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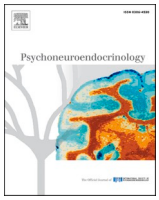

Elevated testosterone and prosocial behavior in female patients with borderline personality disorder independent of social exclusion

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ABSTRACT

Objective: Borderline personality disorder (BPD) is marked by unstable relationships and fear of abandonment. Earlier studies suggest that patients with BPD are highly sensitive to social exclusion and show deficits in trust and cooperation. The hormone testosterone influences such prosocial behavior and regulates aggressive and caring behavior. Previous studies show elevated testosterone levels in female patients with BPD at baseline and after psychosocial stress, while results after social exclusion are missing.

Method: We investigated the effects of social exclusion on prosocial behavior (sharing and punishment) and salivary testosterone in female patients with BPD. Ninety-eight patients with BPD and 98 healthy females matched for menstrual cycle were randomly assigned to an overinclusion or exclusion condition of the virtual ballgame Cyberball. Afterwards, participants played two games in which they could share money with a fictional player ("dictator game") and accept or reject (= punish) offers from a player ("ultimatum game").

Results: Female patients with BPD displayed higher testosterone levels than the control group before and after Cyberball. Social exclusion did not affect testosterone levels. Patients with BPD exhibited more prosocial behavior by sharing more money than controls and punished co-players for unfair offers equally often.

Conclusion: We replicated previous findings of elevated testosterone in female patients with BPD and showed that it is not affected by experimentally induced social exclusion. Regardless of social exclusion, patients with BPD showed more prosocial behavior, which may reflect a status-seeking strategy to secure their social standing.

1. Introduction

Next to impulsive behavior and difficulty in regulating aggression, borderline personality disorder (BPD) is characterized by disturbed interpersonal relationships (Gunderson, 2007). Patients with BPD often have difficulty maintaining stable relationships and experience reduced trust during interpersonal interactions (Unoka et al., 2009). Individuals with BPD frequently report to experience stress (Stiglmayr et al., 2008), which causes worsening of many symptoms such as impulsivity and non-suicidal self-injury (Bourvis et al., 2017) and impairs social cognition. In one of our previous studies, female patients with BPD showed reduced emotional empathy and higher acute dissociation after a psychosocial stressor including elements of social exclusion and evaluation (Wingenfeld et al., 2018). Healthy individuals, in contrast, react to stress with enhanced prosocial behavior including trust and cooperation (Wolf et al., 2015) as well as increased sharing behavior (von Dawans et al., 2019). The results in patients with BPD suggest a "fight-or-flight" response to psychosocial stress, instead of a more prosocial "tend-and-befriend" reaction to stress seen in healthy individuals. One component of social stress can be social exclusion, which individuals with BPD are especially sensitive to (Cavicchioli and Maffei, 2020). Based on these results, we propose that the stressful component of social

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exclusion might explain alterations in prosocial behavior after stress in female patients with BPD.

Social behaviors are partly guided by the sex hormone testosterone, which is regulated by the hypothalamic-pituitary-gonadal (HPG) axis. It is released in situations related to dominance, competition, and status seeking and many studies indicate an association with aggression (Cheng and Kornienko, 2020). Previous studies have reported increased levels of salivary, serum and hair testosterone in female patients with BPD (Bertsch et al., 2018; Bonfig et al., 2022; Dettenborn et al., 2016; Rausch et al., 2015; Roepke et al., 2010). Such heightened levels of testosterone are related to negative health outcomes like obesity and are integral to polycystic ovary syndrome (Roepke et al., 2010; Ruth et al., 2020). Although several studies have explored the involvement of oxytocin or cortisol (Jobst et al., 2014; Reinhard et al., 2022), studies investigating testosterone release in patients with BPD in stressful (social) situations are scarce. One study showed an increase in testosterone after psychosocial stress in both female patients with BPD and healthy controls (HC) (Deuter et al., 2021). To date there are no studies investigating testosterone release and prosocial behavior in response to social exclusion. In the present study we want to fill this gap.

1.1. Prosocial behavior and social exclusion in patients with BPD

Many studies investigating prosocial behavior use economic decision-making games. In a dictator game, one player usually decides how much money of a predefined stake to share with another player, who does not have an active role in the game. In an ultimatum game, the index player has to accept or reject (= punish) monetary offers made by a co-player. Humans have a strong preference for fairness, which often results in making fair splits in dictator games and rejecting unfair offers in ultimatum games (Fehr and Fischbacher, 2003). Previous dictator game studies showed that individuals with borderline personality (BP) (features) shared fair amounts of money with co-players (50:50), just as healthy individuals did (Hepp et al., 2018; Thielmann et al., 2014). Ultimatum game results are mixed. In one study, patients with BPD accepted more offers than HC including unfair offers (Polgár et al., 2014). In another study, individuals with higher BP features rejected more offers than individuals with lower BP features (Thielmann et al., 2014). Others found no differences between HC and patients with BPD in punishment of unfair offers (De Panfilis et al., 2019; Jeung et al., 2020; Wischniewski and Brüne, 2013), but a higher level of rejection of fair offers in patients with BPD (De Panfilis et al., 2019). Taken together, these results suggest unimpaired active cooperation (sharing), but impaired reactive cooperation (punishment) in individuals with BPD. Studies investigating the effects of psychosocial stress and social exclusion on economic decision-making games in individuals with BPD are currently lacking.

