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

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

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

Across species, differences in levels of aggression tend to predict individuals’ behavioral and physiological responses to competition. In lines of mice bred for low or high aggression, the low-aggression mice tend to exhibit a passive coping style characterized by freezing behaviors. This passive coping style is associated with a large increase in glucocorticoids surrounding a competitive event in the low aggression mice, whereas mice bred for high aggression show a lesser change in glucocorticoids when competing [5]. The prediction that aggression should mediate physiological response to competition is compelling in the case of humans’ closest living relatives, chimpanzees (Pan troglodytes) and bonobos (Pan paniscus). Bonobos have been characterized as less aggressive than chimpanzees, particularly in the severity of male aggression [6-9]. In competition over food in particular, chimpanzees are likely to respond aggressively
while bonobos are more likely to share, both in the wild and in experimental manipulations [10, 11]. Given the evidence from the selection experiments on mice, bonobos may demonstrate a passive coping style and show heightened cortisol sensitivity relative to chimpanzees. Alternatively, chimpanzees may be more sensitive to competition than that of bonobos because of their more rigid hierarchies [12, 13]. In human competition, individuals with heightened sensitivity to status, or a greater “power motive,” display larger shifts in testosterone [2, 14]. Therefore, it is unclear which species will show more pronounced rapid shifts in a competitive event.

Previous work supports the hypothesis that chimpanzees and bonobos will in fact differ in their immediate changes in testosterone and cortisol in competitive events. First, testosterone and cortisol levels over the long term are positively correlated with social dominance rank in several primates including chimpanzees and bonobos [15-17]. Thus it is clear that hormones are involved in mediating dominance behaviors in these apes as in other species. Secondly, in rhesus macaques, the winner of an aggressive interaction exhibits a post-contest elevation in testosterone while the loser exhibits a decrease in testosterone levels, indicating that the same steroid hormones found to exhibit rapid shifts surrounding competition in humans may be associated with similar shifts in other primates [18]. Finally, bonobos were found to exhibit an anticipatory rise in cortisol prior to a competition over limited amounts of food, with an even greater increase in cortisol when the food was visibly difficult to share [19]. These studies suggest that the cognitive abilities necessary to anticipate the outcome of competition are present in our closest living relatives. However, quick hormone changes in chimpanzees have not yet been investigated, making the comparison to both bonobos and humans impossible.
Here we compared the endocrine shifts surrounding competition in chimpanzees and bonobos. We predicted that the two species would differ in their steroid hormone profiles in a similar competitive situation. We presented these apes with an experimental dyadic food competition and measured testosterone and cortisol levels prior to and after the competitive event. We had three hypotheses of how bonobos and chimpanzees would differ in their steroid hormone shifts. Each hypothesis applied to both anticipation of the test and response to the test.

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

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

**Hypothesis 3. Differential reactivity:** both species will react to the competitive event, but will do so differently. This hypothesis suggests that competition over food is important to both species and posits that the physiological correlates of the competitive behavior will differ
between the two, given the differing behavioral outcomes seen in the two species. If the two species exhibit distinct coping styles, they may show differing cortisol responses to the test. This prediction would imply that bonobos show greater cortisol shifts, associated with a passive coping style. In turn, if chimpanzees show a greater power motive in seeking dominance, they should show larger testosterone changes than bonobos, among whom the motivation to seek dominance is not as strong.

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

Pairs were presented with three separate days of food competitions, with three conditions (one per day) varying the monopolizability of the food (the order of the conditions was counterbalanced across species, sex, and age). On each day, pairs participated in 3 trials of one condition (thus there were 9 total trials, 3 trials for each of the 3 conditions). In each trial, a controlled amount of food was placed in a specific configuration (according to condition) in a testing room, then the pair was released into the room and allowed to eat the food. After the pair
finished eating, the experimenter immediately placed the food for the subsequent trial. In addition to the paired food competitions, each subject was also presented with a solo condition that replicated the procedure of the paired conditions exactly except that individuals were tested alone rather than in a pair.

