

Original Article**Endothelial function in male body builders taking anabolic androgenic steroids**

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**ABSTRACT**

**Background:** Adverse cardiovascular events have been reported in body builders taking anabolic steroids. Adverse effects of AAS on endothelial function can initiate atherosclerosis. This study evaluates endothelial function in body builders using AAS, compared with non-steroids using athletes as controls.

**Methods:** We recruited 30 nonsmoking male body builders taking AAS, 14 in build up phase, 8 in work out phase, and 8 in post steroid phase, and 30 nonsmoking male athletes who denied ever using steroids. Serum lipids and fasting plasma glucose were measured to exclude dyslipidemia and diabetes. Brachial artery diameter was measured by ultrasound at rest, after cuff inflation, and after sublingual glyceriltrinitrate (GTN) to determine flow mediated dilation (FMD), nitro mediated dilation (NMD) and ratio of FMD to NMD (index of endothelial function).

**Result:** Use of AAS was associated with higher body mass index (BMI) and low density lipoprotein-cholesterol (LDL-C). Mean ratio of flow mediated dilatation after cuff deflation to post GTN dilatation of brachial artery (index of endothelial function) in body builders taking AAS was significantly lower than control group (0.96(0.05) versus 1(0.08); p=0.03). After adjusting BMI, age and weight, no significant difference was seen in index of endothelial function between two groups (p=0.21).

**Conclusion:** Our study indicates that taking AAS in body builders doesn't have direct effect on endothelial function. Future study with bigger sample size and measurement of AAS metabolites is recommended.

**Key words:** endothelium, lipids, anabolic steroids, body builders

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Self-administration of anabolic-androgenic steroids (AAS) for increasing muscular strength and lean body mass has been a widespread practice among athletes, although the use of these drugs is considered a serious health risk. Side effects of AAS include gynecomastia, erythrocytosis, psychological disorders, coagulation activation, hepatotoxicity, virilization, and sudden cardiac death. They also induce an atherogenic lipoprotein profile by decreasing high density lipoprotein-cholesterol (HDL-C) and increasing low density lipoprotein-cholesterol (LDL-C) <sup>1</sup>.

Endothelial dysfunction is an early finding in experimental studies of atherogenesis, pre-

ceding plaque formation <sup>2</sup> and the occurrence of clinical manifestations <sup>3</sup>.

Healthy arteries conduit are capable of accommodating to changes in blood flow by increasing their internal diameter, a phenomenon termed as flow-mediated vasodilatation (FMD). In vitro studies have shown that this physiological vessel response is endothelium-dependent <sup>4</sup> and that the nitric oxide is crucial mediator <sup>5,6</sup>. FMD of large arteries has been used as an in vivo index of endothelial function <sup>7,8,9</sup>. FMD can be induced by hyperemia and can be measured non-invasively, accurately and

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reproducibly by high-resolution ultrasound of the brachial artery<sup>4</sup>. Nitro mediated dilatation (NMD) or endothelial independent dilatation is due to direct effect of exogenous nitric oxide.

Loss of FMD occurs in early stage of atherosclerosis (even before it can be detected by angiography). FMD has been used widely for different clinical purposes<sup>2, 10</sup>, children with familial hypercholesterolemia<sup>11</sup>, patients with a family history of premature coronary arterial disease (CAD)<sup>12</sup>, or with established CAD<sup>13</sup>, and both active and passive smokers<sup>14</sup>.<sup>15</sup> have been shown to display a reduced capacity for large arteries dilatation in response to increased blood flow. Studies on FMD in patients with diabetes<sup>16,17,18,19</sup> and hypertension<sup>20, 21</sup> have yielded controversial results<sup>5</sup>.

In one study that was performed by C.F. Ebenbichler, AAS users had higher body mass index, muscular strength and diminished FMD<sup>22</sup>. In another study from Australia, use of AAS per se was not associated with significant abnormalities of arterial structure or function<sup>23</sup>.

Because of extensive use of AAS in body builders and several reports of premature cardiovascular events in AAS users and controversies in previous studies about AAS effects on endothelial function, we designed a controlled study to investigate endothelial function in body builders taking anabolic-androgenic steroids.

## Subjects and Methods

### *Study population*

We studied endothelial function in 30 male body builders taking AAS and 30 volunteer male athletes as control group (they were working judo for at least 2 years and denied taking AAS). They were recruited from training centers in Isfahan, Iran.

Both groups were nonsmoker. They had similar physical activity with no history of ischemic heart disease (IHD), diabetes, hypertension, hyperlipidemia and family history of premature coronary artery disease that might affect on endothelial function. Exclusion criteria included low-density lipoprotein (LDL)>140

mg/dl, triglycerides (TG)>250mg /dl and fasting plasma glucose (FPG)>110 mg/dl.

