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Hailey Hernandez

April 7, 2019

Hormones and Academic Stress: Coupling and Reactivity of Testosterone, Estradiol, and Cortisol
to an Academic Examination

by

Hailey Hernandez

David Edwards
Adviser

Psychology Department

David Edwards
Adviser

Patricia Brennan
Committee Member

Jennifer Sarrett
Committee Member

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An abstract of
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Abstract

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Hormone coupling is the degree to which fluctuations in hormone levels occur in parallel. The purpose of the present study was to explore hormone coupling and reactivity of testosterone, cortisol, and estradiol in the context of the real-world stressor of an academic examination. In the present study, participants were undergraduate men and women who provided salivary samples on a neutral-day and before and after taking two course exams. Participants provided a measure of subjective stress and completed a brief survey intended to explore the involvement of person-factors on the endocrine and psychological response to stress. Four major findings were observed. First, there were significant sex differences in subjective stress and hormone levels across all time points. Second, hormone reactivity to the first and second exam was significantly associated in men for all three hormones suggesting there are individual differences in hormone reactivity that are carried over from one exam to the next. Third, positive coupling of testosterone and estradiol, and testosterone and cortisol were observed in the majority of individuals. Finally, prestige was positively correlated with mean estradiol level and predicted estradiol and cortisol reactivity in women. These findings suggest that taking an academic exam is a real-world stressor capable of eliciting strong psychological and hormonal responses. These results should encourage research designed to explore exam-related individual differences in fluctuating levels of testosterone, estradiol, and cortisol recognizing that there may be benefits, yet to be revealed, of matching fluctuations in two (or more) hormone pairs.

Keywords: estradiol testosterone cortisol hormone coupling reactivity academic exam

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Table of Contents

Abstract	2
Introduction	3
Material and methods	5
2.1 Participants	5
2.2 Procedural overview	6
2.3 Saliva samples and hormone assays	7
2.4 Online Survey	8
2.5 Statistical Analysis	9
Results	10
3.1 Subjective stress	10
3.2 Person factors, subjective stress, and mean hormone levels	13
3.3 Oral contraceptive use and sex differences in hormone levels	13
3.4 Exam-related hormone reactivity	17
3.5 Hormone coupling	20
Discussion	22
4.1 Subjective stress and hormones	22
4.2 Sex differences in hormone levels	23
4.3 Person-factors and hormones	25
4.4 Individual differences in hormone reactivity	25
4.5 Hormone coupling	26
4.6 Strengths and limitations	27
Conclusion	28

REFERENCES 29

APPENDICES

Appendix 1 34

Appendix 2 35

Appendix 3 36

Appendix 4 37

Appendix 5 38

Appendix 6 39

Appendix 7 40

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Hailey Hernandez, David Edwards

Emory University

Abstract

Hormone coupling is the degree to which fluctuations in hormone levels occur in parallel. The purpose of the present study was to explore hormone coupling and reactivity of testosterone, cortisol, and estradiol in the context of the real-world stressor of an academic examination. In the present study, participants were undergraduate men and women who provided salivary samples on a neutral-day and before and after taking two course exams. Participants provided a measure of subjective stress and completed a brief survey intended to explore the involvement of person-factors on the endocrine and psychological response to stress. Four major findings were observed. First, there were significant sex differences in subjective stress and hormone levels across all time points. Second, hormone reactivity to the first and second exam was significantly associated in men for all three hormones suggesting there are individual differences in hormone reactivity that are carried over from one exam to the next. Third, positive coupling of testosterone and estradiol, and testosterone and cortisol were observed in the majority of individuals. Finally, prestige was positively correlated with mean estradiol level and predicted estradiol and cortisol reactivity in women. These findings suggest that taking an academic exam is a real-world stressor capable of eliciting strong psychological and hormonal responses. These results should encourage research designed to explore exam-related individual differences in fluctuating levels of testosterone, estradiol, and cortisol recognizing that there may be benefits, yet to be revealed, of matching fluctuations in two (or more) hormone pairs.

Keywords: **estradiol testosterone cortisol hormone coupling reactivity academic exam**

1. Introduction

Cortisol is produced in the adrenal cortex which also secretes the androgenic steroid testosterone and androgen precursors. These secretions are regulated by the hypothalamic-pituitary-adrenal (HPA) axis. Testosterone is additionally produced and secreted by the testes and ovaries which also secrete estradiol and other estrogens. These processes are controlled by the hypothalamic-pituitary-gonadal (HPG) axis. Additional amounts of estradiol may be contributed by the peripheral aromatization of testosterone. While cortisol and testosterone can inhibit the axis of the other (Viau, 2002), the activation and deactivation of the two systems appear to be positively “coupled” in a variety of settings. That is, within-person fluctuations of cortisol and testosterone levels occur in parallel: increases and decreases in one hormone are associated with corresponding increases and decreases in the other. In humans, positive cortisol/testosterone coupling has been reported for incarcerated adolescent boys (Dismukes, Johnson, Vitacco, Iturri, & Shirtcliff, 2015), a non-institutionalized sample of adolescent boys and girls (Marceau et al., 2015), and a mixed-sex, lifespan sample of individuals ranging in age from 11-88 years (Harden et al., 2016). Cortisol/testosterone coupling may be particularly evident in situations involving social/evaluative stress. Turan, Tackett, Ledhtreck and Browning (2015) reported positive within-person coupling for adult men and pre/early pubertal boys and girls responding to age-appropriate forms of the Trier Social Stress Test (TSST).

In laboratory studies using the TSST, the magnitude of positive coupling between cortisol and testosterone can be affected by person factors such as dominance, anxiety, and negative affect (Turan et al., 2015). In a small sample of incarcerated adolescent males, psychopathology and callousness were related to cortisol/testosterone coupling. Specifically, hormone uncoupling

was associated with callousness and tighter coupling positively associated with psychopathology (Johnson et al., 2014).

Correlations between testosterone and estradiol assayed from facial and axillary perspiration in men and women are high (Elliot, Muir, & de Catanzaro, 2017; Muir et al., 2008). In naturally cycling women, serum testosterone and estradiol levels peak together at mid-cycle (Rothman et al., 2011) raising the possibility that within-individual fluctuations in these two hormones may be positively coupled.

A university environment can be stressful. Stress can impair performance by disrupting working memory and recall (Buchanan & Tranel, 2008; Oei, Everaerd, Elzinga, van Well & Bermond, 2006) and increase the risk of developing depression, anxiety, and substance use disorders (Beiter et al., 2015; Hysenbegasi, Hass, Rowland, 2005; Ibrahim, Kelly, Adams, & Glazebrook, 2013). González-Cabrera, Fernández-Prada, Iribar-Ibabe, and Peinado (2014) measured salivary levels of cortisol, state-trait anxiety, and perceived stress of Spanish medical graduates preparing for the medical specialty training position (MIR) exam and reported significant increases in salivary levels of cortisol, anxiety and perceived stress across the several month study period. Similarly, Weekes et al. (2006) found that taking an academic exam was associated with increases in salivary cortisol levels (“the stress hormone”) and psychological measures of stress in undergraduate students. Surprisingly, cortisol and psychological measures of stress were not related to one another.

An expanded analysis of endocrine responses to academic stress would be a first step in understanding individual differences in stress reactivity in academic settings. This information could be useful in understanding why some individuals under stress are more prone to negative psychological reactions and the development of psychopathologies than others. The present

study was designed to study the potential coupling of testosterone, estradiol, and cortisol in undergraduate men and women during the presumably stressful time leading up to and completing each of two course examinations that would figure in determining final grades for the course. Based off research suggesting that personality traits may be associated with biological stress reactivity (Bibby, Carroll, Roseboom, Phillips, de Rooij, 2013), the study included a questionnaire component intended to explore the possible involvement of a variety of person-factors in the endocrine and psychological responses to academic stress.

2. Material and Methods

2.1 Participants

The study population consisted of 43 undergraduate men ($n = 16$) and women ($n = 27$) enrolled in one section of a fall 2018 introductory psychology course at Emory University. Forty-two percent of the participants self-identified as Caucasian/European-American, 28% as African/African-American, 19% as Asian/Asian-American and 12% identified as Hispanic/Latino or other. One student (female) failed to complete all parts of the study. Her data was not included in the analysis. Eight students (1 man and 7 women) withdrew from the course before completing the study; to the extent possible, data from these students were included in statistical analyses.