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

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

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

**Anticipatory cortisol**

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

A GLM of chimpanzees’ pre-test log cortisol values with *sex* and *outcome* as factors revealed no significant main effects and no interaction. In contrast, a GLM of bonobos’ pre-test log cortisol values with these two factors demonstrated a significant *sex* *outcome* interaction.
(Wald Chi-Square (1) = 11.070, p = 0.001). To examine this interaction, we performed separate analyses according to sex in each species, using an alpha threshold of 0.013 for significance. In chimpanzees, neither sex showed a significant effect of outcome on cortisol. In bonobos, a significant effect was present only in males – cortisol was higher when the dominant was going to obtain significantly more food than when the two individuals were going to share/the dominant was going to obtain less (Wald Chi-Square (1) = 13.766, p<0.001) (Figure 1). This effect was not significant in bonobo females (Wald Chi-Square (1) = 2.045, p = 0.153). Therefore, it appears that male bonobos anticipated the outcome of the interaction based on the individual with whom they were paired. We performed several control analyses that ensured that these patterns were present in bonobo males regardless of dominance status, the order of the test day, and the type of pair the individual was in (male-male versus male-female) (see Supplemental Online Material for details). However, it was also necessary to show that these results were not simply trait characteristics of the individuals in a given pair, but rather reactions of those individuals to the situation of being paired.

**Anticipatory cortisol relative to baseline cortisol**

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

We performed a GLM analysis on these residuals separately by species, in males only (with an alpha level of 0.013) – since the results were not significant in females of either species.
controlling for individual and with outcome as a factor. This analysis revealed that in bonobo males, there was a significant effect of outcome on changes in cortisol relative to baseline (Wald Chi-Square (1) = 10.635, p = 0.001) (Figure 2). There was no significant effect of outcome in chimpanzee males. These residual analyses suggest that, relative to their own solo values, bonobo males’ cortisol decreased when they were in a pair that was going to share, and increased when they were in a pair where the dominant was going to obtain significantly more food. Meanwhile, chimpanzee males’ cortisol levels did not differ based on outcome, and did not vary greatly between the baseline and test days.

This species difference was especially pronounced when there was going to be an asymmetry in the obtaining of food. We performed separate GLM analyses for each outcome in males with individual and species as factors (setting the alpha level for significance at 0.013). This analysis showed a significant effect of species when one individual was going to monopolize more food (Wald Chi-Square (1) = 9.356, p = 0.002), but not when the pair was going to share (Wald Chi-Square (1) = 1.260, p = 0.26). This suggests that the species difference likely derived from bonobos’ cortisol increasing prior to a situation where sharing was not going to occur, while chimpanzees’ cortisol remained similar to baseline in this instance.

These residual analyses indicate that the observed changes in cortisol prior to the test were associated with individual pairings, rather than simply reflecting either anticipation of food being presented or baseline cortisol differences between individuals.

These results are similar to those found in past work [19] in suggesting both that anticipation of food sharing increases bonobo cortisol levels, and that cortisol increases differentially based on the predicted outcome. Unlike the previous paradigm, however, in our test the 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].

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

**Testosterone**

We began by performing a GLM analysis of post-test log-transformed testosterone (T) values with log pre-test testosterone as a covariate, *individual* as a subject factor, and *species*, *sex*, and *outcome* as between-subject factors, examining all main effects and 2- and 3-way interactions. This analysis revealed only a significant effect of log pre-test testosterone (Wald Chi-Square (1) = 17.461, p<0.001) on post-test testosterone. We then performed a similar
residual analysis of the post-test values as described for cortisol above; this analysis further supported the notion that any distinctions in post-test T levels were simply due to pre-test differences (discussed in Supplemental Online Material). Given our predictions from the cortisol results, we performed further analyses on the pre-test T values to assess whether any anticipatory T patterns were present.

**Anticipatory testosterone**

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

The significant interaction between *species* and *outcome* was present in males only (Wald Chi-Square (1) = 5.857, p = 0.016). In contrast, in females, there was only a significant main effect of *species* (Wald Chi-Square (1) = 5.433, p = 0.020), in that bonobo females had higher pre-test T than chimpanzee females, but no effect of *outcome* or interaction. This suggests that males of the two species showed differing testosterone in anticipation of varying outcomes, while alterations in females’ testosterone levels were less consistent, as was the case for cortisol.

To investigate this further, we performed separate analyses by species in males, using an alpha value of 0.013.
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 274 was going to obtain more food than when the two individuals were going to share. In contrast, in 275 bonobo males, there was no significant effect of outcome (Wald Chi-Square (1) = 1.290, p = 2760.26) (Figure 3).

