

Blood testosterone levels in sickness and in health: Male chimpanzee testosterone levels decrease in face of an immune challenge

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Abstract

As an integral part of the immune response, testosterone secretion is inhibited when an individual is confronted with an immune challenge. Testosterone-mediated physiological, morphological, and behavioral traits are compromised at times of impaired health. Nevertheless, males of some species seem to maintain high levels of testosterone when confronted with an immune challenge, upholding competitive strength but compromising their immune response. It has been argued that this phenomenon will occur only in species living in social systems with high degrees of male-male competition over mating opportunities. Male chimpanzees contest over access to fertile females and dominants sire the majority of offspring. This male mating pattern makes chimpanzees a candidate species where we could expect males to maintain high testosterone levels, compromising their immune response, to ensure immediate reproductive success. We measured blood testosterone levels in male and female chimpanzees, who expressed clinical symptoms (symptomatic) or showed no evidence of clinical disease on assessment (asymptomatic). For females, we expected to find lower testosterone levels in symptomatic individuals than in asymptomatic subjects. In males, we would predict lower testosterone levels in symptomatic individuals than in asymptomatic males, if the immune response leads to a decrease in testosterone secretion. Alternatively, males could have equal levels of testosterone when symptomatic and asymptomatic, upholding competitive strength. Our results show that male chimpanzees exhibit lower levels of testosterone when confronted with an immune challenge than when being asymptomatic. This suggests that male testosterone secretion is suppressed as part of the immune response, which potentially increases survival and lifetime reproductive success. It will, however, negatively impact momentary competitive ability. Also, males may employ different mating strategies, some of which are less testosterone-driven (e.g., affiliative strategies). Consequently, in some individuals, the costs of maintaining high testosterone levels may not outweigh the potential gain in reproductive success.

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1 | INTRODUCTION

Different life history traits compete with each other for limited resources, and therefore, usually have a negative association with one another (Stearns, 1989, 1992; Zera & Harshman, 2001). Classic examples are the trade-off between a delayed onset of reproduction beyond maturity for increased longevity (e.g., Mouroucq et al., 2016), or the suppression of reproduction when confronted with an immune challenge (e.g., Bribiescas & Ellison, 2007; Spratt, 2001). Hormones are involved in mediating this balance between different life history trade-offs (e.g., growth, reproduction, and maintenance; Zera & Harshman, 2001). They produce pleiotropic effects and regulate behavioral and physiological transitions between life history stages (Finch & Rose, 1995; Hau, 2007; Zera & Harshman, 2001).

Testosterone, a sex steroid hormone, mediates relationships between physiology, morphology, and behavior (Hau, 2007). As such, it regulates behavioral and physiological properties modulating male reproduction (Bribiescas & Ellison, 2007; Muehlenbein & Bribiescas, 2005). In male vertebrates, circulating testosterone levels affect spermiogenesis, body mass, as well as competitive and sexual behavior (Bribiescas, 2001; Lipshutz et al., 2019; Muehlenbein & Bribiescas, 2005; Schlatt & Ehmkcke, 2014; Wingfield et al., 1990). Variation in testosterone levels coincide with individual- and species-differences in mate competition, the nature and intensity of aggression, territoriality, and paternal behavior (*Lemur catta*: Gould & Ziegler, 2007; *Junco hyemalis*: McGlothlin et al., 2007; *Pan troglodytes*: Muller, 2017). Accordingly, in seasonal breeders testosterone levels increase during the mating season (for primates e.g., *L. catta*: Gould & Ziegler, 2007; *Eulemur fulvus rufus*: Ostner et al., 2002). In non-seasonal breeders, they vary as a function of intrasexual competition, with higher testosterone levels in species with high levels of male-male competition (e.g., in howler monkeys (*Alouatta palliata*): Cristóbal-Azkarate et al., 2006; in colobus monkeys (*Colobus vellerosus*): Teichroeb & Sicotte, 2008).

In female vertebrates, testosterone is also the most important androgen. It is produced primarily by converting androstenedione into testosterone, but is also produced in the ovaries, the adrenal cortex, and, during pregnancy, small amounts of testosterone can be produced in the placenta (e.g., in humans: Burger, 2002). Female testosterone is quickly converted into estrogen. However, the fact that females have androgen receptors in neural and peripheral tissues suggests that testosterone also plays a role in females beyond the conversion process into estrogen (Staub & De Beer, 1997). For instance, testosterone seems to play a role in female sexual behavior (in humans: e.g., Davis et al., 1995; chimpanzees (*P. troglodytes*): Nadler et al., 1985; mole rats (*Cryptomys hottentotus pretoriae*): Lutermann et al., 2012), aggression and dominance (in humans: Beehner et al., 2005; Davis et al., 1995; in baboons (*Papio hamadryas ursinus*): Grant & France, 2001; in lizards (*Sceloporus jarrovi*): Woodley

& Moore, 1999), as well as in health (e.g., testosterone enhances positive effects of estradiol on bone density in women: Davis et al., 1995). Although the testosterone-behavior correlations in females are similar to those found in males, testosterone plays a much smaller role in reproductive behavior, competition, and reproductive success of females (e.g., Hau, 2007; Stanton et al., 2009).

