



Differential Changes in Steroid Hormones Prior to Competition in Bonobos and Chimpanzees

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2 **Differential changes in steroid hormones prior to competition in bonobos and chimpanzees**

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22research, V.W., B.H., S.L., R.W., and P.E. analyzed data, P.E. contributed new reagents/analytic
23tools, and V.W., B.H., S.L., R.W., and P.E. wrote the paper.

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Abstract

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25 A large body of research has demonstrated that variation in competitive behavior across
26species and individuals is linked to variation in physiology. In particular, rapid changes in
27testosterone and cortisol during competition tend to differ based on an individual's or species'
28typical psychological and behavioral responses in competition. Our species' closest living
29relatives, chimpanzees (*Pan troglodytes*) and bonobos (*Pan paniscus*), exhibit marked
30differences in competitive behavior and its underlying social psychology. This suggests that the
31two species may differ in how their testosterone and cortisol shift during such competitions. We
32tested individuals of both species in a dyadic food competition and measured salivary
33testosterone and cortisol before and after the event. We found that males of both species shifted
34in their steroid hormones in anticipation of the competition, and did so differentially based
35whether they were paired with a tolerant or intolerant partner. However, bonobo males showed
36differential changes only in their cortisol levels, while chimpanzees showed differential changes
37only in their testosterone levels. The results indicate that in anticipation of competition bonobos
38and chimpanzees perceive the situation differently in showing differential endocrine shifts,
39perhaps in line with viewing the event as a stressor in the case of bonobos or a dominance
40contest in the case of chimpanzees. Further work with nonhuman apes can reveal the degree to
41which our species' physiological responses to competition are shared with other apes or have
42been shaped by our own unique evolutionary history.

43Introduction

44 In numerous species, including humans, males engaged in competition tend to show acute
45 shifts in their levels of testosterone and cortisol. These hormones can change on a time-scale of
46 minutes, and tend to do so according to psychological perceptions of the competition, whether
47 anticipation of its perceived difficulty or evaluation of the result [1, 2]. One psychological factor
48 that appears particularly influential is an individual's coping style, or how he responds
49 physiologically across stressful events such as competition. Within and between species,
50 distinctions in coping style affect the relative magnitude and nature of changes in testosterone
51 and cortisol surrounding a competitive event [3, 4]. Differential serotonin receptor sensitivity has
52 been found among individuals of distinct coping styles, indicating a means by which the changes
53 in cortisol or testosterone could be associated with psychological and behavioral effects
54 [reviewed in 3]. Thus given the same competitive event, individuals that psychologically
55 appraise the event differently also show different testosterone and/or cortisol shifts.

56 Across species, differences in levels of aggression tend to predict individuals' behavioral
57 and physiological responses to competition. In lines of mice bred for low or high aggression, the
58 low-aggression mice tend to exhibit a passive coping style characterized by freezing behaviors.
59 This passive coping style is associated with a large increase in glucocorticoids surrounding a
60 competitive event in the low aggression mice, whereas mice bred for high aggression show a
61 lesser change in glucocorticoids when competing [5]. The prediction that aggression should
62 mediate physiological response to competition is compelling in the case of humans' closest
63 living relatives, chimpanzees (*Pan troglodytes*) and bonobos (*Pan paniscus*). Bonobos have been
64 characterized as less aggressive than chimpanzees, particularly in the severity of male aggression
65 [6-9]. In competition over food in particular, chimpanzees are likely to respond aggressively

66while bonobos are more likely to share, both in the wild and in experimental manipulations [10,
6711]. Given the evidence from the selection experiments on mice, bonobos may demonstrate a
68passive coping style and show heightened cortisol sensitivity relative to chimpanzees.
69Alternatively, chimpanzees may be more sensitive to competition than that of bonobos because
70of their more rigid hierarchies [12, 13]. In human competition, individuals with heightened
71sensitivity to status, or a greater “power motive,” display larger shifts in testosterone [2, 14].
72Therefore, it is unclear which species will show more pronounced rapid shifts in a competitive
73event.

74 Previous work supports the hypothesis that chimpanzees and bonobos will in fact differ
75in their immediate changes in testosterone and cortisol in competitive events. First, testosterone
76and cortisol levels over the long term are positively correlated with social dominance rank in
77several primates including chimpanzees and bonobos [15-17]. Thus it is clear that hormones are
78involved in mediating dominance behaviors in these apes as in other species. Secondly, in rhesus
79macaques, the winner of an aggressive interaction exhibits a post-contest elevation in
80testosterone while the loser exhibits a decrease in testosterone levels, indicating that the same
81steroid hormones found to exhibit rapid shifts surrounding competition in humans may be
82associated with similar shifts in other primates [18]. Finally, bonobos were found to exhibit an
83anticipatory rise in cortisol prior to a competition over limited amounts of food, with an even
84greater increase in cortisol when the food was visibly difficult to share [19]. These studies
85suggest that the cognitive abilities necessary to anticipate the outcome of competition are present
86in our closest living relatives. However, quick hormone changes in chimpanzees have not yet
87been investigated, making the comparison to both bonobos and humans impossible.

88 Here we compared the endocrine shifts surrounding competition in chimpanzees and
89 bonobos. We predicted that the two species would differ in their steroid hormone profiles in a
90 similar competitive situation. We presented these apes with an experimental dyadic food
91 competition and measured testosterone and cortisol levels prior to and after the competitive
92 event. We had three hypotheses of how bonobos and chimpanzees would differ in their steroid
93 hormone shifts. Each hypothesis applied to both anticipation of the test and response to the test.

94 **Hypothesis 1.** *Only chimpanzees react:* chimpanzees will react strongly to the
95 competitive event, while bonobos will show little change in their steroid hormone levels. This
96 hypothesis is based on the evidence that bonobos share food more readily in competitions than
97 chimpanzees and carry out behaviors to reduce tension and facilitate sharing such as non-
98 conceptive sex [20]. Furthermore bonobos' dominance hierarchies are more fluid than those of
99 chimpanzees [12], suggesting that the status-determining nature of the competition may be more
100 salient to chimpanzees than to bonobos.