An experimental task frequently used to induce social exclusion is the virtual ball- game Cyberball, which usually consists of an inclusion and an exclusion condition (Williams and Jarvis, 2006), where the co-players toss the ball without the index player. Individuals with BPD are more negatively affected by social exclusion (De Panfilis et al., 2015; Gutz et al., 2015) and report higher threat to fundamental needs such as belonging or control than healthy individuals (Seidl et al., 2020; Weinbrecht et al., 2018). Further, individuals with BPD report feeling more rejected than HC after exclusion (Seidl et al., 2020) but also after inclusion (De Panfilis et al., 2015; Weinbrecht et al., 2018). In healthy individuals, social exclusion often results in prosocial behavior, such as tossing the ball more often to an excluded player (van der Meulen et al., 2016) and even to the excluding player (Dewald-Kaufmann et al., 2021; Reinhard et al., 2022). This is in line with the “tend-and-befriend” hypothesis, which proposes that individuals use prosocial behavior to regain access to their social group. Patients with BPD, in contrast, displayed higher aggressive action tendencies (Gutz et al., 2016) and more other-focused negative emotions than healthy controls after exclusion (Jobst et al., 2014; Seidl et al., 2020). In one study using a partial exclusion paradigm, patients with BPD tossed the ball to the excluding player less than before exclusion (Reinhard et al., 2022). Taken together these results can be interpreted as a “fight-or-flight” response to social exclusion in patients with BPD.

1.2. Effects of social exclusion on testosterone

Testosterone production in the body primarily runs under the regulation of the hypothalamic-pituitary-gonadal (HPG) axis and depends on several organs that are also involved in the physiological stress reaction. The hypothalamus releases gonadotropin-releasing hormone (GnRH), which stimulates the pituitary gland to secrete luteinizing hormone (LH) and follicle-stimulating hormone (FSH), in females prompting the ovaries to produce testosterone. Additionally, the adrenal glands release androgen precursors like dehydroepiandrosterone (DHEA) and androstenedione, which are converted into testosterone in various tissues including the skin and liver (Bienenfeld et al., 2019; Davis and Wahlin-Jacobsen, 2015). This process is tightly regulated by feedback loops that adjust hormone levels based on physiological needs, such as stress or reproductive demands. In contrast, enzymatic activity (e.g., aromatase or 5-alpha-reductase) affects testosterone locally by converting it to other hormones, like estradiol or dihydrotestosterone (DHT), in specific tissues These enzymatic processes are tissue-specific and not subject to the systemic regulation of the HPG axis, making them less dynamic in response to broader physiological changes such as stress or hormonal feedback (Giatti et al., 2020; Martini et al., 1996).

Several studies suggest a bidirectional relationship between the HPG axis and the body’s central stress regulating axis, the hypothalamic-pituitary-adrenal (HPA) axis (Acevedo-Rodriguez et al., 2018; Viau, 2002). Brain regions such as the anterior cingulate cortex, insula, and hippocampus, known for their roles in emotional and social information processing, are involved in activating both axes (Dismukes et al., 2015). This bidirectional relationship is well-supported by studies demonstrating the disrupting impact of stress on reproductive functions (Acevedo-Rodriguez et al., 2018; Whirledge and Cidlowski, 2013)

Cortisol, regulated by the HPA axis, plays a key role in the body’s immediate stress response by mobilizing energy reserves, preparing the body for the "fight-or-flight" reaction (Cain and Cidlowski, 2017; Schwabe and Wolf, 2013; Ulrich-Lai and Herman, 2009). Testosterone involvement in stressful social situations is often linked to perceived threats. Testosterone levels rise in response to challenges to social status, such as social exclusion, and promote behaviors aimed at defending or improving one’s social position (Eisenegger et al., 2011; Losecaat Vermeer et al., 2020). Testosterone’s behavioral effects are primarily mediated by androgen receptors, which modulate physiological and psychological responses (Simmons and Roney, 2011). Additionally, testosterone influences the dopamine system, particularly in the ventral striatum, which is critical for reward processing and competitive behaviors (Losecaat Vermeer et al., 2020; Mehta and Prasad, 2015)

Several studies have explored testosterone release after social exclusion in healthy females, while results in female patients with BPD are missing. Results of one study show a decrease in testosterone in healthy females from before to after exclusion (Seidel et al., 2013) and from before to after both, the inclusion and the exclusion condition (Radke et al., 2018). Additionally, individuals with BPD responded to social exclusion with a reduction of the hormone oxytocin, which promotes prosocial behavior (Jobst et al., 2014; Reinhard et al., 2022). Taken together, these results fit the hypothesis of a “fight-or-flight” response to social exclusion in individuals with BPD.

