A longitudinal analysis of men’s intrasexual competitiveness, state anxiety, salivary testosterone, and salivary cortisol

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**Data files and analysis scripts are publicly available at**
https://osf.io/abqun/

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Abstract
Previous research suggests that competition-induced increases in men’s testosterone levels are associated with increases in their intrasexual competitiveness. Whether these relationships are also evident when considering naturally occurring changes in men’s testosterone levels is an open question, however. To investigate this issue, we carried out a longitudinal analysis of steroid hormone levels and men’s responses on the Intrasexual Competitiveness Scale. We found no evidence that men’s intrasexual competitiveness tracked naturally occurring changes in salivary testosterone, cortisol, or their interaction. However, men did report greater current (i.e., state) anxiety when cortisol was high, replicating previous findings. Our null results for steroid hormones and intrasexual competitiveness suggest that findings for relationships between competition-induced changes in testosterone and men’s intrasexual competitiveness may not necessarily generalize well to relationships with naturally occurring variation in steroid hormones.

Introduction
Results of several studies suggest that increases in men’s testosterone levels due to competitive tasks (hereon referred to as “competition-induced” changes in testosterone) are associated with increases in their intrasexual competitiveness (reviewed in Zilioli & Bird, 2017). For example, men whose testosterone levels increased after competing against another man on a laboratory task (the Point Subtraction Aggression Paradigm, see Geniole et al., 2017 for a review of this method) were more likely to choose to compete again than were men whose testosterone levels did not increase after competing on the initial task (Carré & McCormick, 2008). Similarly, the extent to which men’s testosterone increases after losing a competitive task against another man is positively related to their willingness to compete again (Carré et al., 2009; Mehta & Josephs, 2006).
More recently, it has been hypothesized that some associations between testosterone and competition-related behaviors are moderated by cortisol (see Mehta & Prasad, 2015, for a discussion of evidence for this “Dual Hormone Hypothesis”). For example, Mehta et al. (2015) found that behavior in a competitive bargaining game was predicted by the interaction between changes in testosterone and cortisol. When cortisol decreased, testosterone increases led to greater earnings (Mehta et al., 2015). By contrast, when cortisol increased, testosterone increases led to poorer earnings (Mehta et al., 2015). Failure to consider the moderating role of cortisol could explain null and negative results for relationships between testosterone and competition-related behaviors in some studies (Mehta & Prasad, 2015).

The studies described above suggest that competition-induced changes in steroid hormones are associated with changes in men’s intrasexual competitiveness. Intrasexual competitiveness tracks naturally occurring (i.e., non-induced) within-male changes in testosterone in many non-human primate species (reviewed in Muller, 2017). However, it is an open question whether intrasexual competitiveness also tracks naturally occurring within-individual changes in men’s hormonal status. The current study addresses this issue, using a longitudinal design to investigate whether men’s intrasexual competitiveness tracks changes in their salivary testosterone, salivary cortisol, and/or the interaction between salivary testosterone and cortisol.

We assessed men’s intrasexual competitiveness using Buunk and Fisher’s (2009) Intrasexual Competitiveness Scale. We used this scale for two reasons. First, two recent studies have suggested women’s responses on the Intrasexual Competitiveness Scale track changes in their testosterone (Cobey et al., 2013; Hahn et al., 2016). Second, research suggests that this scale can detect within-subject changes in men’s intrasexual competitiveness. For example, engaging in a competitive task with another man increases men’s scores on the Intrasexual Competitiveness Scale (Buunk & Massar, 2012).
Reported anxiety tracks changes in salivary cortisol (see Kirschbaum & Hellhammer, 1994), increasing when cortisol is high. Consequently, we also investigated the relationship between men’s scores on Spielberger’s (1968/1977) State Anxiety Inventory and hormone levels. This was done primarily as a check on our testing procedures (i.e., as a positive control).