101 **Hypothesis 2.** *Only bonobos react:* bonobos will react strongly to the competitive event,
102 while chimpanzees will show little change in their steroid hormone levels. This hypothesis is
103 based on the finding that bonobos exhibit a rise in cortisol in anticipation of a competition over
104 food [19]. Moreover, the strong dominance hierarchies in chimpanzees may cause the outcome
105 of the food competition to be pre-determined, implying that they will show few endocrine shifts
106 surrounding the competition. In contrast, for bonobos there may be a higher uncertainty of the
107 outcome so endocrine shifts will be greater to mobilize energy or increase cognitive acuity.

108 **Hypothesis 3.** *Differential reactivity:* both species will react to the competitive event, but
109 will do so differently. This hypothesis suggests that competition over food is important to both
110 species and posits that the physiological correlates of the competitive behavior will differ

111between the two, given the differing behavioral outcomes seen in the two species. If the two
112species exhibit distinct coping styles, they may show differing cortisol responses to the test. This
113prediction would imply that bonobos show greater cortisol shifts, associated with a passive
114coping style. In turn, if chimpanzees show a greater power motive in seeking dominance, they
115should show larger testosterone changes than bonobos, among whom the motivation to seek
116dominance is not as strong.

117

118**Results**

119 In the dyadic food competitions subjects were tested in pairs, with each individual
120represented in the sample as a member of a single pair. Before the food competitions, subjects
121participated in a dominance test with their partner to assess which individual was dominant in a
122dyadic food context. The results of this test were correlated with performance in the food
123competitions, in that there was a significant relationship between the number of trials where an
124individual obtained the piece of food in the dominance test and the number of trials where that
125individual monopolized more than half of the food during the food competitions (linear
126regression, $r^2 = 0.37$, $p < 0.001$). Thus, in each pair, there was a pre-assigned dominant and
127subordinate individual.

128 Pairs were presented with three separate days of food competitions, with three conditions
129(one per day) varying the monopolizability of the food (the order of the conditions was
130counterbalanced across species, sex, and age). On each day, pairs participated in 3 trials of one
131condition (thus there were 9 total trials, 3 trials for each of the 3 conditions). In each trial, a
132controlled amount of food was placed in a specific configuration (according to condition) in a
133testing room, then the pair was released into the room and allowed to eat the food. After the pair

134 finished eating, the experimenter immediately placed the food for the subsequent trial. In
135 addition to the paired food competitions, each subject was also presented with a solo condition
136 that replicated the procedure of the paired conditions exactly except that individuals were tested
137 alone rather than in a pair.

138 For each condition (test day), a variable *outcome* denoting relative food sharing was
139 scored. A “1” was scored for *outcome* if the dominant individual obtained significantly more
140 food than the subordinate over the course of the 3 trials, while a “0” was scored if this did not
141 occur (individuals shared the food equally or the dominant obtained less). The differing potential
142 results of *outcome* occurred with roughly the same frequency in the two species (approximately
143 50% of the time in each) ($\chi^2 = 1.33$, $p = 0.25$). Thus chimpanzees and bonobos showed equal
144 frequencies of the dominant monopolizing food on 2 or more of the 3 trials for a given condition.
145 This suggested that any species differences in endocrine patterns according to this factor were
146 not simply a result of the two groups being unequally represented in the two outcome categories.
147 The two species did show significant differences in other behavioral measures in this task, such
148 as in the amount that individuals fed simultaneously at the same pile [21], but were similar in the
149 *outcome* measure.

150 Saliva samples were taken immediately prior to the first trial of the food competition,
151 before the food was presented but after individuals were placed in their pairing. Samples were
152 then collected again 15 minutes after the three trials were finished. The samples were analyzed
153 for testosterone and cortisol using previously validated radioimmunoassay procedures [22]. The
154 values of testosterone and cortisol were log-transformed to normalize the data and to allow the
155 use of parametric statistics.

156 ***Cortisol***

157 First, we investigated the effects of pre-versus post-test, *species*, *sex*, and *outcome* on
158cortisol levels in the 3 paired conditions. We used a generalized linear model (GLM) on the log-
159transformed cortisol values, controlling for the repeated subject variable *individual* (since each
160individual was represented in the data set 3 times for each of the 3 food competition conditions).
161We entered log post-test cortisol as the dependent variable, with log pre-test cortisol as a co-
162variate, and *species*, *sex*, and *outcome* as factors. In this model, we examined all main effects, 2-
163way interactions, and 3-way interactions. This analysis revealed a significant effect of log pre-
164test cortisol on log post-test cortisol (Wald Chi-Square (1) = 67.637, $p < 0.001$), a significant *sex**
165*outcome* interaction (Wald Chi-Square (1) = 9.285, $p = 0.002$), and a significant *sex** *outcome*
166*log pre-test cortisol interaction (Wald Chi-Square (1) = 9.859, $p = 0.002$). Further analyses of
167the post-test values can be found in the Supplemental Online material.

168Anticipatory cortisol

169 We performed a GLM analysis including only the pre-test values of cortisol, controlling
170for *individual* and having *species*, *sex*, and *outcome* as between-subject factors. This analysis
171revealed a significant main effect of *species* (Wald Chi-Square (1) = 11.618, $p = 0.001$), in that
172bonobos tended to have higher pre-test cortisol than chimpanzees, an interaction between *sex**
173*outcome* (Wald Chi-Square (1) = 6.036, $p = 0.014$), and a 3-way interaction between *species*, *sex*,
174and *outcome* (Wald Chi-Square (1) = 8.908, $p = 0.003$). To further investigate the 3-way
175interaction between *species*, *sex*, and *outcome*, we performed split analyses for each species. To
176correct for this multiple testing, we used an alpha threshold of 0.025 for significance.

