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Sex steroid levels temporarily increase in response to acute psychosocial stress in healthy men and women

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ABSTRACT

It is well known that acute psychosocial stress activates the hypothalamic–pituitary–adrenal (HPA) axis and the sympathetic nervous system (SNS). However, the effect of acute psychosocial stress on the hypothalamic–pituitary–gonadal (HPG) axis and levels of sex steroids are less known. The aim of this study was to investigate the effect of acute psychosocial stress on serum concentrations of sex steroids in healthy men and women. Twenty men and 19 women (age 30–50 years) underwent Trier Social Stress Test (TSST), a tool for investigating psychobiological stress responses in a laboratory setting. Blood samples were collected before, directly after the stress test, and after 30 min of recovery. Concentrations of androgens were measured with high specificity LC-MS/MS method; concentrations of cortisol, estradiol and sex hormone-binding globulin were determined using immunoassays. In both men and women we observed significantly elevated levels of testosterone, estradiol, androstenedione and sex hormone binding globulin along with significantly increased adrenocorticotropic hormone (ACTH), serum cortisol, heart rate, systolic blood pressure (SBP), and diastolic blood pressure (DBP) as a response to the stressor. Thus, even though the HPG axis and the production of sex steroids may be inhibited during prolonged periods of stress, the sex steroid levels may increase in the initial phase of acute psychosocial stress.

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1. Introduction

It is well known that acute psychosocial stress activates the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic nervous system (SNS), as a response to overcome the stressor. However, the effect of acute psychosocial stress on the hypothalamic–pituitary–gonadal (HPG) axis and levels of sex steroids is less known. The HPA axis and HPG axis are competitive system and during prolonged periods of stress, the HPG axis and the production of sex steroids could be inhibited (Theorell, 2008). In the initial phase of the acute stress, the response may however be different. Most of the research on sex steroids levels in response to acute stress has been conducted on male mice or rats with focus on the levels of testosterone. While animals studies have consistently shown that chronic stress decreases testosterone levels in males (Hardy et al., 2005), studies on the effect of the acute stress are inconsistent, since both decreased and increased levels of testosterone have been reported (Chichinadze and Chichinadze, 2008). Both increased and unchanged levels of estradiol have been observed in response to acute stress in female rats (Shors et al., 1999).

In humans, several studies have investigated changes in testosterone levels in response to competition-induced stress, demonstrating that testosterone levels may increase prior to and during competitions with or without a physical component (Booth et al., 1989; Hasegawa et al., 2008). It has been suggested that the increase in testosterone level has a preparatory role in stress challenges by inducing competitive and dominant behaviour (Booth et al., 1989). However, there are few studies available that have investigated sex steroid levels in response to acute psychosocial stress. Gerra et al. (2000) investigated stress hormone responses to controlled experimental acute stress in peripubertal males and found that the levels of testosterone in healthy participants were unchanged. In males, stress related to oral presentation on a scientific conference did not affect testosterone or estradiol levels (Heinz et al., 2003); and examination stress in males did not affect estradiol levels (Phillips, 1992). Schoofs and Wolf (Schoofs and Wolf, 2011) studied levels of testosterone and estradiol before and after controlled experimental acute stress and did not find any changes in levels in the male or female participants.

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Thus, the few existing studies on acute psychosocial stress did not show any stress-induced changes of testosterone and estradiol as seen in some animal studies. The inconsistent results seen in animals and humans may depend on that different studies investigate sex steroid levels in response to different types of stressors and that the length of stress exposure and time points for measurements differs among the studies. The Trier Social Stress Test (TSST) is often used as a controlled laboratory stressor, when studying biological responses to acute psychosocial stress in humans. It is a validated and well-established tool for investigating psychobiological stress responses in a laboratory setting. Among the large number of publications on studies employing the TSST only two investigated levels of sex steroids in response to the TSST. One of these (Schoofs and Wolf, 2011) measured testosterone and estradiol in saliva which may be uncertain (Lewis, 2006). The other (Gerra et al., 2000) investigated testosterone response in peripubertal males, whose endocrine response may differ from adult males. As mentioned above, most animal studies are conducted on males. The few studies conducted on humans have predominantly been performed on men. The aim of this study was to investigate changes in sex hormone levels in serum in response to acute psychosocial stress in healthy men and women using the TSST.