2.2 Procedural Overview

The study was approved by the institutional review board (IRB) of Emory University. Consenting participants completing all parts of the study received five credits which counted towards fulfilling the human subjects' research requirement for students enrolled in the course.

Participants were recruited using the Emory SONA system, an online database where undergraduate psychology students can sign up to participate in ongoing research studies, and through an announcement during one class period at the beginning of the semester. Interested participants reported to the Psychology building in the late afternoon two weeks prior to their first exam and were informed about the purpose and procedures of the study. Once participants provided written consent, they were asked to rate “How much stress have you felt during the past 24-hours?” on a scale from 0-9. This number was used as a measure of the individual’s baseline level of subjective stress. Participants then provided a saliva sample. Hormone values for this sample were used as a non-stress baseline. During this session, participants were provided a link to the online survey; they were asked to complete the survey in one sitting at their own convenience before the first exam. On exam day two weeks later, participants reported to the same place around the same time and provided another measure of subjective stress and the second saliva sample before taking the first course exam. A third sample was collected following the completion of the exam. This procedure was repeated for the second course exam which took place exactly six weeks later at the same time and location. Samples were quickly frozen and stored for later hormone assay (details below). Figure 1 shows a timeline for the collection of saliva samples.

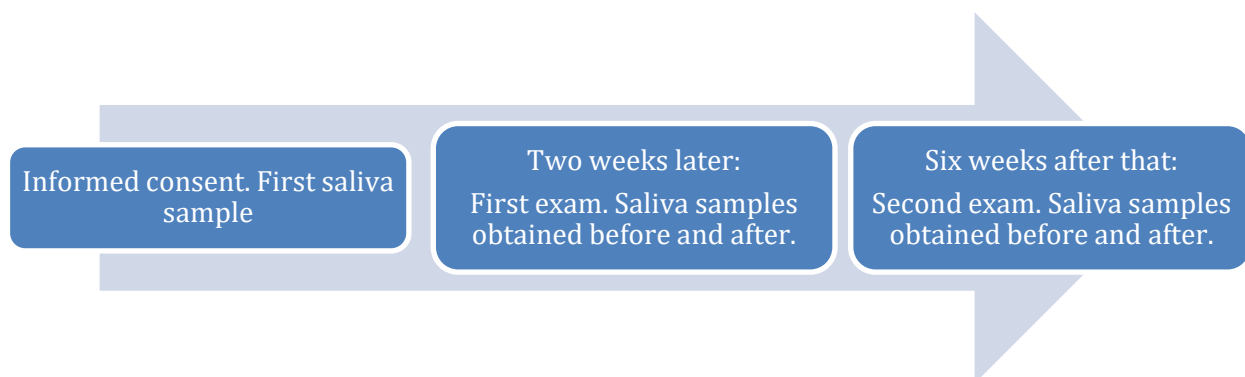


Figure 1. Timeline for the collection of saliva samples.

2.3 Saliva samples and hormone assays

Five saliva samples were collected from each participant. The first sample was obtained between 5 - 6 PM two weeks prior to the first exam. The second sample was obtained between 5:30 - 5:45 PM before the first exam which began at 6 PM. The third sample was obtained immediately after participants finished their exam. There was no time limit for the exam, so the amount of time between the before- and after-exam samples varied between students and ranged from 1.5 – 2.5 hours. The fourth and fifth samples were collected in connection with the second exam and were, as for the first exam, obtained before the start of and immediately after students completed the exam. For the second exam, the collection and timing procedures were the same as the first exam.

Participants were required to thoroughly rinse their mouths with water immediately before giving a saliva sample. Then they were asked to fill, by passive drool through a straw, a 2 ml collection vial (Salimetrics) to the 1.8 ml line marked on the side. Collection of a single sample took approximately 4-7 minutes. Samples were placed in an on-site freezer immediately following collection and later that evening transferred to a larger freezer and stored at -80°C. Approximately three weeks after the final collection round, the frozen samples were shipped to Salimetrics (Philadelphia, Pennsylvania) and assayed for testosterone, estradiol, and cortisol. The testosterone assay had a range of 6.1 pg/mL to 600 pg/mL and had a minimum sensitivity of 1 pg/mL. The intra-assay CV was 4.6% and the inter-assay CV was 9.85%. The estradiol assay had a range of 1 pg/mL – 32 pg/mL and had a minimum sensitivity of 0.1 pg/mL. The intra-assay CV was 7.13% and in the inter-assay CV was 7.45%. The cortisol assay had a range of 0.012 µ/dL – 3.0 µ/dL and had a minimum sensitivity of .007 µ/dL. The intra-assay CV was 4.6% and in the inter-assay CV was 6%.

2.4 Online survey

Each participant was provided a unique, 4-digit ID number. This code was used by participants to access the Qualtrics-formatted online survey. Participants were queried about their sex, race and ethnicity. In addition, women were asked “Are you taking any form of hormonal contraceptive/birth control?” and if they answered “yes” they were asked to specify the method, (“What kind of hormonal contraception are you taking?”), and provided with options that included oral contraceptives (i.e. pill), hormonal patch or injection, intrauterine device (IUD), or implant. The survey included six different inventories of person-factors that could plausibly be involved in hormonal and/or psychological responses to academic stress (see below). The entire survey took approximately 15 – 20 minutes to complete. Participants had two weeks from the time they completed the first sample to before the first exam to complete the survey.

1. **Resilience.** The short form Connor-Davidson Resilience scale (CD-RISC) scale, is a 10-item validated Likert scale adapted by Campbell-Sills and Stein in 2007 from the original 25-item scale developed by Kathryn M. Connor at Duke University in 2003. The short form is designed to measure an individual’s trait-like ability to positively adapt to adversity (Campbell-Sills & Stein, 2007).
2. **Competitiveness.** The Competitiveness Scale is a 37-item Likert scale developed by Newby and Klein in 2014 designed to measure trait competitiveness using participants’ self-perceived level of competitiveness (Newby and Klein, 2014).
3. **Power Dominance Systems Scale.** This is a 39-item questionnaire designed to measure participants’ desire for power or dominance over others (Murphy, 2016).

4. ***Prestige/Dominance Scale.*** This is a 17-item Likert scale designed to measure respondent's trait levels of dominance and prestige (Cheng, Tracy, & Henrich, 2010).
5. ***Casto Trait Competitiveness Scale.*** This is a 16-item Likert scale developed by Kathleen Casto (2016) that measures trait competitiveness and self-efficacy.
6. ***Locus of Control.*** The Adult Nowicki-Strickland Internal-External Control is a 40-item scale designed to measure an individual's predisposition to attribute outcomes to either external (e.g., luck or fate) or internal forces (Nowicki and Duke, 1974).

2.5 Statistical Analysis

Because of the relatively small sample size, no attempt was made to analyze results by ethnicity. Oral contraceptives (OC) were the only form of hormonal contraception used by the women participants. Although women using oral contraceptives tended, on average, to have lower estradiol and testosterone levels than non-users, with only a single exception, hormone means for OC users and non-users were not significantly different. So, for purposes of analysis and presentation, no distinction is made between OC users and non-users.

Two-way mixed (between-within) ANOVAs with participant sex as the between group variable were used to analyze ratings of subjective stress and, in separate analyses, hormone values obtained at baseline, before the first exam, and before the second exam. Paired sample t-tests were used to compare means for subjective stress and hormone values between men and women at different time points. Cohen's *d* was calculated to determine the effect size. Pearson bivariate correlations were also used to analyze associations between subjective stress, person factors, and hormone measures.

Hormone reactivity to the anticipation of taking the examination was measured by expressing each participant's before-exam hormone level as a percent of his/her neutral-day baseline level. These calculations were done for testosterone, estradiol, and cortisol. To determine whether individual differences in hormone reactivity were carried over from one examination to the next, Pearson's r was used to determine the degree of relationship between hormone reactivity to the first exam and reactivity to the second exam. To explore exam hormone reactivity during the exam period, after-exam hormone values were expressed as a percent of before-exam values. Pearson's r was then used to determine the degree of the relationship between individuals' hormone reactivity during the first exam with hormone reactivity during the second exam.

