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Sex hormones adjust "sex-specific" reactive and diurnal cortisol profiles

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ABSTRACT

Sex differences in stress hormone functions are presumed to depend on sex hormones. And yet, surprisingly few psychoneuroendocrine studies actually assess within-sex variations of testosterone, estradiol, and progesterone when investigating sex-specific activities of the hypothalamic–pituitary–adrenal axis. In this methodological study of 204 healthy adults (60 men), we assessed whether cortisol profiles would differ between the sexes when unadjusted or adjusted for basal sex hormones among both sexes. Reactive cortisol was sampled using 6 saliva samples measured every 10-min as part of the Trier Social Stress Test that generally activates cortisol among men more than women. Diurnal cortisol was sampled over two days at (1) awakening, (2) 30-min thereafter, (3) 1400 h, (4) 1600 h, and (5) bedtime. Sex hormones were collected at baseline before the psychosocial stressor and on two occasions during diurnal cortisol assessment. Repeated-measures analysis of covariance controlled for key covariates in analyses unadjusted or adjusted for sex hormones. Results revealed that men had higher reactive cortisol than women in unadjusted analysis, but this sex difference was attenuated when adjusting for sex hormones. While diurnal cortisol showed no sex differences in unadjusted models, adjusting for sex hormones revealed that women have higher morning cortisol. Correlations using area under the curve formulae revealed intriguing sex-specific associations with progesterone in men and testosterone in women that we propose have implications for social and affective neuroscience. In summary, our results reveal that adjusting for sex hormones alters "sex-specific" reactive and diurnal cortisol profiles.

1. Introduction

Adrenal and gonadal hormones drive biobehavioral adaptations that ensure survival of organisms and propagation of species. At the epicenter of these evolutionary drives are the hypothalamic–pituitary–adrenal (HPA) axis production of the stress hormone cortisol and the hypothalamic–pituitary–gonadal (HPG) axis production of sex hormones like testosterone, estrogens, and progesterone that have sex-specific functions (Sapolsky, 2004). Until as recently as 1995, females represented only 17% of subjects/participants in HPA-axis studies that systematically excluded them due to their cyclic HPG-axis variations (Taylor et al., 2000). Despite increasing inclusion of women today in the psychoneuroendocrine studies of stress physiology, within-sex variations in sex hormones are *presumed* but rarely actually measured. To address this idiosyncrasy, the current methodological study will assess the extent to which accounting for sex hormones adjust "sex-specific" cortisol profiles.

Sex differences in stress reactive cortisol have a rich history in the stress-disease literature. Over two decades ago, Kirschbaum et al. (1992) reported what appeared to be a strong sex-based differ-

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ence in cortisol stress reactivity. Compared to women, men showed a 2-fold higher cortisol reactivity after public speaking and mental arithmetic in front of an audience (Kirschbaum et al., 1992). In a follow-up study, women using oral contraceptives manifested further attenuation of cortisol stress reactivity (Kirschbaum et al., 1995). Researchers reasoned that this was an estradiol driven effect. Specifically, oral contraceptives contain high levels of estradiol that stimulate the production of cortisol-binding globulin, which in turn removes free cortisol from circulation, therefore resulting in lower net concentrations of active cortisol. Subsequent studies assessed cortisol reactivity at different menstrual cycle phases to further understand HPA-axis and HPG-axis cross-talk. Results revealed that women with low estrogen display reactivity patterns similar to men, while women with high estrogen resemble women using oral contraceptives (Kirschbaum et al., 1999). Thus, acute stimulation of the HPA-axis is related to estrogen regulation in women.

With regards to research on stress reactivity, estrogen and progesterone vary according to the menstrual cycle, hormone-based contraception, and menopause-related hormone therapy later in life (Bouma et al., 2009; Kajantie and Phillips, 2006; Kirschbaum et al., 1999, 1995; Komesaroff et al., 1999; Marinari et al., 1976; Patacchioli et al., 2006; Prinz et al., 2001). Another consideration is that HPA-axis reactivity generally increases with aging, such that stress reactive amplitudes can be three times stronger in postmenopausal women compared to older men (Otte et al., 2005). Whereas estrogen appears to beneficially dampen the HPA-axis in women (Kajantie and Phillips, 2006), the effects of progesterone are usually limited to understanding female reproduction. And yet, both estradiol and progesterone can also modulate neuroendocrine functioning in men (Champagne et al., 2012; Kirschbaum et al., 1996).

