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Use of anabolic androgenic steroids produces greater oxidative stress responses to resistance exercise in strength-trained men

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ABSTRACT

The aim of this study was to determine the effect of anabolic androgenic steroids (AAS) use on oxidative stress responses to a single session of resistance exercise in strength-trained men. Twenty-three strength trained men, with 11 self-reporting regular AAS use and 12 self-reporting never taking AAS (NAAS) volunteered to participate in this study. Blood draws were obtained pre and post resistance exercise in order to evaluate changes in oxidative stress biomarkers levels (i.e., 8-hydroxy-2-deoxyguanosine [8-OHdG], malondialdehyde [MDA], and nitric oxide [NO]), antioxidant defense systems (i.e., glutathione peroxidase [GPx] and catalase [CAT]), and glucose (GLU) levels. The AAS users had higher level of 8-OHdG (77.3 ± 17 vs. 57.7 ± 18.2 ng/mg), MDA (85.6 ± 17.8 vs. 52.3 ± 15.1 ng/mL), and GPx (9.1 ± 2.3 vs. 7.1 ± 1.3 mu/mL) compared to NAAS at pre exercise ($p < 0.05$). Both the experimental groups showed increases in 8-OHdG ($p = 0.001$), MDA ($p = 0.001$), GPx ($p = 0.001$), NO ($p = 0.04$), CAT ($p = 0.02$) and GLU ($p = 0.001$) concentrations after resistance exercise, and the AAS group indicated significant differences in 8-OHdG ($p = 0.02$) and MDA ($p = 0.05$) concentrations compared with NAAS users at post exercise. In conclusion, use of AAS is associated with alterations in immune function resulting in oxidative stress, and cell damage; however, high-intensity resistance exercise could increase greater oxidative stress biomarkers in strength-trained men.

1. Introduction

Anabolic androgenic steroids (AAS) are one of the most commonly used drugs among athletes, frequently in combination with resistance training to improve physical performance, lean body mass, muscle size, strength, protein metabolism, bone metabolism, and collagen synthesis [1–4]. Although amounts of some AAS may be useful in some pathological conditions, they are often abused by competing athletes desiring to build muscle mass and enhance physical performance, and by non-athletes aiming to improve their personal appearance [5].

The AAS abuse may be a serious problem in the United States, United Kingdom as well as other parts of the world [5–7], and during the past 2 decades the number of AAS users increased more than 2000% in the world [5,6,8]. It seems that AAS abuse have some adverse effects on organs and it is associated with detrimental side-effects on the hepatic [9–12], endocrine, and cardiovascular systems [11,12]. For example, previous studies have reported that AAS induced pathological left ventricular hypertrophy [13] with disproportional extracellular collagen accumulation and/or interstitial fibrosis [14]. For the immune function, researchers reported that use of AAS reduced immune cell number and function [15,16]. On the other hand, AAS have been shown to adversely influence lymphocyte differentiation and proliferation, antibody production, Natural Killer Cytotoxic activity and the production of certain cytokines, thereby altering the immune reaction [11–16].

Although previous authors reported side effects of AAS use on physiological variables, the influence of this drug on oxidative stress responses is unclear. Oxidative stress is a condition in which the delicate balance between free radicals production and their subsequent amelioration via the antioxidant defense system becomes skewed in favor of free radical expression [17–19]. Since longitudinal administration of AAS provides dysfunction of mitochondrial respiratory chain complexes (major source of ROS) and mono-oxygenase systems [20], it would be possible that these alterations were accompanied by an increased ROS generation resulting in oxidative stress and cell damage.

With review in literature, some studies have investigated the effects of AAS on oxidative stress responses in liver [21], brain [22], and cardiac [5] in rats and all of them reported negative effects of AAS. For instant, Camiletti-Moiron et al. [22] examined the effects of high-intensity exercise and AAS use on brain redox status in Wistar rats and

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Table 1
Participant characteristics.

Variable	Group	
	AAS (n = 11)	NAAS (n = 12)
Age (yr)	19.4 ± 2.3	20.8 ± 3.6
Height (cm)	179.5 ± 6.7	181.5 ± 5.5
Weight (kg)	88.1 ± 8.3	87.1 ± 9.4
BMI (kg/m^2)	27.3 ± 2.6	25.9 ± 3.6
Strength-training experience (yr)	3.2 ± 2.1	3.2 ± 2.1

AAS: Anabolic-androgenic steroids, NAAS: Non AAS.

found impairments in brain redox status after stranazolol administration.

Since previous studies have used rats to identify the effects of AAS on oxidative stress biomarkers, no information is available on the effects of AAS use on changes in oxidative stress biomarkers and antioxidant defense systems after resistance exercise (RE) in human. Therefore, the purpose of this investigation was to determine the influence of longitudinal use of AAS on changes in oxidative stress biomarkers and antioxidant defense systems after high-intensity RE in men. We hypothesized that the oxidative stress responses to RE is higher in strength-trained men who used AAS.