Hormone coupling was analyzed by using within-person Pearson's r to identify relationships between levels of testosterone and estradiol, testosterone and cortisol, and estradiol and cortisol for every individual. Hierarchical linear modeling is a common analysis used to determine the degree of hormone coupling but we were unable to perform this analysis in the present study.¹

3. Results

3.1 Subjective stress

At consent and before each exam, participants were asked to rate "How much stress have you felt during the past 24-hours?" Mean ratings for men and women are shown in Figure 2. For men and women, stress was significantly higher before each exam than at baseline ($F(2,62) =$

¹ Hierarchical linear modeling (HLM) is typically used in hormone coupling studies but requires a relatively large sample size (at least 100 participants) to conduct a meaningful test of hormone coupling.

39.85, $p < .001$, $\eta p^2 = .56$). The sex by stress interaction was statistically significant ($F(2,62) = 4.44$, $p = .016$, $\eta p^2 = .125$). Subjective stress ratings between exam 1 and exam 2 were not statistically different for men ($t(13) = 1.21$, $p = .247$, $d = .32$) or women ($t(19) = 1.10$, $p = .286$, $d = .25$). For the first and second exam, mean levels of subjective stress were significantly higher for women than comparable means for men (first exam, $t(41) = 2.42$, $p = .02$, $d = .69$; second exam, $t(32) = 2.03$, $p = .051$, $d = .66$).

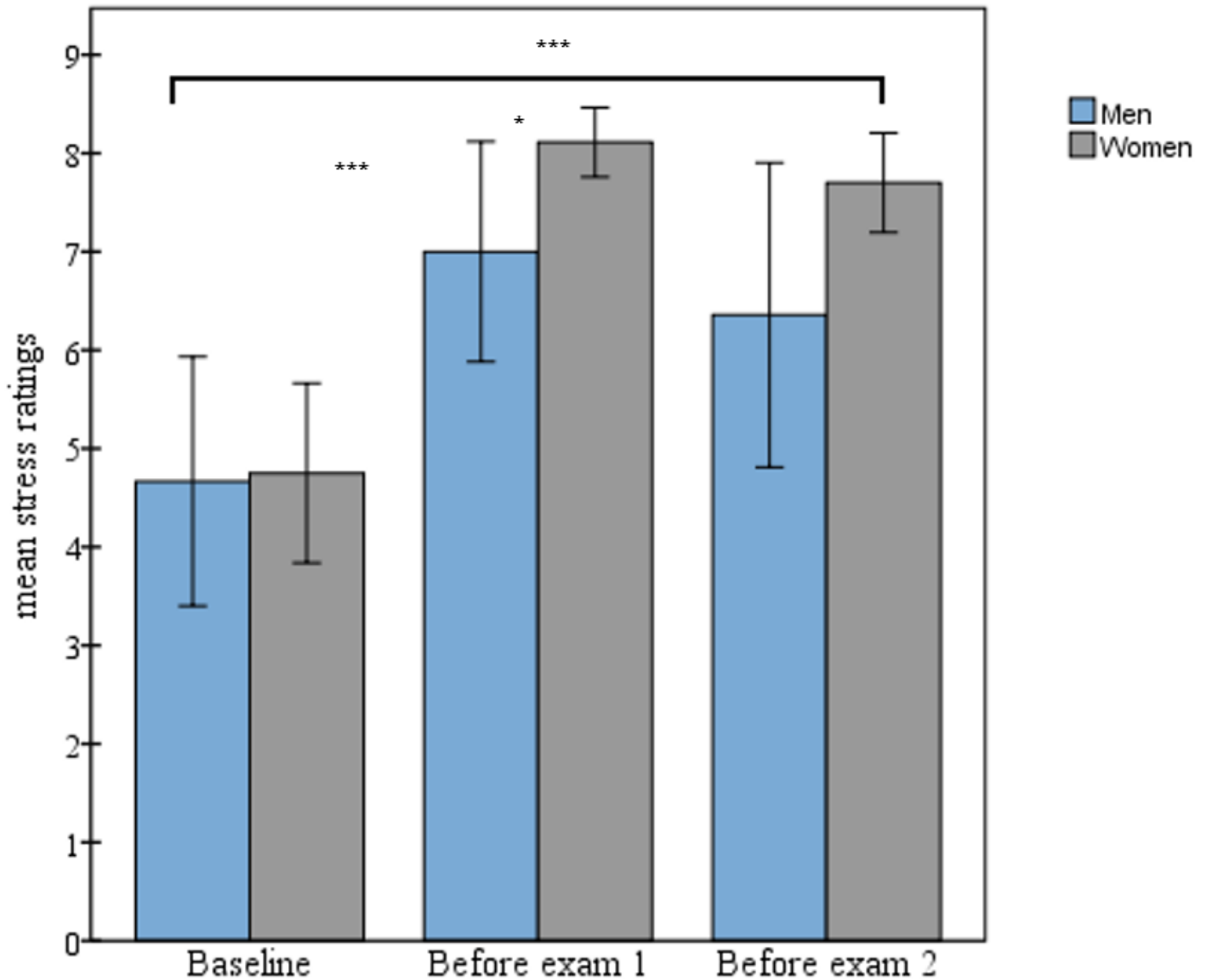


Figure 2. Mean stress ratings at neutral-day baseline and shortly before each of two examinations for men and women. At baseline, $n = 14$ for men and $n = 28$ for women. For the first exam, $n = 15$ for men and $n = 28$ for women. For the second exam, $n = 14$ for men and $n = 20$ for women. Some participants withdrew from the course before the second exam and sample sizes for men and women are correspondingly reduced. One male participant did not provide a measure of subjective stress at baseline so the sample size is less at baseline than at exam 1. Error bars represent 95% confidence intervals. * $p < .05$. *** $p < .001$.

3.2 Associations between person factors, subjective stress, and mean hormone levels

Participants were asked to complete an online survey gathering data on person-factors including resilience, competitiveness, power, prestige, dominance, and locus of control. Locus of control was significantly associated with subjective stress ratings before the first exam for men and women (men: $r(14) = -.51, p = .045$; women: $r(25) = .41, p = .034$). No other person-factors were significantly associated with subjective stress at any of the time points where this was assessed. For each individual, hormone values were averaged to give mean values for that person's testosterone, estradiol, and cortisol². For men, competitiveness ($r(14) = .52, p = .039$) and dominance ($r(14) = .50, p = .048$) were positively correlated with mean testosterone levels. For women, prestige ($r(25) = .410, p = .034$) and locus of control ($r(25) = .414, p = .032$) were positively correlated with mean levels of estradiol.

3.3 Oral contraceptive use and sex differences in hormone levels

On average, mean levels of testosterone and estradiol were lower for oral contraceptive (OC) users than non-users, but with the exception of the samples obtained before the first exam, differences in hormone levels (whether for testosterone, estradiol, or cortisol) for oral contraceptive users and non-users were not statistically significant. Data for OC users and non-users were combined for purposes of analysis and presentation. Figure 3 shows mean levels of testosterone, estradiol, and cortisol for each of the time points sampled for men and women participants.

² For the majority of individuals, means are calculated based off values for five saliva samples. For individuals who dropped the course, averages are based on values for only three samples.

Separate two-way mixed ANOVAs were used to analyze sex differences in hormone levels and changes in hormone levels from baseline to before and after each of the two exams.

On average, men had higher levels of testosterone than women (Figure 3). Sex differences were statistically significant for baseline/first exam hormone values ($F(1,41) = 68.69$, $p < .001$, $\eta^2 = .63$) and baseline/second exam values ($F(1,32) = 68.16$, $p < .001$, $\eta^2 = .68$). For men and women, testosterone levels appeared relatively stable with similar means for baseline and before- and after-exam values. The hormone-by-sex interaction did not approach statistical significance (Figure 3).

On average, women had higher levels of estradiol than men (Figure 3). Sex differences were statistically significant for baseline/first exam hormone values ($F(1,41) = 35.33$, $p < .001$, $\eta^2 = .46$) and baseline/second exam values ($F(1,32) = 24.70$, $p < .001$, $\eta^2 = .44$). For men, estradiol levels appeared relatively stable across baseline, before-exam, and after-exam samples for both exams. For women, in contrast, estradiol levels increased from baseline to before-exam levels and continued to increase over the course of the exam period. This was true for both exams (first exam, $F(2,82) = 3.31$, $p = .041$, $\eta^2 = .08$; second exam, $F(2,64) = 7.91$, $p < .001$, $\eta^2 = .20$). The sex-by-hormone changes interaction was statistically significant for the first exam ($F(2,82) = 5.25$, $p = .007$, $\eta^2 = .114$) but did not approach statistical significance for the second exam.

