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Associations of cortisol/testosterone and cortisol/sex hormone-binding globulin ratios with atherosclerosis in middle-age women

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ABSTRACT

Background and aims: The cortisol/testosterone (C/T) ratio has been hypothesized to be a better predictor of atherosclerosis than cortisol alone. No study has assessed whether the C/T and C/sex hormone-binding globulin (SHBG) ratios are associated with atherosclerosis in a U.S. population sample.
Methods: This substudy included 367 women who had both cortisol from year 15 and testosterone and SHBG at year 16 of the Coronary Artery Risk Development in Young Adults study, an ongoing observational cohort in the United States. Of these, intima-media thickness (IMT) was available at follow-up year 20 in 339 (n = 332 with measurement at carotid bulb), and 303 were free of prevalent coronary artery calcium (CAC) at year 15. Area under the curve (AUC) of salivary cortisol was available in 302 individuals. Ratios of AUCs of cortisol to total testosterone, free testosterone, and SHBG were categorized into tertiles. Associations with CAC and IMT were assessed by regression models adjusted for age, race, body mass index, systolic blood pressure, menopause, oral contraceptive use, diabetes, alcohol, and smoking.
Results: Only the highest tertile of the AUC/free testosterone ratio was positively associated with carotid bulb IMT ($\beta = 0.088$, $P = 0.006$). This tertile was also positively associated with new onset CAC between year 15 and 25 (OR 3.45, 95% CI 1.18–10.06). Tertiles of cortisol or testosterone alone were not associated with new onset CAC.
Conclusion: AUC/Free testosterone ratio may be more associated with atherosclerosis in women than either indicator alone. The ratio may serve as a suitable biomarker of cortisol-linked stress.

1. Introduction

The concept of chronic stress as a risk factor for atherosclerosis has been suggested in both animal [1] and human studies [2,3]. Despite many years of research, it remains difficult to precisely assess chronic stress in humans due to measurement errors on self-reported questionnaires (validity concerns) and the inability of short-term measures of stress hormones (e.g., cortisol levels) to reflect the chronic state or trait. These problems may explain why the association between chronic stress and atherosclerosis has been inconsistent in human studies [4].

The cortisol/testosterone (C/T) ratio has been used as a chronic biomarker of stress, and has been suggested to be a better predictor of coronary heart disease than cortisol alone [4]. There are several reasons why the C/T ratio might be a plausible indicator of chronic stress. Testosterone and cortisol are derived from the same biochemical precursor [5], so if cortisol synthesis increases, there will be a corresponding decline in testosterone synthesis because it is a competitive reaction process [4–6]. Cortisol can suppress the activity of the hypothalamic-pituitary-gonadal axis [7]. Cortisol has a catabolic effect, and testosterone has anabolic effects [4].

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Therefore the C/T ratio may more accurately capture endocrine dynamics than cortisol alone.

The aim of this study was to examine the associations of cortisol alone, testosterone alone, and the C/T ratio with subclinical atherosclerosis in women. We also studied C/sex hormone-binding globulin (SHBG) ratio to provide a more complete physiologic assessment because SHBG has a physiologic role of reducing free circulating testosterone in women [8,9]. Subclinical atherosclerosis was assessed using coronary artery calcium (CAC) and carotid intima-media thickness (IMT).

2. Material and methods

2.1. The coronary artery risk development in young adults (CARDIA) study

The CARDIA study is a multicenter, longitudinal cohort study of the development of coronary artery disease risk factors in young adults. A full description of the study design is published elsewhere [10]. The full CARDIA cohort included 5115 black and white adults aged 18–30 years at the year 0 examination (1985–1986), recruited from four metropolitan areas in the United States (Birmingham, AL; Chicago, IL; Minneapolis, MN; and Oakland, CA) [11]. Within each center, the sample was designed to comprise approximately equal numbers of participants by sex, race (self-defined: black or white), age (18–24 or 25–30 years), and education (≤high school or >high school) [11]. Follow-up examinations (follow-up rates) were conducted at years 2 (91%), 5 (86%), 7 (81%), 10 (79%), 15 (74%), 20 (72%), and 25 (72%). All examinations were approved by institutional review boards at each institution, and informed consent was obtained from each study participant [11].