While high levels of testosterone enhance reproductive success and increase the competitive strength of males, there are also immediate and delayed costs associated with high levels of circulating testosterone (Wingfield et al., 2001). One effect of high testosterone concentration is reduced immunocompetence (Foo et al., 2017; Roberts et al., 2004; Sowers et al., 2001), which can have detrimental consequences at times when an individual is confronted with immunological threats. Therefore, downregulation of testosterone levels at times of severe immune challenges can be interpreted as an adaptive response that favors survival over reproductive effort (Bribiescas & Ellison, 2007). There are multiple pathways translating this trade-off between reproduction and immunity (Figure 1). First, immune responses are mediated via cytokines, which have inhibitory influences on the secretion of gonadotropin-releasing hormone in the hypothalamus. Gonadotropin-releasing hormone stimulates the release of luteinizing hormone. The latter in turn stimulates the production of androgens such as testosterone in the testes (Spratt, 2001). Therefore, immune system responses mediated by cytokines suppress testosterone secretion. Second, during illness a further decline in testosterone levels is caused by physiological changes such as decreased stimulation, responsiveness, and inhibition of the Leydig cell function, as well as an increase in the rate of testosterone metabolic clearance (Dong et al., 1992; Spratt, 2001). A third health related decline in testosterone is mediated by increases in glucocorticoid levels during sickness. Chronic or acute elevated circulating glucocorticoid levels lead to rapid declines of testosterone production by the Leydig cells in the testis (Hu et al., 2008; Sapolsky, 1985; Waite et al., 2009).

These mechanisms that downregulate testosterone are essential for a functioning immune response. The immune response can be perturbed by testosterone as it may bind to lymphocyte receptors, affect the action of immunomodulatory hormones like glucocorticoids, and alter energy allocation or behavior (Smyth et al., 2018). In other words, at times of severe immune challenges, the hypothalamic-pituitary-gonadal axis, which controls testosterone release, is inhibited, causing testosterone secretion to decline (Spratt, 2001), so that the potential immunosuppressive effects of testosterone no longer hinder an effective immune response to an immune threat, thereby increasing chances of survival.

Because of this link between reproductive competence and survivorship, it is argued that individual variation in traits signaling superior physical conditions (e.g., body ornaments in birds) provide honest signals to potential mating partners. If testosterone has

