

Effects of longitudinal abuse of anabolic steroids on liver enzymes activity and lipid profiles of male bodybuilders

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Summary. The purpose of this study was to investigate the effects of anabolic steroids (AS) abuse on liver enzymes activity and lipid profiles in male bodybuilders. 40 well-trained bodybuilders, with 20 self-reporting regular AS use and 20 self-reporting never taking AS (NAS) were recruited for this study. Participants reported to the laboratory for blood sampling to assess liver enzymes activity (Aspartate transaminase [AST], Alanine aminotransferase [ALT] and Alkaline phosphatase [AP]), lipid profiles and fasting blood sugar (FBS). Moreover, maximal strength and muscle volume were measured. The results indicated that AS users had higher strength in the bench press (113 ± 11.8 vs. 93.7 ± 13.3 kg) and leg press (329.5 ± 40.4 vs. 248.5 ± 41.0 kg), muscle volume (arm, 41.2 ± 3.5 vs. 35.1 ± 4.2 cm and thigh, 60.6 ± 6.4 vs. 53.7 ± 5.6 cm), LDL (179.2 ± 34.1 vs. 155.8 ± 37.7 mg/dL), TG (166.5 ± 74.4 vs. 126.9 ± 48.2 mg/dL), TC (253.2 ± 59.6 vs. 143.5 ± 48.0 mg/dL), AST (53.2 ± 14.3 vs. 34.5 ± 11.11 IU/L) and ALT (53.5 ± 15.1 vs. 33.3 ± 7.8 IU/L) ($p < 0.05$). However, NAS users indicated higher HDL (43.5 ± 15.2 vs. 30.7 ± 10.0 mg/dL) and AP (82.7 ± 30.6 vs. 75.6 ± 30.1 IU/L) ($p < 0.05$) in comparison to AS users. In conclusion, AS abuse is associated with alterations in liver enzymes function and lipid profiles that, represent an increased risk profile in athletes who used AS.

Key words: anabolic steroids, liver, strength, lipid, bodybuilding.

Introduction

Anabolic steroids (AS) are one of the most commonly used drugs among athletes, especially in strength-trained men, to improve muscular performance, muscle size and increase strength. (1,2). Outside of some physiological advantage of AS, abuse of this drug has become a serious problem in the United States, United Kingdom as well as other parts of the world (2), and during past 2 decades, the number of AS users increased more than 2000% in the world (3). There is an adverse effect of AS in some organs such as hepatic (4), endocrine, and cardiovascular systems (5). For example, it has been shown that AS may induce pathological left ventricular hypertrophy (6) with disproportional extracellular collagen accumulation and/or interstitial fibrosis (7).

Liver is a key organ actively involved in numerous metabolic and detoxifying functions during exercise. During exercise training liver play an important role to release ATP or glucose. Abuse of AS has an adverse effects on liver function. The liver adverse effects are among the most common and serious associated with AS abuse and are virtually always associated with the oral active 17- α alkylated androgens such as methyltestosterone, methandrostenolone, oxandrolone, and stanozolol. In fact, AS allows increased oral absorption and slower hepatic degradation and clearance, so resulting in greater hepatic toxicity (8). Welder et al. (9) showed that AS are directly toxic to rat hepatocytes with increase of liver enzymes levels. Animal studies clearly shown liver alterations induced by AS. Gragera et al. (10) observed ultrastructural alterations of hepatocytes, the most prominent changes being swelling of

mitochondria and marked increase in the number of lysosomes. Saborido et al. (11) and Molano et al. (12) observed that treatment with stanozolol, either with or without concurrent exercise training, affects lysosomal hydrolases and mitochondrial respiratory chain electron transport in rat liver, without modifying classical serum indicators of hepatic function. Acute adaptative changes on the liver tissue (slight to moderate multifocal lobular inflammation with acidophilic degeneration and evident Kuppfer cells reactivity) were observed by Boada et al. (13) in rats administered with stanozolol for a short time in association with minimal to mild variability in the size of cell nuclei and increased mitosis and binucleation. In the majority of the livers from long-term treatment, the researchers observed cytoplasmic vacuolation, and lipidic degeneration; in addition, as in the case of acute AS-treated animals, they found increased mitosis and binucleation and variability in the size of cell nuclei.