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

Anticipatory testosterone relative to baseline testosterone

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

Thus, chimpanzee males showed a greater departure from their solo T values based on the 292 outcome of the test than did bonobos. This distinction was especially pronounced in the sharing 293 condition – 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
pair was going to share (Wald Chi-Square (1) = 9.330, p = 0.002), though not when one individual in the pair monopolized more food (Wald Chi-Square (1) = 0.000, p = 1.0). This suggests that the more pronounced species difference was in chimpanzees’ T decreasing prior to sharing and bonobos’ T increasing in the same situation (though bonobos’ T also rose when there was an asymmetry in the obtaining of food, thus this increase was independent of outcome).

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

Discussion

These results support the Differential reactivity hypothesis: both bonobos and chimpanzees showed an endocrine shift surrounding the competitive event, but the nature of this reaction differed in the two species. Bonobo males’ cortisol increased in anticipation of competition when they were placed with a partner where there would be an asymmetry in success at obtaining food. These cortisol increases were relative to baseline levels and to changes when subjects were placed in a pairing where they would be able to share. Therefore, bonobos appeared to respond to the competition as a stressor when the food would not be shared, exhibiting a passive coping style and an associated large shift in glucocorticoids. In contrast,
chimpanzee males showed an anticipatory increase in testosterone when placed with a partner where the outcome of the food competition would be asymmetrical, relative both to their baseline testosterone levels and to changes in testosterone when they were placed with a partner where sharing would occur. Chimpanzees may have viewed the competition as status-determining, particularly when the outcome of the interaction was unclear (e.g. there was an uncertain dominance relationship) and showed a correlated anticipatory rise in testosterone. There were no independent effects of species or test outcome on the post-competition values of testosterone and cortisol that were unrelated to these anticipatory effects. These patterns were present equally in dominants and subordinates, across test days, and across ages (see Supplemental Online Material). Thus overall, these results indicate a strong relationship between competition and rapid steroid shifts in both of humans’ closest living relatives. Our findings indicate that male bonobos and chimpanzees can predict the results of a dyadic food competition based solely on being paired with another individual. Moreover, these predictions of the competitive outcomes are associated with rapid endocrine changes in males of both species. While it is possible that the endocrine changes seen here in fact determined the behavioral outcomes of the competition rather than resulting from a prediction of the outcome, humans have been shown to exhibit anticipatory changes in steroids prior to competition in numerous situations, even with unknown competitive partners [24-26]. Subjects in this study were paired with known groupmates and apes are known to track their tolerance levels with other individuals [11, 27], suggesting that individuals were likely able to make these predictions. In turn, this suggests that the patterns of anticipatory appraisal seen in humans are not unique to our species.
These data demonstrate that the behavioral differences observed in chimpanzees and bonobos during dyadic food competition are associated with differences in physiological responses in the two species. These findings are the first to show rapid endocrine changes in association with competition in chimpanzees, and replicate evidence of a pre-competition cortisol increase in bonobos [19]. Further, these results suggest that after the divergence of chimpanzees and bonobos, selection may have acted differentially on the endocrine pathways governing rapid shifts in testosterone and cortisol as a result of the two species’ differing ecological circumstances. In particular, selection against aggression in bonobos may have changed their levels of physiological and psychological reactivity, such that they mirror the lines of mice bred for low aggression and exhibit a passive coping style and large glucocorticoid responses [5, 28]. Future research comparing hormonal parameters in the two species can further illuminate the numerous distinctions already seen between the two in morphology, behavior, and cognition [21, 29, 30].

Notably, the “winner effect,” in which testosterone and cortisol rise in human winners across various competitive contexts, was not observed among the chimpanzees and bonobos [31]. It is possible that the lack of post-competition changes seen here was due to the timing of sampling. Our post-test interval of 15 minutes was chosen to match the human literature, where responses to competition have been observed in that length of time [32, 33]. Chimpanzees and bonobos might in fact react to the outcome of the competition but do so more slowly than humans. This would signify a difference between these apes and humans in the speed of response to wins or losses. However, previous work with bonobos showed no response effects in cortisol levels as long as one hour post-competition [19]. Therefore, the lack of a winner effect in food competitions among bonobos and chimpanzees may represent a lack of salience of the outcome.
of such competitions to these apes, physiologically and psychologically. Though competition over food is ecologically relevant, another context such as competition over mates may be more significant for apes. Even if this is the case, these results suggest that humans are derived in possessing endocrine shifts in response to competitions as unrelated to survival or reproduction as a game of chess.