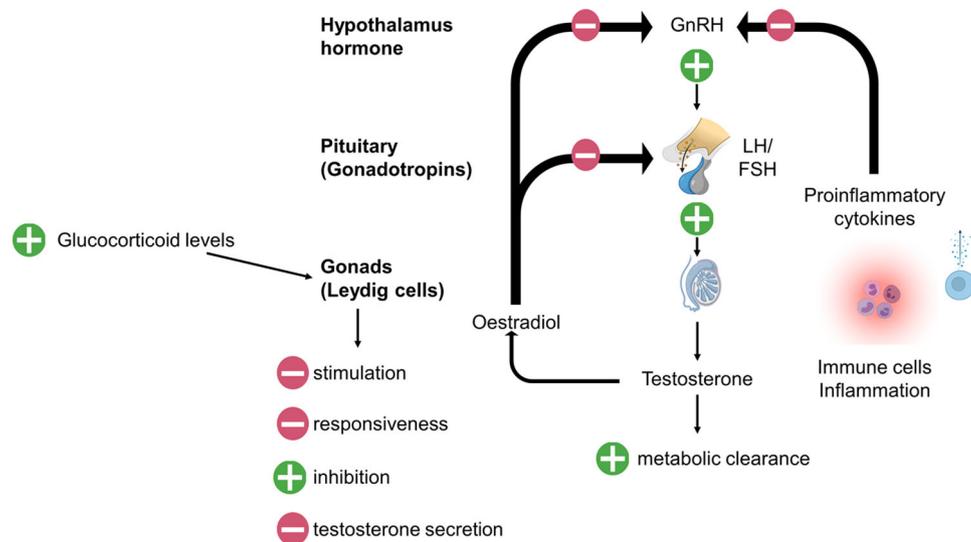


FIGURE 1 Feedback mechanisms on the reproductive axis. Proinflammatory cytokines have inhibitory effects on the secretion of gonadotropin-releasing hormone (GnRH) in the hypothalamus. The release of GnRH stimulates the release of luteinizing hormone (LH) and follicle stimulating hormone (FSH) from the pituitary. LH stimulates androgen production such as testosterone. An increase in glucocorticoid levels renders Leydig cells less responsive to LH stimulation and leads to a decrease in testosterone production. Additionally, an increase in metabolic clearance rate leads to a decrease in circulating testosterone levels. Figure modified after Dwyer & Quinton (2018). Created in BioRender.com

immunosuppressive effects, then keeping testosterone levels high may signal high quality as a mating partner. This idea gave rise to the “immunocompetence handicap hypothesis,” suggesting that changes in testosterone levels mediate the competing demands of reproductive effort and pathogen susceptibility, by reducing immunocompetence to parasites and increasing energetic investment in sexual signals (Folstad & Karter, 1992; Zahavi, 1975; Zuk, 1990). Interestingly, empirical studies investigating the relationship of testosterone and immune function have found both negative as well as positive associations between testosterone levels and immune function markers, such as parasite load, white blood cell counts, or cytokines (e.g., Alonso-Alvarez et al., 2007; Belliure et al., 2004; Bilbo & Nelson, 2001; Roberts et al., 2004). While a first cross-species meta-analysis (including reptile, avian, fish, and mammalian species) did not give strong countenance to the immunocompetence handicap hypothesis (Roberts et al., 2004), an update on this study has found support for immunosuppressive effects of testosterone (a necessary assumption of the immunocompetence handicap hypothesis) (Foo et al., 2017).

Overall, the interactions between testosterone and immunity on the one hand (immunosuppressive effects of testosterone and downregulation of testosterone as an integral part of the immune response respectively), and testosterone and traits of mate competition on the other (with high testosterone levels in species with high degrees of male–male competition), have pleiotropic effects (Hau, 2007; Iserbyt et al., 2017; Muehlenbein & Watts, 2010), making predictions of testosterone level changes to immune challenges a difficult task.

Adult male chimpanzees (*P. troglodytes*) engage in intrasexual affiliations, males dominate females, and males use coercion of females to enhance mate guarding and mating success (Feldblum et al., 2014; Muller et al., 2011; Watts, 1998). Although male chimpanzees respond to the presence of fertile females by increasing testosterone levels and higher testosterone levels are associated with heightened male–male competition, in general the species is considered a nonseasonal breeder and males probably maintain breeding testosterone levels throughout the year as availability of cycling females is unpredictable in nonseasonal breeding systems (Muller & Wrangham, 2004; Sobolewski et al., 2012). Moreover, testosterone also mediates functions that are more indirectly related to male reproductive success in chimpanzees, such as spermiogenesis and muscle mass (Bhasin et al., 1998; Emery Thompson et al., 2012). It is necessary for competitive strength in agonistic interactions and reduces pain perception (Craft et al., 2004). Testosterone further seems to constitute a motivational driver for high status, enhance sensitivity to rank challenges, and reduce fear in contexts when status is threatened (reviewed in Muller & Wrangham, 2004). Similar to studies on seasonally breeding birds (Wingfield et al., 2001), higher ranking male chimpanzees were found to have higher testosterone levels and greater helminth parasite burden than lower ranking peers (Muehlenbein & Watts, 2010). Therefore, according to the immunocompetence handicap hypothesis, male chimpanzees should maintain high testosterone levels independent of health status, if the sex selection for male–male and male–mate competition revoke the cost of the immunosuppressive effects of testosterone (Zuk, 2009). On the other hand, chimpanzee males may respond to an immune

challenge by downregulating circulating testosterone levels (Boonekamp et al., 2008), thereby compromising momentary competitive strength, in favor of survival and future reproductive success (e.g., Muller, 2017). A meta-analysis of the interaction between testosterone and the immune system showed that immune activation per se (initiated by live pathogens or nonpathogenic antigens) suppresses testosterone levels (Boonekamp et al., 2008). Thus, when the immune system gets activated, testosterone is automatically suppressed, generating the often-observed trade-off between immunocompetence and sexual displays. This would in turn lead to decreased competitive strength of the immunologically challenged male, as in chimpanzees, as well as in most other primates, testosterone promotes psychological and physiological mechanisms involved in male competition (Muller, 2017).

