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Diminished cholesterol efflux mediated by HDL and coronary artery disease in young male anabolic androgenic steroid users

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HIGHLIGHTS

- Anabolic androgenic steroids (AAS) abuse impairs the cholesterol efflux mediated by HDL.
- AAS abuse seems to be correlated with lower cholesterol efflux and subclinical coronary artery disease (CAD).
- We found at least 2 coronary arteries with plaques in 25% of AAS users.

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ABSTRACT

Background and aims: Anabolic androgenic steroids (AAS) have been associated with coronary artery disease (CAD). AAS abuse leads to a remarkable decrease in high-density lipoprotein (HDL) plasma concentration, which could be a key factor in the atherosclerotic process. Moreover, not only the concentration of HDL, but also its functionality, plays a pivotal role in CAD. We tested the functionality of HDL by cholesterol efflux and antioxidant capacity. We also evaluated the prevalence of CAD in AAS users.

Methods: Twenty strength-trained AAS users (AASU) age 29 ± 5 yr, 20 age-matched strength-trained AAS nonusers (AASNU), and 10 sedentary controls (SC) were enrolled in this cross-sectional study. Functionality of HDL was evaluated by ^{14}C-cholesterol efflux and the ability of HDL in inhibiting LDL oxidation. Coronary artery was evaluated with coronary computed tomography angiography.

Results: Cholesterol efflux was lower in AASU compared with AASNU and SC (20 *vs.* 23 *vs.* 24%, respectively, $p < 0.001$). However, the lag time for LDL oxidation was higher in AASU compared with AASNU and SC (41 vs 13 vs 11 min, respectively, $p < 0.001$). We found at least 2 coronary arteries with plaques in 25% of AASU. None of the AASNU and SC had plaques. The time of AAS use was negatively associated with cholesterol efflux.

Conclusions: This study indicates that AAS abuse impairs the cholesterol efflux mediated by HDL. Long-term AAS use seems to be correlated with lower cholesterol efflux and early subclinical CAD in this population.

1. Introduction

Anabolic androgenic steroids (AAS) abuse among young people is a widespread problem worldwide. Adverse events like sudden cardiac death and heart attack have been reported in AAS users [1–3], which could be associated with coronary artery disease (CAD) [4].

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Many studies have shown that the illicit use of AAS changes lipid profile, leading to lower high-density lipoprotein (HDL) and higher low-density lipoprotein (LDL) levels [5–8]. It is well known that decreased concentration of HDL is a key factor in the atherosclerotic process [9,10]. More interestingly, not only the concentration of HDL, but also its functionality plays a pivotal role in CAD [11]. Kosmas and collaborators reported that the cholesterol efflux capacity from macrophages is an excellent metric of HDL functionality, because it has a strong inverse association with the risk of CAD, independently of HDL plasma levels [12].

Recently, Baggish and collaborators showed in older adult men that long-term AAS utilization seems to be associated with myocardial dysfunction and CAD [4]. However, whether AAS abuse leads to impairment of HDL functionality is completely unknown.

In the present study, we evaluated the functionality of HDL, which could be one of the mechanisms in the atherosclerotic process. We also evaluated the prevalence of subclinical CAD in AAS users.

2. Materials and methods

2.1. Study population

The local Human Subject Protection Committee approved this cross-sectional study (3945/13/070), and each participant provided written informed consent. Between April 2015 and April 2017, we enrolled 68 health male participants (asymptomatic and without previous cardiovascular disease) between 18 and 45 years of age. Of these, 18 were excluded due to lack of time to participate in the study, obesity, and smoking (see inclusion criteria below). Fifty age-matched participants were evaluated and allocated: 20 AAS users (group AASU), 20 AAS nonusers (group AASNU). Both groups (AASU and AASNU) were recreational weightlifters or amateur bodybuilding athletes who were recruited from gymnasiums. In addition, 10 age-matched sedentary men (without regular exercise training and/or sports, < 150 min/week of physical activity such as walking with light/moderate intensity) without cardiovascular disease (hypertension, diabetes, hypercholesterolemia, obesity [body mass index > 30 kg/m^2]) served as a control group. Exclusion criteria for all participants were with known CAD, smoking, alcohol consumption, use of diuretics, statins and/or antihypertensive medications, and liver and kidney disease.