Body builders taking ASS were following a training cycle, typically consisting of a workout phase, build-up phase and a competition phase. In the workout phase (non-steroid phase) they were performing muscle training and not using AAS currently, but they had history of taking such substances during previous training cycles; however they were free of AAS use within recent 8 weeks. In the build-up phase (steroid phase), athletes actually had both muscle training and taking AAS (Build-up phase was usually 8–12 weeks). In the competition phase (post-steroid phase) they were continuing muscle training but were free of anabolic steroids use. This phase immediately starts after build up phase. At the time of the study, 8 body builders were in workout phase, 14 in build-up phase and 8 in competition phase. Both case and control groups had history of at least 2 years of training (at least 2 times weekly). The following substances were self-administered during build-up phase: nandrolone esters (nandrolone phenylpropionate, nandrolone decanoate), testosterone esters (testosterone propionate, testosterone cypionate, testosterone enanthate) for intramuscular (IM) injection, and winobanin, mesterolone and testosterone undecanoate for oral administration. All bodybuilders take AAS at least for 2 years. The study was approved ethically by Isfahan university of Medical Sciences and both groups gave informed consents. Weight was measured by weighting machine (kg), height by standard meter (m), and body mass index (BMI) calculated by dividing weight (kg) to square of height (m) [kg/m<sup>2</sup>].

### *Laboratory measurements*

Blood sampling was done for antecubital vein after 10 hours of overnight fasting. Plasma total cholesterol, TG, HDL, LDL and FPG concentrations were determined. LDL assayed by Randox kit and total cholesterol, TG, FPG and HDL assayed by Pars Azmun kit (RA-1000, American).

### **Ultrasonic study**

The technique for assessing endothelium-dependent and endothelium-independent vasodilatation by non-invasive ultrasound has been described in details by others <sup>2</sup>. Briefly, the diameter of the brachial artery was measured in triplicate: at rest, during reactive hyperemia and after administration of sublingual GTN (0.4 mg) using a high resolution ultrasound machine with a 7.5 MHz linear array transducer (Dornier -522, Texas.US). Longitudinal images of the brachial artery were obtained proximal to the antecubital fossa. Transmit focus zones were set approximately to the depths of the anterior and the posterior vessel walls. Images were magnified, and depth and gain settings were used to optimize the image of the vessel wall, in particular, the media-adventitia interface. Other investigators have demonstrated that artery conduit dilatation in response to flow increase is endothelium-dependent <sup>24</sup>, whereas the dilational response to glyceryl trinitrate is endothelium-independent <sup>25</sup>.

All patients rested for at least 10 min before the first scan was obtained. Increased flow was then induced by inflation of a pneumatic tourniquet (ALPK2, Japan) placed around the forearm to a pressure of 300 mmHg for 4–5 min. A second scan was obtained 45–60 sec after cuff deflation. After a recovery phase of 15 min, sublingual GTN (0.4 mg) was administered and 3–4 min later, the last scan was obtained.

Flow mediated dilatation (FMD) and GTN-induced vasodilatation (NMD) were adjusted to vessel size by dividing the percentage of FMD or GTN-induced vasodilatation by the baseline diameter of the vessel. For correction of the negative association of vessel size, The percentage of the diameter of the endothelium-dependent dilatation to the at-rest-diameter (EDD) and the percentage of the diameter of the endothelium-independent vasodilatation to the at-rest-diameter (EID) were calculated. The other parameter is the ratio of FMD/NMD as an index of endothelial function.

### **Statistical Analysis**

Descriptive data are expressed as mean (SD). Data of case and control groups were compared by independent student t-tests. As age, BMI and weight were not matched in two groups, covariance analysis was performed to adjust these variables on analysis of the data. p-values of less than 0.05 were considered statistically significant.

### **Result**

#### **Baseline characteristics and laboratory data**

The baseline characteristics, lipid profiles and FPG of the athletes are shown in Table 1. There were no differences in height; and systolic, diastolic and mean arterial blood pressure between case and control groups. The mean age, weight and BMI of control group were lower than AAS users group. Total cholesterol and HDL-cholesterol did not differ between body builders group and athlete controls. LDL-cholesterol was higher in ASS users group than athlete controls.

Workout phase body builders display higher LDL-C versus other groups (p=0.03).

**Table 1.** Baseline characteristics and laboratory data of body builders and athlete controls.

	Athlete controls N=30	AAS users N=30	Result p-value
AGE (age)	22.7(4.9)	26.3(4.1)	0.005
Height (cm)	175.2(6.8)	175.6(6.3)	0.831
Weight (kg)	75.0(14.2)	87.9(12.3)	0.001
BMI (kg/m <sup>2</sup> )	24.3(4.1)	28.4(2.7)	0.001
MBP(mmHg)	85.5(6.2)	86(7.4)	0.779
FPG (mg/dl)	89.7(8)	86.9(9)	0.216
TG (mg/dl)	91.3(26.2)	101.2(49.3)	0.337
Chol (mg/dl)	162.3(28.3)	168.8(33.1)	0.419
LDL (mg/dl)	59.8(20.7)	73.6(31.2)	0.048
HDL (mg/dl)	44.8(8.4)	43.4(12.1)	0.614

BMI=Body Mass Index, MBP=Mean Blood Pressure, FPG=Fasting plasma glucose, TG=triglyceride, Chol=total cholesterol LDL=Low-Density Lipoprotein, HDL=High-Density Lipoprotein

### Vascular Reactivity

Results are shown after adjustment of BMI, age and weight effect on vascular reactivity. Mean basal brachial artery diameter (BBD), FMD, NMD, EDD, EID and FMD/NMD are shown in Table 2.