177 A GLM of chimpanzees' pre-test log cortisol values with *sex* and *outcome* as factors
178revealed no significant main effects and no interaction. In contrast, a GLM of bonobos' pre-test
179log cortisol values with these two factors demonstrated a significant *sex***outcome* interaction

180(Wald Chi-Square (1) = 11.070, $p = 0.001$). To examine this interaction, we performed separate
181analyses according to sex in each species, using an alpha threshold of 0.013 for significance.

182 In chimpanzees, neither sex showed a significant effect of *outcome* on cortisol. In
183bonobos, a significant effect was present only in males – cortisol was higher when the dominant
184was going to obtain significantly more food than when the two individuals were going to
185share/the dominant was going to obtain less (Wald Chi-Square (1) = 13.766, $p < 0.001$) (Figure 1).
186This effect was not significant in bonobo females (Wald Chi-Square (1) = 2.045, $p = 0.153$).

187 Therefore, it appears that male bonobos anticipated the outcome of the interaction based
188on the individual with whom they were paired. We performed several control analyses that
189ensured that these patterns were present in bonobo males regardless of dominance status, the
190order of the test day, and the type of pair the individual was in (male-male versus male-female)
191(see Supplemental Online Material for details). However, it was also necessary to show that
192these results were not simply trait characteristics of the individuals in a given pair, but rather
193reactions of those individuals to the situation of being paired.

194Anticipatory cortisol relative to baseline cortisol

195 To assess individuals' departure from their baseline cortisol when paired, we performed a
196regression analysis comparing the log pre-test day cortisol values with the log pre-solo day
197cortisol values. These measures were highly correlated ($r^2 = 0.25$, $p < 0.001$). We then used the
198unstandardized residuals of this regression to investigate how much an individual's pre-test
199cortisol value on a given test day departed from what would be predicted based on their pre-solo
200day cortisol level.

201 We performed a GLM analysis on these residuals separately by species, in males only
202(with an alpha level of 0.013) – since the results were not significant in females of either species

203—controlling for *individual* and with *outcome* as a factor. This analysis revealed that in bonobo
204males, there was a significant effect of *outcome* on changes in cortisol relative to baseline (Wald
205Chi-Square (1) = 10.635, $p = 0.001$) (Figure 2). There was no significant effect of *outcome* in
206chimpanzee males. These residual analyses suggest that, relative to their own solo values,
207bonobo males' cortisol decreased when they were in a pair that was going to share, and increased
208when they were in a pair where the dominant was going to obtain significantly more food.
209Meanwhile, chimpanzee males' cortisol levels did not differ based on *outcome*, and did not vary
210greatly between the baseline and test days.

211 This species difference was especially pronounced when there was going to be an
212asymmetry in the obtaining of food. We performed separate GLM analyses for each outcome in
213males with *individual* and *species* as factors (setting the alpha level for significance at 0.013).
214This analysis showed a significant effect of *species* when one individual was going to
215monopolize more food (Wald Chi-Square (1) = 9.356, $p = 0.002$), but not when the pair was
216going to share (Wald Chi-Square (1) = 1.260, $p = 0.26$). This suggests that the species difference
217likely derived from bonobos' cortisol increasing prior to a situation where sharing was not going
218to occur, while chimpanzees' cortisol remained similar to baseline in this instance.
219These residual analyses indicate that the observed changes in cortisol prior to the test were
220associated with individual pairings, rather than simply reflecting either anticipation of food being
221presented or baseline cortisol differences between individuals.

222 These results are similar to those found in past work [19] in suggesting both that
223anticipation of food sharing increases bonobo cortisol levels, and that cortisol increases
224differentially based on the predicted outcome. Unlike the previous paradigm, however, in our test
225the bonobos could not see the configuration of food prior to the pre-test saliva sample. Thus, our

findings suggest that on simply being partnered with a given individual, bonobos were able to evaluate the respective tolerance level with their partner, and their cortisol rose in a situation where the two individuals might not share. In contrast, their cortisol decreased when they were likely to share with their partner. The causal relationship between these cortisol changes and the corresponding behavior in the test is unclear. Subjects' cortisol may have increased because they knew that they were not going to share, or their increased cortisol levels may have caused them to be less likely to attempt to share; we elaborate on this point further in the general discussion. However, this does suggest that bonobos' sharing is associated with reduced arousal in their lower cortisol levels, and may in part explain why they voluntarily share food with other individuals [23].

236 This change in cortisol was present only in bonobo males, not in chimpanzee males. We
237 consider two explanations for the species difference. First, chimpanzees possibly do not perform
238 the same anticipatory appraisal, e.g. they might not expect (or respond to the expectation of)
239 differential food sharing. Alternatively, chimpanzees might perform such appraisals without a
240 change in their cortisol levels. To discriminate between these possibilities we performed similar
241 analyses with the testosterone data to assess whether chimpanzees were generally non-reactive to
242 the competition or whether they did not show cortisol shifts in particular.

243 ***Testosterone***

244 We began by performing a GLM analysis of post-test log-transformed testosterone (T)
245 values with log pre-test testosterone as a covariate, *individual* as a subject factor, and *species*,
246 *sex*, and *outcome* as between-subject factors, examining all main effects and 2- and 3-way
247 interactions. This analysis revealed only a significant effect of log pre-test testosterone (Wald
248 Chi-Square (1) = 17.461, $p < 0.001$) on post-test testosterone. We then performed a similar

249residual analysis of the post-test values as described for cortisol above; this analysis further
250supported the notion that any distinctions in post-test T levels were simply due to pre-test
251differences (discussed in Supplemental Online Material). Given our predictions from the cortisol
252results, we performed further analyses on the pre-test T values to assess whether any anticipatory
253T patterns were present.

