQP1 is also involved in angiogenesis, particularly in wound healing, organ regeneration and possibly tumor propagation [28]. AQP1 belongs to a family of proteins expressed on the plasma membrane of cells involved in fluid transport. An increase of AQP1 stimulates endothelial cell migration and angiogenesis [5]. Moreover, this channel is an excellent marker for increased vascular microstructure in some tumors, including prostate cancer, which may be a consequence of angiogenesis and require the removal of tumor edema [23].

Hormonal manipulations can lead to disruption of normal prostate development which can lead to permanent effects on the normal processes of the development and function of the gland. Considering

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age as the determining factor in the development of prostate diseases and because this organ is dependent on the action of hormones, it is extremely relevant to understand whether the use of AAS, relative to physical exercise during the transition period between youth and adulthood, can alter the structure and microvascularization of the ventral prostate during the aging process.

Thus, the present study aimed to evaluate whether the use of AAS, relative to resistance physical exercise during the post-pubertal stage, interferes with the aging morphophysiology of the prostate. We aimed to investigate whether resistance physical exercise and/or AAS lead to changes in the general structure of the prostate and in the expression of AQP1 and VEGF in the prostates of adult and older animals.

Materials and methods

Animals and methods

Fifty-six male Sprague–Dawley rats, obtained from the Multidisciplinary Center for Biological Research of the State University of Campinas (Centro Multidisciplinar para Investigação Biológica da Universidade Estadual de Campinas — CEMIB/UNICAMP), were divided into 8 groups with 7 animals in each group. There were 4 adult groups, euthanized at 140–150 days old, and 4 older groups, euthanized at 300–310 days old. Fig. 1 shows the experimental design.

The SD_1, ED_1, SD_2 and ED_2 groups received intramuscular injections (i.m.) of nandrolone decanoate, Deca Durabolin® (10 mg/kg/week), twice a week (5 mg/kg/each time) for 7 weeks according to the protocol by Shokri et al. [31]. This dosage, according to Pope and Katz [27], corresponds to the improper dose administered by athletes, namely, 10 to 100 times higher than the therapeutic dose. The SV_1, EV_1, SV_2, and EV_2 groups received i.m. injections of a propylene glycol vehicle (0.2 ml/kg body weight) twice a week after training.

The animals in the exercise groups experienced physical training with jumping sessions in a PVC cylinder containing 38-cm deep water at 30 °C for 8 consecutive weeks. In the first week, the rats in the trained groups were adapted to water exercise. After adaptation, the animals performed 4 series of 10 jumps, spaced by 60-second intervals, 3 times per week with a progressive overload of 50 (second and third weeks), 60 (fourth and fifth weeks) and 70% (sixth, seventh and eighth weeks) body weight. The weight overload was placed on the ventral thorax of the animal using a brace.

The 7-week period established for the nandrolone decanoate injections and physical training was defined considering the time required for spermatogenesis, which varies from 48–56 days [21,31]. Thus, this period was considered sufficient for the possible effects on the hypothalamic–pituitary–gonadal axis. The rats were weighed weekly, and water and food consumption were measured daily.

All the experiments and surgical procedures in this study were approved by the Ethics Committee for Animal Use of the IBB/UNESP under protocol No. 385.

Material processing

Forty-eight hours [14] after the final jump session, the animals in the SV_1, SD_1, EV_1 and ED_1 groups were euthanized and composed the adult group. The rats in the SV_2, SD_2, EV_2 and ED_2 groups were euthanized at 300–310 days and composed the older group (Fig. 1). For euthanasia, the animals were placed in a CO_2 chamber and subsequently decapitated with a guillotine. The ventral prostate and epididymal fat were collected and weighed. The change in body weight (BW) for each group was defined as the difference between the final and initial body weights (BW final — BW initial).

Some of the ventral lobes of the prostate were fixed in 10% buffered formalin, embedded in paraplast (Paraplast Plus, St. Louis, MO, USA), cut into 4-μm thick sections and stained with hematoxylin–eosin. For the stereological analyses, two slides from 5 animals per group were used, from which 36 images/animal (200×, AxioVision Zeiss microscope) were obtained for 180 images per group. The stereological data were obtained using Weibel & Paumgartner [37] method of a 168-point grid over the images and analyzed with the ImageJ 1.47 software, Windows version (National Institutes of Health, USA). For the histopathological analyses, five slides per group were analyzed and photographed in an AxioVision (Zeiss) microscope. The pathologies were identified as reported by Shappell et al. [30].

Another section of the ventral prostate was flash frozen in liquid nitrogen and stored at −80 °C. The samples were homogenized in

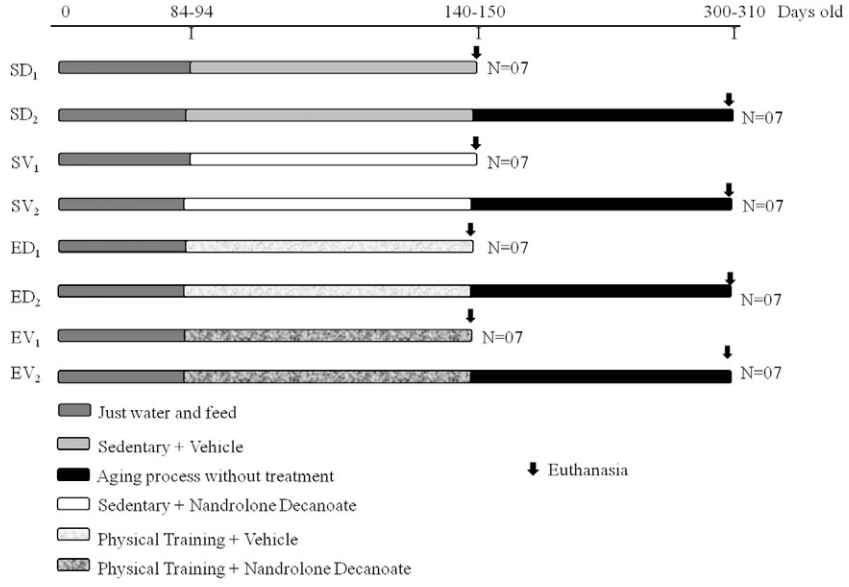

Fig. 1. Experimental design: SV1: Adult control group, sedentary without steroid use; SV2: Older control group, sedentary without steroid use; SD1: Adult group, sedentary with steroid use; SD2: Older group, sedentary with steroid use; EV1: Adult group, subjected to physical exercise without steroid use; EV2: Older group, subjected to physical exercise without steroid use; ED1: Adult group, subjected to physical exercise with steroid use; and ED2: Older group, subjected to physical exercise with steroid use.