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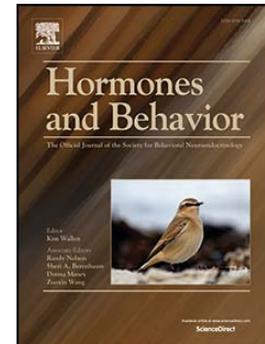
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**Testosterone and androgen receptor gene polymorphism are associated
with confidence and competitiveness in men**

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Abstract**A contribution to a special issue on Hormones and Human Competition**

Studies in non-human animals and humans have demonstrated the important role of testosterone in competitive interactions. Here, we investigated whether endogenous testosterone levels predict the decision to compete, in a design excluding spite as a motive underlying competitiveness. In a laboratory experiment with real monetary incentives, 181 men solved arithmetic problems, first under a noncompetitive piece rate, followed by a competition incentive scheme. We also assessed several parameters relevant to competition, such as risk taking, performance, and confidence in one's own performance. Salivary testosterone levels were measured before and 20 minutes after the competition task using mass spectrometry. Participants were also genotyped for the CAG repeat polymorphism of the androgen receptor gene, known to influence the efficacy of testosterone signaling in a reciprocal relationship to the number of CAG repeats. We observed a significant positive association between basal testosterone levels and the decision to compete, and that higher testosterone levels were related to greater confidence in one's own performance. Whereas the number of CAG repeats was not associated with the choice to compete, a lower number of CAG repeats was related to greater confidence in those who chose to compete, but this effect was attributable to the polymorphism's effect on actual performance. An increase in testosterone levels was observed following the experiment, and this increase varied with self-reported high-school math grades. We expand upon the latest research by documenting effects of the androgen system in confidence in one's own ability, and conclude that testosterone promotes competitiveness without spite.

Introduction

The androgen testosterone is theorized to promote dominance in humans, i.e. it promotes an individual's motivation to seek and maintain social status (Cashdan, 1995; Eisenegger et al., 2011; Grant and France, 2001; Josephs et al., 2006; Mazur, 2005; Mazur and Booth, 1998; Mehta et al., 2015). Across a large number of non-human animal species, including rodents, wolves, cattle, and non-human primates, testosterone levels relate positively to social rank and dominant behaviors, especially when the status hierarchy is unstable (Beaver and Amoss, 1982; Beehner et al., 2005; Boissy and Bouissou, 1994; Cavigelli and Pereira, 2000; Coe et al., 1979; Collias et al., 2002; Grant and France, 2001; Harrington and Asa, 2010; Muehlenbein and Watts, 2010; Oliveira et al., 1996; Wingfield et al., 1990).

In humans, social status is often pursued by choosing to compete with others (Archer, 2006; Edwards, 2006; Mazur, 2005; Mazur and Booth, 1998). Research into the role of testosterone in human competition has so far focused on two dominant models, i.e. a basal model and a reciprocal model of testosterone effects (Mazur and Booth, 1998). The former assumes that individuals' testosterone measurements represent short-term fluctuations around a characteristic basal level. Accordingly, basal testosterone is moderately stable when measured at the same time of day and is assumed to represent an individual's stable concern for status, similar to a personality trait (Sellers et al., 2007). Testosterone seems to be related to concerns for status outside of conscious awareness, and thus represents an implicit motive (Stanton and Schultheiss, 2009; Terburg et al., 2012). Empirical support for the basal model stems from studies showing, for example, that basal testosterone levels correlate positively with psychometric measures such as the self-reported ability to win in competition (Suay et al., 1999), and overbidding strategies in auctions (Van Den Bos et al., 2013). Other studies, however, employing two-stage competition designs have revealed no relationship between basal testosterone and an individual's decision to compete in the second stage (Carré and McCormick, 2008; Mehta and Josephs, 2006). The reciprocal model assumes that testosterone is responsive to competition in the sense that it should rise after victory and drop after defeat in competitive interactions (Mazur and Booth, 1998). This is generally interpreted in the way that an increase in the level of this hormone encourages, while a decrease in level of this hormone discourages the decision to compete further (Mazur and Booth, 1998). Support for the reciprocal model stems from real-world sports competitions and rigged laboratory competitions, for example (Gladue et al., 1989; Mazur et al., 1992; Mazur and Lamb, 1980; McCaul et al., 1992). Several studies, however, observed that testosterone levels tended to be higher after competitions than before, usually independent of match outcome (Bateup et al.,

2002; Casto et al., 2014; Edwards and Kurlander, 2010; Edwards et al., 2006; Gonzalez-Bono et al., 1999; Hamilton et al., 2009; Steiner et al., 2010; Suay et al., 1999), and for recent reviews see (Oliveira and Oliveira, 2014), (Carré and Olmstead, 2015) and (Hamilton et al., 2015). An important variable determining the direction of the change in testosterone levels appears to be how individuals appraise the competition, i.e. the perception of a challenge favors a competition-related increase in testosterone, while perception of threat does not. (Gonzalez-Bono et al., 1999; McCaul et al., 1992; Salvador, 2005). In the context of competitive performance, this suggests that perceived skill might be crucial in shaping the testosterone response.

Virtually all previous research on the role of testosterone in human competition have used zero-sum games in which one player's win is strictly the other player's loss (Tauer and Harackiewicz, 2004). As a result, the winner gains status, while the opponent loses it. Although an opponent's loss might be the desirable outcome for spiteful individuals (Morgan et al., 2003), this might not be true for others (Niederle and Vesterlund, 2007). Hence, in zero-sum competitions, an individual may choose to compete in order to lower their competitors' probability of winning. However, there is also evidence that some individuals are averse to being spiteful in competition, effectively undermining their motivation to compete (Niederle and Vesterlund, 2007).

Interestingly, recent research has shown that testosterone may also promote reconciliation after a competition in women (Casto and Edwards, 2016), and following competitive interactions in men, as well as affiliative behaviors during interactions with women (van der Meij et al., 2012). Testosterone administration also appears to reduce aggressive calling behavior during competition in a poker paradigm (van Honk et al., 2016). Although that evidence is indirect, it might suggest that basal testosterone levels are related to competitiveness without spite.