254Anticipatory testosterone

255 We began by analyzing the pre-test testosterone values in a GLM with *species*, *sex*, and
256*outcome* as factors. This GLM analysis revealed a significant effect of *species* (Wald Chi-Square
257(1) = 8.845, $p = 0.003$), with bonobos' pre-test testosterone higher than that of chimpanzees, a
258significant effect of *sex* (Wald Chi-Square (1) = 8.610, $p = 0.003$), showing that males' T was
259higher than that of females, and a significant *species** *outcome* interaction (Wald Chi-Square (1)
260= 4.339, $p = 0.037$). Given the known differences in T levels and responsiveness to competition
261between males and females in humans [24] and the prediction from the cortisol findings that
262effects would be more pronounced in males, we performed separate analyses for each sex. We set
263the alpha value for significance at 0.025.

264 The significant interaction between *species* and *outcome* was present in males only (Wald
265Chi-Square (1) = 5.857, $p = 0.016$). In contrast, in females, there was only a significant main
266effect of *species* (Wald Chi-Square (1) = 5.433, $p = 0.020$), in that bonobo females had higher
267pre-test T than chimpanzee females, but no effect of *outcome* or interaction. This suggests that
268males of the two species showed differing testosterone in anticipation of varying outcomes,
269while alterations in females' testosterone levels were less consistent, as was the case for cortisol.
270To investigate this further, we performed separate analyses by species in males, using an alpha
271value of 0.013.

272 In chimpanzee males, the impact of *outcome* approached significance (Wald Chi-Square
273(1) = 4.618, $p = 0.03$). Specifically, males tended to show higher pre-test T when the dominant
274was going to obtain more food than when the two individuals were going to share. In contrast, in
275bonobo males, there was no significant effect of *outcome* (Wald Chi-Square (1) = 1.290, $p =$
2760.26) (Figure 3).

277 Again, we performed several controls and found that this pattern was present equally in
278dominants and subordinates, across test days, and regardless of the sex of the partner (discussed
279in the Supplemental Online Material). As with the cortisol analyses, we next ascertained whether
280these T differences were traits of the individuals in the pairs or reactions to being in the paired
281test situation.

282Anticipatory testosterone relative to baseline testosterone

283 We took T residuals in the same way described for cortisol above, and found that log pre-
284test T was highly correlated with log pre-solo T ($r^2 = 0.13$, $p < 0.001$). We then performed GLM
285analyses on the unstandardized residuals of this regression, separately by species and sex (thus at
286an alpha level of 0.013) with *outcome* as a factor. In chimpanzee males, the effect of *outcome* on
287the residuals was only marginally significant at this level (Wald Chi-Square (1) = 5.241, $p =$
2880.02), in that males' T tended to be lower than baseline when they were going to share, and
289higher than baseline when the dominant was going to obtain significantly more. In bonobo
290males, the effect of *outcome* was not significant (Figure 4).

291 Thus, chimpanzee males showed a greater departure from their solo T values based on the
292outcome of the test than did bonobos. This distinction was especially pronounced in the sharing
293condition – performing a GLM analysis in males for only this condition, with *individual* and
294*species* as factors (and an alpha level of 0.013) showed a significant effect of *species* when the

295pair was going to share (Wald Chi-Square (1) = 9.330, $p = 0.002$), though not when one
296individual in the pair monopolized more food (Wald Chi-Square (1) = 0.000, $p = 1.0$). This
297suggests that the more pronounced species difference was in chimpanzees' T decreasing prior to
298sharing and bonobos' T increasing in the same situation (though bonobos' T also rose when there
299was an asymmetry in the obtaining of food, thus this increase was independent of outcome).

300 In contrast to the cortisol results above, where bonobos showed stronger differential
301shifts based on outcome than did chimpanzees, in testosterone the pattern of anticipatory change
302was stronger in chimpanzees and non-significant in bonobos. This suggests that the initial
303cortisol results do not reflect an enhanced ability of bonobos to predict the outcome of a food
304competition based on pairing or that bonobos are more reactive to being paired with certain
305individuals. Instead, both species appear to be able to anticipate the outcome of the test (as
306quantified by the *outcome* variable) based on simply being placed in a pair, but they differ in
307their associated endocrine shifts.

308

309Discussion

310 These results support the *Differential reactivity* hypothesis: both bonobos and
311chimpanzees showed an endocrine shift surrounding the competitive event, but the nature of this
312reaction differed in the two species. Bonobo males' cortisol increased in anticipation of
313competition when they were placed with a partner where there would be an asymmetry in
314success at obtaining food. These cortisol increases were relative to baseline levels and to changes
315when subjects were placed in a pairing where they would be able to share. Therefore, bonobos
316appeared to respond to the competition as a stressor when the food would not be shared,
317exhibiting a passive coping style and an associated large shift in glucocorticoids. In contrast,

318 chimpanzee males showed an anticipatory increase in testosterone when placed with a partner
319 where the outcome of the food competition would be asymmetrical, relative both to their
320 baseline testosterone levels and to changes in testosterone when they were placed with a partner
321 where sharing would occur. Chimpanzees may have viewed the competition as status-
322 determining, particularly when the outcome of the interaction was unclear (e.g. there was an
323 uncertain dominance relationship) and showed a correlated anticipatory rise in testosterone.
324 There were no independent effects of species or test outcome on the post-competition values of
325 testosterone and cortisol that were unrelated to these anticipatory effects. These patterns were
326 present equally in dominants and subordinates, across test days, and across ages (see
327 Supplemental Online Material). Thus overall, these results indicate a strong relationship between
328 competition and rapid steroid shifts in both of humans' closest living relatives.