Female vertebrates (and some invertebrates) are usually considered the more immunocompetent sex (Nunn et al., 2009), which is explained by sexual dimorphism in immunosuppressive substances, including testosterone, as well as fundamentally different life-history strategies (e.g., female mammals may increase fitness by investing in immune defense and consequently lengthening lifespan). The link between female testosterone and immune function is poorly studied so far, however. Many studies have manipulated female testosterone levels using pharmacological doses, but this approach has limited importance for the physiological range of testosterone concentrations occurring within ecological and evolutionary contexts (Goymann & Wingfield, 2014), and the biological relevance of changes in testosterone levels in females remains largely unknown. However, there is evidence that in humans, females exposed to severe pathogen challenges have reduced testosterone levels as compared to healthy controls (Sinha-Hikim et al., 1998). Therefore, in contrast to males, female chimpanzees are expected to exhibit decreasing levels of testosterone when they are immunologically challenged. As part of the immune response, testosterone secretion should be inhibited to optimize the reaction to an immunological threat.

To test this, we compared blood testosterone levels measured in samples of zoo-housed male and female chimpanzees that showed signs of clinical disease (symptomatic) to blood testosterone measures of samples of other individuals that were collected at times when subjects were without obvious symptoms (asymptomatic).

2 | MATERIALS AND METHODS

2.1 | Ethics statement

Blood sample collection and associated procedures were undertaken under normal clinical care by zoo veterinarians. Apes were never anaesthetized for the purpose of this study. All blood samples used in this study were obtained for management reasons (e.g., health checks, implantation of contraception, transfer to other facilities, or treatment of disease or injuries). We adhered to the ASP Principles for Ethical Treatment of Non-Human

Primates. All procedures were in accordance with relevant national guidelines for the care and use of laboratory animals. We conformed to the Directive 2010/63/EU.

2.2 | Animals and sample collection

Testosterone was measured in 58 plasma samples obtained from zoo-housed chimpanzees (37 females, 21 males). Data were collected at ten different zoos. Age of subjects ranged 10–49 years (median age: female 24 years, males 27 years). Only individuals older than ten years of age were included in our sample, to ensure that testosterone levels had already increased with puberty (Anestis, 2006; Behringer et al., 2014). All individuals were housed in mixed-sex social groups, and were fed with a mix of fruits and vegetables several times per day and had *ad libitum* access to fresh water.

We analyzed hormone levels in blood samples that were taken by veterinarians whenever apes were handled for management reasons, including regular health checks, transfer to another facility, implantation of contraception, and treatment of acute diseases or injuries. All samples obtained under the first three circumstances (health checks, transfer to another facility, implantation of contraception) were derived from individuals with no obvious signs of disease or injury and no evidence of clinical disease on assessment. Therefore, these individuals were scored as “asymptomatic,” and data were combined, although we cannot rule out entirely that an individual experienced symptoms that were missed in the clinical examination. All samples scored “symptomatic,” were collected when a veterinarian considered an intervention necessary due to severe symptoms of impaired health (individuals treated for acute diseases or injuries). The symptomatic group included a variety of health issues ranging from chronic nose infections, inflammation of wounds, severe respiratory disease, to phlegmon, including viral as well as bacterial infections. Samples derived from 27 asymptomatic females, 14 asymptomatic males, ten symptomatic females, and seven symptomatic males.

All samples were collected in appropriate blood tubes for plasma separation (plasma, not serum samples were analyzed for hormone levels). After blood collection, samples were centrifuged in the veterinary facilities of the respective zoos or in the Max Planck Institute for Evolutionary Anthropology (MPI-EVA) in Leipzig, Germany. Supernatant was transferred into a plastic vial, which was then labelled with the individual's name and the date of plasma sample collection. Samples were stored at -20°C at the MPI-EVA or at the zoo the sample originated from. Samples were transported frozen from the zoos to the MPI-EVA.

2.3 | Extraction procedure

The extraction of testosterone from plasma followed the extraction protocol as described in previous publications (Hauser et al., 2011; Preis et al., 2011) with some modifications. We mixed 200 μl plasma

with 400 μ l acetonitrile and 5 μ l internal standard (a mixture of all internal standards (Hauser et al., 2008) at 20 pg/ μ l in 30% acetonitrile). We then centrifuged the mixture for 10 min at 13,000 rpm (18,000g). Supernatant was pipetted into a test tube and diluted with 6 ml water. We performed a solid phase extraction. A total of 3 ml HR-X cartridges were conditioned with 3 ml methanol and 3 ml deionized water (high performance liquid chromatography [HPLC] grade, Mallinckrodt Baker), followed by the application of the sample to the cartridge (2 \times 3 ml). Then we washed the cartridges with 3 ml water and 3 ml of 20% methanol. Testosterone was eluted with 3 ml methanol and evaporate to dryness at 45°C under nitrogen stream. Afterwards, we washed the walls of the glass tubes with 300 μ l acetonitrile, centrifuged them for 2 min, evaporated them again at 45°C, and reconstituted them in 50 μ l of 30% acetonitrile. Finally, we transferred the extracts into HPLC vial inserts and stored them until measurement at -20°C.