AASU and AASNU groups had been involved in strength training for at least 2 years. AASU should be self-administering AAS in periodic cycles lasting from 8 to 12 weeks for at least 2 years with 2–4 cycles per year. All AASU were on a cycle over the course of the study.

The doping urine test was performed by ultra-performance liquid chromatography with tandem mass spectrometry detection (UPLC-MS/MS) in all groups to document the presence of AAS. In addition, the testosterone/epitestosterone (T/E) concentration ratio in the urine was determined as previously reported [13]. A T/E ratio greater than six indicates exogenous testosterone use [13].

2.2. Measures and procedures

All the participants abstained from sports supplements, caffeine-containing products, and exercise training for 48 h before the exams. Arterial blood pressure was measured using the auscultatory method with the participant in a seated position after 10 min of rest in a quiet, temperature-controlled (21 °C) room.

2.3. Blood measures

Blood sample was collected in the morning (between 8:00–10:00 a.m.) after 12 h fasting and after 30 min of resting for lipid assessment (total cholesterol, LDL, HDL, and triglycerides), glucose, and high-sensitivity C-reactive protein (hs-CRP), hepatic and kidney biomarkers, and hormonal parameters.

2.4. Determination of cholesterol efflux mediated by HDL

The cholesterol efflux was evaluated by ^{14}C-cholesterol efflux mediated by HDL with macrophages cultured cells from mice. Cholesterol efflux assays were done in quadruplicate wells in one single experimental batch in order to avoid inter assay variability. The mean value of basal efflux (considering all plaques) was subtracted from the efflux value obtained with the addition of patient's HDL. Then, it was obtained the cholesterol efflux specifically mediated by HDL. The percentage of 14C-cholesterol efflux was calculated as follow; 14C-cholesterol in medium/14C cholesterol in medium +14C cholesterol in cells x 100. Detailed determination of cholesterol Efflux is described in the Supplementary Data.

2.5. HDL antioxidant capacity

Low-density lipoproteins (LDL), isolated from a pool of healthy human plasma donors, were dialyzed against PBS without EDTA and then diluted to obtain a concentration of 40 mg of LDL protein in 500 μL of deionized H_2O. The time (min) of LDL resistance to oxidation (lag time) was calculated between the start of the reaction and the intersection of time with the extrapolated line of the propagation phase, and the maximum rate of conjugated diene formation determined by maximum absorbance/min [14]. Detailed determination of HDL antioxidant capacity is described in the Supplementary Data.

2.6. Coronary computed tomography angiography image acquisition

To assess coronary artery plaque and calcification, all participants underwent coronary computed tomography angiography according to the guidelines of the Society of Cardiovascular Computed Tomography (SCCT) [15]. Detailed CTA method is described in the Supplementary Data.

2.7. Body composition

Body composition was assessed by dual-energy X-ray absorptiometry (DXA), (Discovery DXA system, Hologic Inc) to measure total fat-free mass, fat mass, and fat percentage in all participants. DXA was used to exclude possible bias of body mass index (BMI) among the participants.

2.8. General cardiovascular risk

To assess the clinical cardiovascular risk score, we used the Castelli Index to assess the risk of development of coronary artery disease (CAD) [16] and Framingham Heart Study Score that predicts risk of specific atherosclerotic cardiovascular disease (CVD risk) [9]. Detailed general cardiovascular risk is described in the Supplementary Data.

2.9. Statistical analysis

The sample size calculation was based on difference of 5% alpha error and 20% beta error to detect a mean difference in CAD (primary outcome) in any of the two groups. Thus, the delta gradient is 100% for 17 patients for each group (AASU and AASNU) with a statistical power of 84%. In addition, 10 sedentary men (group SC) were included. Data are presented as mean ± standard deviation (SD) or median (interquartile range – IQR – 25%–75%). The Kolmogorov-Smirnov test was used to evaluate the normal distribution of the variables studied. The parametric data were obtained by one-way ANOVA analysis of variance. When a significant difference was found, the Scheffe post-hoc comparison test was used. Kruskal Wallis and Dunn's multiple comparison tests were used for nonparametric data. The chi-square test was used for categorical variables. Bivariate correlation test (Spearman) was also used. $p < 0.05$ indicates statistical significance. The Statistical

Table 1
Physical characteristics, clinical biomarkers, and hormonal profile in anabolic androgenic steroid users (AASU), anabolic androgenic steroid nonusers (AASNU), and sedentary control (SC).