The mean index of endothelial dysfunction (FMD/NMD) without adjusting BMI, age and weight was significantly lower in total body builders taking AAS in compare with athlete controls suggesting that endothelial function was markedly impaired in body builders taking AAS (0.96(0.05) versus 1(0.08);  $P=0.03$ ) but after adjustment of these variants no significant difference was seen in index of endothelial function between two groups ( $P=0.21$ ).

The difference in mean FMD/NMD between three sub groups of body builders taking AAS and control group was not statistically significant ( $P=0.18$ ).

**Table 2.** Vascular variables of body builders and athlete controls, adjusted by age, weight and BMI.

	Athlete controls N=30	AAS users N=30	Result p-value
BBD	4.10(.49)	5.17(.64)	0.001
FMD	4.43(.46)	5.48(.70)	0.001
NMD	4.43(.44)	5.67(.67)	0.001
EDD	1.08(.08)	1.06(.04)	0.2
EID	1.08(.08)	1.10(.07)	0.79
FMD/NMD	1(.09)	.96(.06)	0.21

BBD= Basal Brachial artery Diameter, FMD=Flow Mediated Dilatation, NMD=Nitro Mediated Dilatation, EDD (Endothelial Dependent Dilatation) =FMD/BBD, EID (Endothelial Independent Dilatation)=NMD/BBD, FMD/NMD = index of Endothelial function

### Discussion

Androgenic anabolic steroids are used by a considerable proportion of the community to enhance their physique and performance. More than one million of Americans use or have used anabolic steroids <sup>26</sup>. From this large

population, there have been case reports association of AAS and premature cardiovascular complications including thrombo-embolic disease (myocardial infarction, stroke and pulmonary embolism), cardiomyopathy, ventricular arrhythmias and LV hypertrophy <sup>27, 28</sup>, but it remains to be clarified if these associations are more than chance observations.

Arterial reactivity is an important functional determinant of vascular health or disease <sup>29</sup>. In previous human studies, androgens have had variable effects on arterial reactivity. High-dose androgen use by genetic females (female-to-male transsexuals) may be associated with impaired vascular reactivity <sup>30</sup>, and androgen deprivation in men who have undergone castration for prostate cancer is associated with enhanced vascular reactivity <sup>31</sup>. Acutely administered testosterone, however, results in vasodilatation in both animal and human studies, through an endothelium-independent mechanism (probably via ATP-sensitive potassium channels) <sup>32, 33, 34</sup>.

C.F.Ebenbichler evaluated the endothelium-dependent and endothelium-independent vasodilatation in body builders as a group of athletes prone to using anabolic-androgenic steroids in order to increase lean body mass and muscular strength. Body builders group had a history of steroid intake for 4.7 years for work-out phase body builders, 3.7years for build-up phase body builders, and 4.6 years for competition phase body builders. The results showed diminished FMD in all body builders groups as a marker of endothelial dysfunction <sup>22</sup>.

In another study from Australia, use of AAS per se was not associated with significant abnormalities of arterial structure or function <sup>23</sup>.

In our study, we have investigated endothelial function on AAS taking body builders. Since all of the athletes evaluated in this study didn't have any history of smoking, HLP, HTN, DM and IHD, therefore we excluded dyslipidemia and other factors that might affect on endothelial function. Initially, our study showed that endothelial function was impaired markedly in body builders taking AAS

in comparison with other non AAS taking athletes. After adjustment of BMI, weight and age, no significant difference was noted in endothelial function between two groups. Therefore, presumably AAS itself doesn't have direct effect on vascular reactivity and may affect on endothelial function throughout weight gain.

No direct effect of AAS on endothelial function was seen in our study, in comparison to C.F. Ebenbichler study, which may be due to longer duration of AAS usage in their body-builders group or, in our study, the control group who denied any AAS taking, may had AAS using.

Significantly higher LDL-C was observed in AAS users before and after BMI, weight, age and adjustment that can be suggestive of direct effect of AAS on increasing LDL level, but this higher LDL in AAS users was not associated with endothelial dysfunction that may be related to exclusion of athletes with high LDL (over 140) from our study.

Also the sample size in our study may not be enough for evaluation of direct effect of AAS on endothelial function, therefore other study with bigger sample size and measurement of AAS metabolites may be required on future.

### ***Limitation of study***

We found that body builders use AAS routinely, therefore we were unable to find control group from body builders. So, we took other athletes that their training was isometric (judo), as the control group. Moreover, we were unable to find any available laboratory test to detect AAS metabolites in urine for exclusion of hormone users from the control group, therefore we selected the control group from Judo athletes with no history of AAS consumption and we accepted their claim for not taking AAS.

### **Conclusion**

Our study indicates that intake of AAS in body builders doesn't have any direct effect on endothelial function. Future study with bigger sample size and measurement of AAS metabolites is recommended.

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