2.4 | Analytical methods

We measured plasma testosterone levels using liquid chromatography-tandem mass spectrometry with a Waters Acquity UPLC separation module equipped with a binary solvent manager, sample manager, and a column oven (Waters). Separation was performed on a reverse phase C-18 column (Acquity UPLC BEH C18 1.7 μ m, 2.1 \times 100 mm) protected by an in-line filter unit.

The composition of eluent A and B was water containing 0.1% formic acid, and acetonitrile, respectively. The gradient was 25% B (0–1.5 min), linear increase to 65% B (1.5–8.5 min), 95% B (8.5–10 min), 25% B (10–12 min). Flow rate was 0.35 ml/min. A total of 10 μ l of the extract were injected.

Mass spectrometric analyses were carried out on a Xevo TQ-S tandem quadrupole mass spectrometer (Waters) with electrospray ionization in positive mode. We excluded samples that had an internal standard recovery of more than \pm 50% from the expected values from our analysis and to less volume to repeat the measurement ($N = 3$). We quantified data with MassLynx (Version 4.1; TargetLynx-Software).

2.5 | Statistical analyses

To assess sex-specific patterns of plasma testosterone levels in asymptomatic and symptomatic individuals, we fitted a Linear Mixed Model with a Gaussian error structure (Baayen, 2008). The response variable testosterone was log-transformed. Individuals' age was included into the model as a control variable to account for age-specific variation in testosterone levels (Bribiescas, 2020; Schaeb's et al., 2017). The interaction term of health state (a binary variable indicating whether the individuals were asymptomatic or symptomatic at the time of sample collection) and sex (male or female) was added to the model as a predictor term. This two-way interaction allows to test for differences in plasma testosterone levels in

asymptomatic and symptomatic individuals depending on sex. All predictor and control variables were z-transformed (centered to a mean of zero with a standard deviation of one) to obtain comparable estimates. As samples of individuals originated from different collections and various site-specific factors might affect testosterone levels, we included a random intercept to indicate the zoo at which each individual was housed.

To detect potential issues with multicollinearity between predictor variables, we tested the variance inflation factor (VIFs) of the standard linear model, excluding interaction terms and random effects (function "vif" of the R package "car"; Fox, 2011). We found no indications of collinearity (maximum VIF = 1.102). To test significance of the full model we used a likelihood ratio test (Dobson & Barnett, 2008), comparing the full model that included the three-way-interaction predictor term, the control variable, and random effects, with the null model that included only the control variable and random effects (Forstmeier & Schielzeth, 2011). If the two models did not differ significantly from each other (the threshold for statistical significance was set at $p = 0.05$), we considered the simpler model (which would be the null model), as the final model, and results and would base discussions on the simple model structure. If the full and the null model did differ significantly from each other, we would reduce model complexity by eliminating the two-way-interaction from the initial model (thus, the reduced model would contain main effects of health state and sex, as well as the control variable age). Likelihood ratio tests were used to compare competing models with each other (i.e., model containing the two-way interaction with the simplified model containing main effects). Quantile-quantile plots and distribution of residuals plotted against fitted values were inspected to check model assumptions. Models were fitted in RStudio, Version 1.3.959 (2009-2020, R Core Team, 2021) using the function "lmer" (R package "lme4", Bates et al., 2015).

The data that support the findings of this study are available from the corresponding author upon reasonable request.

3 | RESULTS

Models included data of 58 chimpanzees from ten different zoos. Table 1 gives an overview of means, SD, medians and ranges of plasma testosterone levels (ng/ml) in male and females chimpanzees with and without obvious signs of disease at the time of sample collection.

We found a significant difference between the full model, which contained the two-way interaction term between z-transformed health state and sex as well as the z-transformed control variable age and the random effect, and the null model, which contained only the z-transformed control variable age and the random effect ($\chi^2 = 80.36$, $df = 3$, $p < 0.001$). Therefore, we proceeded with model reduction (as described in the methods section). The final model was the full model, containing the interaction term between health state and sex, as well as the control variable age and the random intercept for zoo (Table 2). The interaction term of health state and sex was a significant

	Signs of disease	Mean (ng/ml)	SD (ng/ml)	Median (ng/ml)	Range (ng/ml)
Male chimpanzees	absent	5.84	±3.63	5.67	1.57–14.50
	present	1.06	±0.85	1.04	0.14–2.65
Female chimpanzees	absent	0.40	±0.23	0.34	0.13–1.00
	present	0.32	±0.26	0.28	0.05–0.98

Note: The table shows means, SDs, medians, and ranges of plasma testosterone levels (ng/ml) in males and females with and without obvious signs of disease.