Variables	AASU (n = 20)	AASNU (n = 20)	SC (n = 10)	*p*
Age (years)	29 ± 5	29 ± 5	29 ± 3	0.861
Weight (kg)	97.4 (90.1–104.9) *†	82.0 (74.0–88.0)	74.8 (70.0–87.5)	0.003
Height (m)	1.78 ± 0.04	1.80 ± 0.09	1.76 ± 0.08	0.841
BMI (kg/m^2)	31.11 ± 3.45 *†	25.45 ± 1.92	25.70 ± 3.38	< 0.001
SBP (mmHg)	130 (130–140) *†	120 (100–120)	120 (110–120)	< 0.001
DBP (mmHg)	90 (80–90) *†	80 (70–80)	80 (70–80)	< 0.001
TC (mg/dL)	186 (143–208)	155 (134–188)	189 (175–200)	0.07
HDL-c (mg/dL)	19 (13–25) *†	44 (41–54)	50 (40–55)	< 0.001
LDL-c (mg/dL)	144 (105–179) *†	96 (81–125)	122 (105–132)	0.001
Non-HDL-c (mg/dL)	157 (121–198)†	111 (94–139) *	147 (128–152)	0.03
Triglycerides (mg/dL)	74 ± 23	75 ± 35	98 ± 45	0.15
Glucose (mg/dL)	90 ± 7	90 ± 6	92 ± 8	0.66
Urea (mg/dL)	36 ± 8	33 ± 6	30 ± 9	0.13
Creatinine (mg/dL)	1.30 ± 0.16 *†	1.11 ± 0.21	1.02 ± 0.12	< 0.001
GOT (U/L)	41 (34–55) *†	23 (20–32)	23 (19–29)	< 0.001
GPT (U/L)	63 (54–88) *†	36 (30–51)	35 (29–54)	0.001
Gamma GT (U/L)	26 (21–36) *	29 (24–44)	36 (27–53)	0.02
TSH (uUl/mL)	1.91 (1.11–2.49)	1.73 (1.26–2.24)	1.54 (1.00–2.77)	0.74
FSH (mUl/mL)	0.10 (0.10–0.10) *†	2.88 (1.90–4.91)	2.30 (2.48–4.60)	< 0.001
LH (mUl/mL)	0.10 (0.10–0.10) *†	2.73 (2.03–3.49)	3.55 (2.57–6.09)	< 0.001
Prolactin (ng/mL)	6.70 (4.67–9.61)	7.66 (5.21–8.47)	6.13 (4.40–7.49)	0.45
Lean mass (kg)	82.05 ± 9.18 *†	62.81 ± 7.15 *	53.94 ± 7.38	< 0.001
Fat (kg)	12.0 (9.9–15.1) *†	15.5 (11.7–18.1) *	19.5 (15.2–26.8)	0.033
Fat percentage (%)	13.18 ± 5.62 *†	19.27 ± 4.33 *	27.59 ± 7.49	0.005
BMD (kg)	3.51 ± 0.32 *	3.33 ± 0.44	3.02 ± 0.36	0.007

Data are presented as mean ± SD or median ± IQR (25%–75%; IQR = interquartile range).
BMI = body mass index, BMD = bone mineral density, SBP = systolic blood pressure, DBP = diastolic blood pressure, TC = total cholesterol, HDL-c = high-density lipoprotein cholesterol, LDL-c = low-density lipoprotein cholesterol, GOT = glutamic-oxalacetic transaminase; GPT = glutamic-pyruvic transaminase; Gamma GT = gamma-glutamyl transferase; TSH = thyroid stimulating hormone; FSH = follicle stimulating hormone; LH = luteinizing hormone.
*$p < 0.05$ *vs.* SC; †$p < 0.05$ *vs.* AASNU.