Furthermore, it would be relevant to further break down competitiveness into constituents such as certain social-emotional as well as motivational and reward processes. For instance, risk taking is a critical aspect of competitiveness (Niederle and Vesterlund, 2007). Additional factors are one's ability to perform the task for which one is competing, and having overly optimistic beliefs about own performance (i.e. overconfidence) have been shown to be strong predictors of competitiveness (Lichtenstein et al., 1977; Niederle and Vesterlund, 2007).

It might thus be possible that both risk taking and overconfidence mediate the effects of basal testosterone levels on competitiveness. This is likely, as basal levels of testosterone correlate positively with risk-taking measures (Apicella et al., 2008; Sapienza et al., 2009; Stanton et

al., 2011); but see (Stanton et al., 2011), and with performance in competitive settings (Mehta et al., 2009; Vermeer et al., 2016). Indirect evidence for testosterone's role in overconfidence stems from a study showing that the 2D:4D digit ratio (a marker for prenatal testosterone exposure) is associated with the extent to which individuals overestimate their own performance (Dalton and Ghosal, 2014). However, no study to date has investigated whether basal testosterone levels and competitiveness are correlated directly or whether this correlation is indirect, i.e. mediated via risk-taking or confidence.

Finally, it is firmly established that many of testosterone's behavioral effects are mediated by androgen receptors (though aromatization effects are also likely to be important, see (Eisenegger et al., 2012; van Honk et al., 2012); these are expressed in diverse regions in the brain, including the amygdala (Rubinow and Schmidt, 1996). When activated by testosterone, one signaling pathway involves androgen receptors exerting transcriptional control of androgen-dependent genes by binding to androgen response elements within gene regulatory sequences in the nucleus. Transactivation of target genes by the androgen receptor, however, varies with the relative expansion of a poly-glutamine stretch in the N-terminal domain of the androgen receptor protein, which is encoded by a trinucleotide (CAG) repeat polymorphism in exon 1 of the X-chromosome androgen receptor gene (Chamberlain et al., 1994; Zitzmann and Nieschlag, 2003). There is substantial inter-individual variability in the number of CAG repeats and hence of the androgen receptor's capacity to induce or repress gene transcription, which appears to drop in gradual relation to an increasing number of CAG repeats (Zitzmann and Nieschlag, 2003). Thus, the lower the number of CAG repeats, the higher testosterone's efficiency is in exerting its effects via the androgen receptor. A lower number of CAG repeats has been linked to human aggressive behavior (Rajender et al., 2008), to greater upper body strength, and higher self-report measures of dominance (Simmons and Roney, 2011). The polymorphism is also hypothesized to play a role in cognitive skills (Manning, 2007), which bears on the performance aspects of the cognitive tasks often used in laboratory competitions. However, no research has investigated the role of the androgen receptor CAG repeat polymorphism in competitiveness so far.

Therefore, we investigated the role of basal testosterone and the androgen receptor CAG repeat polymorphism in individuals' decisions to compete in a competition paradigm in which spite does not influence competitiveness. We investigated whether effects operate via risk, performance, and confidence in one's own performance. We also tested the effects of competition on testosterone level changes, and whether these changes depend on one's own skills.

We hypothesized that basal testosterone levels correlate positively with the decision to compete, and that competition is associated with testosterone increases. Moreover, we hypothesized that the androgen receptor polymorphism explains variance in an individual's performance and the decision to compete.

Methods

Participants

In total, 181 male white university students of German descent with a mean age of 22.5 years (± 2.9 SD) were recruited to participate in a study about "Hormones and Behavior". Exclusion criteria were history of psychiatric disorder, chronic or acute illness, medication or substance abuse, and studying psychology. The study was approved by the Ethics Committee of the University of Freiburg, Germany. Six participants were excluded after performing the experiment due to self-reported psychoactive medication intake, as revealed in a final questionnaire. Three additional participants were excluded because salivary testosterone levels could not be determined, giving a total of 172 participants.

General procedure

All experiments were performed between 10.00 and 11.00 a.m. to control for diurnal variations in testosterone secretion. The experiment was designed for groups of four individuals, so participants arrived at the laboratory in groups of four, eight, or twelve. Assignment to groups was randomized. Participants did not know each other and were instructed not to communicate with one another for the study's duration. Participants were seated at computers in individual cubicles so they could not see the other people and did not know with whom they were interacting. Following informed consent, the first saliva sample was taken to assess basal testosterone levels. Then, the competition task was explained with standard instructions presented on the computer screen, and instructions were repeated by the experimenter. Then the competition task started (see below), and only at the end was feedback given about task performances. Finally, participants were asked to complete questionnaires, and thereafter, the second saliva sample was taken (45 minutes after the first saliva sample; 20 minutes after completion of the competition task). A total of 45 groups of four participated in the experiment. Each participant received €10 for completing the experiment. Depending on their task performance, additional compensation could be earned.

Procedure of competition task, confidence and risk taking measure

The computer-based experiments were run with the Zurich Toolbox for Readymade Economic Experiments (z-Tree: Fischbacher, 2007). The experiment involves a multi-stage competition design involving an incentivized cognitive real-effort task (see below for technical details). In the first three stages, payment was contingent on correctly adding up as many sets of five two-digit random numbers within five minutes. Participants were allowed to use scratch paper, but no calculator. The numbers were randomly drawn and presented on the computer screen (see supplementary figure 4), and participants had to type in their answers on the keyboard. Once the participant submitted an answer, a new problem appeared jointly with information on whether the previous answer had been correct. A record of the number of correct and wrong answers was kept on the screen. Participants had to go through four stages, and were only informed about the rules of payment in each stage immediately before performing the real-effort task.

In the first stage, all participants performed the real-effort task and received the same monetary reward for every correctly solved equation (“piece rate”).

In the second stage, participants performed a forced competition, always in groups of four, and were only paid if they were the winner in their group (“forced competition”). Payment for each correctly solved mathematical problem for the winner was four times higher than in the “piece rate” compensation scheme.