329 Our findings indicate that male bonobos and chimpanzees can predict the results of a
330 dyadic food competition based solely on being paired with another individual. Moreover, these
331 predictions of the competitive outcomes are associated with rapid endocrine changes in males of
332 both species. While it is possible that the endocrine changes seen here in fact determined the
333 behavioral outcomes of the competition rather than resulting from a prediction of the outcome,
334 humans have been shown to exhibit anticipatory changes in steroids prior to competition in
335 numerous situations, even with unknown competitive partners [24-26]. Subjects in this study
336 were paired with known groupmates and apes are known to track their tolerance levels with other
337 individuals [11, 27], suggesting that individuals were likely able to make these predictions. In
338 turn, this suggests that the patterns of anticipatory appraisal seen in humans are not unique to our
339 species.

340 These data demonstrate that the behavioral differences observed in chimpanzees and
341 bonobos during dyadic food competition are associated with differences in physiological
342 responses in the two species. These findings are the first to show rapid endocrine changes in
343 association with competition in chimpanzees, and replicate evidence of a pre-competition
344 cortisol increase in bonobos [19]. Further, these results suggest that after the divergence of
345 chimpanzees and bonobos, selection may have acted differentially on the endocrine pathways
346 governing rapid shifts in testosterone and cortisol as a result of the two species' differing
347 ecological circumstances. In particular, selection against aggression in bonobos may have
348 changed their levels of physiological and psychological reactivity, such that they mirror the lines
349 of mice bred for low aggression and exhibit a passive coping style and large glucocorticoid
350 responses [5, 28]. Future research comparing hormonal parameters in the two species can further
351 illuminate the numerous distinctions already seen between the two in morphology, behavior, and
352 cognition [21, 29, 30].

353 Notably, the “winner effect,” in which testosterone and cortisol rise in human winners
354 across various competitive contexts, was not observed among the chimpanzees and bonobos
355 [31]. It is possible that the lack of post-competition changes seen here was due to the timing of
356 sampling. Our post-test interval of 15 minutes was chosen to match the human literature, where
357 responses to competition have been observed in that length of time [32, 33]. Chimpanzees and
358 bonobos might in fact react to the outcome of the competition but do so more slowly than
359 humans. This would signify a difference between these apes and humans in the speed of response
360 to wins or losses. However, previous work with bonobos showed no response effects in cortisol
361 levels as long as one hour post-competition [19]. Therefore, the lack of a winner effect in food
362 competitions among bonobos and chimpanzees may represent a lack of salience of the outcome

363of such competitions to these apes, physiologically and psychologically. Though competition
364over food is ecologically relevant, another context such as competition over mates may be more
365significant for apes. Even if this is the case, these results suggest that humans are derived in
366possessing endocrine shifts in response to competitions as unrelated to survival or reproduction
367as a game of chess.

368 Similar to what is seen in humans, we found the strongest effects of the competition on
369steroid hormones in males, whereas females did not exhibit any significant patterns. Steroid
370shifts surrounding competition in women are inconsistent and only observed in some studies [24,
37134, 35]. The sex differences in rapid endocrine changes in humans appear to be more pronounced
372in reaction to psychological stimuli than to exercise or other physiological stimulation where
373both sexes show steroid shifts, suggesting that women do not simply show generally muted
374endocrine responsivity [36, 37]. The lack of significant patterns in the endocrine shifts of female
375chimpanzees and bonobos indicates that the lessened response of women to psychological status
376competitions or stressors may have deep evolutionary roots.

377 Overall, the present results suggest that our closest living relatives have the capacity to
378anticipate and appraise the results of dyadic food competitions and that their physiology changes
379accordingly. Further, they indicate that the shifts in testosterone and cortisol prior to competition
380seen in humans are evolutionarily inherited. Given that chimpanzees shifted in testosterone and
381not cortisol, while for bonobos the pattern was the opposite, independent mechanisms may
382govern the sensitivity of testosterone and cortisol to the anticipation of competition in these
383species and humans as well. Further, anticipatory shifts were more relevant to these apes than the
384outcome of the competitive events. Future work can tease apart the psychological factors and
385physiology mediating anticipatory versus response changes. Our results pave the way for

understanding how different selection pressures have promoted species differences in behavioral endocrinology, including comparisons with our own species.

Methods

Subjects

The subjects for this experiment were 24 bonobos living at Lola ya Bonobo Sanctuary in the Democratic Republic of Congo and 33 chimpanzees living at Tchimpounga Chimpanzee Sanctuary in the Congo Republic. The bonobo subjects ranged in age from 4 to 21 years old, with a mean age of 8.5 years. Eleven males and 12 females were sampled for steroid analysis, but enough sample volume for testosterone analysis was only obtainable for 7 of these females. One bonobo male participated in the behavioral testing but it was not possible to obtain a sufficient volume of saliva from him to perform hormonal analysis. The chimpanzee subjects ranged from 5 to 19 years old, with a mean age of 9.4 years old. 16 males and 17 females were sampled for both cortisol and testosterone. More information about the subjects' living circumstances and rearing history can be found in the Supplemental Online Material.

There were 12 bonobo pairs and 24 chimpanzee pairs tested. Pairs were balanced in terms of age, in that equal numbers of adult and juvenile pairs were used. The age of the two individuals in a pair was matched as closely as possible. Pairs were also balanced with respect to sex (equal numbers of male-male, male-female, and female-female pairs were tested in each species). Certain chimpanzees participated in repeated pairs, but for the analyses reported here, only the first pair that these subjects participated in was used so as to represent every individual in the sample equally. The second individual in that subject's repeated pair was still included as a subject, resulting in 24 bonobos and 33 chimpanzees in the sample.

409 *Food competitions*

410 Subjects were presented with 3 paired food competition conditions and a solo condition
411 where they underwent the same procedure alone. The procedure of these conditions and the
412 dominance test are described in the Supplemental Online Material. A subject's solo condition
413 was either prior to the three paired conditions or after the three paired conditions, with this
414 placement (before or after) counterbalanced across species, sex, and age. The paired conditions
415 and the solo condition were videotaped for behavioral coding.