Package for the Social Sciences (SPSS) version 23 was used to perform all the statistical analyses.

3. Results

Fifty age-matched participants were evaluated and allocated: 20 AAS users (group AASU), 20 AAS nonusers (group AASNU) and10 sedentary men (group SC). Physical characteristics, clinical biomarkers, and hormonal profile are shown in Table 1. Cumulative lifetime duration of strength training, AAS use and types of AAS used are shown in Supplementary Table 1.

The cholesterol efflux mediated by HDL was significantly lower in AASU compared with AASNU and SC (Fig. 1A). On the contrary, the lag time of LDL oxidation was higher in AASU than in AASNU and SC (Fig. 1B).

In addition, AASU had a modified composition of HDL particles with reduced HDL-cholesterol, HDL-triglycerides, HDL-apo AI, and HDL-phospholipids compared with AASNU and SC (Fig. 2A–D, respectively).

We found at least 2 coronary artery segments with lipid, fibro-lipid, and/or calcium plaques in 25% of AASU (Table 2). In contrast, none of the AASNU and SC participants had CAD. In those AASU who had subclinical CAD, fibro-lipid plaques were in 58%, followed by 27% with lipid plaques, and 15% with calcium plaques (Table 2). The mean total plaque volume was 274.4 mm^3, with negative index remodelling of 97.8%, and degree of stenosis from 30 to 50% (Table 2). Left anterior descending artery (LAD), circumflex artery (CX), right artery (RCA), diagonal artery (DI), and first diagonal artery (D1) were the most affected segments (Table 2).

Supplementary Figs. 1A and B shows calcified plaque in the left anterior descending artery (LAD) in a 27-year-old man AAS user, and a mixed plaque in the LAD in a 43-year-old man AAS user, respectively. Moreover, it is interesting to note that one 41-year-old AASU (24 cumulative years of AAS use) had a coronary ulcer in the left anterior descending artery; and one 43-year-old AASU (11 cumulative years of AAS use) underwent cardiac catheterization, but without coronary angioplasty.

We found that the time of AAS use was negatively associated with cholesterol efflux mediated by HDL (Fig. 3A), HDL-cholesterol

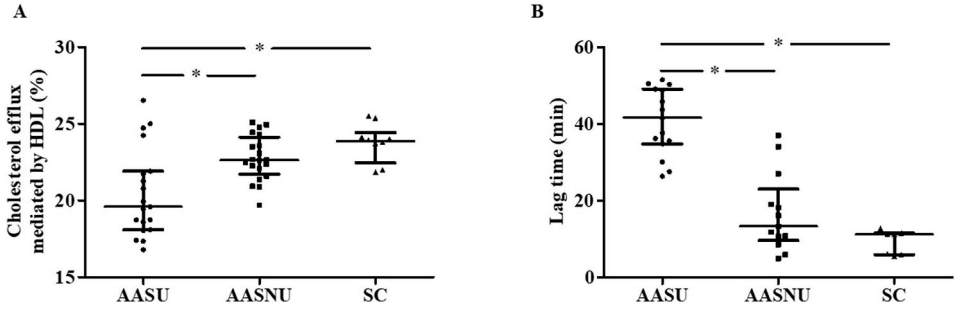

Fig. 1. (A) Cholesterol efflux mediated by high-density lipoprotein (HDL), AASU (n = 19), AASNU (n = 20), and SC (n = 10), and (B) lag time for low-density lipoprotein (LDL) oxidation. AASU (n = 15), AASNU (n = 13), and SC (n = 7).