On average, cortisol levels for men and women were similar and cortisol means for men and women (Figure 3) did not significantly differ (t-test for independent groups) for any of the sampling times or by a two-way mixed ANOVA with cortisol as the within-group variable and sex as the between group variable. For men and women, before-exam cortisol levels were little different from baseline values, but after-exam cortisol levels for men and women were typically

lower than baseline or before-exam levels, giving a statistically significant cortisol effect for the first exam ($F(2,82) = 9.0, p < .001, \eta^2 = .18$) and second exam ($F(2,64) = 8.79, p < .001, \eta^2 = .22$). The sex-by-cortisol interaction was not statistically significant for the first exam but approached statistical significance for the second ($F(2,64) = 3.08, p = .053, \eta^2 = .09$) due to the fact that the decrease in cortisol level for men was greater than that for women.

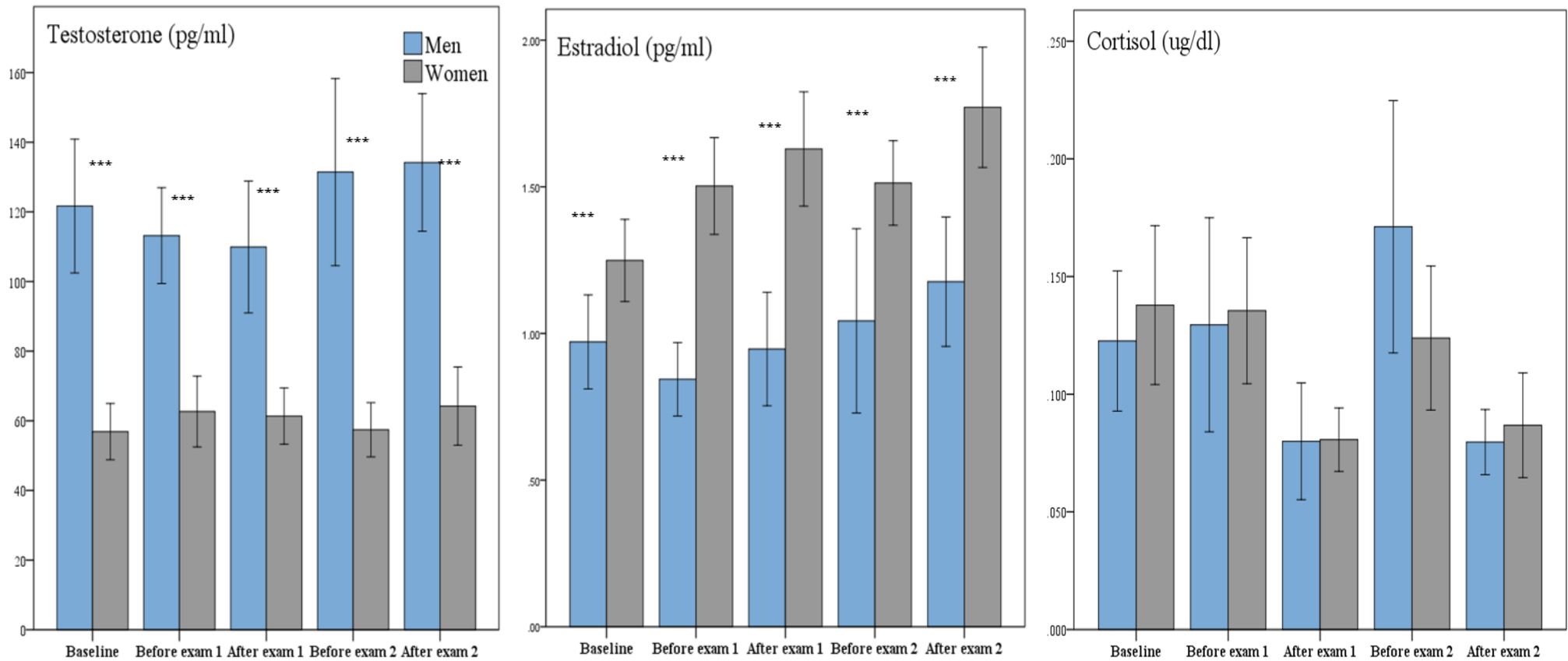


Figure 3. Mean levels of salivary testosterone, estradiol, and cortisol levels at neutral-day baseline, and before and after exams 1 and 2 for men and women. For Exam 1, $n = 16$ for men and $n = 27$ for women. For Exam 2, $n = 14$ for men and $n = 20$ for women. Error bars represent 95% confidence intervals. Significance levels refer to t-test (independent groups) of the difference in means between men and women participants. * $p < .05$. ** $p < .01$. *** $p < .001$.

3.4 Exam-related hormone reactivity

There were large individual differences in hormone reactivity to the exams, with some participants showing an increase, others showing a decrease, and others showing little or no change relative to values for neutral-day baseline. To determine the extent to which hormone reactivities are stable from one exam to another, before-exam hormone levels were expressed as a percent of neutral-day baseline levels for each hormone for each individual. Figure 4 shows correlations in hormone reactivities for the men and women who gave saliva samples in connection with both the first and second exam. For men, hormone reactivity for the first exam was significantly correlated with hormone reactivity for the second exam, and this was true for testosterone ($r(12) = .78, p = .001$), estradiol ($r(12) = .53, p = .050$), and cortisol ($r(12) = .74, p = .002$). For women, before-exam estradiol reactivity for the first exam was significantly correlated with before-exam reactivity to the second exam ($r(18) = .66, p = .002$). Correlations between hormone reactivities for the first and second exam were not statistically significant for either testosterone ($r(18) = .37, p = .11$) or cortisol ($r(18) = .27, p = .27$).

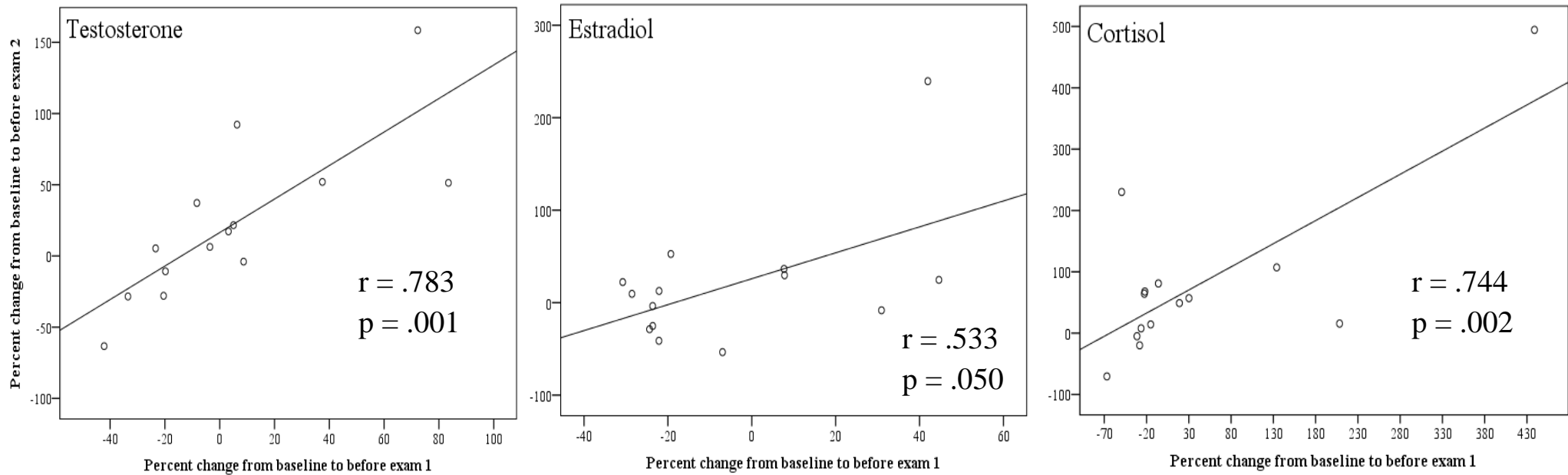
Men

Figure 4. Pearson correlations of testosterone, estradiol, and cortisol reactivity from baseline to before exam 1 ($n = 16$) and before exam 2 ($n = 14$). Reactivity is expressed as a measure of percent change from baseline levels to before exam levels for each exam. Each data point represents hormone change values for a single individual. $*p < .05$.