In the third stage of the experiment, participants were asked to decide according to which of the two payment schemes they wanted to perform the real-effort task (“competition choice”). If a participant chose to compete in the third stage, then his own performance was compared with that of the three other participants in the group in stage two. In other words, each participant competed with the past “forced competition” performance of the other group members. This means that each participant could win, provided he outperforms the other group member in stage two. This task feature ensures that participants choosing the competition option are competing against the scores of others also performing under the competition payout conditions. Hence, in stage three, participants faced a decision problem that has no impact on the ability of the other participants in the same group to win. In other words, a given participant with strong mathematical abilities cannot exert spite and make others more likely to lose by choosing the competitive incentive scheme in stage three (Niederle and Vesterlund, 2007). Therefore, participants do not perform in a zero-sum competition in stage three.

In the final stage four (“control measure”), participants did not have to perform again, but instead they had to choose which compensation scheme (competitive versus piece rate) they wanted to apply to their past performance in stage one (“piece rate”). Thus, a participant’s compensation in stage four depended on the number of correct answers they had provided in stage one. This final stage served to control for additional factors related to competition, but not competitiveness *per se*. For instance, participants may be averse to receiving feedback about their own performance in relation to others, and such feedback would be provided to participants only if they chose the competition compensation scheme. Risk taking is another aspect of competitions in general, and the decision in stage four is inherently a risky decision. Importantly, however: the decision in stage four is not influenced by competitiveness, as there is no performance thereafter.

After these four stages, participants had to provide an estimation of their own performance (“confidence measure”). This subjective measure helps to clarify whether competitiveness as assessed in stage three is driven by an overly optimistic view of one’s own ability.

Participants then had to perform a final risk-taking measure, a well-known risk-taking task (Holt and Laury, 2002). This was added to check for risk-taking propensity, without any social element in it (“risk-taking measure”). Not until these two measures were complete was feedback given about their own performance, namely whether they had won or lost the competitions and the risk-taking task’s outcome. Therefore, while participants knew their absolute performance in a given stage, i.e., how many problems they had solved correctly, they were not informed about their relative performance, i.e. the performance compared to the other four players, until all four stages had ended and the additional control measures had been recorded.

At the end, a random number from one to four was drawn by the computer program to determine which of the four stages in the competition task would be selected for earnings. In addition, one of the six decisions in the risk measure was also randomly selected for earnings. All three confidence measures were paid (see below). This experiment lasted about forty-five minutes, and in addition to the €10 show-up fee participants earned on average €16.1 (SD: €21.8) in the competition task and €2.8 (SD: €1.7) in the risk-taking task.

Details of competition task and control measures

1. Piece rate: Participants were informed that if stage one was selected for payment, they would receive €0.50 per correct answer. Participants then performed the real effort task for five minutes.

2. *Forced competition*: Participants were informed that if stage two was selected for payment, the participant with the highest number of correct problems in the group of four would receive €2 per correct answer while the others would receive nothing. They then performed the real effort task for five minutes.

3. *Competition choice*: Participants were informed that if stage three was selected for payment that the amount of their payment would depend on their decision as to which of the two compensation schemes they wanted applied to their future performance - the piece rate (i.e., €0.50 for each correct answer), or the competitive incentive scheme (€2 per correct answer). They were informed that if they chose the competitive incentive scheme, they would get €2 per correct answer only if their score in stage three was higher than that of the other group members in stage two (the one they just completed), and that they would otherwise receive nothing (in case of ties, the winner was chosen randomly). Participants were first required to decide on one of these two options, and then to perform the real-effort task for five minutes.

4. *Control choice*: Participants were informed that if stage four was selected for payment, their payment would depend on their decision as to which of the two compensation schemes they wanted applied to their past performance in stage one, the piece rate (i.e., €0.50 for each correct answer) or the competitive incentive scheme (€2 per correct answer). They were informed that they would receive €2 per correct answer if their stage one piece-rate performance had been the highest among the participants in their group; otherwise, they would receive no payment (in case of ties, the winner was chosen randomly). Participants were reminded of their stage one piece-rate performance, and were then required to decide for one of the two options. They did not perform a real-effort task in stage four.

Confidence measure. Participants were asked to guess their rank in each stage (one to three separately) at the end of stage four. Each participant chose a rank between one and four that reflected their presumed performance in the respective stage, and was paid €0.50 for each correct choice.

Risk-taking measure. Participants took six decisions and in each, they had to choose between a risky option (50% chance of winning €10 or 50% of winning €0.50) and a safe option. The safe option was €2, €3, €4, €5, €6, or €7.50. Before they made their decisions, they were told, that one option would be randomly selected for payment. We counted the number of times a subject chose the risky option (zero – six times) and used this sum as our measure of risk taking.

Hormonal assessment and genotyping

Saliva samples to assess testosterone were collected with Salivettes (Sarstedt, Nümbrecht, Germany). Since interference effects with cotton-based collection methods have been reported, we used Salivettes with synthetic swabs. Testosterone concentrations were determined by liquid chromatography tandem mass spectrometry (LC—MS/MS). The salivette collection tubes were centrifuged and 100 μ L saliva was mixed with 50 μ L internal standard, and 150 μ L methanol/water containing 50mg/mL ZnSO₄ (v/v:50/50) and vortexed for 1 min. Thereafter, the tube was centrifuged at 12000r/min for 5 min. 200 μ L of the supernatant were then injected into a Shimadzu HPLC system (Shimadzu, Canby, OR, USA) coupled to an AB Sciex API 5000 Turbo-ion-spray(R) triple quadrupole tandem mass spectrometer (AB Sciex, Foster City, CA, USA). The system was controlled by AB Sciex Analyst(R) software (version 1.5.1).

DNA was extracted from saliva in Oragene collection vials (DNA Genotek, ON, Canada) by desalting procedure following the manufacturer's protocol. The androgen receptor CAGn repeat polymorphism was genotyped with PCR as described previously (Zitzmann and Nieschlag, 2003). CAGn repeat numbers could not be determined in another 2 participants. We included those two participants in all those analyses not involving the CAGn repeat polymorphism.

Statistical analyses

In all statistical analyses, basal testosterone levels were treated as a continuous variable. For the figures we use a median split of basal testosterone levels, for illustrative purposes.