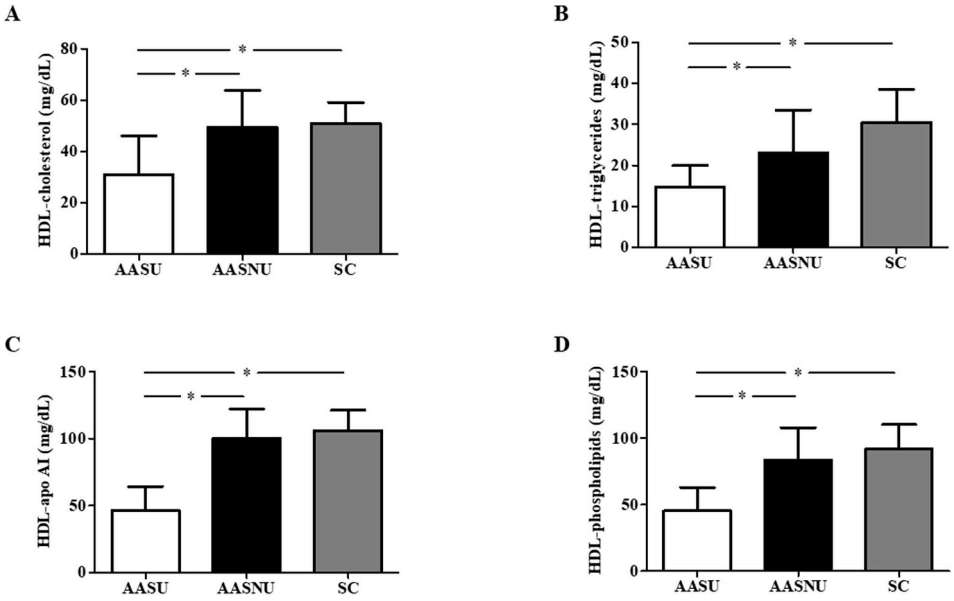

Fig. 2. Composition of a high-density lipoprotein (HDL) particle. AASU (n = 20), AASNU (n = 20), and SC (n = 10).

Table 2
Computed tomography coronary angiography in anabolic androgenic steroid users (AASU), anabolic androgenic steroid nonusers (AASNU), and sedentary control (SC).

	AASU (20)	AASNU (20)	SC (10)	*p*
Coronary arterial plaque, n	5 (25%) * †	0	0	0.019
History familiar of CAD, n	1 (5%)	1 (5%)	1 (5%)	0.814

AASU	Vessel	Location	Lipid plaque, mm^3	Fibro lipid plaque, mm^3	Calcium plaque, mm^3	Total volume, mm^3	‡ Degree of stenosis, %	Index remodelling, %
27-year-old (3 years of AAS use)	LAD	Proximal	35.3 (20.2%)	85.9 (49.2%)	53.5 (30.6%)	174.7	2	81
	LAD	Medial	3.5 (24.0%)	8.7 (60.3%)	2.3 (15.7%)	14.5	1	88
27-year-old (6 years of AAS use)	LAD	Proximal	18.5 (30.4%)	35.4 (58.2%)	6.9 (11.4%)	60.8	1	126
	LCx	Proximal	51.1 (13.4%)	190.7 (50.0%)	139.4 (16.6%)	381.2	1	138
39-year-old (18 years of AAS use)	RCA	Proximal	216.2 (23.4%)	676.6 (73.3%)	29.8 (3.2%)	922.6	2	68
	RCA	Medial	216.2 (23.4%)	242.4 (49.8%)	12.3 (25.7%)	470.9	2	92
	LCx	Medial	22.7 (44.4%)	28.4 (55.6%)	0	51.1	2	88
	LAD	Medial	77.9 (21.5%)	260.0 (72.0%)	25.0 (12.0%)	362.9	2	58
41-year-old (24 years of AAS use)	LCx	Proximal	26.0 (20%)	107.0 (87.0%)	18.0 (15.0%)	151.0	1	38
	LAD	Medial	113.8 (38.2%)	179.2 (60.2%)	0	293.0	1	118
43-year-old (11 years of AAS use)	LAD	Proximal	64.3 (26.2%)	136.1 (55.5%)	44.6 (18.2%)	245.0	4	131
	DI	Proximal	126.6 (39.9%)	147.0 (46.4%)	43.4 (13.7%)	317.0	4	87
	DI	Proximal	56.9 (29.9%)	114.1 (60.1%)	19.0 (10.0%)	190.0	1	132
	RCA	Distal	57.5 (27.7%)	70.1 (33.8%)	79.6 (38.4%)	207.2	1	124
Mean			77.6 (27.3%)	163.0 (58.0%)	33.8 (15.0)	274.4	2	97.8

‡ Represents the degree of stenosis at specific coronary artery segment on a scale of 0–5, where 0 = 0% stenosis, 1 ≤ 30%, 2 = 30%–50%, 3 = 51%–70%, 4 = 71%–99%, and 5 = 100%.
CAD = coronary artery disease; LAD = left anterior descending artery; LCX = left circumflex artery; RCA = right coronary artery; DI = diagonal branch artery.