Methods

Participants
Fifty-nine heterosexual men participated in the study (mean age=21.97 years, SD=3.14 years). None of these men were currently taking any form of hormonal supplement or had taken any form of hormonal supplement in the 90 days prior to participation. Participants took part in the study as part of a larger project investigating hormonal correlates of voice and face perception (Kandrik et al., 2016, 2017).

Procedure
Participants completed up to five weekly test sessions, which took place between 2pm and 5pm to minimize diurnal variation in hormone levels (Papacosta & Nassis, 2011). Fifty-three of the participants completed all five test sessions.

During each test session, participants provided a saliva sample via the passive drool method (Papacosta & Nassis, 2011). Participants were instructed to avoid consuming alcohol and coffee in the 12 hours prior to participation and to avoid eating, smoking, drinking, chewing gum, or brushing their teeth in the 60 minutes prior to participation.

In each test session, participants also completed Buunk and Fisher's (2009) Intrasexual Competitiveness Scale (M=2.94, SD=0.98). Because it is not known whether reported intrasexual competitiveness tracks naturally occurring (i.e., non-induced) changes in men’s hormonal status, we sought to validate our testing procedures by replicating the well-established finding that reported anxiety tracks changes in salivary cortisol (see Kirschbaum &
Consequently, participants also completed Spielberger’s (1968/1977) State Anxiety Inventory (M=36.2, SD=8.68) in each test session.

The Intrasexual Competitiveness Scale is a 12-item questionnaire on which participants indicate how applicable each item is to them using a one to seven scale. Example items include, “I want to be just a little better than other men” and “I tend to look for negative characteristics in men who are very successful”. Higher scores on this scale indicate greater intrasexual competitiveness.

The State Anxiety Inventory is a 12-item questionnaire on which participants indicate how applicable each item is to them right now (i.e., at the time of responding) using a one (not at all) to four (very much so) scale. Example items include, “I feel strained” and “I am tense”. Higher scores on the State Anxiety Inventory indicate greater state (i.e., current) anxiety.

**Assays**

Saliva samples were immediately frozen and stored at -32°C until being shipped, on dry ice, to the Salimetrics Lab (Suffolk, UK) for analysis. There they were assayed using the Salivary Testosterone Enzyme Immunoassay Kit 1-2402 (M = 177.6 pg/mL, SD = 42.1 pg/mL, sensitivity<1.0 pg/mL, intra-assay CV=4.60%, inter-assay CV=9.83%) and Salivary Cortisol Enzyme Immunoassay Kit 1-3002 (M = 0.19 µg/dL, SD = 0.11 µg/dL, sensitivity<0.003 µg/dL, intra-assay CV=3.50%, inter-assay CV=5.08%).

Hormone levels more than three standard deviations from the sample mean for that hormone or where Salimetrics indicated levels were outside the sensitivity range of the relevant ELISA were excluded from the dataset (<1% of hormone measures were excluded for these reasons). The descriptive statistics given above do not include these excluded values.

Values for each hormone were centered on their subject-specific means to isolate effects of within-subject changes in hormones. They were then scaled so the majority of the distribution for each hormone varied from -.5 to .5 to
facilitate calculations in the linear mixed models. Since hormone levels were centered on their subject-specific means, men with only one value for a hormone could not be included in the analyses. After these exclusions, our final data set contained 54 men.

**Results**

We used linear mixed models to test for possible effects of hormonal status on intrasexual competitiveness and state anxiety. Analyses were conducted using R version 3.3.2 (R Core Team, 2016), with lme4 version 1.1-13 (Bates et al., 2014) and lmerTest version 2.0-33 (Kuznetsova et al., 2013). Data files and analysis scripts are publicly available at https://osf.io/abqun/.

The dependent variable was questionnaire score (separate models were run for the Intrasexual Competitiveness Scale and the State Anxiety Inventory). Predictors were the scaled and centered testosterone and cortisol levels and their interaction. Random slopes were specified maximally following Barr et al. (2013) and Barr (2013). Full model specifications and full results for each analysis are given in our Supplemental Information.