CAGn repeat number, confidence in one's own performance, self-reported math grade, and the risk measure were treated as ordinal variables. Performance measures were treated as continuous variables. See supplementary tables 1 and 2 for summary statistics and a correlation matrix for all variables reported here. We tested the association between basal testosterone levels and the decision to compete in stage three using a non-parametric Mann-Whitney test, statistically a conservative approach.

We then estimated several logistic regression models with basal testosterone levels as predictor and choice of compensation scheme in stage three as dependent variable, and included several control variables (please see supplementary tables 3 and 4 for a detailed description of this model). Briefly, in model one we included basal testosterone levels as the *sole* predictor. In models two to five, we added either the actual performance in stage one and changes in performance from stage one to stage two, or reported confidence in one's own performance in stage two, or risk-taking or the self-reported math grades as predictors. In

model six, we added all these predictors together with a further control variable, i.e. the decision in stage four. Finally, we also added the CAGn repeat number as a predictor. We report odds ratios and conditional marginal effects throughout the analyses, which are evaluated at the mean values.

To test effects on performance, we conducted univariate ANOVAs for each stage separately. We tested the association between basal testosterone and performance, and the interaction between basal testosterone and choice of compensation scheme on performance using basal testosterone, number of CAGn repeat number, math grade and choice of compensation scheme as predictors, and number of correctly solved problems as dependent variables (see supplementary table 5).

Our confidence measure is the inverse guessed rank, i.e., someone who guessed he would be first has a confidence value of 3 and someone who guessed he would be fourth has a confidence value of 0. As this is a rank-ordered variable, we used an ordered logit regression model to test the association between basal testosterone levels and the confidence in one's own performance, as well as the interaction between testosterone levels and choice of compensation scheme on confidence. We used basal testosterone, number of CAGn repeat numbers, performance and the choice of compensation scheme in stage three as predictors and confidence as the dependent variable (see supplementary table 6).

Because our risk-taking measure is an ordinal scale, we used an ordered logit regression to test for the relationship between basal testosterone levels and risk-taking. We report the conditional marginal effect of always choosing the risky option evaluated at the mean basal testosterone value. Finally, we used a univariate ANOVA to assess changes in testosterone levels over the entire experiment, with performance and math grade as predictors and change in testosterone levels as the dependent variable. Preliminary analysis showed that neither testosterone levels nor the number of CAGn repeat numbers were associated with math abilities (Spearman rank correlation: $\rho > .861$). Furthermore, the number of CAG repeats was not associated with testosterone levels (Spearman rank correlation: $\rho = -.008$; $p = .919$).

Results

Is testosterone and androgen receptor gene variation related to competitiveness?

Of the 172 participants, 89 chose the piece rate as compensation scheme in stage three, whereas 83 individuals chose to compete. This choice was significantly associated with baseline testosterone levels (continuous variable), in that the higher levels were related to a

greater likelihood of choosing the competition scheme (Mann-Whitney test: $z=2.06$, $p=0.039$). For illustrative purposes, our sample was divided by median split into high and low testosterone groups (Figure 1). We also conducted a t -test, which yielded almost the same result (with even a lower p -value than with the Mann-Whitney test).

Having established that there is a clear relationship between basal testosterone levels and the decision to compete, we tested whether this relationship would remain significant if we controlled for other predictors of the decision to enter the competition in stage three. We run six logit regressions and we report the marginal effects and odds ratios (OR) of the included independent variables (see supplementary tables 3 and 4). The odds ratio is an unstandardized effect size statistic. In the first regression, we found that a ten-fold higher basal testosterone level increased the likelihood of choosing the competition in stage three by 4.4 percentage points ($p=.018$, $OR=1.018$). An odds ratio of 1.018 in this analysis indicates that the odds of entering the tournament increase by 1.8% if the baseline testosterone level is 1 pg/ml higher. In the second regression we observed that on average, each additional problem solved in stage one increased the likelihood that a participant had chosen to compete in stage three by 3.6 percentage points ($p=.003$, $OR=1.156$). Similarly, each problem solved in stage two beyond the number of problems solved in stage one increased the likelihood to enter competition in stage three by 5.6 percentage points ($p<.001$, $OR=1.261$). In the third regression, we found that on average a participant with high confidence in his own performance in stage two was 59 percentage points more likely to choose to compete in stage three than someone with low confidence ($p<0.001$, $OR=16.96$). An individual with medium confidence in his own performance in stage two was 33 percentage points more likely to compete than someone with low confidence ($p<0.001$, $OR=3.97$). In the fourth regression we added risk-taking as a predictor for the decision to compete, and noted that participants who were risk-seeking were 20 percentage points more likely to compete in stage three than risk-averse participants ($p=0.037$, $OR=2.21$). Medium-risk-averse participants did not differ significantly from high-risk-averse participants in their decision to compete ($p=0.852$, $OR=1.08$). In the fifth regression we found that participants who reported the highest possible math grade were 45 percentage points more likely to compete than a participant with a low math grade ($p<0.001$, $OR=7.70$). Participants with the second and third highest math grade were 34 and 33 percentage points more likely to compete than participants with a low math grade (second highest $p=0.001$, $OR=5.01$; third highest $p=0.005$, $OR=4.76$). In the sixth regression we also added the choice in the control condition (stage four) as an additional predictor ($p<0.001$, $OR=5.75$).

It is noteworthy that controlling for all these predictors alone (regressions 2-5) or jointly (regression 6) does not change the significance or the effect size of basal testosterone levels on competitiveness. The odds ratio of this main effect even rises from 1.018 to 1.022, compared to regression 1 (when not controlling for any other variable), suggesting that the relationship between basal testosterone and the decision to compete is independent of actual performance, confidence in one's own ability, risk preferences, and math skills.

Finally, for regression 7, we added the androgen receptor CAG repeat polymorphism as an additional predictor (leaving all other predictors in the model), which did not change the relationship between basal testosterone levels and the decision to compete ($p=0.014$; $OR=1.023$). However, the androgen receptor CAG repeat polymorphism itself did not predict the decision to compete ($p>.165$). This suggests that the relationship observed between basal testosterone and the decision to compete is independent of the CAG repeat polymorphism. We also analyzed interactions effects between the CAG repeat and testosterone. Please see supplementary table 9 for the results.