(Fig. 3B), and HDL-apo AI (Fig. 3C). Moreover, the time of AAS use was positively associated with total coronary artery plaque volume (Fig. 3D).

Finally, we also calculated 2 different clinical cardiovascular risk scores, both Castelli Index and Framingham Heart Study scores were higher in AASU compared with AASNU and SC. Vascular aging and hs-CRP were also higher in those men who used AAS (Supplementary Table 2).

4. Discussion

To the best of our knowledge, this is the first study to assess the effect of illicit use of AAS on the function of HDL as a possible mechanism involved in CAD in young men. We found that AAS users have impaired efflux cholesterol capacity mediated by HDL when compared with that in sedentary men or the weightlifters who did not use AAS. In our cohort, about 1 in 4 weightlifters (25%) who used AAS had signs of subclinical CAD on CT. In contrast, none of the AAS nonusers and the sedentary participants had subclinical CAD. In additional, a negative correlation between the time of AAS use with efflux cholesterol was found.

The illicit use of AAS has several cardiovascular implications, such as autonomic imbalance, increased blood pressure, and endothelial dysfunction [7,17,18]. Moreover, AAS abuse is also linked to lower HDL concentration, high levels of coronary artery calcium [19], and increased coronary artery plaque volume [4]. However, the mechanisms involved in subclinical CAD in this population are completely unknown. Santora and collaborators were the first to show an association between

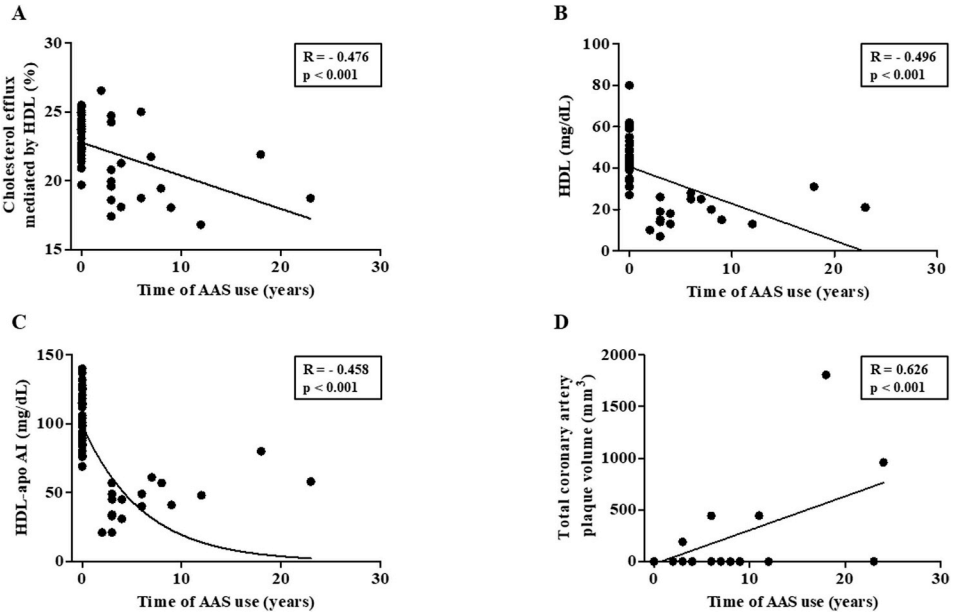

Fig. 3. (A) Time of AAS use and cholesterol efflux mediated by high-density lipoprotein (HDL) AASU (n = 19), (B) time of AAS use and HDL concentration AASU (n = 20), (C) time of AAS use and HDL-apo AI AASU (n = 20), and (D) time of AAS use and total coronary artery plaque AASU (n = 20).