Although there are several reports concerning the physiological abnormalities induced by AS abuse, the liver enzymes activity after using this drug in human subjects is unclear. Since previous studies used Rats to identify the effects of AS on liver enzymes activity and lipid profile, the information about the effects of longitudinal AS abuse on changes in liver enzymes and lipid profiles in human subjects especially in strength-trained men is scarce. Therefore, the purpose of this investigation was to determine the influence of longitudinal abuse of AS on liver enzymes activity and lipid profiles of men bodybuilders.

Methods

Subjects

The subjects of this study were 40 weight trained men, with 20 self-reported regular AS use and 20 self-reported never taking AS (NAS) (Table 1). Inclusion criteria were resistance training history of minimum of 5 yr with four to five training sessions per week. The specific inclusion criterion for the AS group was a documented self-reported history of AS abuse for 1 to 3 years and inclusion criterion for the NAS group was self-reported history of never taking AS. 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 Guilan university human research ethics committee.

Design

A cross-sectional cohort design was used for the study, with participants required to make a single visit to laboratory. Initially, subjects completed self-report questionnaires related to general health, training status, and history as well as detailed accounts of AS abuse. This was followed by assessment of body composition, arm and thigh circumferences, strength test and a venous blood sample. All tests were conducted on the participants after an overnight fast, as well as a 24-h abstention from resistance training.

History of AS use

The participants in the AS group had experience of AS abuse at least 1 to 3 years. The types of AS currently being used by some of the AS participants included trenbolone (number of use (N) = 4), testosterone (N = 2), sustanon (N = 3), boldenone (N = 1), nandrolone (N = 3), oxandrolone (N = 3), and stanozolol (N = 4). Of those in the AS group who provided sufficient information to perform an analysis of their daily usage (n = 20), we found that the mean AS dose was 220 mg.d⁻¹ with a SD of 152 mg.

Body composition

Height was measured using a wall-mounted stadiometer (Seca 0123, Germany) to the nearest centimeter. Body mass was measured to the nearest 0.1 kg using a medical scale (Seca 760102, Germany). Percentage of body fat was measured using 3-site skin fold thickness (chest, abdominal, and thigh). The measurement was used according to the method by Jackson and Pollock (14). All skin fold measurements

Table 1. Subjects characteristics (mean±sd).

	AS (n=20)	NAS (n=20)	P value
Age (y)	25±2.9	24.2±3.1	0.12
Height (cm)	172.7±5.9	174.1±5.4	0.31
Body mass (kg)	81.1±10.3	74.6±6.7	0.04
Body fat (%)	18.1±4.5	15.7±3.9	0.08

were taken using Lafayette caliper (Skin Fold Caliper, Model 01127-INDIANA). Skinfold thickness was based on the average of the two trials. If the two skinfold measurements at the same site differed by more than 0.5 mm, a third measurement was obtained and the mean value used.

Muscle circumference

The circumferences of mid thigh and mid arm of the right side were assessed during full muscle contraction using tape measure with nearest to 0.1 cm (15).

Strength assessment

Strength was measured using the one repetition maximum (1RM) bench press and leg press exercises. The 1RM testing was performed according to method previously described by Kraemer and Fry (15). The participants performed a warm-up set of 8 to 10 repetitions at a light weight. A second warm-up consisted a set of three to five repetitions with a moderate weight, and third warm-up included one to three repetitions with a heavy weight. After the warm-up, each subject was tested for the 1RM by increasing the load during consecutive trials until the participants were unable to perform a proper lift, complete range of motion and correct technique. The 1RM test was determined by ~5 sets of one repetition, with 3–5 minutes of rest among attempts. Spotters and investigators were present to provide verbal encouragement and safety for the subjects.

