Physiology & Behavior 99 (2010) 8–16

Contents lists available at ScienceDirect

Physiology & Behavior

journal homepage: www.elsevier.com/locate/phb

Salivary testosterone, cortisol, and progesterone: Two-week stability, interhormone correlations, and effects of time of day, menstrual cycle, and oral contraceptive use on steroid hormone levels ☆

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ARTICLE INFO

Article history:
Received 24 June 2009
Received in revised form 19 September 2009
Accepted 2 October 2009

Keywords:
Testosterone
Cortisol
Progesterone
Steroid hormones
Stability
Test–retest reliability
Human
Interhormone correlations
Oral contraceptives
Menstrual cycle
Time of day

ABSTRACT

With salivary assessment of steroid hormones increasing, more work is needed to address fundamental properties of steroid hormone levels in humans. Using a test–retest design and radioimmunoassay assessment of salivary steroids, we tested the reliability of testosterone, cortisol, and progesterone levels across two weeks, as well as the effects of oral contraceptives, menstrual cycle phase, and time of day on steroid hormone levels. Testosterone and cortisol were found to be highly reliable in both sexes. Progesterone was found to be reliable after collapsing across sex. Oral contraceptive use was associated with lower levels of testosterone, but did not affect cortisol. Contrary to expectations, oral contraceptives also did not affect progesterone. Menstrual cycle was found to affect levels of progesterone, but not testosterone or cortisol. Time of day had an effect on cortisol, on progesterone only at one testing time, and no effect on testosterone. We explored the interhormone correlations among testosterone, progesterone, and cortisol. All three hormones were positively correlated with one another in men. In women, progesterone was positively correlated with testosterone and cortisol, but testosterone and cortisol were uncorrelated.

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1. Introduction

The ability to obtain valid measures of bioactive steroid hormones from human saliva has led to an increase in the use of hormones in psychological research. This increased attention on salivary hormones has raised issues heretofore not thoroughly addressed in the human literature, specifically the stability of basal hormone levels over time. The influence of interhormone relationships [34], circadian rhythms [14], menstrual cycle [3,15], and the use of oral contraceptives [20] on endogenous salivary hormone levels have all been researched on their own, but their impact on the stability of basal steroid levels has been mostly neglected. With the increased use of salivary steroid hormones in psychological research, more basic research is needed to assure researchers that salivary assessments of hormones actually represent what they are interpreted as representing (e.g. baseline measurements are reliable and relatively stable, individual differences in basal levels are reasonably static, etc.).

Currently, there is a dearth of research on the stability of steroid hormone levels in human populations. In order for psychologists to use salivary steroid hormones as a trustworthy assessment, research into the reliability of these assessments is essential. Just as self-report questionnaires are subject to thorough psychometric testing (e.g. [11,31]), salivary assessments of hormones must be subject to the same scrutiny if they are to be used as markers of stable properties of individuals' endocrine systems. To date, only two studies have specifically addressed the stability of salivary testosterone in an adult population [4,29]. Both studies found testosterone to be highly reliable over a variety of time periods, but neither took into consideration important factors that could potentially influence steroid hormone levels. While Dabbs [4] examined the stability of testosterone levels over a variety of time periods, oral contraceptive use was not considered, and reliability was calculated after collapsing across sex, which is problematic given the large differences between men and women's testosterone levels [29]. Sellers et al. [29] tested the stability of testosterone without consideration of time of day,

☆ "Stability" here refers to the consistency of basal levels of endogenous hormones over time. Other fields may refer to this same effect as "reproducibility," but in the context of the current study we use "stability" to refer to the consistency of hormone levels across assessment periods over time.

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0031-9384/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.physbeh.2009.10.001

menstrual cycle, or oral contraceptive use. Both studies examined testosterone in isolation, without measuring any other steroid hormones, such as cortisol or progesterone.

The primary use of cortisol in psychological research has been as a biomarker of the stress response [6], and most research on the stability of cortisol has focused on the reliability of cortisol levels in the morning (e.g. [7,24,40]) and the reliability of its diurnal pattern [41]. Though the morning reliability of cortisol and its response to stressors has been thoroughly studied, there is very little research on the stability of salivary cortisol levels in an adult population, and the little research that has been conducted has generally focused on methodological sources of variability in cortisol levels [16,17]. This limited previous research has found cortisol to be somewhat less stable than other steroid hormones. For instance, Pearson correlation coefficients ranging from 0.20 to 0.25 were found when testing cortisol levels over a six week time span [19].

Unlike testosterone and cortisol, progesterone is a generally understudied steroid hormone in the context of human social behavior, though recent work has started to explore its role in affiliation motivation and social closeness (e.g. [2,28]). The majority of research on the fundamentals of salivary progesterone levels has been conducted in children, and focused almost solely on circadian rhythms, not the stability of progesterone over varying time periods [14]. There is some research on the relationship between progesterone and behavior, but no tests of the basic stability of basal progesterone levels in an adult population. Thus, all three steroid hormones that we have discussed are used in psychological research, but all three lack sufficient research to establish that they are stable enough to warrant their use as dispositional measures.

