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Original Article

Sex steroids mediate discrete effects on HDL cholesterol efflux capacity and particle concentration in healthy men

Katya B. Rubinow, MD*,[1], Tomas Vaisar, PhD[1], Jing H. Chao, MD, Jay W. Heinecke, MD, Stephanie T. Page, MD, PhD

Center for Research in Reproduction and Contraception, Seattle, WA, USA (Drs Rubinow, Chao, and Page); and Diabetes Institute, Division of Metabolism, Endocrinology, and Nutrition, Department of Medicine, University of Washington School of Medicine, Seattle, WA, USA (Drs Rubinow, Vaisar, Heinecke, and Page)

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BACKGROUND: Exogenous testosterone decreases serum concentrations of high-density lipoprotein cholesterol (HDL-C) in men, but whether this alters cardiovascular risk is uncertain.

OBJECTIVE: To investigate the effects of testosterone and estradiol on HDL particle concentration (HDL-Pima) and metrics of HDL function.

METHODS: We enrolled 53 healthy men, 19 to 55 years of age, in a double-blinded, placebo-controlled, randomized trial. Subjects were rendered medically castrate using the GnRH receptor antagonist acyline and administered either (1) placebo gel, (2) low-dose transdermal testosterone gel (1.62%, 1.25 g), (3) full replacement dose testosterone gel (1.62%, 5 g) or (4) full replacement dose testosterone gel together with an aromatase inhibitor for 4 weeks. At baseline and end of treatment, serum HDL total macrophage and ABCA1-specific cholesterol efflux capacity (CEC), HDL-Pima and size, and HDL protein composition were determined.

RESULTS: Significant differences in serum HDL-C were observed with treatment across groups ($P = .01$ in overall repeated measures ANOVA), with increases in HDL-C seen after both complete and partial testosterone deprivation. Medical castration increased total HDL-Pima (median [interquartile range] 19.1 [1.8] nmol/L at baseline vs 21.3 [3.1] nmol/L at week 4, $P = .006$). However, corresponding changes in total macrophage CEC and ABCA1-specific CEC were not observed. Change in serum 17β-estradiol concentration correlated with change in total macrophage CEC ($\beta = 0.33$ per 10 pg/mL change in serum 17β-estradiol, $P = .03$).

CONCLUSIONS: Testosterone deprivation in healthy men leads to a dissociation between changes in serum HDL-C and HDL CEC. Changes in serum HDL-C specifically due to testosterone exposure may not reflect changes in HDL function.

Introduction

Low circulating testosterone concentrations associate with increased cardiovascular disease (CVD) risk in men,[1] but whether this association reflects a causal relationship remains uncertain. Similarly, the impact of testosterone replacement therapy (TRT) on CVD risk is unclear and

[1] K.B.R. and T.V. are co-first authors and contributed equally to this work.

* Corresponding author. University of Washington School of Medicine, Box 358055, 815 Mercer Street, Seattle, WA 98109, USA.
E-mail address: rubinow@uw.edu

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particularly controversial with regard to its use in older men with late-onset hypogonadism.[1,2] Concern stems in part from the observation that exogenous testosterone administration to men often modestly lowers serum concentrations of high-density lipoprotein cholesterol (HDL-C),[3] a well-established negative risk factor for CVD.[4]

Measurement of HDL-C concentrations may have limited utility as a predictor of CVD risk. Despite the strong, inverse association between HDL-C and CVD risk in epidemiologic studies, recent clinical trials have demonstrated that pharmacologic treatments designed to increase HDL-C have failed to confer cardiovascular protection despite substantially raising HDL-C concentrations.[5,6] Thus, the possibility exists that TRT also could result in a dissociation between HDL-C and CVD risk. Increasingly, alternative metrics of HDL composition and function have been used as indices of the relationship between HDL and CVD.[7] The use of these alternative metrics may offer key insights into HDL-associated mechanisms through which testosterone may influence CVD risk in men.