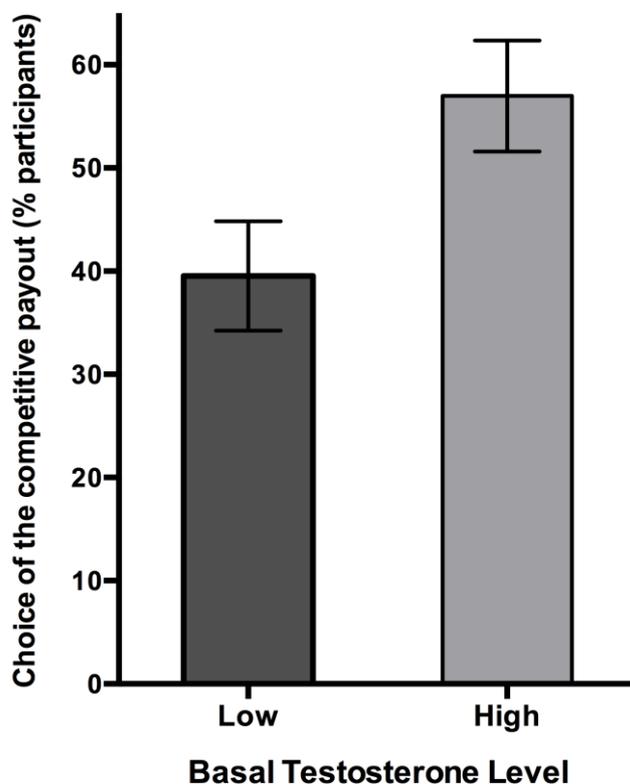


Figure 1. Graphic representation of the relationship between basal testosterone levels and competitiveness. Participants with higher basal testosterone chose to compete more often than those with lower levels ($p=0.039$). The error bars represent the standard errors of the mean.

Grouping in low and high levels is based on a median split for illustrative purposes (total $n=172$).

Is testosterone related to performance?

For the stage three analysis, we added choice in stage three, its interactions with basal testosterone, and CAGn repeat number terciles as independent variables. These analyses (see also supplementary table 5) revealed that basal testosterone levels were not related to performance in any stage (stage one: $F_{1,162}=0.00$, $p=.973$; stage two: $F_{1,162}=0.21$, $p=.649$; stage three: $F_{1,158}=0.75$, $p=.387$). There was also no significant interaction with choice in stage three ($F_{2,158}=2.00$; $p=.159$). CAGn repeat number was related neither to performance in stage one ($F_{1,162}=0.03$; $p=.973$) nor in stage two ($F_{1,162}=0.48$; $p=.621$). However, in stage three we observed significant interaction between the decision to compete and CAGn repeat number terciles ($F_{2,158}=3.89$; $p=.022$; $\eta^2 = .047$), but no main effect ($F_{2,158}=1.37$; $p=.257$; $\eta^2 = .02$). More specifically, a participant who chose to compete in stage three with a lower CAGn repeat number (<19) solved 3.4 problems more than one with a medium CAGn repeat number (20-23) ($F_{1,158}=4.89$; $p=.028$), and 4.5 problems more than a participant with a higher CAGn repeat number (>23) ($F_{1,158}=7.77$; $p=.006$). Given that participants who chose to compete in stage three solved on average 11.5 problems, these are relatively large effects. The performance did not vary with CAGn repeat numbers in participants who chose not to compete in stage three ($ts<1.19$; $ps>.23$).

Is testosterone related to confidence in own performance?

Before receiving feed-back about their own performance, participants were asked to guess their rank in performances in stages one to three. They were rewarded if their guesses were correct and guesses correlated significantly with performance in all stages ($\rho^1=0.59$ $\rho^2=0.47$ $\rho^3=0.47$; $ps<.001$). In the first logit regression we used basal testosterone levels, CAGn repeat number terciles and the choice in stage three as predictors of confidence (supplementary table 6). The estimates show that a tenfold-higher basal testosterone level was associated with an increase in the likelihood of being highly confident by 3.3 percent ($p=.048$; $OR=1.015$). A tenfold-higher testosterone level here relates to a comparison of the participants with the lowest testosterone levels with participants with the highest levels observed in our study sample. However, CAGn repeat number terciles did not affect confidence (2nd tercile $p=.660$; 3rd tercile $p=.653$). In addition, we found a significant interaction effect between the choice in stage three and the 2nd and 3rd CAGn repeat number terciles (2nd tercile $p=.020$; $OR=0.064$; 3rd

tercile $p=.008$; $OR=0.04$). We did not detect an interaction effect of baseline testosterone levels and the choice in stage three ($p=.186$).

As the CAGn repeat number effect on confidence seemed to be driven by participants who chose to compete in stage three, we analyzed those participants separately. This analysis confirmed that participants in the 2nd CAGn repeat number tercile were 48 percent less likely to report highest confidence in their own performance than participants in the 1st tercile of CAGn repeat numbers ($p=.011$; $OR=0.11$). Participants in the 3rd tercile were 53 percent less likely to report highest confidence than those in the 1st tercile ($p=.004$; $OR=0.09$).

Interestingly, when we added actual performance in stage three as a control variable, CAGn repeat numbers no longer predicted confidence (2nd tercile $p=.171$; $OR=0.23$; 3rd tercile $p=.131$; $OR=0.20$).

What is the mediator of CAGn repeat number on confidence?

We ran a mediation analysis to test whether the effect of CAGn repeat numbers on confidence is mediated by actual performance in those who decided to compete in stage three, which showed that 47% of the effect of CAGn repeat numbers on confidence is indirect via performance. The total indirect effect ($z=2.24$ $p=.025$) as well as the indirect effects of the 2nd and 3rd CAGn repeat number terciles are significant and negative (2nd: $z=1.92$ $p=.054$; 3rd: $z=2.39$ $p=.017$). Regarding performance, we found that for each additional problem solved, the likelihood that a participant would report highest confidence increases by 7 percentage points ($p=.002$; $OR=1.32$). In contrast, participants who chose not to compete revealed no significant effect of CAGn repeat number ($ps>.29$).