416 *Coding of behavioral variables*

417 Videos of behavior in the test were coded by the first author. A randomly chosen 20% of
418 the trials were also coded for reliability by a second coder who was blind to the hypotheses of the
419 study. On each trial, the presence or absence (0/1) of a given behavior was scored. For this
420 analysis, only one behavioral variable was used (the results of other behavioral analyses on this
421 data set can be found in [21]). This variable denoted whether the dominant obtained more than
422 half of the food in a given trial. If the dominant obtained less of the food or approximately half, a
423 "0" was scored, while a "1" was scored if the dominant clearly obtained more of the food on that
424 trial. The reliability for this measure was high (Cohen's kappa = 0.88, $p < 0.001$).

425 The *outcome* variable used in the analyses was derived from this behavioral coding. A
426 "1" was scored for *outcome* if the dominant obtained noticeably more food on 2 or 3 trials out of
427 the 3 total trials of the condition. If the dominant obtained more food on only 1 or 0 trials, this
428 was scored as a "0" for that condition. The *outcome* measure was usually consistent within a
429 given pair, in that a dominant would obtain significantly more food across each of the 3 food
430 competition conditions, but could vary across condition within each pair. Thus each individual in
431 a pair was represented in the data set 3 times, once for each condition. Importantly, the scores for

432outcome were the same for both individuals in the pair (the dominant and the subordinate), thus
433this variable represented asymmetry versus sharing in the distribution of feeding rather than a
434win or loss.

435*Hormonal sampling*

436 Before the food competition on a given day, subjects were placed with their partner and
437the preliminary saliva sample was taken. Samples were taken again 15 minutes after the 3 test
438trials, with subjects remaining with their partners during this interval. The 15 minute interval
439began at the start of the last trial, so that the time subjects took to eat their food in this trial did
440not alter the time of the saliva collection. Subjects waited in the testing room with their partner
441for the 15 minute interval, and were not permitted to eat any food during this time. Subjects were
442observed during this 15 minute period, and any instances of socio-sexual behavior, play,
443aggression, or ingestion of feces that might affect the endocrine measurements were recorded. In
444the solo condition, subjects were alone when their pre-test sample was taken, and they waited
445alone in the testing room for the 15 minute post-test interval.

446 To control for the effect of time of day on hormone levels, a given pair was always run
447within the same two-hour time window, minimizing any circadian variation that might influence
448within-pair patterns. Further, the number of pairs in each age and sex category tested in the
449morning and the afternoon was counterbalanced as best as possible. It was not feasible to do this
450for all pairs due to constraints of the testing facilities. All tests were carried out between 8:00
451AM and 4:00 PM, reducing the probability that the high levels of testosterone and cortisol
452observed immediately after waking in chimpanzees influenced results [38], since apes of both
453species were awake for several hours prior to the start of the tests. These tests were not
454physiologically demanding for subjects, making it unlikely that exertion affected the endocrine

455changes seen. Further, any changes that occurred as a result of being fed would also be present in
456the solo condition – thus, though eating may have affected cortisol levels, the paired test would
457not be biased relative to the solo test in this dimension.

458 Saliva samples were collected while subjects were in the test rooms, which were familiar
459rooms of their dormitory. The experimenter or caretaker first washed and disinfected his/her
460hands, then poured ground Sweet Tarts candy onto a cotton round. This specific candy was used
461to stimulate saliva because it has been shown not to alter measurements of cortisol in humans
462[39, 40]. The experimenter/caretaker then stood next to the mesh of the dormitory, and if the
463subject approached her, she placed the cotton round inside the subject's lip so that it could suck
464on the cotton and ingest the Sweet Tarts while the cotton absorbed its saliva. Once the cotton
465round had taken in enough saliva, it was placed into a syringe and squeezed to express the saliva
466into a test tube. Though using cotton as a collection implement may affect measurements of
467steroids, cotton has been shown to introduce fairly uniform rates of error across samples [39, 41].
468This means that while the absolute results presented here might not be comparable to those
469obtained without stimulation, the comparisons within this subject pool are effective since the
470method was consistent across subjects. The collection period for any particular sample did not
471span longer than 20 minutes.

472 Fifty microliters of 0.1% sodium azide solution was added to samples immediately after
473collection to prevent contamination and to allow samples to be kept at room temperature until
474they were returned to the laboratory [22]. The saliva samples were analyzed in the Reproductive
475Ecology Laboratory at Harvard University. Salivary testosterone measurements were made using
476an I-125 based radioimmunoassay kit (#4100, Diagnostic Systems Laboratories, Webster, TX,
477USA) with the following modifications: standards were prepared in assay buffer and run at six

478 concentrations from 2 to 375 pg/ml. Samples were added in 100 µl amounts together with 300 µl
479 of assay buffer. First antibody (20 µl) and labeled steroid (50 µl) were added to each tube to yield
480 a total reaction volume of 470 µl per tube. After overnight incubation at 4° C, 500 µl of second
481 antibody was added to each reaction tube. Reaction tubes were subsequently centrifuged for 45
482 minutes; after aspiration of the supernatant, tubes were counted in a gamma counter for two
483 minutes. In pilot assays, the ape testosterone values using the standard aliquot for human assays
484 (200 µl) were too high to be readable in the assay range. Thus, we used only 100 µl of the
485 chimpanzee and bonobo saliva for the T assays, with the same standard curve as employed in the
486 human testosterone radioimmunoassay protocol.