Our analysis of scores on the Intrasexual Competitiveness Scale revealed no significant effects of testosterone (estimate=0.05, t=0.22, p=.829), cortisol (estimate=0.11, t=0.52, p=.610), or their interaction (estimate=2.58, t=1.65, p=.101).

Our analysis of scores on the State Anxiety Inventory revealed no significant effect of testosterone (estimate=3.01, t=0.83, p=.413). There was a significant positive effect of cortisol (estimate=6.81, t=2.40, p=.018), indicating that men reported greater anxiety when cortisol was higher. The interaction between cortisol and testosterone was not significant (estimate=0.59, t=0.02, p=.981).

**Discussion**

The current study used a longitudinal design to investigate the hormonal correlates of changes in men’s reported intrasexual competitiveness and state anxiety. Consistent with previous research (see Kirschbaum & Hellhammer,
1994), men reported greater anxiety when salivary cortisol was high. Contrary to our predictions, however, we observed no significant relationships between intrasexual competitiveness and men’s testosterone, cortisol, or their interaction. Thus, our data provide no support for the hypothesis that men’s intrasexual competitiveness tracks naturally occurring changes in these steroid hormones.

In contrast, there is good evidence that competition-induced changes in men’s testosterone influence their intrasexual competitiveness (reviewed in Zilioli & Bird, 2017). Importantly, our null result for testosterone and intrasexual competitiveness does not appear to be a consequence of smaller changes in testosterone across test sessions in our study compared with competition-induced changes in testosterone levels in previous research. In fact, the mean difference between our participants’ maximum and minimum testosterone levels (M = 55.5 pg/mL, SD = 25.0 pg/mL) is greater than the mean competition-induced testosterone changes reported in previous research (Carré & McCormick, 2008; Carré et al., 2009; Mehta & Josephs, 2006). For example, the mean change in testosterone reported in Carré et al. (2009) was 24.7 pg/mL.

Since cortisol and its interaction with testosterone were included as predictors in our model, our null result for testosterone and intrasexual competitiveness cannot be explained by unexamined effects of cortisol. We also suggest that our null result for testosterone and intrasexual competitiveness is unlikely to be due to our measure of intrasexual competitiveness (Buunk & Fisher's Intrasexual Competitiveness Scale) measuring trait-like, rather than state-like, aspects of intrasexual competitiveness. Previous work has shown that engaging in a competitive task with another man alters men’s responses on this scale (Buunk & Massar, 2012). Our results are consistent with the suggestion that intrasexual competitiveness may not track naturally occurring changes in male testosterone in species where males compete for long-term access to mates, as is the case in humans (Muller, 2017). Alternatively, links between changes in testosterone and intrasexual competitiveness in men may be specific to situations where there is an explicit social challenge (see
Zilioli & Bird, 2017). Directly testing these possibilities is likely to be a fruitful avenue for research.

We found no significant effects of men’s hormonal status on intrasexual competitiveness. However, there was a weak and non-significant (p=.10) interaction between the effects of testosterone and cortisol for intrasexual competitiveness. This interaction suggested that there was a weak positive relationship between testosterone and intrasexual competitiveness when cortisol was low, but not when it was high. While we emphasize again here that this interaction was not significant, we also note that the pattern is consistent with the Dual Hormone Hypothesis (see Mehta & Prasad, 2015, for a discussion).

Collectively, our results suggest that the effects of competition-induced change in testosterone on men’s intrasexual competitiveness (assessed using the Point Subtraction Aggression Paradigm, or similar) do not necessarily generalize to naturally occurring changes in men’s testosterone and responses on Buunk and Fisher’s (2009) Intrasexual Competitiveness Scale. This raises the possibility that correlations between competition-induced changes in testosterone and men’s intrasexual competitiveness do not reflect direct effects of testosterone on behavior. Testosterone-administration studies would clarify this issue.