Confidence in stages one and two was not related to basal testosterone levels or the CAGn repeat number ($ps>.31$).

Together, this suggests that the androgen receptor CAG repeat polymorphism influenced confidence primarily via its effect on actual performance in stage three. This does not apply to basal testosterone levels, as the hormone seems to account for additional variance in confidence, beyond actual performance.

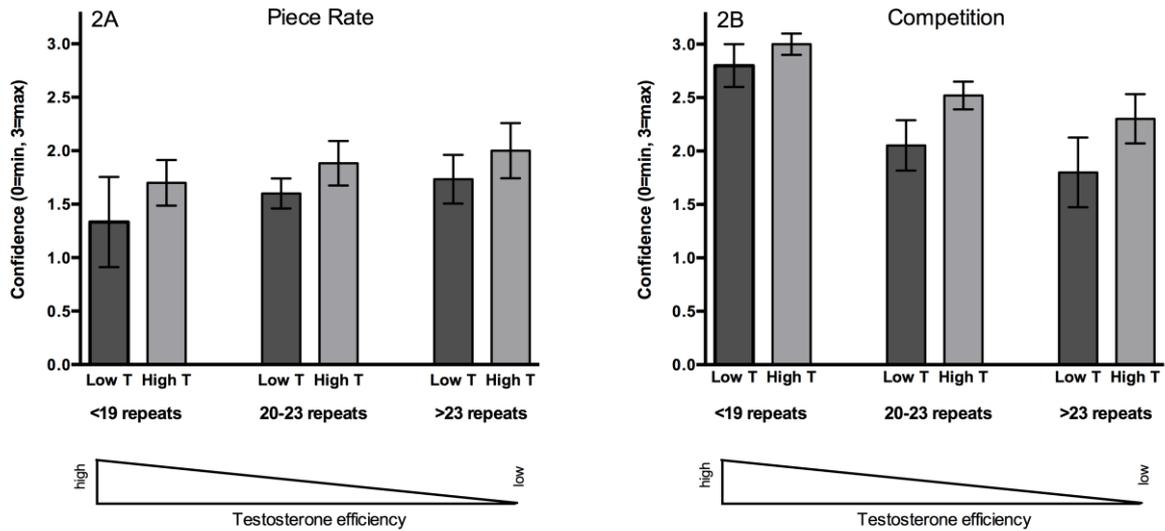


Figure 2. Participants' confidence in their own performance in relation to basal testosterone levels and CAGn repeat numbers. Participants with higher testosterone levels were more confident in stage three ($p=.048$) than those with lower levels. In those who chose to compete, we also observed that a lower CAGn repeat number was associated with greater confidence, compared to those with a higher number of repeats ($p=.044$). Y-axes indicate confidence (belief that one is ranked worst = 0, belief that one is ranked first = 3). The error bars represent the standard error of the mean. The number of observations for the left graph is 88 and for each bar from left to right: 6, 10, 30, 17, 15 and 10. For the right graph the number of observations is 82 and for each bar from left to right: 5, 3, 19, 25, 10 and 20.

Is testosterone related to risk taking?

An ordered logistic regression revealed that basal testosterone levels related positively to risk-taking ($p=.048$; OR=1.01). The probability of always choosing the risky option rose by 1 percentage point for every tenfold-higher basal testosterone level (supplementary table 7). Adding the CAGn repeat number terciles as further predictors revealed no significant effects ($ps>.65$).

Do testosterone levels increase after competition?

We noted a significant increase in testosterone levels from pre- to post-competition (40.39 pg/ml \pm 21.90 SD vs 50.54 pg/ml \pm 30.69 SD; Wilcoxon signed-rank test: $z=5.46$, $p<.001$). The increase in testosterone levels was not associated with performance in any of the three stages (supplementary table 8: all $F_s<.36$; all $p_s>.55$), nor with the choice to compete in stage three ($F_{1,166}=.06$; $p=.812$). However, the self-reported high school math grade did relate

significantly to the change in testosterone levels, in that participants with low math abilities demonstrated a decline in testosterone levels, suggesting a lack of engagement in the task due to the poor chance of performing well (see Figure 3; $F_{4,166}=2.56$; $p=.040$; $\eta^2 = .058$). Winning the competition in stage 2 or 3 had no effect on changes in the testosterone levels (stage 2: $F_{1,170}=.02$; $p=.899$; stage 3: $F_{1,170}=.00$; $p=.954$). We also used the established regressor variable method (Mehta and Josephs, 2006; Wirth et al., 2006) to analyze how self-reported math grade relates to testosterone level changes. This alternative analysis also yielded similar results, albeit only a statistical trend in the same direction ($F_{4,166}=2.35$; $p=.056$; $\eta^2 = .054$).

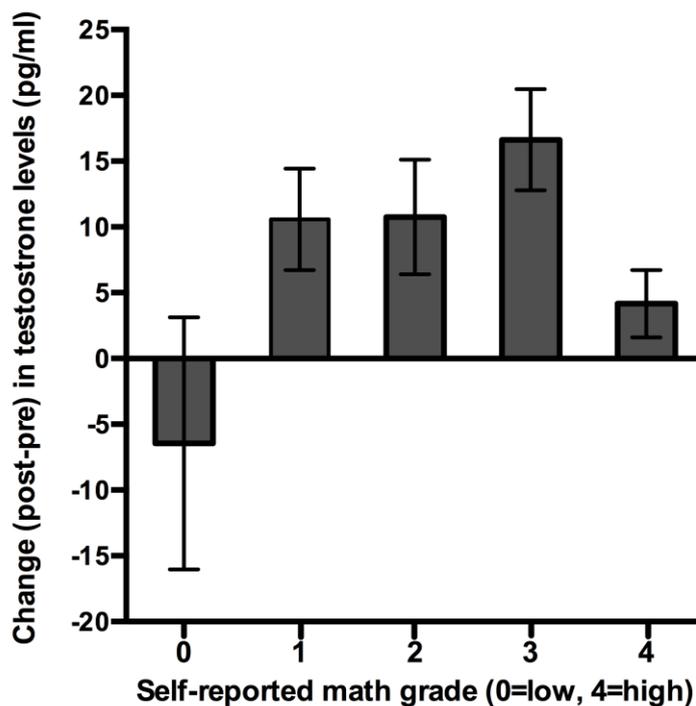


Figure 3. Change in testosterone levels over the course of the experiment against the self-reported math grade in high school (failed = 0, passed > 0). Participants exhibited a significant increase in testosterone levels after the competition compared to baseline, but this varied significantly with the high school math grade ($p=.040$). The error bars represent the standard error of the mean. The number of participants for each math grade from low to high is: 6, 25, 32, 58 and 51 participants.