2. Methods

2.1. Participants

Twenty-three strength-trained men were recruited through local gyms, and personal contacts (Table 1). For inclusion in the study, participants had to have a minimum of 2 yr of resistance training experience with four to five training sessions per week and age between 18 and 25 yr. Exclusion criterions for the study was the presence of known respiratory, cardiovascular, or musculoskeletal disease. A specific inclusion criterion for the AAS-using group (AAS: n = 11) was a documented self-reported history of AAS use for at least 1 yr (Table 2). An inclusion criterion for the non-AAS (NAAS) group (n = 12) was self-reported history of never taking AAS. The participants in the AAS group had similar dose and type of steroids through the 1 yr ago and all of them were during the methenolone enanthat use. Before taking part in the study, the participants were notified about the potential risks involved and gave their written consent. This study was approved by the university's human research ethics committee.

2.2. Study design

A cross-sectional design was used for this study. The participants recruited to the laboratory four times. Initially, participants completed self-report questionnaires related to general health, training status, and history of AAS use. During this session, height (Seca 222, Terre Haute, IN) and weight (Tanita, BC-418MA, Tokyo, Japan) for each athlete was measured and then the participants performed one repetition of maximum (1RM) test for the bench press and lat-pull down exercise. On day two, 1RM of arm curl and back squat were assessed. On day three, 1RM of knee extension and knee flexion were measured. On day four, the participants performed a single session of high-intensity RE. Pre and post RE blood samples were obtained to analyze changes in oxidative stress biomarkers and antioxidant defense systems. All tests and RE sessions were conducted on the same time of day (i.e., morning, 9:00–12:00 A.M.), under similar weather and laboratory conditions. The subjects were advised to avoid any vigorous activities on the day before the test and during the study.

Table 2
Anabolic-androgenic steroids used by participants.

	Drug	Number of Times Reported
Testosterone cypionate	(Depo testosterone)	3
Nandrolone phenpropionate	(Durabolin)	1
Methandrostenolone	(Dianabol)	2
Oxandrolone	(Anavar)	1
Methenolone enanthate	(Depo premabalon)	3

2.3. Muscle strength assessment

For prescription of RE, the 1RM of each exercise (i.e., bench press, lat-pull down, arm curl, back squat, knee extension and knee flexion) was determined. One RM testing was performed using procedures described in detail elsewhere [23]. Spotters were present to provide verbal encouragement and ensure safety.

2.4. Resistance exercise program

A trained researcher supervised exercise session carefully so that exercise prescriptions were correctly administered during session (i.e., rest and or velocity of movement). Before the exercise session, the participants performed a general warm-up consisting of 10 min running, 5 min dynamic stretching, and 5 min of ballistic movements to increase blood circulation and temperature of the involved muscle groups. Also, a specific warm-up of 1 set of 5 repetitions at 50–60% of 1RM was performed before an exercise. The participants performed high-intensity RE including 5 sets with 80% of 1RM to failure for the bench press, lat-pull down, arm curl, back squat, knee extension and knee flexion, respectively. The rest period between exercises and sets were 60 and 30 s, respectively.

2.5. Blood sampling and analysis

At before (pre exercise) and after (at least 1 min post exercise) RE, venous blood samples (5 mL) were collected from a forearm vein. Then, the blood was collected into EDTA-containing tubes and centrifuged immediately at 1370 × *g* for 10 min at 4 °C, and the plasma was collected. The blood was allowed to clot at room temperature for 30-min and centrifuged at 1500 × *g* for 10 min. The serum layer was removed and frozen at −20° C in multiple aliquots for further analyses.

2.6. Markers of oxidative stress

Oxidative DNA damage, 8-OHdG excretion, was measured by using the enzyme-linked immunosorbent assay (ELISA). The assay was carried out in duplicate using the manufacturer's instructions (New 8-OHdG Check, ELISA, Japan Institute for the Control of Aging; Catalog No: KO G-200S/E). Plasma MDA was measured by using a spectrophotometric assay for MDA. The assay was carried out in duplicate; intraassay coefficients of variances were 3.4%, and detection limit was 0.0088 μM, using themanufacturer's instructions (Bioxytech®MDA-586TM, Spectrophotometric Assay for MDA. Catalog Number 21044; OXIS Research, Portland, OR, USA). NO was measured using ELISA kit (Nitrate/Nitrite Colorimetric Assay Kit, Cayman Chemical, and Ann Arbor, Catalog: 780001 USA) and manufacturer's instructions.

2.7. Markers of defense system

GPx were analysed by using a Glutathione Peroxidase Assay Kit (catalog no. 703102; Cayman Chemical Co., Ann Arbor, Michigan, USA) according to the manufacturer's instruction. The CAT assay kit was obtained from Cayman Chemical (catalog no. 707002, Cayman Chemical Co., Ann Arbor, Michigan, USA) and the assays were conducted according to their instructions. The kit consists of assay buffer, sample buffer, formaldehyde standard, catalase, potassium hydroxide, methanol, hydrogen peroxide, purpald, and potassium periodate. After