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```r
options(scipen=999)
library(tidyverse)
library(lmerTest)
library(viridis)
sessionInfo()
```
R version 3.3.2 (2016-10-31)
Platform: x86_64-apple-darwin13.4.0 (64-bit)
Running under: OS X Yosemite 10.10.5

locale:
[1] en_GB.UTF-8/en_GB.UTF-8/en_GB.UTF-8/C/en_GB.UTF-8/en_GB.UTF-8

attached base packages:
[1] stats     graphics  grDevices utils     datasets  methods   base

other attached packages:
[1] viridis_0.4.0 viridisLite_0.2.0 lmerTest_2.0-33 lme4_1.1-13 Matrix
_1.2-11 dplyr_0.7.2
[7] purrr_0.2.3   readr_1.1.1 tidyr_0.7.1   tibble_1.3.4   ggplot
 2_2.2.1 tidyverse_1.1.1

loaded via a namespace (and not attached):
[1] Rcpp_0.12.12 lubridate_1.6.0 lattice_0.20-35 assertthat_0.2.0
digest_0.6.12
[6] psych_1.7.5   R6_2.2.2     cellranger_1.1.0 plyr_1.8.4
backports_1.1.0
[11] acepack_1.4.1 httr_1.3.1   rlang_0.1.2      lazyeval_0.2.0
   readxl_1.0.0
[16] minqa_1.2.4   data.table_1.10.4 nloptr_1.0.4 rpart_4.1-11
checkmate_1.8.3
[21] splines_3.3.2 stringr_1.2.0 foreign_0.8-69 htmlwidgets_0.9
munsell_0.4.3
[26] broom_0.4.2   modelr_0.1.1 pkgconfig_2.0.1 base64enc_0.1-3
mnormt_1.5-5
[31] htmltools_0.3.6 nnet_7.3-12    gridExtra_2.3 htmlTable_1.9
Hmisc_4.0-3
[36] MASS_7.3-47   grid_3.3.2    nlme_3.1-131 jsonlite_1.5
gtable_0.2.0
[41] magrittr_1.5   scales_0.4.1 stringi_1.1.5 reshape2_1.4.2
bindrcpp_0.2
[46] latticeExtra_0.6-28 xml2_1.1.1 Formula_1.2-2 RColorBrewer_1.1-2
tools_3.3.2
[51] forcats_0.2.0 glue_1.1.1 hms_0.3      parallel_3.3.2
survival_2.41-3
[56] colorspace_1.3-2 cluster_2.0.6 rvest_0.3.2 knitr_1.17
bindr_0.1
[61] haven_1.1.0
# calculate standard errors
se <- function(x, na.rm = FALSE) {
  if (na.rm) {
    the.SE <- sqrt(var(x, na.rm = TRUE) / length(na.omit(x)))
  } else {
    the.SE <- sqrt(var(x, na.rm = FALSE) / length(x))
  }

  return(the.SE)
}

Load Data

data_hormones <- read_csv("hm_intrasexual_comp_anon.csv")

Descriptives

The number of sessions completed per man

data_hormones %>%
  group_by(hm_id) %>%
  summarise(
    sessions = n_distinct(date)
  ) %>%
  group_by(sessions) %>%
  summarise(
    n = n()
  ) %>%
  spread(sessions, n) %>% t()

Mean age for the sample
data_hormones %>%
  group_by(hm_id, date, age) %>%
  summarise(n = n()) %>%
  ungroup() %>%
  group_by() %>%
  summarise(
    mean_age = mean(age, na.rm = TRUE),
    sd_age = sd(age, na.rm = TRUE),
    se_age = se(age, na.rm = TRUE)
  ) %>%
  gather("stat", "value", 1:length(.)) %>%
  mutate(value = round(value, 4)) %>%
  separate(stat, c("stat", "")) %>%
  spread(stat, value)

| <chr> | mean | sd   | se   |
|-------|------|------|------|
| age   | 21.9679 | 3.1446 | 0.1932 |

1 row

Exclusions

Exclude men with only 1 session

data_multisession <- data_hormones %>%
  group_by(hm_id) %>%
  filter(n_distinct(date) > 1) %>%
  ungroup()

Exclude hormone outliers
test_mean <- mean(data_multisession$test)
test_sd <- sd(data_multisession$test)
cort_mean <- mean(data_multisession$cort)
cort_sd <- sd(data_multisession$cort)
data_NA_outliers <- data_multisession %>%
mutate(
  test = ifelse (test > test_mean + 3*test_sd |
                 test < test_mean - 3*test_sd, NA, test),
  cort = ifelse (cort > cort_mean + 3*cort_sd |
                 cort < cort_mean - 3*cort_sd, NA, cort)
)
data_NA_outliers %>%
group_by(hm_id, date) %>%
summarise(
  t = is.na(mean(test)),
  c = is.na(mean(cort))
) %>%
ungroup() %>%
select(t:c) %>%
gather('hormone', 'na', t:c) %>%
group_by(hormone) %>%
summarise(
  'valid' = n() - sum(na),
  'excluded' = sum(na)
) %>%
arrange(hormone)

| hormone | valid | excluded |
|---------|-------|----------|
| c       | 260   | 1        |
| t       | 257   | 4        |

2 rows

Exclude participants with < 2 valid hormone values after outlier exclusion

data_final <- data_NA_outliers %>%
group_by(hm_id) %>%
filter(sum(!is.na(test)) > 1 & sum(!is.na(cort)) > 1) %>%
ungroup()
# centre hormones within-subject, scale variables

data_scaled <- data_final %>%
group_by(hm_id) %>%
mutate(
  test.s = (test-mean(test, na.rm=TRUE)) / 180,
  cort.s = (cort-mean(cort, na.rm=TRUE)) / 0.5,
  avg.partner = mean(partner,na.rm=TRUE)
)
data_scaled %>%
group_by(hm_id, date, test.s, cort.s) %>%
summarise(n = n()) %>%
ungroup() %>%
gather("hormone", "value", test.s:cort.s) %>%
ggplot(aes(value, colour=hormone)) +
geom_density(alpha=.5) +
scale_x_continuous(limits = c(-1,1))

Mean hormone levels
data_scaled %>%
group_by(hm_id, date, age, test, cort) %>%
summarise(n = n()) %>%
ungroup() %>%
group_by() %>%
summarise(
  mean_test = mean(test, na.rm = TRUE),
  sd_test = sd(test, na.rm = TRUE),
  se_test = se(test, na.rm = TRUE),
  mean_cort = mean(cort, na.rm = TRUE),
  sd_cort = sd(cort, na.rm = TRUE),
  se_cort = se(cort, na.rm = TRUE)
) %>%
gather("stat", "value", 1:length(.)) %>%
mutate(value = round(value, 4)) %>%
separate(stat, c("stat", "hormone")) %>%
spread(stat, value)

| hormone | mean   | sd     | se     |
|---------|--------|--------|--------|
| cort    | 0.1868 | 0.1064 | 0.0066 |
| test    | 177.5778 | 42.1499 | 2.6344 |

2 rows

Analyses

Men’s changes in intrasexual competitiveness

Descriptives
## LMEM Analysis