Outliers

The mean testosterone level is 40 pg/ml with a standard deviation of 21.9. We identified two outliers (3 standard deviations below or above the mean) with testosterone levels of 106 and 116 pg/ml. All these results (with the exception of the relation between testosterone and risk)

are robust, excluding the two outliers. Excluding the outliers – though barely affecting the odds ratio – renders the relationship between risk-taking and testosterone insignificant ($p=.143$; $OR=1.01$).

Discussion

Testosterone is known to play an important role in competitive interactions. Here, using an experimental paradigm with real monetary incentives, we observed that individuals who had higher basal testosterone levels were more likely to decide to compete. Our results were stable after controlling for a number of possible confounds, and demonstrate that the association between basal testosterone levels and competitiveness was significant even when controlled for other important factors such as task-related skills, actual performance, confidence in one's own performance, and risk-taking behavior. Because all these additional factors can be influenced by basal testosterone as well, the stability of our effect is remarkable. We also show that higher testosterone levels were related to participants' greater confidence in their own performance. The sizes of the effect of baseline testosterone on competitiveness and confidence, however, are relatively small. We detected no significant association between the androgen receptor CAG repeat polymorphism and the decision to compete, but did observe that a lower CAGn repeat number (associated with more efficient testosterone signaling) was related to higher confidence in those who chose to compete. This effect seems to arise, however, from a significant association between the polymorphism and actual performance, i.e. 47% of the effect of CAGn repeat numbers on confidence was *indirect* via performance. We also observed a significant increase in testosterone levels following the experiment, but only in those who reported to have passed high-school math.

Our main finding is that basal testosterone levels relate positively to the decision to compete in an experimental setting in which spite plays no role. While previous research applying two-stage competition designs has revealed no relationship between basal testosterone and an individual's decision to compete in the second stage (Carré and McCormick, 2008; Mehta and Josephs, 2006), those studies were primarily designed to test the reciprocal aspects of the biosocial theory of status (Mazur and Booth, 1998), i.e., how the competition outcome induced testosterone fluctuations affect the decision to compete in a second stage. The task designs in the latter studies also differed from ours in that they were not monetarily incentivized, and the competitions were zero-sum games. In addition, the outside options (to not compete) were different, i.e., they involved interactions with either the experimenter (Carré and McCormick, 2008), or completing a questionnaire on food, music, and

entertainment preferences (Mehta and Josephs, 2006). These differences preclude making a precise comparison with our findings, but future studies might test whether testosterone is related to the subjective value of the outside options in competition designs. Another explanation for the discrepancy in findings might be that we used mass spectrometry to determine testosterone concentrations, which is assumed to be more precise and valid than immunoassay (Welker et al., 2016).

Although our design is not suited to test the reciprocal model because of the multi-stage nature of the task, it is interesting that we failed to observe that the competition outcome in the second (or among those who decided to compete in the third stage) exerted any influence on testosterone level changes. This adds to the somewhat inconsistent evidence on whether winning or losing a competition is in itself enough to cause testosterone levels to modulate. Instead, our findings regarding testosterone level changes are in line with theoretical and empirical evidence suggesting that the direction of testosterone level changes is moderated by cognitive and motivational factors (Salvador, 2005; Salvador and Costa, 2009). Earlier research has shown, for example, that the motivation to win (Suay et al., 1999), high power motivation (Schultheiss et al., 2005), mood (Booth et al., 1989; McCaul et al., 1992), and even opponent self-efficacy (van der Meij et al., 2012) are important moderators of the testosterone response to competition. In our study, a sufficient math grade (“passed”) can most likely be interpreted as the significant chance of winning the mental arithmetic contest, and the testosterone increase associated with this may reflect a positive appraisal associated with the competition. This does not apply, however, to those with the lowest math grades (“failed”).

We did not observe a significant association between basal testosterone levels and the CAGn repeat numbers. The assumption is that a higher CAGn repeat number results in diminished androgen sensitivity, which in turn leads to increased androgen production due to negative feedback regulation. Several studies have indeed observed that the CAGn repeat number correlates closely with basal testosterone levels, e.g., (Crabbe et al., 2007; Manuck et al., 2010; Travison et al., 2010; Walsh et al., 2005), while others have not observed this (Alevizaki et al., 2003; Canale et al., 2005; Goutou et al., 2009; Harkonen et al., 2003; Krithivas et al., 1999; T'Sjoen et al., 2005; Van Pottelbergh et al., 2001). The discrepant findings on CAG repeat length and basal levels of testosterone may be attributed to differences in study subject selection criteria or different genetic background between populations, or as postulated by others, that the main determinant of this polymorphism is an increased estrogen/androgen ratio (Huhtaniemi et al., 2009). Although we do not replicate

previous findings with regards to the significant link of CAGn repeat number and basal testosterone levels, the fact that the two measures do not correlate in our study allowed us to treat them statistically as independent predictors of our behavioral measures.

The interesting finding of ours - that basal testosterone effects on confidence are independent of actual performance, while those of the androgen receptor CAG repeat polymorphism are driven by actual performance - is intriguing from the perspective of the androgen system's organizational versus its activational role. Organizational effects refer to the ability of steroids to sculpt nervous system structure during development, and their ability to program activational responses to steroids later in life (Sisk and Zehr, 2005). Among men, genetically determined variation in the function of the androgen receptor is thought to explain part of the variability in structural and functional organization of brain circuits underlying testosterone-related social behaviors (Baron-Cohen et al., 2005). In line with this, significantly lower number of CAG repeats in the androgen receptor gene in intellectually gifted boys have been observed (Celec et al., 2013). This is not entirely true for basal testosterone levels, as some studies report positive (Azurmendi et al., 2005; Kutlu et al., 2001; Muller et al., 2005), while others report negative associations with intelligence (Celec et al., 2013). Thus, one could speculate that the androgen receptor CAG repeat polymorphism is more strongly involved in the brain's organizational aspects of performing certain cognitive skills, whereas adult testosterone levels are more strongly involved in context-dependent beliefs surrounding one's own performance in competitive interactions.

