

Increased concentration of 7-ketocholesterol and 7 β -hydroxycholesterol in HDL from young anabolic androgenic steroid users

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Short report

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Abstract

Background:

Oxysterols are cholesterol oxidative derivatives that play an important role in cell cholesterol homeostasis. However, HDL particles enriched with oxysterols, especially the 7-ketocholesterol, have lower ability to mediate cell cholesterol removal by the reverse cholesterol transport. Anabolic androgenic steroids (AAS) misuse is associated with diminished cholesterol efflux and increased atherogenesis in young men. Unknown is whether the concentration of oxysterols is altered in AAS users.

Methods:

In this subanalysis, we evaluated six AAS users (AASU) and 6 AAS nonusers (AASNU) involved in strength training, and 6 sedentary men (SC). Oxysterols were evaluated by gas chromatography-mass spectrometry in HDL particles isolated by ultra centrifugation.

Results:

AASU presented higher levels of 7-ketocholesterol (104.1 ± 64.0 vs. 43.7 ± 8.3 and 43.2 ± 9.1 ng/mg C; $p < 0.05$) and 7 β -hydroxycholesterol (15.5 ± 5.6 vs. 10.1 ± 2.0 and 8.1 ± 1.0 ng/mg C; $p < 0.05$) in HDL compared to AASNU and SC, respectively. 7 α -hydroxycholesterol (10.7 ± 4.2 vs. 8.0 ± 1.5 and 6.8 ± 2.2 ng/mg C; $p = 0.089$) and 27 hydroxycholesterol (25.6 ± 7.2 vs. 28.0 ± 7.0 and 21.8 ± 3.2 ng/mg C; $p = 0.235$) were similar among groups.

Conclusion:

This pilot study suggests that AAS abuse increases 7-ketocholesterol and 7 β -hydroxycholesterol in HDL that may be related to HDL impaired function and increased atherosclerotic coronary disease in young AAS users. The clinical implication of this finding should be considered in future, larger, long-term studies.

Trial registration:

This is a cross-sectional pilot observational study from a retrospectively registered study NCT03450837.

Introduction

Oxysterols are derived from the enzymatic and/or non-enzymatic oxidation of the ring structure and side chain portion of the cholesterol molecule that plays an important role in cell cholesterol homeostasis(1–3). The 27-hydroxycholesterol (27-OH) is related to cell cholesterol homeostasis by controlling the expression of genes involved in cholesterol biosynthesis and excess cholesterol exportation. Moreover, 27-OH is considered an adding route to the reverse cholesterol transport due to the solubility through the plasma membrane(4).On the other hand, 7-ketocholesterol (7-K), 7 β -hydroxycholesterol (7 β -OH), and 7 α -hydroxycholesterol (7 α -OH) are the most prevalent oxysterols in atherosclerotic lesions and closely

related to inflammation, oxidation, monocyte recruitment and apoptosis in the arterial wall compartment. Moreover, increased 7-K alters cellular plasma membrane leading to an impairment of the cholesterol efflux, which is the first step of the reverse cholesterol transport (RCT) that drives cholesterol to the liver for excretion into bile and feces(5).

Anabolic androgenic steroids (AAS) have been associated with several cardiovascular injuries(6–10). Recent studies show the presence of subclinical coronary arterial disease(11, 12) and diminished cholesterol efflux capacity mediated by high-density lipoprotein (HDL) in young AAS users(12). Increased oxysterols may be one of the mechanisms involved in the lower HDL functionality in young AAS users. However, this hypothesis remains to be tested.

In the present study, we evaluated the oxysterols 7-K, 7 α -OH, 7 β -OH and 27-OH in HDL particles isolated in a subgroup of current AAS users and nonuser involved in strength training, and sedentary men.

Methods

This is a subanalysis from a previous cross-sectional study(12). We evaluated a subgroup of 18 participants (29 ± 5 year): six AAS users (AASU), six AAS nonusers (AASNU) and six sedentary control men (SC). Both groups (AASU and AASNU) were recreational weightlifters or amateur bodybuilding athletes who were recruited from gymnasiums. The inclusion and exclusion criteria were previously described(12).