Akin to research on "female" hormones, testosterone has been extensively studied in men. Notwithstanding, growing evidence reveals that androgens interact with social contexts among both sexes. Beyond reproductive functions, testosterone interacts with various HPA-axis modulators (e.g., dehydroepiandrosterone antagonizes cortisol) which can contribute to multi-systemic pathophysiology (Juster et al., 2011a). For reasons explained in part by basal differences in HPG-axis functioning, men show the most pronounced differences in HPA-axis responsivity to laboratory-based stressors that elicit social-evaluative threat (Dickerson and Kemeny, 2004; Kajantie and Phillips, 2006; Kirschbaum et al., 1992). Yet for both sexes, enhanced HPG-axis activities like testosterone production are also indicative of challenge and anticipation (Chichinadze and Chichinadze, 2008; Chichinadze et al., 2012a,b), suggesting that the HPG-axis is involved in preparedness to respond for both sexes in stress paradigms.

Complementary to reactive cortisol paradigms, naturalistic variations in HPA-axis functioning can be assessed non-invasively in day-to-day contexts. Upon awakening, the *cortisol awakening response* (CAR) represents a normal surge in cortisol levels reaching maximal concentrations approximately 30 min after awakening (Pruessner et al., 1997). This surge is followed by gradually declining cortisol concentrations throughout the day as pulsatile secretion decreases in amplitude and frequency (Clow et al., 2010). The nadir usually occurs around midnight, and cortisol levels start to rise again in the early morning hours (Kudielka et al., 2012; Loucks et al., 2008). Unbeknownst is whether HPA-axis circadian rhythmicity differs between the sexes (Kudielka et al., 2012). Like studies assessing stress reactivity, studies of diurnal cortisol have rarely assessed the functioning of the HPG-axis that may influence inter-individual differences in cortisol profiles.

To address these lacunas, our methodological study explored how sex differences in reactive and diurnal cortisol differ according to general linear models that are (1) unadjusted for sex hormones but otherwise control for key covariates or that are (2) adjusted for testosterone, estradiol, and progesterone sampled among both sexes. Our general hypothesis was that cortisol sex differences in unadjusted models would be statistically attenuated when adjusting for sex hormones. In secondary analyses, we explored the directionality of HPG-axis and HPA-axis associations to further understand cross-talk between these systems by calculating area under the curve scores for key cortisol time-windows in reactive and diurnal conditions. Anticipatory distress was also assessed in relation to HPG-axis and reactive HPA-axis activities.

2. Methods

2.1. Participants

Participants included 60 men and 144 women of diverse reproductive status (cycling: $n = 55$; contraceptive: $n = 47$; postmenopausal: $n = 42$). Participants were recruited from among the employees of the *Institut universitaire en santé mentale de Montréal*, the largest psychiatric hospital in the Canadian province of Quebec.

Table 1 lists sample information. Inclusion criteria were liberal in order to best represent the health of various professions all from within the same workplace. Women were more likely than men to have a diagnosed mental health condition ($p = .002$), psychotropic use ($p = .010$), and past psychiatric sick leave ($p = .007$). By contrast, men were more likely than women to use illicit drugs ($p = .001$). Main analyses therefore controlled for illicit drugs and mental health condition that correlated with psychotropic use ($r = .91$, $p < .0001$) and past psychiatric history ($r = .568$, $p < .001$). This heterogeneity in sample characteristics was consistently related to age that we additionally controlled for in main analyses.

2.2. General protocol

This study was approved by the local research ethics board of the *Institut universitaire en santé mentale de Montréal* and adheres to the Declaration of Helsinki.

Testing was conducted by two women (N.D. and A.B.D.) and two men (R.P.J. and O.B.) between October 2011 and December 2012 at the Centre for Studies on Human Stress (Montreal, Quebec, Canada). Access to the participant pool was enabled by hospital administrators who allowed workers to participate during their working hours without prejudice and in full confidentiality. In partnership with our communications department, recruitment was promoted via conferences, intranet advertisements, large banners, face-to-face visits to units, and word of mouth. Prospects were instructed to contact our laboratory for a 15-min screening interview prior to scheduling appointments.

During their first laboratory visit between 13h00 and 18h00 (arrival time: $M = 13$h35, $SE = 0{:}51$ min) that lasted 90 min, participants (a) were requested to read and sign the consent form; (b) were instructed to provide saliva samples every 10 min at seven occasions throughout the visit to assess baseline sex hormones and reactive cortisol; (c) completed a cognitive task (data not reported here); (d) were exposed to the Trier Social Stress Test; (e) were instructed in the use of our internet-based questionnaire system; (f) were provided instructions for diurnal salivary collection to assess day-to-day variations in sex hormones and cortisol; and finally (g) were debriefed. Between visits, participants collected saliva samples at home and completed electronic questionnaires that took about 45 min to finish. During their second visit, participants returned materials and partook in a blood draw and physical examination to assess allostatic load (data not reported here). Participants received 50$ CAD as compensation for their contribution.