Women

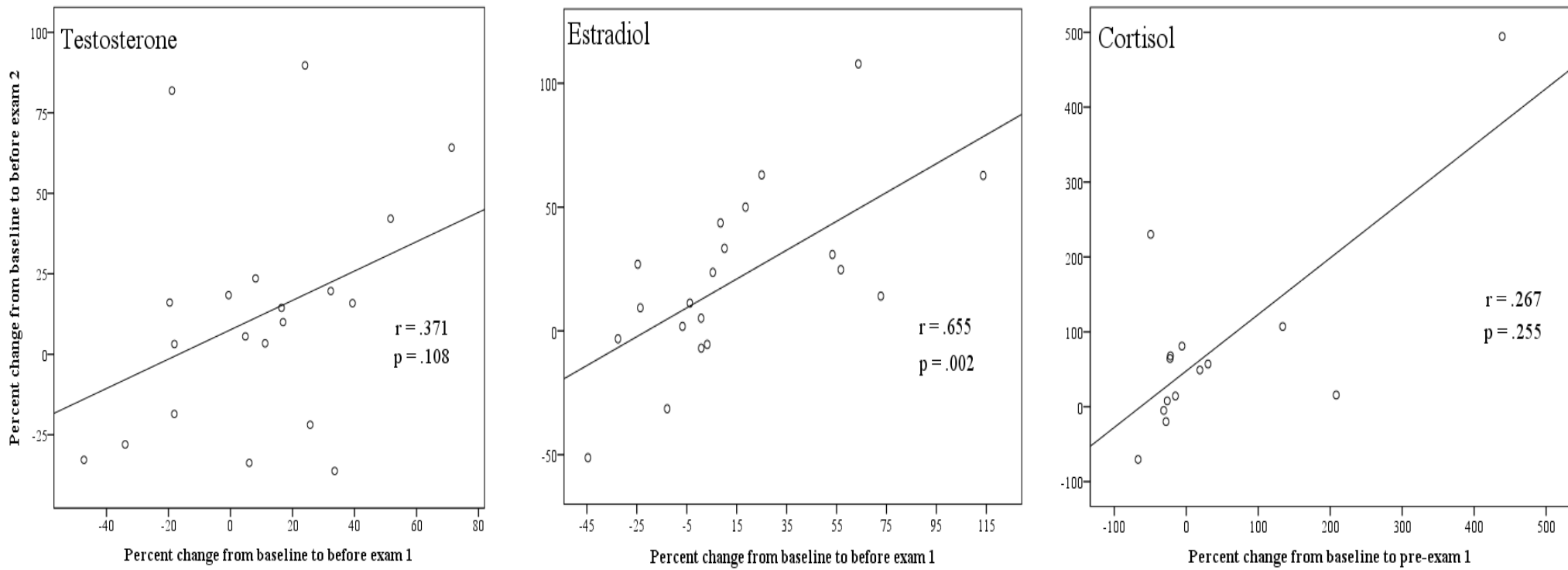


Figure 4. Pearson correlations of testosterone, estradiol, and cortisol reactivity from baseline to before exam 1 ($n = 27$) and before exam 2 ($n = 20$). Reactivity is expressed as a measure of percent change from baseline levels to before exam levels for each exam. Each data point represents hormone change values for a single individual. $*p < .05$.

3.4.1 Reactivity during the exam

Reactivity during the exam was expressed as a percent change of individuals' after-exam hormone levels from their before-exam levels. For all three hormones, reactivity during the first exam was not significantly associated with reactivity during the second exam for men (testosterone, $r(12) = -.10$, $p = .73$; estradiol, $r(12) = .18$, $p = .53$; cortisol, $r(12) = .29$, $p = .32$) or women (testosterone, $r(28) = -.05$, $p = .835$; estradiol, $r(18) = .41$, $p = .07$; cortisol, $r(18) = .04$, $p = .88$).

3.5 Hormone coupling

3.5.1 Within-person Pearson correlations

Within-person Pearson correlations between testosterone, estradiol, and cortisol across the different time points for saliva sampling for each participant are shown in Figure 5. The majority of individuals (88%) show positive correlations between levels of salivary testosterone and estradiol, and positive correlations between testosterone and cortisol (81%). Individual differences in correlations between estradiol and cortisol are more mixed. For men, the relationship is positive in 57% cases and negative in 43% of the cases. For women, the relationship is positive in 37% of the cases and negative in 62% of the cases. For any given hormone pair, there are some individuals in which variation in the level of one hormone is almost perfectly linked with variation in another. In other cases, the two hormones changed in opposite directions or did not appear to change in relation to each other at all. There were no apparent differences in coupling of any of the hormones according to sex. Coupling was also not predicted by any of the person-factors measured in the study.

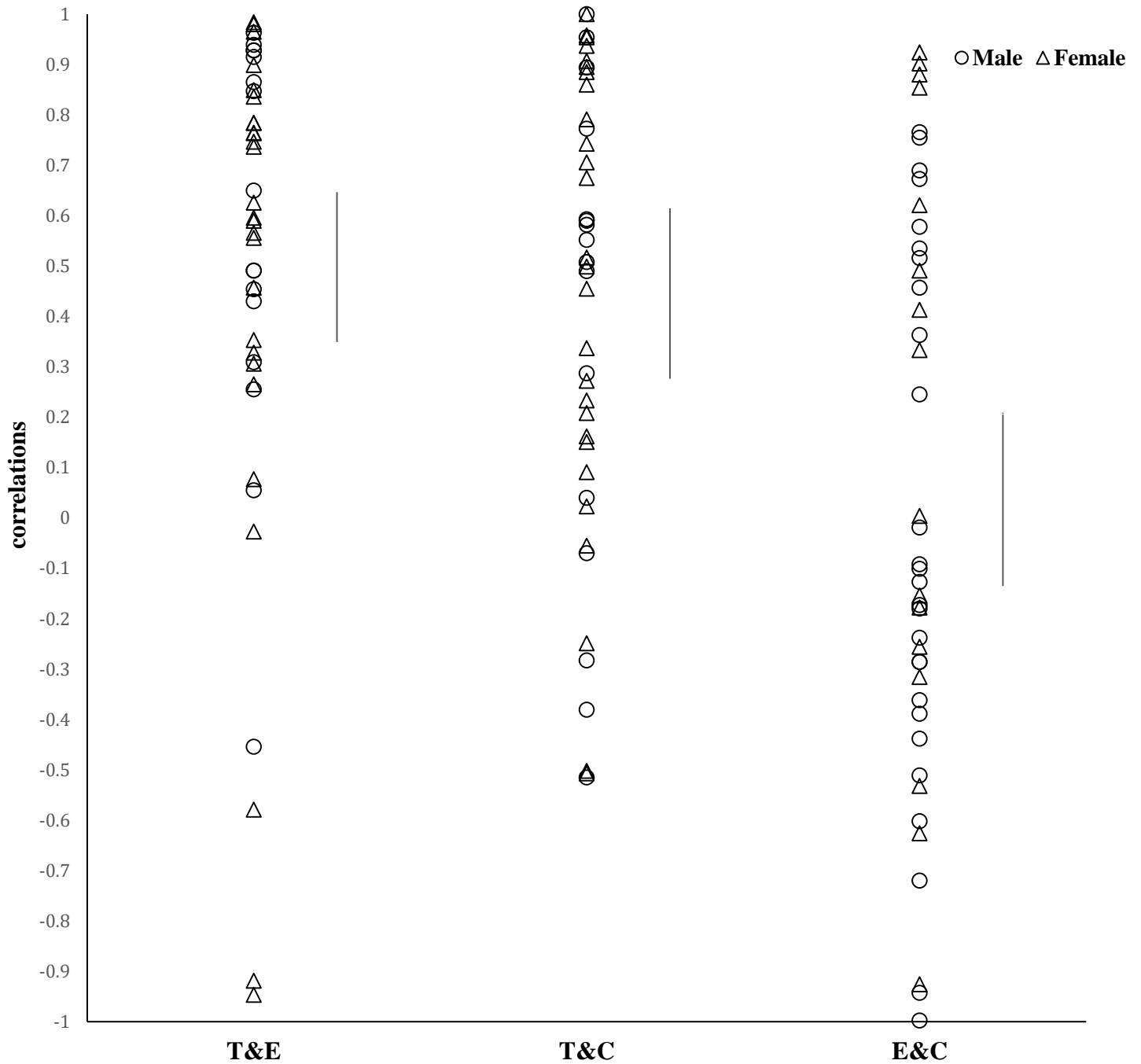


Figure 5. Each data point represents the Pearson correlation between levels of two hormones for a single individual ($n = 43$). Correlations are shown for testosterone and estradiol (T&E), testosterone and cortisol (T&C), and estradiol and cortisol (E&C). Vertical bars represent 95% confidence intervals.