AAS abuse with lower HDL concentration and coronary artery calcification [19]. More recently, Baggish and collaborators reported left ventricular dysfunction and premature subclinical CAD in weightlifters who used AAS [4]. However, both studies enrolled middle-aged men with an age range from 28 to 55 years (mean age around 40 years old). In our study, we enrolled only asymptomatic young men, without a history of cardiovascular disease, with the mean age of 29 years. We have demonstrated that even in younger men, the illicit use of AAS has a potential to provoke subclinical CAD.

The illicit use of AAS, mainly those administrated orally, leads to greater hepatic lipase (HL) activity [6]. HL plays a key role in facilitating the uptake of lipoproteins by the liver, which is one the main mechanisms to decrease HDL-cholesterol and apo AI in AASU [6]. In our study, we found a remarkable decrease in HDL plasma concentration, HDL-cholesterol and apo AI. Moreover, HDL-triglycerides and HDL-phospholipids were lower in AAS users. Taken together, these findings show that AAS also alters the structure of HDL, which may impair HDL functionality. In fact, we found that weightlifters who used AAS had a diminished cholesterol efflux, that means a lowered ability of HDL to accept cholesterol from macrophages.

Despite the impairment in cholesterol efflux mediated by HDL, the antioxidant capacity of HDL is higher in AAS users compared with sedentary and AAS nonusers. This finding suggests that HDL protects LDL oxidation for a longer time. However, we only studied the lag time for LDL oxidation. Thus, we do not know how much LDL was oxidized (quantitatively). This issue deserves future study. For instance, in young men without a history of AAS use, resistance training decreases the oxidized LDL in the plasma [20]. The HDL particle is composed of apolipoproteins and enzymes, such as the paraoxonase-1 (PON1), which seems to be influenced by testosterone replacement in older hypogonadal men [21]. PON1 has an antioxidant capacity and may be increased by the supraphysiologic doses of testosterone. However, this hypothesis should be addressed in AAS users.

This study has clinical implications. First, we found that AAS users have decreased cholesterol efflux capacity by HDL, which could be, at last in part, one of the mechanisms associated with subclinical CAD. Second, 25% of young AAS users had signs of subclinical CAD with high-volume coronary plaque and even coronary luminal stenosis that would not be expected in young men. Third, the most-used general cardiovascular risk, the Castelli Index (ratio of TC:HDL cholesterol) and Framingham Heart Study, were worse in AAS users.

4.1. Limitations

We recognize limitations in our study. We studied only men; therefore, the results should be interpreted with caution in women. The cholesterol efflux capacity mediated by HDL was only evaluated in vitro. Our study was correlational, and the mechanism of the cholesterol efflux capacity mediated by HDL on coronary disease should be addressed in future studies. Despite of limitations on the quantitative plaque characterization, specially the overlap between pixel densities of lipid and fibrotic plaque, this technique is widely used in the literature and has demonstrated the ability to detect plaques that has prognostic value [22].

4.2. Conclusions

This study indicates that AAS abuse impairs the cholesterol efflux mediated by HDL. Long-term AAS use seems to be correlated with lower cholesterol efflux mediated by HDL and early subclinical CAD in this population.

Conflicts of interest

The authors declared they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

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Mauricio Yonamine (Doping measurements);

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Carlos Eduardo Rochitte (Data collection and final discussion);

Maria Janieire de Nazaré Nunes Alves (Study design, writing and final discussion).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.atherosclerosis.2019.02.006.

References

[1] R.A. McNutt, G.S. Ferenchick, P.C. Kirlin, et al., Acute myocardial infarction in a 22-year-old world class weight lifter using anabolic steroids, Am. J. Cardiol. 62 (1988) 164.

[2] V. Fineschi, I. Riezzo, F. Centini, et al., Sudden cardiac death during anabolic steroid abuse: morphologic and toxicologic findings in two fatal cases of body-builders, Int. J. Leg. Med. 121 (2007) 48–53.

[3] P.J. Angell, T.F. Ismail, A. Jabbour, et al., Ventricular structure, function, and focal fibrosis in anabolic steroid users: a CMR study, Eur. J. Appl. Physiol. 114 (2014) 921–928.