Blood sampling and analysis

Blood samples were drawn (10 cc) from the antecubital vein into plain evacuated test tubes. All the blood samples were drawn after 12 h of fasting and 8 h of sleeping. 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. Assessment of fasting blood sugar (FBS), total cholesterol (TC), HDL, LDL, and triglycerides (TG) were performed using the Daytona RS blood analysis machine (Randox, Co., Antrim, N. Ireland). Liver enzymes (i.e., AST, ALT and AP) were analyzed using conventional spectrophotometric methodology using a DxC 600 autoanalyzer (instrument and reagents from Beckman Coulter, Fullerton, CA).

Statistical analysis

All data were subjected to tests of normality. Differences between AS and NAS participants were analyzed using paired t-tests. The level of significant was set at $p \leq 0.05$. Statistical analysis of data was performed using statistical software package SPSS Version 16 (SPSS, Inc., Chicago, IL).

Results

Although height did not differ between groups, participants in the AS group were significantly heavier than NAS group ($p < 0.05$). However, body fat percentage was not significantly different between groups.

The participants in AS group indicated greater strength in the bench and leg press exercises than NAS group ($p < 0.05$), and there were also differences in the arm and thigh circumferences between groups ($p < 0.05$) (Table 2).

LDL and HDL were significantly elevated and reduced, respectively, in the AS group compared with NAS group ($p < 0.05$). FBS, TG and TC were higher in the AS group in comparison to NAS group ($p < 0.05$). There was little difference in partial thromboplastin time (PTT) between groups (33.4±5.7 vs. 32.9±5.3 sec) (Table 2).

AST and ALT levels were significantly elevated in the AS group than NAS group, whereas the AP level was higher for the NAS group ($p < 0.05$) (Table 2).

Discussion

The aim of this study was to compare the effects of longitudinal abuse of AS on liver enzymes activity, lipid profiles, strength and muscle volume in men bodybuilders. The results indicated that body mass, strength and muscle volume were greater for the athletes who used AS. However, the liver enzymes activity and lipid profiles were higher in the AS users than NAS group.

The findings of this study indicated that the AS users were heavier and the arm and thigh circumferences were greater in the AS users compared to NAS group. Moreover, the AS users were stronger than NAS users in the 1RM of bench press and leg press

Table 2. Data for the strength, muscle volume, lipid profiles and liver enzymes activity in AS and NAS groups (mean±SD).

	AS (n=20)	NAS (n=20)	P value
1RM bench press (kg)	113±11.8*	93.7±13.3	0.02
1RM leg press (kg)	329±40.8*	248.5±41	0.003
Arm circumference (cm)	41.2±3.5*	35.1±4.2	0.04
Thigh circumference (cm)	60.6±6.4*	53.7±5.6	0.03
PTT (sec)	33.4±5.8	32.9±5.3	0.13
FBS (mg/dL)	87±11.9*	81.3±9.8	0.05
TG (mg/dL)	166.5±74.4*	126.9±48.2	0.04
TC (mg/dL)	253.2±59.6*	143.5±48	0.01
HDL (mg/dL)	30.7±10*	43.5±15.2	0.02
LDL (mg/dL)	179.2±34.1*	155.8±37.7	0.04
AST (IU/L)	53.2±14.3*	34.5±11.1	0.02
ALT (IU/L)	53.5±15.1*	33.3±7.8	0.02
AP (IU/L)	75.6±30.1	82.7±30.6†	0.05

1RM: 1 repetition maximum, PTT: partial thromboplastin time, FBS: fasting blood sugar, TG: triglyceride, TC: total cholesterol, HDL: high density lipoprotein, LDL: low density lipoprotein, AST: Aspartate transaminase, ALT: Alanine aminotransferase, AP: Alkaline phosphatase. *Significant differences compared with NAS group ($p \leq 0.05$); †Significant differences compared with AS group ($p \leq 0.05$).