Previous research has suggested that part of testosterone's effects on competition might be explained via potential effects on the processing of the incentive value of monetary rewards. The important role of testosterone in modulating activity of the mesolimbic reward system, in which dopamine is centrally involved in signaling the incentive values of rewards (Robbins and Everitt, 1996), has long been established. Depleted testosterone levels, for instance via castration, lower the concentration of dopamine in the striatum in rodents, an effect that can be prevented via supplementation with testosterone (Alderson and Baum, 1981; Mitchell and Stewart, 1989). Moreover, the administration of testosterone in gonadally-intact adult male rats increases the dopamine concentration (Silva et al., 2009) and dopamine turnover in the striatum (Thiblin et al., 1999). In rhesus macaques, circulating testosterone levels were found to correlate positively with concentration of striatal tyrosine hydroxylase, the rate-limiting step in dopamine synthesis (Morris et al., 2010). In humans, single-dose testosterone administration in healthy female subjects increases BOLD activation in the ventral striatum during reward anticipation, which is most pronounced in women with low appetitive

motivation (Hermans et al., 2010). In line with this, a field study has shown, for instance, that in stock market competitions, traders with higher morning testosterone levels made higher profits during the day (Coates and Herbert, 2008), which lends support to the idea that testosterone might raise the incentive value of financial rewards. However, since in our study the expected monetary profit for correctly solving a mathematical problem is identical in the competitive versus piece rate payment schemes, our findings suggest that basal testosterone effects on competitiveness are not driven by changes in the incentive processing of financial rewards. One might argue that this does not apply to highly confident individuals, because from their perspective, choosing the competitive incentive scheme in stage three is associated with higher expected profit. However, since the relationship between basal testosterone levels and competitiveness remains statistically significant when controlling for confidence, we suggest that in the present study, basal testosterone does not increase competitiveness by increasing the incentive of monetary rewards. This is also supported by recent findings of a testosterone-administration study showing an increase in status-seeking during competition, even when this was financially costly (van Honk et al., 2016). However, future studies will need to address the role of monetary incentives during competition in more detail, for instance by employing competition designs with and without monetary incentives.

Finally, we observed that basal testosterone levels were positively related to risk-taking, beyond effects on the decision to compete. Keeping in mind that this result was not robust when testosterone level outliers were removed (Pollet and van der Meij, 2016) our result fits in with the generally mixed findings on the role of testosterone in risk taking (Apicella et al., 2008; Sapienza et al., 2009; Stanton et al., 2011). This seems to be the case also in pharmacological studies aiming at exogenously manipulating testosterone levels. Such studies either reported no effect on risk taking (Boksem et al., 2013; Wu et al., 2016; Zethraeus et al., 2009), or increased risk taking in tasks with feedback (van Honk et al., 2004), or in risk tasks with unknown probabilities (Goudriaan et al., 2010).

In addition, a recent study reported that in men whose testosterone concentrations increased in response to a competition were less risk-averse than men whose testosterone concentrations dropped (Apicella et al., 2014). We did not observe an association between testosterone level changes and risk-taking, which is interesting given that in both studies testosterone level changes were independent of competition outcome. It should be noted, however, that we used a multi-stage competition task and thus changes in testosterone levels can not be directly compared with the ones elicited by the competition task in (Apicella et al., 2014).

Thus in sum, our study results suggest that baseline testosterone is positively related to competitiveness without spite, and that this is independent of potential effects of basal testosterone on confidence, performance and financial risk-taking. A limitation is that the precise nature of this main effect remains elusive. One possibility is that basal testosterone relates positively to competitiveness, but negatively to spite. In addition, future neuroimaging studies might elucidate the role of reward-processing regions in driving the motivation to compete that does not entail monetary incentives. Our study has limitations in that we have assessed confidence after the competition (before the competition's outcome was apparent); it would be interesting to observe effects of basal testosterone on confidence assessed before a competition performance. In addition, our measure of testosterone level changes is confounded in the sense that not all the participants shared the same experience when making the choice in stage three. Our measure of real-effort might be adapted in future studies, for instance, it would be interesting to see whether more basic measures of performance (e.g., motor-based measures of real-effort) would produce the same results (Vermeer et al., 2016). In addition, given differences in competitiveness across gender (Niederle and Vesterlund, 2007), future studies might look into the role of baseline testosterone in competitiveness in females using the same behavioral design. Finally, our results provide correlative evidence only, and should therefore be confirmed both in larger samples and by hormone administration protocols (Bos et al., 2012; Eisenegger et al., 2013; Goetz et al., 2014; Welling et al., 2016).

Conclusions

Our study provides evidence for an association between testosterone levels and competitiveness, and variability of the androgen gene with performance under competition. We found that men with high basal testosterone levels are more likely to compete. In those who deliberately chose to compete, we observed that higher testosterone levels are associated with more confidence in one's own performance, while more efficient testosterone signaling is associated with a superior actual performance. It appears that basal testosterone levels are related to measures of competitiveness in men in a design in which spite cannot influence behavior, and this remains significant even when controlling for testosterone effects on other factors such as task-related skills, actual performance, confidence and risk-taking behavior.

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Highlights

- Baseline testosterone levels are positively associated with the decision to compete in a mathematical skills-based task in men
- Effect is specific to competitiveness and not driven by risk taking, performance, or confidence in one's own performance
- In those who chose to compete, higher testosterone levels are associated with higher confidence in own performance
- Lower number of repeats of the androgen receptor CAG repeat polymorphism, associated with more efficient testosterone signaling, is associated with higher actual performance
- Testosterone levels increase over the competition following the experiment, and this increase varies with self-reported high-school math grades