[4] A.L. Baggish, R.B. Weiner, G. Kanayama, et al., Cardiovascular toxicity of illicit anabolic-androgenic steroid use, Circulation 135 (2017) 1991–2002.

[5] M.A. Santos, C.V. Oliveira, A.S. Silva, Adverse cardiovascular effects from the use of anabolic-androgenic steroids as ergogenic resources, Subst. Use Misuse 49 (2014) 1132–1137.

[6] A.T. Morikawa, R.C. Maranhao, M.J. Alves, et al., Effects of anabolic androgenic steroids on chylomicron metabolism, Steroids 77 (2012) 1321–1326.

[7] M.J. Alves, M.R. Dos Santos, R.G. Dias, et al., Abnormal neurovascular control in anabolic androgenic steroids users, Med. Sci. Sports Exerc. 42 (2010) 865–871.

[8] F.R. Souza, A.R.K. Sales, M.R. Dos Santos, et al., Retrograde and oscillatory shear rate in young anabolic androgenic steroid users, Scand. J. Med. Sci. Sports 29 (2019) 422–429.

[9] RB, Sr D'Agostino, R.S. Vasan, M.J. Pencina, et al., General cardiovascular risk profile for use in primary care: the Framingham Heart Study, Circulation 117 (2008) 743–753.

[10] P.W. Wilson, R.B. D'Agostino, D. Levy, et al., Prediction of coronary heart disease using risk factor categories, Circulation 97 (1998) 1837–1847.

[11] A.V. Khera, M. Cuchel, M. de la Llera-Moya, et al., Cholesterol efflux capacity, high-density lipoprotein function, and atherosclerosis, N. Engl. J. Med. 364 (2011) 127–135.

[12] C.E. Kosmas, G. Christodoulidis, J.W. Cheng, et al., High-density lipoprotein functionality in coronary artery disease, Am. J. Med. Sci. 347 (2014) 504–508.

[13] D.H. van de Kerkhof, D. de Boer, J.H. Thijssen, et al., Evaluation of testosterone/epitestosterone ratio influential factors as determined in doping analysis, J. Anal. Toxicol. 24 (2000) 102–115.

[14] H. Esterbauer, G. Striegl, H. Puhl, et al., Continuous monitoring of in vitro oxidation of human low density lipoprotein, Free Radic. Res. Commun. 6 (1989) 67–75.

[15] S. Abbara, P. Blanke, C.D. Maroules, et al., SCCT guidelines for the performance and acquisition of coronary computed tomographic angiography: a report of the society of cardiovascular computed tomography guidelines committee: endorsed by the north American society for cardiovascular imaging (NASCI), J Cardiovasc Comput Tomogr 10 (2016) 435–449.

[16] W.P. Castelli, Cholesterol and lipids in the risk of coronary artery disease–the Framingham Heart Study, Can. J. Cardiol. 4 (1988) Suppl A:5A-10A.

[17] C.B. Severo, J.P. Ribeiro, D. Umpierre, et al., Increased atherothrombotic markers and endothelial dysfunction in steroid users, Eur J Prev Cardiol 20 (2013) 195–201.

[18] M.R. dos Santos, R.G. Dias, M.C. Laterza, et al., Impaired post exercise heart rate recovery in anabolic steroid users, Int. J. Sports Med. 34 (2013) 931–935.

[19] L.J. Santora, J. Marin, J. Vangrow, et al., Coronary calcification in body builders using anabolic steroids, Prev. Cardiol. 9 (2006) 198–201.

[20] J.L. da Silva, C.G. Vinagre, A.T. Morikawa, et al., Resistance training changes LDL metabolism in normolipidemic subjects: a study with a nanoemulsion mimetic of LDL, Atherosclerosis 219 (2011) 532–537.

[21] K.B. Rubinow, T. Vaisar, C. Tang, et al., Testosterone replacement in hypogonadal men alters the HDL proteome but not HDL cholesterol efflux capacity, J. Lipid Res. 53 (2012) 1376–1383.

[22] S. Motoyama, M. Sarai, H. Harigaya, et al., Computed tomographic angiography characteristics of atherosclerotic plaques subsequently resulting in acute coronary syndrome, J. Am. Coll. Cardiol. 54 (2009) 49–57.