TABLE 2 Results of the final model

	Estimate	SE	<i>p</i> value
Intercept	−0.2130	0.1371	
Health state (symptomatic)	−1.0014	0.1787	
Sex (male)	2.2191	0.1808	
Age at sampling	0.1831	0.2175	
Health state × sex (interaction)	−1.4433	0.3738	<i>p</i> < 0.001
	Variance	SD	
Zoo	0.07142	0.2672	
Residual	0.41865	0.6470	

Note: The table shows estimates and standard errors for all variables included in the model. We present *p* values with significance levels set at *p* < 0.05 for the relevant predictors. The final model contained the interaction term between health state (asymptomatic vs. symptomatic) and sex (female vs. male), the age of the subject at the time of sample collection, and a random intercept for zoo. The interaction term health state × sex significantly predicted plasma testosterone levels. Asymptomatic males had significantly higher testosterone levels as compared to symptomatic males. Females had no substantial differences in testosterone levels between the two health states.

predictor of plasma testosterone levels (estimate ± SE = −1.44 ± 0.37, *p* < 0.001, see Figure 2). In particular, males had an almost sixfold decrease in testosterone levels when symptomatic as compared to when they were symptom free. Females did not show pronounced differences in testosterone levels between the two health states (Figure 2).

4 | DISCUSSION

Although many studies have found empirical indication that immune function directly affects testosterone secretion, the immunosuppressive effects of testosterone are still debated. This is partly due to the fact that most of these studies were observational rather than experimental (Roberts et al., 2004). However, results so far show that testosterone promotes behaviors in males that increase their pathogen susceptibility and affects genes that influence infection resistance (Klein, 2000; Zuk, 2009). Thus, the effects of testosterone in combination with the behaviors, that are stimulated by this

TABLE 1 Description of plasma testosterone data in male and female chimpanzees with and without obvious signs of disease

hormone, make males the “sicker sex” (Zuk, 2009). In this study, we measured testosterone levels obtained from blood samples of chimpanzees, who showed no clinical signs of disease, and compared them with testosterone levels measured in samples from subjects who showed severe disease symptoms. We found that male chimpanzees with disease symptoms had significantly lower testosterone levels compared to asymptomatic males, while females had no detectible shift in testosterone levels depending on their health state.

Our finding that male testosterone levels in individuals with pathologic symptoms were lower than in asymptomatic subjects is in line with reports from studies on humans and rodents (Dong et al., 1992; Spratt, 2001). Our results support other studies indicating that, in adult males, during illness, physiological mechanisms lower testosterone levels, diverting energy from reproductive effort into maintenance and finally, survival. A decline in testosterone levels during sickness can be advantageous: inhibiting energetically costly behaviors and metabolic processes and promoting immune functioning.

The immunocompetence handicap hypothesis predicts that, if testosterone has immunosuppressive effects, males in societies with high degrees of male–male competition should maintain high levels of testosterone at times of immune challenges to signal good genes to the choosing females (Folstad & Karter, 1992). Chimpanzees constitute a classic example of a primate species with high degrees of male–male competition (Boesch et al., 2006; Mitani, 2009). Anecdotal reports from the wild describe how a male, showing obvious symptoms of impaired health, was immediately challenged by another male and lost his rank position (Boesch & Boesch-Achermann, 2000). Such dynamics would suggest that a male cannot afford reduced testosterone levels to maintain high rank and, therefore, would have to bear testosterone-related costs. However, our results show that symptomatic males have considerably lower blood testosterone levels than males who are symptom-free. A possible explanation for the lack of evidence for the immunocompetence handicap principle in chimpanzees is that another crucial assumption of the hypothesis might not be met in these primates: high testosterone levels at times of immune challenges are assumed to be a trait selected for by female choice (Folstad & Karter, 1992; Foo et al., 2017; Lochmiller, 1996; Roberts et al., 2004; Viney et al., 2005). In chimpanzees, male coercion, rather than female choice might be the main determinant for reproductive success in males (Muller et al., 2011), thus violating a