```r
model.IC.TbyC <- lmer(intr_cmpt ~ 1 + test.s * cort.s + (test.s * cort.s || hm_id),
                       data = data_scaled, REML = FALSE)
summary(model.IC.TbyC)
```
Linear mixed model fit by maximum likelihood t-tests use Satterthwaite approximations to degrees of freedom [lmerMod]

Formula: intr_cmpt ~ 1 + test.s * cort.s + ((1 | hm_id) + (0 + test.s | hm_id) + (0 + cort.s | hm_id) + (0 + test.s:cort.s | hm_id))

Data: data_scaled

AIC      BIC   logLik deviance df.resid
443.2    475.0   -212.6    425.2      244

Scaled residuals:

Min       1Q   Median       3Q      Max
-2.74743 -0.53734 -0.01468  0.52107  2.57212

Random effects:

Groups   Name          Variance Std.Dev.
hm_id    (Intercept)   0.7636   0.8738
hm_id.1  test.s        0.3089   0.5558
hm_id.2  cort.s        0.3906   0.6250
hm_id.3  test.s:cort.s 0.0000   0.0000
Residual               0.1468   0.3831

Number of obs: 253, groups:  hm_id, 54

Fixed effects:

Estimate Std. Error   df  t value          Pr(>|t|)
(Intercept)  2.94997  0.12167 54.44000 24.245 <0.0000000000000002 ***
test.s       0.04926  0.22677 38.26000   0.217               0.829
cort.s      0.11120  0.21597 34.95000   0.515               0.610
test.s:cort.s 2.57927  1.56516 162.98000   1.648               0.101

---

Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Correlation of Fixed Effects:

  (Intr) test.s cort.s
test.s    -0.002
  cort.s   0.015   -0.209
tst.s:crt.s -0.066   0.017   -0.197

Men’s changes in anxiety

Descriptives
```r
data_scaled %>%
  group_by() %>%
  summarise(
    mean_StateAnxiety = mean(st_anx, na.rm = TRUE),
    sd_StateAnxiety = sd(st_anx, na.rm = TRUE),
    se_StateAnxiety = se(st_anx, na.rm = TRUE)
  ) %>% gather("stat", "value", 1:length(.)) %>%
  mutate(value = round(value, 4)) %>%
  separate(stat, c("stat", "measure")) %>%
  spread(stat, value)
```

| measure     | mean | sd  | se  |
|-------------|------|-----|-----|
| StateAnxiety| 36.2412 | 8.6799 | 0.5414 |

1 row

**LMEM Analysis**