4. Discussion

4.1 Subjective stress and hormones

Self-report ratings of stress were typically higher in the hour preceding the two exams than for neutral-day baseline. Consistent with other studies (Helbig & Backhaus, 2017; Brougham et al., 2009), women reported higher levels of exam-related stress than men. Exactly why this is so is a matter of frank speculation. Some suggest these differences are related to differences in how men and women perceive stressors (Hamaideh, S.H., 2012) in that women tend to perceive things as more stressful than men do.

Locus of control was the only person-factor that predicted subjective stress ratings for men and women before the first exam but not for the second exam. For men, this relationship was negative and for women it was positive. This suggests that, at least for women, external control beliefs, or the degree to which the individual believes things happen by outside agents, is related to greater subjective feelings of stress in response to taking an academic exam. Men and women in this study also did not significantly differ with respect to stress ratings obtained at neutral day baseline, but did significantly differ (or nearly so) for ratings of subjective stress obtained immediately before the first and second exam ($t(41) = 2.42, p = .02, d = .69$, and $t(32) = 2.03, p = .051, d = .66$). Differences in subjective stress appear to be context dependent, a fact that suggests that strategies intended to ameliorate exam-related stress are probably best if focused specifically on exam strategies rather than more general strategies for alleviating non-specific stress.

Subjective stress was also not associated with levels of hormones at any time point for men and women. This is particularly surprising since cortisol is often touted as the “stress hormone” because it is (for example) typically elevated in stressful settings. With a meta-

analysis of 208 laboratory studies of acute psychological stressors and tests, [Dickerson and Kemeny \(2004\)](#) argue that “motivated performance tasks elicited cortisol responses if they were uncontrollable or characterized by social-evaluative threat (p.355).” While academic exams of the sort used in the present study are psychologically stressful, they involve no obvious “social” evaluation and their outcomes are, with an effective study strategy, to a large extent controllable.

4.2 Sex differences in hormone levels

4.2.1 Testosterone

Men had higher levels of salivary testosterone than women, undoubtedly owing to the hormone contribution of the testes. For men and women, testosterone levels appeared relatively stable across baseline, before-exam, and after-exam periods for both exams.

4.2.2 Estradiol

Women had higher levels of salivary estradiol than men, presumably owing to the contribution of the ovaries. For men, estradiol levels appeared similarly stable for both exams. In contrast, for women, estradiol levels increased from baseline to before-exam levels and continued to increase over the course of the exam period. This was true for both exams. Estradiol levels remained elevated during the exam period but how long they remained elevated after that cannot be determined from the data at hand. The functional significance (if any) of this elevation is unknown. Estradiol has been shown to increase in women in connection with the anticipation of athletic competition but not the competition itself ([Edwards & Turan, 2019 unpublished](#)). Estradiol has been little studied in the context of stress. The present result should encourage

research intended to explore the variety of “stressful” settings and circumstances under which estradiol levels predictably vary and the functional consequences of this variation. The proximate cause(s) of exam-related increases in salivary levels of estradiol remain to be determined.

Possibilities include secretion due to the activation of the HPG axis or sympathetic activation of the ovary, decreased metabolic clearance, and hemocentration (Edwards & Casto, 2013).

4.2.3 Cortisol

There were no significant sex differences in the levels of cortisol at any of the time points sampled. Before-exam cortisol levels for men and women were little different from neutral-day baseline values which is consistent with other reports (Kirschbaum, Wüst, & Hellhammer, 1992). Cortisol levels also typically fell while men and women were taking the exam. This is perhaps reflecting a degree of psychological relief from the anticipation of taking an exam where the outcome cannot be predicted until the exam is in front of the individual to review.

There were also no significant sex differences found for exam-related cortisol reactivity for either the first or second exam, a result not in keeping with other reports that found greater cortisol reactivity in men to psychological stressors such as the Trier Social Stress Test (Liu et al., 2017). The striking absence of exam-related cortisol responses suggest that cortisol responses may vary according to context and that not all “stressors” produce a cortisol response.

4.3 Person-factors and hormones

Three person-factors were found to be associated with mean levels of hormones: competitiveness, dominance, and prestige. Competitiveness and dominance were positively related to mean level of testosterone in men. This is consistent with other studies that have found

strong links between dominance and testosterone (Mazur & Booth, 1998). That said, competitiveness and dominance did not predict subjective stress or hormone reactivity to either of the two exams for any of the hormones measured in this study. The prestige subscale of the Dominance-Prestige scale (Cheng et al., 2010) is designed to measure the respondent's perception of how peer group others view him/her and to some extent, their self-esteem. Sample questions included: "Members of my peer group respect and admire me"; "I am held in high esteem by those I know"; and "My unique talents and abilities are recognized by others." For women respondents in the present study, prestige was positively correlated with mean estradiol level; prestige was the only person-factor that predicted mean estradiol level. Prestige also predicted estradiol reactivity to the first and second exam (first exam: $r(25) = .47, p = .013$; second exam: $r(18) = .41, p = .073$) and cortisol reactivity to the second exam ($r(18) = .51, p = .023$). That prestige predicted estradiol and cortisol reactivity suggests that it may moderate some hormone responses to an academic stressor.

4.4 Individual differences in hormone reactivity

In men, whether for testosterone, estradiol, or cortisol, before-exam levels (relative to baseline) for the first and second exam were strongly associated. That is, hormonal changes to the first exam strongly predicted changes to the second exam. For women, before-exam estradiol reactivity for the first exam was significantly correlated with before-exam reactivity to the second, but testosterone and cortisol reactivity for the first and second exam were not.

For men and women there are large individual differences in how an individual responds to the immediate prospect of taking an important examination. For any given hormone, levels in

reaction to academic stress may go up, down, or stay the same. Idiosyncratic hormone responses for men are carried over from one exam to the other suggesting that whatever a man's pattern, it is relatively stable over time. The extent to which an individual's pattern of response to academic stress carries over to other kinds of stressors remains to be determined and is likely to be related to some (as yet unknown) person-factors.

4.5 Hormone coupling

Coupling of testosterone, estradiol, and cortisol was explored in two ways. For the majority of individuals, within-person correlations between levels of testosterone and estradiol, and testosterone and cortisol were positive (Figure 5). These positive correlations were not significantly different for men and women. The positive association between salivary testosterone and estradiol levels is consistent with studies demonstrating positive associations between testosterone and estradiol in facial and axillary perspiration (Muir et al., 2008; Elliot et al., 2017). The positive within-person associations between testosterone and cortisol and, to a somewhat lesser extent, testosterone and estradiol in the present study are consonant with results reported by others in different contexts (Edwards & Kurlander, 2010; Turan et al., 2015). In keeping with an earlier report (Edwards & Turan, 2019 unpublished), there was no clear evidence to support coupling of estradiol and cortisol.

For any given pair of hormones there were large individual differences in the strength and direction of coupling (Figure 5). Person variables such as dominance, anxiety, negative affect, and psychopathological traits can affect the strength of coupling between cortisol and testosterone (Johnson et al., 2014; Marceau et al., 2015). None of the person-factors measured in

this study predicted coupling of any of the hormones. The extent to which these, or any other, variables affect coupling with estradiol remains to be determined.