Similar to what is seen in humans, we found the strongest effects of the competition on steroid hormones in males, whereas females did not exhibit any significant patterns. Steroid shifts surrounding competition in women are inconsistent and only observed in some studies [24, 34, 35]. The sex differences in rapid endocrine changes in humans appear to be more pronounced in reaction to psychological stimuli than to exercise or other physiological stimulation where both sexes show steroid shifts, suggesting that women do not simply show generally muted endocrine responsivity [36, 37]. The lack of significant patterns in the endocrine shifts of female chimpanzees and bonobos indicates that the lessened response of women to psychological status competitions or stressors may have deep evolutionary roots.

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

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

Coding of behavioral variables

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

The outcome variable used in the analyses was derived from this behavioral coding. A “1” was scored for outcome if the dominant obtained noticeably more food on 2 or 3 trials out of the 3 total trials of the condition. If the dominant obtained more food on only 1 or 0 trials, this was scored as a “0” for that condition. The outcome measure was usually consistent within a given pair, in that a dominant would obtain significantly more food across each of the 3 food competition conditions, but could vary across condition within each pair. Thus each individual in a pair was represented in the data set 3 times, once for each condition. Importantly, the scores for
outcome were the same for both individuals in the pair (the dominant and the subordinate), thus this variable represented asymmetry versus sharing in the distribution of feeding rather than a win or loss.

**Hormonal sampling**

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

To control for the effect of time of day on hormone levels, a given pair was always run within the same two-hour time window, minimizing any circadian variation that might influence within-pair patterns. Further, the number of pairs in each age and sex category tested in the morning and the afternoon was counterbalanced as best as possible. It was not feasible to do this for all pairs due to constraints of the testing facilities. All tests were carried out between 8:00 AM and 4:00 PM, reducing the probability that the high levels of testosterone and cortisol observed immediately after waking in chimpanzees influenced results [38], since apes of both species were awake for several hours prior to the start of the tests. These tests were not physiologically demanding for subjects, making it unlikely that exertion affected the endocrine
changes seen. Further, any changes that occurred as a result of being fed would also be present in
the solo condition – thus, though eating may have affected cortisol levels, the paired test would
not be biased relative to the solo test in this dimension.

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

Fifty microliters of 0.1% sodium azide solution was added to samples immediately after
collection to prevent contamination and to allow samples to be kept at room temperature until
they were returned to the laboratory [22]. The saliva samples were analyzed in the Reproductive
Ecology Laboratory at Harvard University. Salivary testosterone measurements were made using
an I-125 based radioimmunoassay kit (#4100, Diagnostic Systems Laboratories, Webster, TX,
USA) with the following modifications: standards were prepared in assay buffer and run at six
concentrations from 2 to 375 pg/ml. Samples were added in 100 µl amounts together with 300 µl of assay buffer. First antibody (20 µl) and labeled steroid (50 µl) were added to each tube to yield a total reaction volume of 470 µl per tube. After overnight incubation at 4º C, 500 µl of second antibody was added to each reaction tube. Reaction tubes were subsequently centrifuged for 45 minutes; after aspiration of the supernatant, tubes were counted in a gamma counter for two minutes. In pilot assays, the ape testosterone values using the standard aliquot for human assays (200 µl) were too high to be readable in the assay range. Thus, we used only 100 µl of the chimpanzee and bonobo saliva for the T assays, with the same standard curve as employed in the human testosterone radioimmunoassay protocol.

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

The average intra-assay coefficient of variation was 8% for testosterone and 8% for cortisol, and average inter-assay coefficient of variation was 16% for testosterone and 25% for cortisol. Though this inter-assay CV for cortisol is on the higher end of the acceptable range, all 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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References


Figure Legends

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

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

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

**Figure 4.** Pre-test testosterone (T) values according to species and outcome, males only. These values are expressed as residuals of the pre-test values relative to the pre-solo values.
Bars denote standard error of the mean. Chimpanzee males decreased in T relative to their solo values when they were in a pair that was going to share food, and increased when in a pair where one individual was going to monopolize the majority of the food. Bonobo males did not show such a distinction in T based on outcome. The bonobo sample size here is smaller than in the previous analyses because some bonobos completed the food competitions but did not produce enough saliva in the solo condition to be analyzed for testosterone and so had no baseline with which to compare their T values during the test.