487 Salivary cortisol measurements were made using an I-125 based radioimmunoassay kit
488 (#2000, Diagnostic Systems Laboratories, Webster, TX, USA) with the following modifications:
489 Standards were prepared in assay buffer and run at six concentrations from 35 to 2000 pg/ml.
490 Samples were added in 25 µl amounts together with 200 µl of assay buffer. Antibody complex
491 and labeled steroid were diluted 1:2 and added to each tube in 150 µl amounts to yield a total
492 reaction volume of 525 µl per tube. After overnight incubation at 4° C, 500 µl of second
493 antibody was added to each reaction tube. Reaction tubes were subsequently centrifuged for 45
494 minutes; after aspiration of the supernatant, tubes were counted in a gamma counter for two
495 minutes.

496 The average intra-assay coefficient of variation was 8% for testosterone and 8% for
497 cortisol, and average inter-assay coefficient of variation was 16% for testosterone and 25% for
498 cortisol. Though this inter-assay CV for cortisol is on the higher end of the acceptable range, all
499 of the samples for a given individual were run in the same assay, meaning that any within-

individual variation would not have been affected by inter-assay variation. We counter-balanced the individuals whose samples were run in each assay according to species, sex, and age.

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516References

5171. Oyegbile T, Marler C (2005) Winning fights elevates testosterone levels in California
518 mice and enhances future ability to win fights. *Hormones and Behavior* **48**, 259-267.
5192. Booth A, Granger DA, Mazur A, Kivlighan KT (2006) Testosterone and social behavior.
520 *Soc. Forces* **85**, 167-191.
5213. Koolhaas JM, de Boer SF, Buwalda B, van Reenen K (2007) Individual variation in
522 coping with stress: A multidimensional approach of ultimate and proximate mechanisms.
523 *Brain, Behavior and Evolution* **70**, 218-226.
5244. Salvador A, Costa R (2009) Coping with competition: Neuroendocrine responses and
525 cognitive variables. *Neuroscience and Biobehavioral Reviews* **33**, 160-170.
5265. Veenema AH, Koolhaas JM, De Kloet ER (2004) Basal and stress-induced differences in
527 HPA axis, 5-HT responsiveness, and hippocampal cell proliferation in two mouse lines.
528 *Annals of the New York Academy of Sciences* **1018**, 255-265.
5296. Kano T (1992) *The Last Ape: Pygmy Chimpanzee Behavior and Ecology* (Stanford
530 University Press, Stanford, California).
5317. Wrangham R (1999) Evolution of coalitionary killing. *Yearbook of Physical*
532 *Anthropology* **42**, 1-30.
5338. Hohmann G (2001) Association and Social Interactions Between Strangers and Residents
534 in Bonobos (*Pan paniscus*). *Primates* **42**, 91-99.
5359. Muller M, Wrangham R (2009) *Sexual Coercion in Primates and Humans: An*
536 *Evolutionary Perspective on Male Aggression Against Females* (Harvard University
537 Press, Cambridge, MA).

53810. Fruth B, Hohmann G (2002) How bonobos handle hunts and harvests: why share food?
539 In *Behavioural diversity in chimpanzees and bonobos*, eds. Boesch C, Hohmann G, &
540 Marchant L (Cambridge University Press, Cambridge), pp. 231-243.
54111. Hare B, Melis AP, Woods V, Hastings S, Wrangham R (2007) Tolerance allows bonobos
542 to outperform chimpanzees on a cooperative task. *Curr. Biol.* **17**, 619-623.
54312. Vervaecke H, de Vries H, van Elsacker L (2000) Dominance and its Behavioral Measures
544 in a Captive Group of Bonobos (*Pan paniscus*). *International Journal of Primatology* **21**,
545 47-68.
54613. Muller M (2002) Agonistic relations among Kanyawara chimpanzees. In *Behavioural*
547 *diversity in chimpanzees and bonobos*, eds. Boesch C, Hohmann G, & Marchant L
548 (Cambridge University Press, Cambridge), pp. 112-123.
54914. Schultheiss O, Wirth M, Torges C, Pang J, Villacorta M, Welsh K (2005) Effects of
550 Implicit Power Motivation on Men's and Women's Implicit Learning and Testosterone
551 Changes After Social Victory or Defeat. *Journal of Personality and Social Psychology*
552 **88**, 174-188.
55315. Muehlenbein M, Watts D, Whitten P (2004) Dominance Rank and Fecal Testosterone
554 Levels in Adult Male Chimpanzees (*Pan troglodytes schweinfurthii*) at Ngogo, Kibale
555 National Park, Uganda. *American Journal of Primatology* **64**, 71-82.
55616. Marshall A, Hohmann G (2005) Urinary Testosterone Levels of Wild Male Bonobos (*Pan*
557 *paniscus*) in the Lomako Forest, Democratic Republic of Congo. *American Journal of*
558 *Primatology* **65**, 87-92.

55917. Sapolsky R (2000) How Do Glucocorticoids Influence Stress Responses? Integrating
560 Permissive, Suppressive, Stimulatory, and Preparative Actions. *Endocrine Reviews* **21**,
561 55-89.
56218. Bernstein I, Rose R, Gordon T (1974) Behavioral and environmental events influencing
563 primate testosterone levels. *Journal of Human Evolution* **3**, 517-525.
56419. Hohmann G, Mundry R, Deschner T (2008) The Relationship Between Socio-Sexual
565 Behavior and Salivary Cortisol in Bonobos: Tests of the Tension Regulation Hypothesis.
566 *American Journal of Primatology* **70**, 1-10.
56720. de Waal F (1987) Tension regulation and nonreproductive functions of sex in captive
568 bonobos (*Pan paniscus*). *National Geographic Research Reports* **3**, 318-335.
56921. Wobber V, Wrangham R, Hare B (in press) Bonobos exhibit delayed development of
570 social behavior and cognition relative to chimpanzees. *Curr. Biol.*
57122. Lipson S, Ellison P (1989) Development of Protocols for the Application of Salivary
572 Steroid Analyses to Field Conditions. *American Journal of Human Biology* **1**, 249-255.
57323. Hare B, Kwetuenda S (in press) Bonobos voluntarily share their own food with others.
574 *Curr. Biol.*
57524. Kivlighan K, Granger D, Booth A (2005) Gender differences in testosterone and cortisol
576 response to competition. *Psychoneuroendocrinology* **30**, 58-71.
57725. Suay F, Salvador A, Gonzalez-Bono E, Sanchis C, Martinez M, Martinez-Sanchis S,
578 Simon V, Montoro J (1999) Effects of competition and its outcome on serum testosterone,
579 cortisol and prolactin. *Psychoneuroendocrinology* **24**, 551-566.
58026. Filaire E, Maso F, Sagnol M, Ferrand C, Lac G (2001) Anxiety, hormonal responses, and
581 coping during a judo competition. *Aggressive Behav.* **27**, 55-63.