In some individuals, changing salivary levels of testosterone, estradiol, and cortisol appear to be coordinated in relationship to each other (Figure 5). That within-individual fluctuations in cortisol and testosterone are positively related is perhaps because the adrenal cortex is the principal source for testosterone as it fluctuates on a short-term basis. Alternatively, when event-related cortisol and testosterone responses are correlated, it could be because the adrenal glands and gonads are responding similarly to the physical and/or psychological elements of the event. The positive within-individual correlations between fluctuating levels of testosterone and estradiol suggest the gonads are a common source for these hormones.

4.6 Strengths and limitations

The present study is apparently the first, to our knowledge, to examine hormone coupling in the context of the real-world stressor of an academic exam. The inclusion of estradiol, a hormone not typically studied in the context of hormonal reactivity to stress, is a strength. Given the relatively small sample size, results should be interpreted with caution. That said, the statistically robust increase in estradiol relative to neutral-day baseline seen in connection with the first and second exam is a novel finding that warrants further exploration. For women, the positive relationship found between prestige, mean estradiol, and estradiol and cortisol reactivity to the exam, suggests that prestige is an idiosyncratic person-factor that predicts and may moderate hormonal responses to an academic stressor. The extent to which this is true in other contexts remains to be determined. Individual differences in hormone reactivity and coupling

appear to be stable (Figure 4 and 5). The basis for these differences warrants further investigation.

Conclusions

Anticipating and taking an academic examination is a real-world stressor capable of eliciting strong psychological and hormonal responses. This study provides evidence for exam-related increases in salivary estradiol levels and the coupling of testosterone and estradiol, and testosterone and cortisol in at least some individuals. Particularly for men, individual differences in hormonal reactivity to an academic exam appear to be relatively stable, carrying over from one exam to another given six weeks later. In women, greater prestige appeared to predict mean estradiol level and estradiol reactivity to both academic exams suggesting that prestige is one of the idiosyncratic person-factors predicting hormone changes, particularly estradiol, to an academic stressor. These results should encourage research designed to explore the basis for and functional significance of exam-related individual differences in fluctuating levels of testosterone, estradiol, and cortisol recognizing that there may be benefits, yet to be revealed, of matching fluctuations in two (or more) hormone pairs.

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Appendix I

Table 1

Means and Standard Deviations for scores on the Connor-Davidson Resilience Scale (CDRISC), Competitiveness scale, Power and Dominance Systems scale (PDSS), Prestige-Dominance scales (PD), Casto Trait Competitiveness Scale, and the Adult Nowicki-Strickland Internal-External of Control (ANSIE) measure for men and women.

Measure	Men (<i>n</i> = 16)		Women (<i>n</i> = 28)	
	<i>M</i>	<i>SD</i>	<i>M</i>	<i>SD</i>
CDRISC	4.01	.59	3.57*	.73
Competitiveness	3.46	.67	2.91*	.72
PDSS	4.31	.57	4.19	.71
PD				
Prestige	5.16	.62	5.12	.87
Dominance	3.65	.68	3.45	1.40
Casto Trait Competitiveness	3.56	.63	3.06*	.59
ANSIE	11.13	5.97	13.36	5.06

Note. For the CDRISC, Competitiveness, PDSS, PD, and Casto Trait Competitiveness, higher mean scores are indicative of individuals possessing more of the traits the construct assessed. ANSIE is scored in the external direction. Higher mean scores are indicative of more externally controlled individuals. * $p < .05$.

Appendix II

Table 2

Summary of Intercorrelations for scores on the CDRISC, Competitiveness scale, Power and Dominance Systems scale, Prestige and Dominance scale, Casto Trait Competitiveness scale, and the Adult Nowicki-Strickland Internal-External control measure (ANSIE).

Measure	1	2	3	4	5	6	7
1. CDRISC	—	.15	.10	.49**	-.04	.34*	-.09
2. Competitiveness	.15	—	.56**	.20	.58**	.88**	-.01
3. PDSS	.10	.56**	—	.41**	.70**	.62**	.07
4. PD Prestige	.49**	.20	.41**	—	.26	.32**	.05
5. PD Dominance	-.04	.58**	.70**	.26	—	.58**	.13
6. Casto Trait Competitiveness	.34*	.88**	.61**	.32*	.58**	—	.10
7. ANSIE	-.09	-.01	.07	.05	.13	.10	—

Note. * $p < .05$, ** $p < .01$.

Appendix III

Table 3

Correlations between questionnaires and mean level of testosterone, estradiol, and cortisol.

Measure	Men			Women		
	Testosterone	Estradiol	Cortisol	Testosterone	Estradiol	Cortisol
1. CDRISC	.166	-.073	.075	-.215	.022	-.021
2. Competitiveness	.520*	.214	.476	.051	.021	-.037
3. PDSS	.230	-.140	.037	.121	.242	.013
4. PD Prestige	-.095	-.111	-.212	.168	.410*	.166
5. PD Dominance	.501*	.127	.165	.273	.087	.078
6. Casto Trait Competitiveness	.429	.261	.397	.052	.223	.081
7. ANSIE	-.097	.368	-.132	.414*	.310	.068

Note. * $p < .05$.

Appendix IV

Table 4

Means and Standard Deviations for scores on the Connor-Davidson Resilience Scale (CDRISC), Competitiveness scale, Power and Dominance Systems scale (PDSS), Prestige/Dominance scale (PD), Casto Trait Competitiveness Scale, and the Adult Nowicki-Strickland Internal-External of Control (ANSIE) measure for individuals who remained enrolled in the course and completed all parts of the study and those who withdrew from the course and did not complete all parts of the study (1 man and 7 women).

Measure	Enrolled ($n = 35$)		Withdrew ($n = 8$)	
	<i>M</i>	<i>SD</i>	<i>M</i>	<i>SD</i>
CDRISC	3.83	.67	3.3*	.75
Competitiveness	3.13	.79	3.02	.52
PDSS	1.21	.64	4.37	.77
PD				
Prestige	5.07	.77	5.39	.80
Dominance	3.45	1.07	3.83	1.64
Casto Trait Competitiveness	3.24	.68	3.23	.47
ANSIE	12.36	5.38	13.38	6.05

Note. Individuals who withdrew from the course scored significantly lower on the CDRISC than those who remained in the course.

* $p < .05$.

Appendix V

Table 5

Men correlations between testosterone, estradiol, and cortisol hormone reactivities for the first exam. Exploratory analyses were conducted to explore whether individual differences in reactivity for one hormone are related to individual differences in reactivity in another hormone.

	1	2	3	4	5	6
Hormone reactivity						
1. Testosterone AB	—	.676**	.073	.569**	.336	-.032
2. Estradiol AB	.676**	—	-.098	.561*	.773**	.073
3. Cortisol AB	.073	-.098	—	.310	-.063	.474
4. Testosterone AC	.569*	.561*	.310	—	.498*	.500*
5. Estradiol AC	.336	.773**	-.063	.498*	—	.091
6. Cortisol AC	-.032	.073	.474	.500*	.091	—

Note. AB represents baseline to before-exam levels of the corresponding hormone and AC represents baseline to after-exam levels of the corresponding hormone. For men, there were significant positive correlations between corresponding testosterone and estradiol reactivities. * $p < .05$. ** $p < .01$.

Appendix VI

Table 6

Women correlations between testosterone, estradiol, and cortisol hormone reactivities for the first exam. Exploratory analyses were conducted to explore whether individual differences in reactivity for one hormone are related to individual differences in reactivity in another hormone.

	1	2	3	4	5	6
Hormone reactivity						
1. Testosterone AB	—	.261	.753**	.444**	.154	.467*
2. Estradiol AB	.261	—	-.091	.291	.903**	.068
3. Cortisol AB	.753**	-.91	—	.252	-.125	.388*
4. Testosterone AC	.444**	.291	.252	—	.517**	.851**
5. Estradiol AC	.154	.903**	-.125	.517**	—	.231
6. Cortisol AC	.467*	.068	.388*	.851**	.231	—

Note. AB represents baseline to before-exam levels of the corresponding hormone and AC represents baseline to after-exam levels of the corresponding hormone. For women, there were significant positive correlations between corresponding testosterone and cortisol reactivities. * $p < .05$. ** $p < .01$.

Appendix VII

Part One (CD-RISC). Please indicate the degree with which the following items accurately describe you.