2.2. Study population

For this sub-study, we utilized data from two CARDIA ancillary studies that measured cortisol, testosterone, and SHBG to test our hypothesis. One ancillary study was conducted in year 15 at the Oakland and Chicago sites and included salivary cortisol measurement [12]. The second ancillary study was conducted in year 16 and included sex hormones and SHBG measurements. Together, the 2 studies provided a total of 367 women who defined the sub-study population. Of these 367 women (age range 32–51, mean age 40 years old), 28 were missing all data on IMT measurements (resulting N = 339). Additionally 5 were missing data on confounding variables, and 27 were missing some but not all IMT measures. For the CAC analysis, 320 women had CAC data from both years 15 and 25, of whom 17 already had positive CAC findings at year 15. Therefore, 303 women were included in the analysis assessing CAC incidence from year 15 to year 25. Fig. 1 illustrates the study population.

2.3. Cortisol, testosterone, and ratios

The detailed cortisol measurement protocol was described elsewhere [13]. Participants were given materials and instructed regarding the collection of saliva samples at the conclusion of their year 15 follow-up CARDIA clinic visit [13]. Samples were collected from participants on a single weekday, in most cases the Monday after a Friday or Saturday clinic visit [13]. Participants were instructed not to eat, brush their teeth, or drink liquids for at least 15 min before collecting a sample [14]. They were provided six saliva sample containers to be used over the course of the day: upon awakening ("when your eyes open and you are ready to get up"), 45 min, 2.5 h, 8 h, and 12 h after awakening, and at bedtime ("right before getting into bed") [13]. Participants were instructed to record the time they woke up in a log and were provided alarm watches (preset to their regular wakeup time) to remind them to collect samples; they were also given a form that allowed them to easily recalculate the desired sample times if they woke up at a different time than anticipated [13]. Cortisol concentrations were determined by time-resolved immunoassays with fluorometric end point detection [13]. Intra- and interassay variabilities were each less than 12%.

Area under the curve (AUC) for cortisol, a time-adjusted measure of total cortisol exposure while awake, is thought to reflect cumulative tissue exposure to cortisol across the day; persistently high total daily output may create "wear and tear" on various body tissues, resulting in structural or functional changes that could affect disease vulnerability [15]. On the other hand, the additional use of diurnal slopes attempts to capture cortisol circadian fluctuation patterns. The slope is usually operationally defined as the line resulting from regression of cortisol values collected across the day onto hours since awakening excluding the morning awakening response [15]. A negative diurnal slope is generally considered indicative of healthy hypothalamus-pituitary-adrenal (HPA) axis function, with a flattened or positive diurnal slope suggestive of potential HPA axis dysfunction [15]. AUC and slope were chosen for the chronic cortisol indices because they have greater 12-month stability than cortisol awakening response [15]. One study reported 12-month intra-class correlation coefficients for AUC of about 0.5 and for slope of about 0.25 [15].

The AUC for cortisol was calculated as the plot of log-transformed cortisol values against collection times from the first to the last sample [13]. The AUC was computed only for those people who had data for the first sample and a minimum of 12 h between their first and last samples [13]. The cortisol slope was calculated for those who had the first sample and the sixth sample as reported previously [13]. All 367 women in the substudy had both 1st and 6th samples. The slope was estimated by separately fitting a linear regression line for each participant that predicted the log-transformed cortisol concentrations from time (hours) since awakening [13]. To minimize the impact of morning rise in cortisol on slope estimation, the second saliva sample was excluded from the slope calculation [13].

Detailed testosterone and SHBG measurement protocols are published elsewhere [16]. Briefly, SHBG, total testosterone, and free testosterone were measured in a single batch on serum specimens collected at year 16 in the CARDIA Women's Study [16]. SHBG was determined using equilibrium dialysis on a Sephadex G-25 column [16]. This method estimates the amount of testosterone capable of being bound by SHBG [16]. Total testosterone was measured with a competitive immunoassay (Bayer Diagnostics, Tarrytown, NY) that employed direct chemiluminescent technology on the ACS:180 automated chemiluminescent system (Beckman Coulter, Brea, CA). The coefficient of variation for the total testosterone sample (80 ng/dL) was 5.9%. The manufacturer, Beckman Coulter, reports that the coefficient of variation (CV) for this assay is less than 10% for total testosterone >50 ng/dL, while The College of American Pathologists document an inter-assay CV of 13.4% for 30 ng/dL samples with the Beckman Coulter system, the lowest of 16 systems surveyed [16]. Due to imprecision at the lower end of the detection limit of the assay, total testosterone levels below 10.0 ng/dL were all reported as 5 ng/dL [16]. Free testosterone which is used for estimation of free testosterone level in women [9] was calculated based on measured total testosterone and SHBG levels using the method described by Pearlman [16,17]. The analyses only included measures obtained from women who reported that they were not pregnant.

The following ratios of AUC and of slope of cortisol to total testosterone, free testosterone, and SHBG were computed: AUC/