Oxysterols Measurement In Hdl

Venous blood was collected after 12 h fasting and plasma was immediately isolated in a refrigerated centrifuge, following the addition of preservatives and antioxidants. HDL was isolated from plasma by discontinuous density ultracentrifugation. After dialysis, the amount of oxysterols in HDL was determined by gas chromatography-mass spectrometry (GC/MS). HDL (800 μ L) was added with deuterated internal standards (7 α -hydroxycholesterol-d7, 7 β -hydroxycholesterol-d7, 7-ketocholesterol-d7, 27-hydroxycholesterol-d6; Avanti Polar Lipids, Alabaster, USA) and hydroxytoluenebutylated (BHT; 5 μ g/mL). After alkaline hydrolysis (10 mL of absolute ethanol and 0.4 M of potassium hydroxide, for 2 h at room temperature, pH 7.0) lipids were extracted with 20 mL of chloroform and 6 mL of 0.9 M NaCl following vigorous agitation and centrifugation (750 \times g for 15 min at 4 $^{\circ}$ C). The organic phase was evaporated and diluted in 1 mL of toluene. Oxysterols and cholesterol were isolated by solid phase extraction (Sigma-Aldrich Supelclean LC-Si SPE Tubes SUPELCO, Bellefonte, USA). The sample was applied to a column previously conditioned in 2 mL of hexane, following a wash with 1 mL of hexane. Cholesterol and oxysterols were sequentially eluted by using 8 mL of 1.5% isopropanol in hexane and 6 mL 30% isopropanol in hexane. The solvent was evaporated, and the lipid extract was derivatized by using pyridine: trimethylsilyltrifluoroacetamide (BSTFA; Sigma-Aldrich, St. Louis, USA) (1:1, v/v) at 60 $^{\circ}$ C for 1 h. One μ L of sample was injected into a gas chromatograph coupled to a mass spectrometer (Shimadzu GCMS-QP2010, Kyoto, Japan). The separation was performed on a Restek capillary column (100%

dimethyl polysiloxane—Rxi®-1 ms. Cat. #13323), 30 m, internal diameter 0.25 mm, for 30 min, using helium as mobile phase, with a constant linear velocity of 44.1 cm/sec. The oven started at 240 °C for 7 min with an increment of 5 °C/min, up to 290 °C. The mass spectrometer was operated in single ion monitoring, an ionization voltage of 70 eV with the temperature of the ion source at 300 °C(13, 14). The intra assay coefficient of variation was 19%.

Statistical Analysis

Data are presented as mean \pm standard deviation (SD). The Shapiro-Wilk test was used to evaluate the normal distribution of the variables. The parametric data were analyzed by one-way ANOVA analysis of variance. When a significant difference was found, the Scheffé post-hoc comparison test was used. $P < 0.05$ indicates statistical significance.

Results

HDL isolated from AASU showed higher levels of 7-K (104.1 ± 64.0 vs. 43.7 ± 8.3 vs. 43.2 ± 9.1 ng/mg C; $p < 0.05$; Fig. 1A) and 7β -OH (15.5 ± 5.6 vs. 10.1 ± 2.0 vs. 8.1 ± 1.0 ng/mg C; $p < 0.05$; Fig. 1B) when compared to AASNU and SC, respectively. However, 7α -OH (10.7 ± 4.2 vs. 8.0 ± 1.5 vs. 6.8 ± 2.2 ng/mg; $p = 0.089$; Fig. 1C) and 27-OH (25.6 ± 7.2 vs. 28.0 ± 7.0 vs. 21.8 ± 3.2 ng/mg; $p = 0.235$; Fig. 1D) were not different among AASU, AASNU and SC, respectively.

Discussion

Oxysterols participate in the regulation of several physiological process including those related to intracellular cholesterol homeostasis(1–3). On the other hand some species of oxysterols are more related to inflammation, apoptosis and atherosclerotic plaque instability. Increased oxysterols plasma concentrations are observed in individuals with hypercholesterolemia and atherosclerosis and are associated with the degree of coronary stenosis(15). High oxysterols concentrations have been reported in the atherosclerotic plaque. In addition, they are associated with the progression of the lesion(16). The accumulation of 7-K is closely related to the expression of endoplasmic reticulum stress markers in atherosclerotic macrophages as well as to the rupture prone areas in lesion(17). Furthermore, previous studies showed that HDL particles enriched with oxysterols have lower ability to remove cellular cholesterol(4). The intracellular accumulation of 7-K alters plasma membrane leading to the impairment in cholesterol efflux(5). In the present study, we found increased levels of 7β -OH and 7-K in HDL isolated from AAS users. This response may contribute to the lower ability of HDL in removing cell cholesterol and trigger a higher pro-oxidant and pro-inflammatory effect in young male under AAS abuse. In fact, we previously found a diminished cholesterol efflux mediated by HDL and coronary artery disease in young male AAS users(12).

