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Anabolic androgenic steroids reverse the beneficial effect of exercise on tendon biomechanics: An experimental study

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ABSTRACT

Background: The effect of anabolic androgenic steroids on tendons has not yet been fully elucidated. Aim of the present study was the evaluation of the impact of anabolic androgenic steroids on the biomechanical and histological characteristics of Achilles tendons.
Methods: Twenty-four male Wistar rats were randomized into four groups with exercise and anabolic steroids (nandrolone decanoate) serving as variables. Protocol duration was 12 weeks. Following euthanasia, tendons' biomechanical properties were tested with the use of a modified clamping configuration. Histological examination with light and electron microscopy were also performed.
Results: In the group of anabolic steroids and exercise the lowest fracture stress values were observed, while in the exercise group the highest ones. Histological examination by light and electron microscopy revealed areas of collagen dysplasia and an increased epitendon in the groups receiving anabolic steroids and exercise.
Conclusions: These findings suggest that anabolic androgenic steroids reverse the beneficial effect of exercise, thus resulting in inferior maximal stress values.

1. Introduction

Anabolic Androgenic Steroids (AAS) are synthetic derivatives of testosterone. Since the discovery of the molecule of testosterone in 1935 [1], the use of androgenic compounds as ergogenic aids has attracted the attention not only of professional athletes but also of the greater masses [2–4]. This extensive AAS consumption has raised a lot of interest regarding their impact on the organism. Under this scope, many of the aspects of the systemic deleterious actions of AAS have been already elucidated [5,6]. As far as the impact of AAS on tendons is concerned, the first evidence on a relationship between AAS and tendon injuries came from case reports [7–11]. The studies that followed could be characterized to a certain extent contradicting, mainly due to methodological differences. The extrapolation of reliable data from biomechanical testing of small laboratory animals' tendons, without affecting their biomechanical characteristics is technically challenging. In order to overcome this difficulty the modified cryo-jaw technique for biomechanical testing has been proposed in the literature [12,13]. The aim of the present study was the determination of the effect of the AAS use on the biomechanical and histological parameters of the Achilles tendon (AT) in Wistar rats.

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2. Materials and methods

2.1. Laboratory animals

Twenty-four male 12-week-old Wistar rats were used for the needs of the present study (200–250 g). The animals were housed under conditions of controlled temperature (23 ± 2 °C) and humidity (60%). There was a 12 h light/dark circle and access to food and water was *ad libitum*. A positive vote was granted by the Ethics Committee of the local Veterinary Directorate and all procedures were conducted in accordance with ethical recommendation of the European Communities Council Directive of November 24, 1986 (86/609/EEC). Prior to study inclusion, all animals were kept for a week in the laboratory premises in order to minimize stress. The animals were randomized into four equal groups with AAS treatment and exercise serving as variables: (1) Control Group ($n = 6$): No intervention; (2) AAS group ($n = 6$): AAS administration/no exercise; (3) Exercise Group ($n = 6$): Exercise/no AAS; (4) AAS and Exercise Group ($n = 6$): Combination of AAS administration and exercise. The four groups did not differ in terms of size and weight ($p > 0.05$). At the end of the protocol statistically significant differences were seen among the groups ($p = 0.006$). The control group showed the highest weight (mean weight: 384 g, SD 37), followed by the exercise group (mean weight: 370 g, SD 32), the anabolic group (mean weight: 320 g, SD 43) and anabolic and exercise group: 314 g, SD 31).

2.2. AAS compound and administration protocol

Nandrolone Decanoate was administered intramuscularly (i.m.) at the gastrocnemius twice a week at a dosage of 5 mg/kg for a total period of 12 weeks. This is a mega-dose, equivalent to that taken by professional athletes and bodybuilders [2]. In the exercise and in the control group, the vehicle of Nandrolone Decanoate (sterilized sesame oil) was administered at the same site and at the same time points, as placebo. Injections were made by turns at both legs in order to minimize soft-tissue irritation.

2.3. Exercise protocol

The exercise protocol that also lasted for 12 weeks consisted of training in a custom-made motorized running wheel. A period of one week preceded the start of the exercise protocol, in order to ensure that the rats would get acquainted with the exercise procedure. The animals were exercised for 30 min each day, five days per week at a speed of 0.5 m/s. The constant speed did not permit a more intense exercise for anyone of the groups. The level of activity of the animals when not exercising was not quantified; however, no behavioral abnormalities were observed during the protocol between the different groups.