2. Method

2.1. Participants

Thirty-nine healthy subjects (20 men and 19 women), aged 30 to 50 years, were included in the study. The mean age for males was 37 years (SD 5) and for females 38 years (SD 5). The subjects were recruited from a cohort study, surveying psychosocial work environment and health, or through advertising in a local daily newspaper. To be included in the study, subjects had to be between 30 and 50 years of age, and not suffer from chronic stress problems; only individuals reporting “no stress at all” or “very little stress” on a single perceived stress item (Elo et al., 2003) were included in the study. Exclusion criteria were; having a body mass index less than 18.5 kg/m^2 or over 30 kg/m^2, high blood pressure, infection, vitamin B-deficiency (high homocysteine), known systemic disease such as diabetes, thyroid disease or known psychiatric disease. Beside from anthropometric measures and screening interview, blood samples were taken and analysed to confirm that above exclusion criteria's were not fulfilled. Because the varying levels of sex hormones during menstrual cycle and the use of estrogens are known to affect the physiological response to acute stress (Kirschbaum et al., 1999; Kajantie and Phillips, 2006), women taking oral contraceptives including estrogens and postmenopausal women were excluded. Subjects who were taking psychoactive medications or any medications that may affect the HPA axis function were also excluded. Other exclusion criteria were pregnancy or nursing. The study was approved by the Regional Ethical Review Board in Göteborg, Sweden, and was conducted according to the Helsinki Declaration. All participants gave written informed consent before entering the study.

2.2. Study procedure

The TSST was set up according to the original design of Kirschbaum (Kirschbaum et al., 1993). The stress task in TSST consists of a simulated job interview and a mental arithmetic task, both in front of a committee (two men and one woman), a video camera, and a microphone. Subjects were instructed to abstain from hard physical exercise 24 h before the stress test. Subjects were also instructed to avoid beverages containing caffeine at least 2 h before the stress test and to eat a standardized lunch. Smoking and using snuff were accepted but not on the test day. For female subjects, the stress tests were conducted during follicular phase of the menstrual cycle (self-reported, between the 5th and 10th days). The phase of the menstrual cycle was confirmed by measuring serum levels of estradiol and progesterone in samples collected before the stress test. The stress tests were performed at the Institute of Stress Medicine of Region Västra Götaland in Göteborg. The total test time for each subject was 2 h, including preparations and measurements after completing the test, and the test procedure was conducted between 1300 h and 1700 h (to avoid circadian rhythm effects). At arrival, an intravenous catheter was inserted in the subject's forearm (−30 time point). The first blood sample was drawn at the −10 time point. The second blood sample was drawn directly before the TSST started (0 time point). Between these two measurements, the participants rested (approximately 7 min). At the start of the TSST, the participants were introduced to the tasks and asked to prepare for the simulated job interview (10 min). After this, the participants underwent a simulated job interview (5 min) and thereafter performed a mental arithmetic task (5 min). Directly after the end of the stress test (the +20 time point), a third blood sample was drawn. 10 and 20 min into the 30 minute recovery period, the fourth and fifth blood samples were drawn (the +30 and +40 time points). A final blood sample was drawn at the end of the recovery period (+50 time point). Cardiovascular responses (heart rate, systolic blood pressure, and diastolic blood pressure) were electronically recorded (CardioPerfect Workstation, Welch Allyn) every fifth minute from 10 min before the TSST started until 30 min after the TSST ended (from the −10 time point to the +50 time point).

2.3. Hormone assays

A total of 122 mL of blood was collected from each of the participants during the TSST. Blood samples at six time points (−10, 0, +20, +30, +40, and +50) were collected (7 mL at each point) for measurements of plasma ACTH and serum cortisol. Blood samples at four of the six time points (−10, 0, +20, and +50) were collected (20 mL at each point) for measurement of sex hormones. The samples were collected in two different tubes; pre-chilled tubes containing EDTA and serum separator tubes. After the tubes have been centrifuged, plasma and serum was stored at −80 °C until assayed. Concentrations of testosterone and androstenedione were determined using Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS) method, as extensively described elsewhere (Kushnir et al., 2006; Kushnir et al., 2010). The detection limit for both androstenedione and testosterone was 35 pmol/L. The assays showed within-run variation less than 10% and between-run variation less than 12% (Kushnir et al., 2006; Kushnir et al., 2010). Calibration curves were generated with every set of samples using six calibration standards; three quality control samples were included with every set of samples. Concentrations of free testosterone were calculated based on binding constants with SHBG and albumin (Vermeulen et al., 1999). Plasma concentrations of ACTH were measured by immunoradiometric assay (limit of detection (LOD), 0.4 pmol/L) (CIS bio International, Gif-sur-Yvette Cedex, France). Serum concentrations of cortisol were measured by electrochemiluminescence immunoassay (LOD, 20 nmol/L). Serum concentrations of estradiol were measured by radioimmunoassay (RIA) (LOD, 0.04 nmol/L) from DiaSorin (Saluggia, Italy) and of sex steroid binding globulin (SHBG) using an IMMULITE 2000 analyser (Siemens, Los Angeles, CA). Inter-assay coefficients of variation were below 10% for ACTH, below 11% for cortisol, below 12% for estradiol at 0.4 nmol/L and below 16% at 0.04 nmol/L.

2.4. Statistical analysis

Baseline values for concentrations of free and total testosterone, estradiol, androstenedione, SHGB, ACTH, cortisol; and values of the heart rate, systolic blood pressure and diastolic blood pressure were calculated by taking the means of the two baseline values at the −10 time point and the 0 time point. Three women and one man had missing values for heart rate, systolic and diastolic blood pressure

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