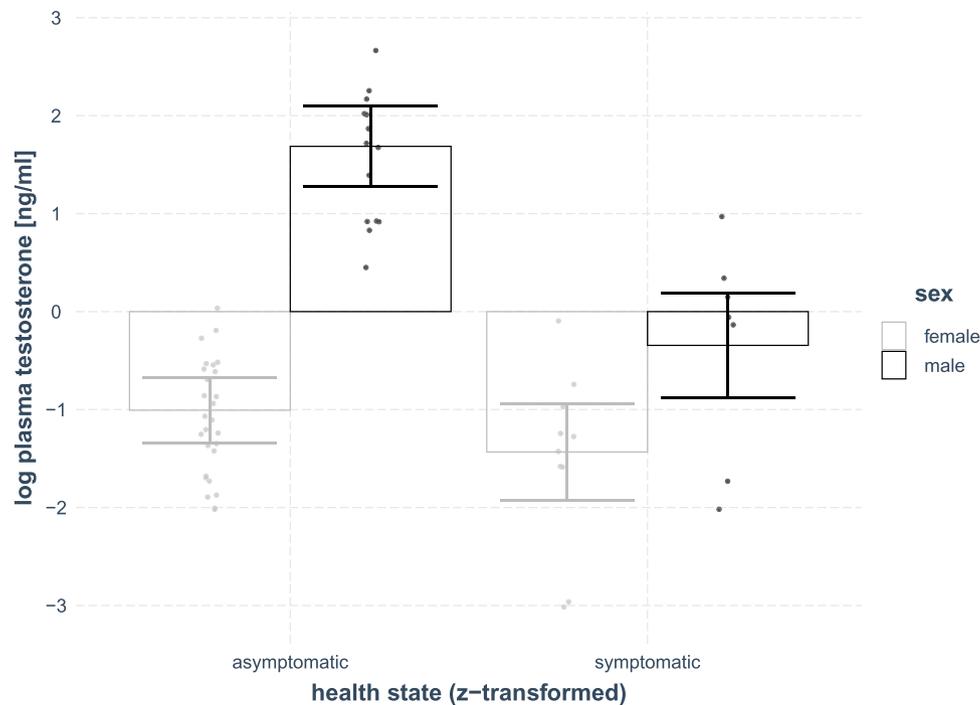


FIGURE 2 Plasma testosterone levels by health state and sex. Log-transformed plasma testosterone levels (ng/ml) are shown on the y-axis. Confidence intervals are presented. Dots represent individual measures. Asymptomatic (grey box on the left) and symptomatic (grey box on the right) females show similar plasma testosterone levels. Asymptomatic males (black box on the left) have significantly higher plasma testosterone levels than symptomatic males

necessary prerequisite for the immunocompetence handicap principle. Furthermore, we could not control for the presence of fertile females at the timepoint of sample collection in our model, because this information was not available. This might have an impact on our results, as previous studies have showed that male testosterone levels increase in the presence of fertile females (Muller & Wrangham, 2004). According to the immunocompetence hypothesis, the presence of fertile females is essential as only then the male can gain immediately from signaling good health. In chimpanzees, the presence of cycling females is highly unpredictable (but see Wallis, 1995). High testosterone levels are predictive of male dominance, which in is a predictor of reproductive and mating success in chimpanzees. Also, dominance rank is quickly challenged and not easily regained once lost. Therefore, male chimpanzees are expected to maintain breeding levels of testosterone throughout the year. Alternatively, our finding may be due to an effect of different mating-strategy phenotypes that depend to differing degrees on testosterone: while the majority of offspring are, in fact, sired by dominant male chimpanzees, lower ranking males often produce offspring with nulliparous, young females, who seem to be less attractive to dominant males, and therefore there is less male–male competition over mating access to them (Reddy et al., 2021; Wrublewski et al., 2009). One could argue, that lower-ranking males will profit from down-regulating testosterone levels at times of immune challenges to ensure survival, compromising potential momentary reproductive success with infant-rearing-inexperienced females. Furthermore, apart from male coercion, the strength of affiliative bonds between a

male and a female predicts reproductive success (Reddy et al., 2021). In males that affiliate with females to ensure mating opportunities, testosterone may play a lesser role than in coercive males. The dataset of the current study did not allow us to investigate the effect of different male mating strategies, dominance rank, and degrees of affiliation. Future studies taking these factors into account may find that the immunocompetence handicap principle holds for dominant, coercive males (who would be expected to keep high testosterone levels even at times of immune challenges), but is violated in males adopting other mating strategies.