2.4. Biomechanical analysis

All animals were euthanized at 12 weeks under ether anesthesia. The ATs along with the gastrocnemius muscles and foot from both legs were harvested, as previously described [12]. An alternative clamping technique employing rapid freezing was developed for the biomechanical study of rat bone-Achilles tendon-muscle units. The clamping device consisted of two separate parts (Fig. 1). The first was a pincers-like clamp for bone fixation, while the second a modified cryo-jaw. The modified cryo-jaw comprised of a liquid nitrogen cup mounted on the inferior load frame and a structure that formed a cavity with adjustable dimensions. The latter was placed in the medial axis of the device at a higher level with respect to the liquid nitrogen cup. The muscle was placed and fixed inside the cavity, ensuring that the musculotendinous junction was a few millimeters distant to the cryo-jaw. Subsequently, the bone was fixed to the pincers-like clamp and mounted on the upper part of the load frame. For the present study a MTS MiniBionix 858 (MTS System Corp., Eden Prairie, MN, USA) load frame was utilized. Liquid nitrogen was poured inside the cup in order to achieve rapid freezing of the muscle. Afterwards, the mechanical testing was initiated with a displacement rate of 1 mm/min. The axial force exerted on the specimen was measured using a 500 N Instron Tensile Load Cell (Instron, Canton, MA, USA). The required liquid nitrogen volume was accurately determined in a series of pre-tests by placing a T-type thermocouple probe inside the tendon tissue at the musculotendinous transition area, providing real-time temperature measurements. Use of 75 cm^3 of liquid nitrogen resulted in a total temperature drop of the tendon of about 10 °C, while the muscle was frozen to a satisfactory degree in order to withstand loads until tendon failure, without any noticeable slippage. During the biomechanical testing, room temperature remained constant at 25 ± 2 °C.

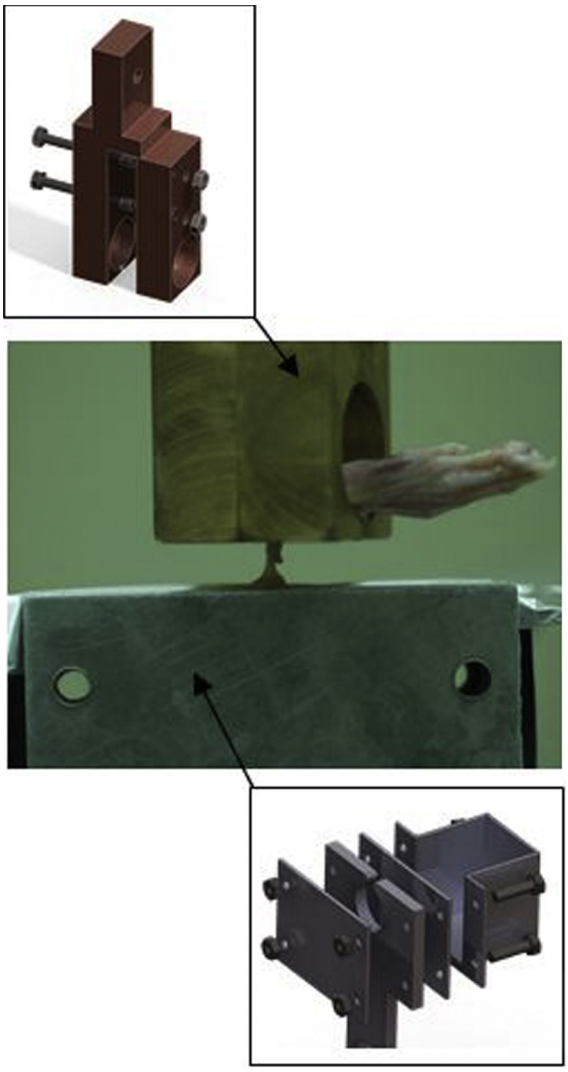

Fig. 1. The biomechanical testing device setting with the two separate parts of the clamping device are depicted.

2.5. Histological examination

ATs were subjected to light and electron microscopy examination. For the needs of light microscopy the tendons were fixed in 10% formalin at room temperature. Subsequently, the tissues were