The present study has limitations. This is an exploratory and observational analysis of a subgroup of participants. We included only men and the results should be interpreted with caution in women. However,

the results were highly significant and relates to previous data regarding HDL loss of function.

Conclusion

Anabolic androgenic steroids abuse increases the amount of 7 β -OH and 7-K in HDL, which may explain, at least in part, the impairment in macrophage cholesterol efflux mediated by HDL and atherosclerotic coronary artery disease previously reported in young AAS users. The clinical implication of this finding should be considered in future, larger, long-term studies.

Abbreviations

AASU

anabolic androgenic steroids users

AASNU

anabolic androgenic steroids nonusers

HDL

high-density lipoprotein

SC

sedentary control

SD

standard deviation

27-OH

27-hydroxycholesterol

7-K

7-ketocholesterol

7 β -OH

7 β -hydroxycholesterol

7 α -OH

7 α -hydroxycholesterol

Declarations

Ethics approval and consent to participate

The local Human Subject Protection Committee approved this cross-sectional study (3945/13/070), and each participant provided written informed consent.

Consent for publication

All authors have read the manuscript and agreed for the submission.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

FRS (Study design, statistics, data collection, writing and final discussion);

MRS (Study design, statistics, writing and final discussion);

GWPF (Data collection, writing and final discussion);

GSF (Oxysterols measurement and final discussion)

CEN (Writing and final discussion);

MP (Data collection, writing and final discussion);

MJNNA (Study design, writing and final discussion).

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Figures

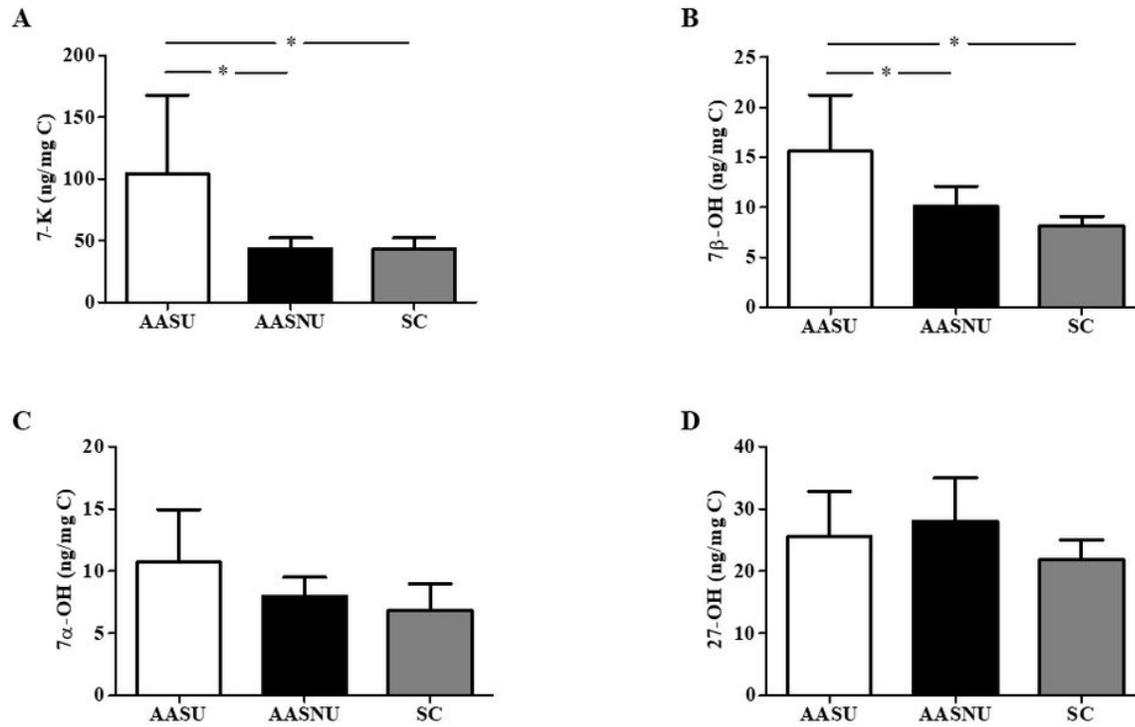


Figure 1

Concentration of oxysterols, 7-ketocholesterol (7-K), 7β-hydroxycholesterol (7β-OH), 7α-hydroxycholesterol (7α-OH) and 27-hydroxycholesterol (27-OH) in HDL isolated from anabolic androgenic steroids users (AASU), anabolic androgenic steroids nonusers (AASNU) and sedentary controls (SC). The amount of oxysterols was corrected per mg of cholesterol in HDL; *= p < 0.05.