58227. Melis AP, Hare B, Tomasello M (2006) Chimpanzees recruit the best collaborators.
583 *Science* **311**, 1297-1300.
58428. Wrangham R, Pilbeam D (2001) African apes as time machines. In *All apes great and*
585 *small*, eds. Galdikas B, Briggs N, Sheeran L, Shapiro G, & Goodall J (Kluwer
586 Academic/Plenum Publishers, New York), pp. 5-18.
58729. Rosati A, Stevens J, Hare B, Hauser M (2007) The evolutionary origins of human
588 patience: Temporal preferences in chimpanzees, bonobos, and human adults. *Curr. Biol.*
589 **17**, 1663-1668.
59030. Lieberman DE, Carlo J, Ponce de Leon M, Zollikofer C (2007) A geometric
591 morphometric analysis of heterochrony in the cranium of chimpanzees and bonobos.
592 *Journal of Human Evolution* **52**, 647-662.
59331. Mazur A, Booth A (1998) Testosterone and dominance in men. *Behavioral and Brain*
594 *Sciences* **21**, 353-363.
59532. Elias M (1981) Serum Cortisol, Testosterone, and Testosterone-Binding Globulin
596 Responses to Competitive Fighting in Human Males. *Aggressive Behav.* **7**, 215-224.
59733. Gladue M, Boechler M, McCaul K (1989) Hormonal Response to Competition in Human
598 Males. *Aggressive Behav.* **15**, 409-422.
59934. Bateup H, Booth A, Shirtcliff E, Granger D (2002) Testosterone, cortisol, and women's
600 competition. *Evolution and Human Behavior* **23**, 181-192.
60135. van Anders S, Watson N (2007) Effects of ability- and chance-determined competition
602 outcome on testosterone. *Physiology and Behavior* **90**, 634-642.

60336. Kirschbaum C, Klauer T, Filipp S, Hellhammer D (1995) Sex-specific effects of social
604 support on cortisol and subjective responses to acute psychological stress. *Psychosomatic*
605 *Medicine* **57**, 23-31.
60637. Kudielka BM, Kirschbaum C (2005) Sex differences in HPA axis responses to stress: a
607 review. *Biol. Psychol.* **69**, 113-132.
60838. Muller M, Lipson S (2003) Diurnal patterns of urinary steroid excretion in wild
609 chimpanzees. *American Journal of Primatology* **60**, 161-166.
61039. Smider N, Essex M, Kalin N, Buss K, Klein M, Davidson R, Goldsmith H (2002)
611 Salivary cortisol as a predictor of socioemotional adjustment during Kindergarten: a
612 prospective study. *Child Dev.* **73**, 75-92.
61340. Talge N, Donzella B, Kryzer E, Gierens A, Gunnar M (2005) It's not that bad: Error
614 introduced by oral stimulants in salivary cortisol research. *Developmental Psychobiology*
615 **47**, 369-376.
61641. Granger D, Shirtcliff E, Booth A, Kivlighan K, Schwartz E (2004) The "trouble" with
617 salivary testosterone. *Psychoneuroendocrinology* **29**, 1229-1240.
- 618
- 619

620 **Figure Legends**

621 **Figure 1.** Pre-test log cortisol values according to species and outcome of the food
622 competitions, males only. Bars denote standard error of the mean. Chimpanzee males showed
623 no differential cortisol before sharing than before conditions where the dominant obtained
624 significantly more food. In contrast, bonobo males' pre-test cortisol was higher if the
625 dominant were going to obtain significantly more food than if individuals were going to
626 share.

627

628 **Figure 2.** Pre-test cortisol values according to species and outcome, males only. These values
629 are expressed as residuals of the log pre-test values relative to the log pre-solo values. Bars
630 denote standard error of the mean. Bonobo males increased in cortisol relative to their solo
631 values when they were in a pair that was going to show a disparity in the obtaining of food,
632 while they showed a decrease when they were in a pair that was going to share.
633 Chimpanzees' values did not differ greatly from those shown in the baseline, or vary based
634 on outcome.

635

636 **Figure 3.** Pre-test log testosterone (T) values according to species and outcome, males only.
637 Bars denote standard error of the mean. Chimpanzee males tended to show differential pre-
638 test testosterone based on whether their pair was going to share food or not, with higher T in
639 the non-sharing pairs. In contrast, there was no difference in T in bonobo males according to
640 this measure.

641

642 **Figure 4.** Pre-test testosterone (T) values according to species and outcome, males only.
643 These values are expressed as residuals of the pre-test values relative to the pre-solo values.

57

58

644 Bars denote standard error of the mean. Chimpanzee males decreased in T relative to their
645 solo values when they were in a pair that was going to share food, and increased when in a
646 pair where one individual was going to monopolize the majority of the food. Bonobo males
647 did not show such a distinction in T based on outcome. The bonobo sample size here is
648 smaller than in the previous analyses because some bonobos completed the food
649 competitions but did not produce enough saliva in the solo condition to be analyzed for
650 testosterone and so had no baseline with which to compare their T values during the test.

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