Above and beyond a need to document the stability of steroid hormones, a more nuanced understanding of key contributing factors to variations in hormone levels, as well as how levels of salivary hormones are interrelated, is critical. Previous research has shown that among female research participants, factors such as phase of menstrual cycle, use of oral contraceptives, and relationship status can all affect steroid hormone levels and their relationship with psychological constructs [25,32]. Previous research has also shown that there is a complex and dynamic relationship between endocrine axes. The antagonistic relationship between the hypothalamic–pituitary–adrenal (HPA) and hypothalamic–pituitary–gonadal (HPG) axes, responsible for the situational release of cortisol and testosterone, respectively, has been well-established [34,35], but the nature of interhormonal dynamics in humans requires more research. These dynamics are especially poorly understood outside of the cortisol–testosterone relationship. For instance, very few studies have examined the relationship between salivary cortisol and progesterone [15,37], and we are unaware of any studies reporting the relationship between salivary testosterone and progesterone.

The purpose of the present study was to provide foundational knowledge regarding the stability of three steroid hormones in both sexes over a two-week time span. We measured and tested the stability of testosterone, cortisol, and progesterone over a two-week time period, as well as examined the effects of the menstrual cycle and oral contraceptive use, which were expected to affect progesterone levels in particular, on female participants' salivary levels of all three hormones. Finally, intercorrelations between the three hormones were explored in an attempt to further understand the hormones' relationships to one another.

2. Method

2.1. Participants

One hundred and twenty two students enrolled at the University of Michigan, Ann Arbor participated in the two-session study, with data collection sessions spaced exactly 14 days apart. Participants were recruited via flyers posted in campus buildings, and contacted the experimenters through an email address provided on the flyer. The experimenters scheduled two sessions for the participants to come to the lab to participate in the study. The session dates were scheduled exactly 14 days apart with each data collection session taking place at the exact same time of day, though time of day of participation varied between participants. The study had received approval from the Institutional Review Board at the University of Michigan prior to data collection, and all participants provided informed consent at the time of participation.

From the initial pool of participants, ten did not return for the second part of the study and two participants' data were lost due to a programming error. All were dropped from analysis. To account for daily fluctuations in hormone levels due to circadian rhythms [4], nine participants whose second session was completed at a different time of day (range: 9:30 am to 4:00 pm) were dropped from the analysis. An additional 22 participants' data were not included in the analysis due to unavailability of hormone data (e.g. insufficient or contaminated saliva sample). Of the remaining 79 participants constituting the final participant pool, 55 were women and 24 were men, with a mean age of 19.7 years, and 60.8% self-identified as Caucasian, 29.1% Asian, 3.8% African-American, 2.5% Pacific Islander, and 3.8% other or mixed ethnic groups. From this pool, a few participants were not included in all analyses due to the unavailability of hormone data for each of the three hormones (e.g. insufficient saliva sample for all assays). The progesterone analyses included 74 participants (53 women and 21 men), the testosterone analyses included 75 participants (52 women and 23 men), and the cortisol analyses included 76 participants (53 women and 23 men).

2.2. Procedure and design

The study had a test–retest design, with two data collection sessions spaced 14 days apart. At both testing sessions, participants came into the lab to complete a battery of measures assessing participants' mood, personality and cognitive functioning, and to provide saliva samples for hormone analysis (see [26], for a report on the findings related to personality). Participants also completed a demographic questionnaire regarding age, sex, ethnicity, and information that could impact the viability of the saliva sample (e.g. whether he/she smokes, how long since he/she brushed his/her teeth, how long since he/she consumed caffeine). Female participants also provided information regarding the date of the onset of their most recent menstrual cycle, the average length of their menstrual cycle, and whether or not they were currently using oral contraceptives. Participants completed personality measurements, questionnaires, and provided samples using computerized instruction, though an experimenter was present to oversee data collection.

2.3. Salivary sampling

For each of the saliva samples, participants used a stick of sugar-free chewing gum to stimulate saliva flow and collected up to 7.5 mL of saliva in a sterile polypropylene vial. They discarded the chewing gum after each saliva collection [42,27]. Participants' collection vials were sealed immediately after each collection and placed in frozen storage in accordance with previous research on sample storage [4,16]. Samples were freed from mucopolysaccarides and other residuals by three freeze–thaw cycles followed by centrifugation.

2.4. Assay procedure

Salivary hormone levels were assessed with solid-phase Coat-A-Count ^{125}I radioimmunoassays for testosterone (TKTT), cortisol (TKCO), and progesterone (TKPG) provided by Diagnostic Products Corporation (Los Angeles, CA). To determine salivary hormone

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