In contrast to males, testosterone levels in samples of female chimpanzees were not affected by symptomatic pathologies. However, androgen levels are much lower in females than in males, and measures are often complicated by the sensitivity of the test, making assessments of norm values and symptomatic changes a difficult task (Davison & Davis, 2003). Moreover, in females, like in males, androgens are produced by the gonads, but in women 40%–60% originate from the adrenal gland (Abraham, 1974). Therefore, female testosterone levels should be interpreted with caution.

The health problems in the symptomatic chimpanzees of this study were variable, including wounds, but also bacterial and viral infections. While this obviously introduces some heterogeneity into the dataset, it should not have influential impact on the interpretations and implications of our results because, at least in humans, a reduction of testosterone levels is not disease specific. Decreased testosterone levels can be associated with severe systemic diseases, burns, sepsis, myocardial infarction, vaccination, traumatic or surgical injuries, as well as various types of

infectious pathogens (Handelsman, 2001; Shattuck & Muehlenbein, 2015; Spratt, 2001). This goes as far that the following notion has been made: "*Such transient biochemical androgen deficiency [...] is so common that it should be considered a normal accompaniment of severe acute or chronic illness*" (Handelsman, 2001).

Another aspect that could potentially have introduced a bias in our dataset is the timepoint of sampling during an illness. However, in men, health impairment causes a rapid decline of serum testosterone levels within 24–48 h (Vogel et al., 1985). Thus, the time when samples were collected during the disease period should not have affected our results.

In our study, male chimpanzees who needed medical treatment had lower testosterone levels in comparison to other individuals. This finding is based on a single testosterone measure per individual. Obviously, a single measure presents merely a snapshot of testosterone levels at the timepoint of sample collection. Therefore, we cannot make inferences on long-term effects of low testosterone levels in males. The conservative assumption is that they returned to initial values with convalescence. In men, low testosterone levels spanning longer periods of time have been shown to impair mental health. Men with pathological conditions such as testosterone deficiency syndrome or simply a decline of testosterone level with age suffer from physical impairment, decreased sexual function as well as psychological disturbances and mental disorders, and have an overall lower quality of life (e.g., Ciocca et al., 2016; Tong et al., 2012). Our study design does not permit a statement or conclusions about the relationship of testosterone levels and affective state in the chimpanzees of our study populations. The relationship between testosterone levels and welfare will be an interesting avenue of research for the future.

Another interesting study avenue would be to look at males in wild populations. We know that, for instance, during respiratory diseases, glucocorticoid levels increase significantly in wild chimpanzees (Behringer et al., 2020). Such an increase is expected since proinflammatory cytokines stimulate the hypothalamic–pituitary–adrenal axis to increase glucocorticoid production (Chapman et al., 2013). As a consequence of increased glucocorticoid production, the production of testosterone by the Leydig cells should be suppressed. This suggests that in wild populations we should expect similar patterns as in our study on zoo-housed individuals. However, male–male competition may be more pronounced in wild chimpanzees, increasing the potential gain for a male with high testosterone levels even during times of an immune challenge. In zoos, males are confronted with both, fewer male competitors than in the wild and with fewer females, lowering the level of male–male competition in comparison to wild chimpanzees. Furthermore, females in zoos may be more predictable in terms of cycling frequency (as compared to their wild counterparts) or may even be on contraception, potentially altering both female proceptive behaviors as well as male interest (e.g., Baum et al., 1977; Welling, 2013). Thus, the trade-off between immune responses and competitive strength differ between zoo-housed and wild chimpanzee populations. Studies on parasite loads in wild populations indicate that high ranking male chimpanzees have higher testosterone and higher parasite counts than lower ranking peers (e.g., Muehlenbein & Watts, 2010). This could hint at wild chimpanzee males' investment into reproductive output rather than health.

5 | CONCLUSION

Our study has shown that testosterone levels in chimpanzee males but not in females decline when individuals were symptomatic in comparison to asymptomatic individuals. Many physiological mechanisms such as the release of cytokines during immune responses or increases in glucocorticoids suppress testosterone production by the Leydig cells in males. A testosterone decline during disease is adaptive, because energy is allocated from reproduction into immune function, and reproductive behaviors are reduced to improve survival.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

Ruth Sonnweber: conceptualization (equal); formal analysis (lead); methodology (equal); visualization (equal); writing original draft (equal); writing review and editing (equal). **Jeroen Stevens:** conceptualization (equal); resources (equal); writing review and editing (equal). **Gottfried Hohmann:** conceptualization (equal); writing review and editing (equal). **Tobias Deschner:** conceptualization (equal); methodology (equal); writing review and editing (equal). **Verena Behringer:** conceptualization (equal); funding acquisition (lead); investigation (lead); methodology (equal); resources (equal); writing original draft (equal); writing review and editing (equal).

PEER REVIEW

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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