exercises. These findings are in line with previous studies which reported larger gains in body mass, muscle size and strength performance after AS abuse (17-22). A large number of studies reported that use of AS can increase the body mass (2-5 kg) (17,18). Alen and Hakkinen (23) reported that 6 months AS use induced 5 kg gains in body mass. In relation to muscle size or circumference, some studies reported no alterations of circumferences after AS use (24,25). In contrast, other researchers addressed that use of AS could induce increases in muscle size (20,21,22). The largest gains of muscle circumferences were seen at the thorax, shoulders and upper arm (19). Although, AS have been demonstrated to stimulate protein synthesis (21), the effects on muscle size and circumference could not be established. It has only been in the last decade that clear evidence for the muscle building properties of AS in males and athletes became available (20-23). It seems that these mechanisms could be a reason to greater body mass and muscle size in the AS users.

The most prevalent results for AS abuse is to promote muscle mass and strength. Bhasin et al (26) examined the effects of AS abuse and strength training on muscle size and found that 10 weeks AS use + strength training increased arm and thigh muscle circumferences and these changes were greater than strength training only. Moreover, higher strength for the AS user have been supported in previous studies (21,23,25). It can be concluded that AS administration may increase muscle mass and circumference and whether type I or type II muscle fibers are more profoundly affected is not clear yet. It appears that increase in muscle mass can be attributed to muscle hypertrophy and also the formation of new muscle fibers (20). The key roles seem to be played by satellite cells (i.e., they are enhanced by AS administration) and androgen receptors. Androgen receptors are expressed in myonuclei of muscle fibers and in capillaries and are more present in upper limb than in lower limb. AS administration induced an increase in androgen receptor-containing myonuclei in the muscles and also increase the myonuclear number per fiber in the muscle (19,20). Sinha-Hikim et al. (22) observed that muscle hypertrophy induced by exogenous testosterone administration was associated with an increase in satellite cell number, changes in satellite cell ultra-structure and a proportionate increase in myonuclear number (21,22,23). These observations may explain the regional differences in body mass, muscle fiber adaptation, muscle circumferences and strength development between AS and NAS users.

In addition, we observed an altered lipid profile in AS users. The TC was higher in AS users, the difference between groups was statistically significant, which supports data from Baldo-Enzi et al. (27), but contradicts Sader et al. (28). It can be explained that lipid profiles and overall cholesterol are important when determining cardiovascular and atherosclerotic risk (29). The decrease in HDL in AS users in the present study agrees with past research (30,31). Likewise, an increase in LDL in the current study also supports previous data (31). Supraphysiological doses of AS lead to high hepatic androgen exposure, and high androgen levels can alter levels of lipoprotein, which directly affects the formation of HDL (32) and these changes could increase cardiovascular disease in athletes who use AS.

In accordance with the findings of this study, number of studies reported elevation of liver enzymes activity after AS abuse (10-13). The results indicated that long-term abuse of AS increased basal levels of ASP and ALP in athletes, whereas this drug induced decreases in the AP levels in comparison to NAS athletes. Even though the numbers of subjects were small, the results indicated that in the general collective consciousness of the medical community, use of AS closely associated with liver disease. Previous reports on athletes who use AS have suggested that AS may cause serious hepatic dysfunction using ASP and ALP (12,33). It is presumed that AS are responsible for liver damage (33). Also, these lesions are reversible, at least partially, as has been reported in several case reports, and in some series of long-term follow-up (34,35); however, progression to hepatic insufficiency has been published (36). It would be conclude that long-term abuse of AS induced elevation of liver enzymes activity resulting hepatic toxicity. AS treatment is known to induce hepatic structural and ultrastructural changes (33) that may cause modifications in the liver subcellular fractionation pattern consequently enhances of liver enzymes activity.

In conclusion, the results from this study indicate that longitudinal abuse of AS coupled with strength training in athletes is associated with greater muscle mass, strength and muscle size than NAS user athletes. However, these greater enhancements are in accordance with elevation of lipid profiles and liver enzymes activity resulting liver damage and toxicity.

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