The best characterized function for HDL is its role in reverse cholesterol transport (RCT), the process by which cholesterol from peripheral cells is transferred by HDL to the liver for excretion in the bile. HDL mediates RCT both directly by delivering the cholesterol to liver via scavenger receptor class B type 1 (SR-BI) and indirectly through transfer of cholesterol to apolipoprotein B (apoB)–containing particles and delivery via LDL receptor.[8] In addition to RCT, HDL can also deliver cholesterol to steroidogenic cells, adipocytes, and endothelial cells through interaction with SR-BI.[9,10] HDL cholesterol efflux capacity (CEC), the ability of HDL to accept cholesterol from macrophages—the first critical step of RCT—can be quantified in clinical samples using cell-based assays.[11] Importantly, serum HDL-C concentrations are dissociated from HDL CEC; HDL-C accounts for less than half of the variance in HDL CEC,[11] underscoring the limitations of serum HDL-C as a measure of HDL function.[12] Furthermore, HDL CEC has been shown to be a predictor of both prevalent and incident coronary artery disease independent of HDL-C concentrations.[13,14] HDL particles of different sizes have been shown to preferentially mediate cholesterol efflux through discrete pathways,[15,16] and large HDL subclasses in particular have been associated with lower CVD prevalence and severity, although these findings have not been uniform across studies.[17] Furthermore, lower total HDL particle concentration (HDL-Pima) has been associated with prevalent cerebrovascular disease and incident cardiovascular events.[18–21] HDL-Pima and size distribution have been shown to more closely associate with CVD than HDL-C.[18,19] Thus, quantifying changes in these alternative HDL metrics may generate important new insights into the relationship between testosterone and HDL and thereby into the relationship between testosterone and CVD risk in men.

To investigate the relationship between testosterone and HDL, we measured prespecified HDL-associated metrics using serum samples collected during a randomized, double-blind clinical intervention study in which healthy, eugonadal men underwent sex steroid manipulation for a 4-week treatment period. At baseline and end of treatment, serum HDL CEC, and HDL-Pima, size distribution, and protein composition were quantified to comprehensively characterize the effects of testosterone on HDL in men.

Materials and methods

Study design and subjects

A total of 53 subjects with normal baseline serum testosterone concentrations were enrolled. Of these, 45 completed all study visits and study-related procedures and were included in the analyses. As described elsewhere,[22] enrolled subjects were healthy, eugonadal men between 19 to 55 years of age. Exclusion criteria included a history of diabetes, uncontrolled hypertension, body mass index >33 kg/m^2, and current or recent use of testosterone, glucocorticoids, or lipid-lowering medications including statins.[22]

The GnRH receptor antagonist acyline (300 μg/kg body weight)[23] was administered by subcutaneous injection to all subjects at baseline and study week 2. Subjects were randomized to receive one of the following regimens administered daily over the 4-week treatment period: (1) placebo transdermal gel and placebo tablets (castrate group), (2) low-dose testosterone gel (1.25 g of 1.62% gel; AndroGel, Abbvie Inc; Chicago, IL) and placebo tablets (low testosterone/estrogen [T/E] group), (3) full replacement dose testosterone gel (5g of 1.62% gel) and placebo tablets (normal T/E group), or (4) full replacement dose testosterone gel and the aromatase inhibitor letrozole to confer selective estrogen deprivation (Apotex Corporation; Ontario, Canada) (normal T/low E group). Double-blinded randomization to treatment groups was performed with a block size of 4. At baseline and end of treatment (study week 4), serum HDL CEC was measured by 2 methods with serum depleted of apoB-containing particles. Total HDL-Pima and particle size distribution were quantified by calibrated ion mobility analysis (cIMA).[18] This study was approved by the University of Washington Institutional Review Board and registered at ClinicalTrials.gov (ID NCT01686828). Written informed consent was obtained from all subjects before any study procedures were performed.

Sample collection and processing

Serum sex steroids were measured by liquid chromatography–tandem mass spectrometry as previously described.[22] For measurement of fasting lipid concentrations and HDL analyses, whole blood was collected and allowed to clot for 15 minutes on ice. Samples were then centrifuged at 3000 rpm at 4°C for 10 minutes and immediately stored at −80°C. Samples were kept at −80°C until batched and analyzed together after a single thaw. Fasting lipid concentrations were measured at the Northwest Lipid Metabolism and