	Not at all like me (1)	2 (2)	Sort of like me (3)	4 (4)	Very much like me (5)
I am able to adapt to change. (1)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
I can deal with whatever comes. (2)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
I try to see the humorous side of problems. (3)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Coping with stress can strengthen me. (4)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
I tend to bounce back after illness or hardship. (5)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
I can achieve goals despite obstacles. (6)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
I can stay focused under pressure. (7)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
I am not easily discouraged by failure. (8)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
I think of myself as a strong person. (9)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
I can handle unpleasant feelings. (10)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

Part Two (Competitiveness Scale). Rate the degree with which the following statements apply to you. Please read carefully.

	Strongly Disagree (1)	Slightly Disagree (2)	Neither Agree nor Disagree (3)	Slightly Agree (4)	Strongly Agree (5)
I like to be better than others at almost everything (1)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
I get a lot of enjoyment out of competition (2)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Other people comment on how competitive I am (3)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
I enjoy setting and beating goals through competition (4)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
I don't care if other people are better at things than I am (5)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
No matter what, I try to be better than others at things (6)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
I am a competitive person (7)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

I view almost every situation as a way to prove that I am better at things than others. (8)

I can improve my competence by competing (9)

I put a lot of effort into beating others at things (10)

I love the thrill of competition (11)

Being the best makes me feel powerful (12)

I don't really care if I get beat in a competition (13)

Competition motivates me (14)

For as long as I can remember, I have wanted to outperform others (15)

Competition allows me to judge my level of competence (16)

I do not find competition self-fulfilling (17)

I think a lot about ways to win (18)

I love to compete (19)

I enjoy beating others in almost every area in life (20)

Losing in a competition wouldn't bother me (21)

I enjoy competing against others (22)

It's important for me to outperform others (23)

I wouldn't mind finishing in last place in a competition (24)

I use
competition
as a way to
prove
something to
myself (25)

I think about
competition a
lot (26)

Winning
makes me
feel superior
to others (27)

I like to
challenge
others (28)

Other people
notice how
much I have
to dominate
others in a
competition
(29)

I like being
the best
compared to
other people
(30)

Competing
doesn't really
matter to me
(31)

Competition
allows me to
measure my
own success
(32)

I would
rather not
compete (33)

I perform
better when I
compete
against others
(34)

I try to be the
best person in
the room at
almost
anything (35)

Winning does
not make me
feel superior
to others (36)

Others notice
that I am
competitive
(37)

Part Three (Dominance-Prestige Scale). Rate the degree with which the following statements accurately describe you. Please read carefully.

I am willing to use aggressive tactics to get my way. (7)

I am held in high esteem by those I know. (8)

I try to control others rather than permit them to control me. (9)

I have a forceful or dominant personality. (10)

Others know it is better to let me have my way. (11)

I enjoy having authority over other people. (12)

My unique talents and abilities are recognized by others.

(13)

I am considered an expert on some matters by others. (14)

Others seek my advice on a variety of matters.

(15)

Some people are afraid of me. (16)

Others enjoy hanging out with me. (17)

Part Four (Casto Trait Competitiveness Scale). Rate the degree with which the following statements apply to you. Please read carefully.

	Not at all True of me (1)	Somewhat not true of me (2)	I'm in the middle (3)	Somewhat true of me (4)	Very True of me (5)
I do not give up easily in competition. (1)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
I am better than others at most things that I do. (2)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
I'm not necessarily interested in beating others in order to achieve my goals. (3)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
I hate losing. (4)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
I try to be the best at everything. (5)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
I have what it takes to perform well under pressure. (6)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
I rarely turn down a challenge from another person. (7)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
I want to be better than other people. (8)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

I have skills/qualities that make me better than other competitors. (9)

I am a competitive person. (10)

Other people think I am a competitive person. (11)

Competitions make me uncomfortable. (12)

I can often be stubborn when it comes to being right in an argument. (13)

I'm confident in my ability to perform well on most tasks. (14)

I perform better when a task becomes a competition. (15)

I'd rather compete against myself (against my own personal bests) than others. (16)

I am willing to
endure
discomfort to
be a winner.
(17)



Part Five (Power Dominance Systems Scale). Rate the degree with which you agree with the following statements. Please read carefully.

I have a knack for knowing who is the most powerful person in the group because I notice how others act around them. (20)

I don't have much power compared to other people. (21)

I really don't want to be the boss. (22)

When I work with others, I like to take the lead. (23)

I am very timid around others (24)

Even if I voice my views, people don't pay attention to them. (25)

I do not like
to be a
"follower."
(26)

It is easy for
me to tell
when
someone is
powerful
because I
can see it in
their body
language.
(27)

When I am
in a group, I
try to have
more
influence
than other
people. (28)

I always try
to spot the
dominant
people in
any
situation.
(29)

I like to tell
people what
they should
do. (30)

I like to
make my
presence
felt. (31)

I am not
very good at
knowing
which
people can
be easily
dominated.
(39)



Part Six. (Adult Nowicki-Strickland Internal-External Control). We are trying to find out what men and women your age think about certain things. We want you to answer the following questions the way you feel.

	yes (1)	no (2)
Do you believe that most problems will solve themselves if you don't fool with them? (1)	<input type="radio"/>	<input type="radio"/>
Do you believe that you can stop yourself from catching a cold? (2)	<input type="radio"/>	<input type="radio"/>
Are some people just born lucky? (3)	<input type="radio"/>	<input type="radio"/>
Most of the time, do you feel that getting good grades means a great deal to you? (49)	<input type="radio"/>	<input type="radio"/>
Are you often blamed for things that just aren't your fault? (50)	<input type="radio"/>	<input type="radio"/>
Do you believe that if somebody studies hard enough, he or she can pass any subject? (51)	<input type="radio"/>	<input type="radio"/>
Do you feel that most of the time it doesn't pay to try hard because things never turn out right anyway? (52)	<input type="radio"/>	<input type="radio"/>
Do you feel that if things start out well in the morning that it's going to be a great day, no matter what you do? (53)	<input type="radio"/>	<input type="radio"/>
Do you feel that most of the time parents listen to what their children have to say? (54)	<input type="radio"/>	<input type="radio"/>
Do you believe that wishing can make good things happen? (55)	<input type="radio"/>	<input type="radio"/>
When you get criticized, does it usually seem it's for no good reason at all? (56)	<input type="radio"/>	<input type="radio"/>

Most of the time do you find it hard to change a friend's (mind) opinion? (57)

Do you think that cheering, more than luck helps a team to win? (58)

Do you feel that it is nearly impossible to change your parents' mind about anything? (59)

Do you believe that your parents should allow you to make most of your own decisions? (60)

Do you feel that when you do something wrong there's very little you can do to make it right? (61)

Do you believe that most people are just born good at sports? (62)

Are most of the other people your age and sex stronger than you are? (63)

Do you feel that one of the best ways to handle most problems is just not to think about them? (64)

Do you feel that you have a lot of choice in deciding whom your friends are? (65)

If you find a four-leaf clover, do you believe that it might bring good luck? (66)

Do you often feel that whether or not you do your homework has much to do with what kinds of grades you get? (67)

Do you feel that when a person your age is angry with you, there's little you can do to stop him or her? (68)

Have you ever had a good luck charm? (69)

Do you believe that whether or not people like you depends on how you act? (70)

Will your parents usually help you if you ask them to? (71)

Have you ever felt that when people were angry with you, it was usually for no reason at all? (72)

Most of the time, do you feel that you can change what might happen tomorrow by what you do today? (73)

Do you believe that when bad things are going to happen they are just going to happen no matter what you do to try to stop them? (74)

Do you think that people can get their own way if they just keep trying? (75)

Most of the time, do you find it useless to try to get your own way at home? (76)

Do you feel that when good things happen, they happen because of hard work? (77)

Do you feel that when somebody your age wants to be your enemy, there's little you can do about it? (78)

you feel that it's easy to get friends to do what you want them to do? (79)

Do you usually feel that you have little to say about what you get to eat at home? (80)

Do you feel that when someone doesn't like you there's little you can do about it? (81)

Do you usually feel that it is almost useless to try in school because most other students are just plain smarter than you are? (82)

Are you the kind of person that believes that planning ahead makes things turn out better? (83)

Most of the time, do you feel that you have little to say about what your family decides to do? (84)

Do you think it's better to be smart than lucky? (48)