```r
model.SA.TbyC <- lmer(st_anx ~ 1 + test.s * cort.s + (test.s * cort.s || hm_id),
                       data = data_scaled, REML = FALSE)
summary(model.SA.TbyC)
```
Linear mixed model fit by maximum likelihood t-tests use Satterthwaite approximations to degrees of freedom [lmerMod]
Formula: st_anx ~ 1 + test.s * cort.s + ((1 | hm_id) + (0 + test.s | hm_id) +
    (0 + cort.s | hm_id) + (0 + test.s:cort.s | hm_id))
Data: data_scaled

AIC     BIC   logLik deviance df.resid
1752.5   1784.3   -867.3   1734.5      244

Scaled residuals:
  Min      1Q   Median      3Q     Max
-2.74041 -0.56461 -0.09314  0.42326  3.08526

Random effects:
  Groups   Name          Variance Std.Dev.
  hm_id    (Intercept)     32.06   5.662
  hm_id.1  test.s          95.45   9.770
  hm_id.2  cort.s           0.00   0.000
  hm_id.3  test.s:cort.s 1362.36  36.910
  Residual                 37.82   6.149
Number of obs: 253, groups:  hm_id, 54

Fixed effects:
  Estimate   Std. Error    df  t value  Pr(>|t|)
(Intercept)    36.3340     0.8723  55.0200 41.654 <2e-16 ***
test.s          3.0104     3.6436  45.7200   0.826          0.4130
cort.s          6.8102     2.8402 149.8900   2.398          0.0177 *
test.s:cort.s   0.5898    24.4971  18.5500   0.024          0.9811

---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Correlation of Fixed Effects:
  (Intr) test.s cort.s
test.s     0.006
cort.s   -0.036 -0.219
tst.s:crt.s -0.119 -0.005 -0.224

Graph of relationship between cortisol and state anxiety
data_scaled %>%
  group_by(hm_id) %>%
mute(mean_st_anx = mean(st_anx)) %>%
  ungroup() %>%
  ggplot(aes(cort.s*0.5, st_anx, group = hm_id)) +
  geom_smooth(aes(color = mean_st_anx), method = "lm", alpha = 0.25, show.legend = FALSE) +
  geom_point(aes(color = mean_st_anx), alpha = 0.5, show.legend = FALSE) +
  ylim(0, 100) +
  labs(
    title = "Cortisol-State Anxiety correlation by subject",
    x = "Cortisol (subject-mean centred)",
    y = "State Anxiety Score"
  ) +
  scale_color_viridis()