1.3. Study aim and hypotheses

In the present study, we aim to investigate the effects of social exclusion on prosocial behavior and testosterone release in female patients with BPD. We expect that individuals with BPD will exhibit higher baseline testosterone than HC and show an increase in testosterone after

social exclusion. We propose that due to their elevated testosterone levels patients with BPD will not show “tend-and-befriend” behavior and instead resort to less prosocial "fight-or-flight" behavior after exclusion, i.e., sharing less money and punishing unfair offers at higher rates than HC.

2. Method

The present analyses were part of a larger study. Results on empathy, salivary cortisol and alpha amylase release after social exclusion have been published elsewhere (Graumann et al., 2023).

2.1. Participants

A priori sample size calculation was conducted using G*Power (Faul et al., 2009) for the larger study from which this paper is drawn (Graumann et al., 2023). The primary outcome variables of the whole study were emotional empathy, sharing, and punishment. In a previous study (Wingenfeld et al., 2018), we observed a significant group × stress interaction on empathy, showing an effect size of ηp^2 =.05. Larger effects were found for stress on sharing behavior (d =.57, ηp^2 =.075) (von Dawans et al., 2012). Based on these findings, we expected similar effects in response to the Cyberball paradigm. To detect an effect size of ηp^2 =.07 with α = 0.05 and power (1-β) of 0.90, a total sample of 185 participants was required for a one-way ANOVA with four groups (92 HC and 92 patients with BPD). Because our dependent measures, i.e., empathy, sharing and punishment, were likely not independent from each other, we additionally calculated the required sample size for a MANOVA revealing a slightly lower needed sample size of $N = 128$. To make sure that there would be enough power to conduct additional post-hoc comparisons, we opted for the above-mentioned sample size of 92 HC and 92 patients with BPD. We recruited 98 healthy females and 98 female patients with BPD to account for potential data loss due to technical errors or incomplete data, ensuring that the final sample size would meet the requirements for sufficient statistical power.

The sample comprised participants between the ages of 18 and 50 with a Body-Mass-Index between 17.5 and 30 kg/m^2. All participants were assigned female sex at birth, which was assessed through self-report and corroborated through patient data from our patient data system. HC were matched for age, education, menstrual cycle and intake of hormonal contraception. Menstrual cycle phase was calculated using the calendar method based on self-reported onset of the last period and usual duration of the cycle.

Exclusion criteria for all participants included intake of glucocorticoids, pregnancy and autoimmune, endocrine, metabolic, neurodegenerative, and CNS diseases. Exclusion criteria for patients with BPD included acute major depressive episode, current substance abuse, psychotic symptoms and intake of more than three different psychotropic substances or benzodiazepines. Daily intake of more than three different psychotropic substances or current intake of benzodiazepines led to exclusion in patients with BPD to exclude polypharmacy. The dosage of psychotropic medication had to remain consistent for at least one week prior to testing. Healthy control participants were required to have no history of psychiatric diagnoses, treatment, or medication use throughout their lifetime. Participants were recruited through online postings and flyers distributed within the hospital. Additionally, inpatients diagnosed with BPD were selected from the Department of Psychiatry and Neurosciences at Charité Berlin, Campus Benjamin Franklin, Germany. All participants were informed on the procedure and the voluntary nature of their participation in written and oral form and gave consent before participation. All participants received reimbursement of a minimum of 60 Euros and an additional amount of up to 30 Euros based on dictator and ultimatum game results. The study was approved by the Charité Ethics Committee.

2.2. Procedure

In the first part of the study, participants underwent diagnostic interviews and filled out questionnaires on psychopathological symptoms using the web application RedCap on a tablet or computer in the laboratory. Questionnaires included Beck Depression Inventory (BDI-II) (Beck et al., 1996) assessing depressive symptom severity and the Borderline Symptom List short version (BSL-23) (Bohus et al., 2009) to assess borderline symptom severity.

The experimental sessions took place in the afternoon. Participants played either an exclusion or an overinclusion condition of the virtual ball game Cyberball (Williams and Jarvis, 2006). Afterwards, participants played the dictator game and the ultimatum game and took the Multifaceted Empathy Test (MET) (Dziobek et al., 2011). Before Cyberball and after the tasks, participants rated current mood, wakefulness and nervousness on the Multidimensional Mood State Questionnaire (MDMQ) (Steyer et al., 1997).

2.3. Social exclusion

To induce social exclusion, we used Cyberball (version 5, desktop version). Participants were randomized to either an exclusion or an overinclusion condition as the control condition, because individuals with BPD are known to have a biased perception of inclusion (De Panfilis et al., 2015; Weinbrecht et al., 2018). Both conditions ran for two to three minutes and consisted of 30 ball-tosses each. In the exclusion condition, participants only received the ball twice at the beginning of the game and then no longer. In the overinclusion condition, participants received 45 % of all throws. Before the game, participants received instructions, stating that they would play the game with two real co-players via an internet connection, while in fact these co-players were computer-generated. At the end of the experiment, all participants were debriefed.

After the tasks, participants reported Cyberball induced need threat (e.g., “I felt invisible”) and ostracism intensity (e.g., “I was excluded”), which was assessed using the Need Threat Questionnaire (NTQ) (Grzyb, 2005). Participants additionally had to indicate the extent to which they believed that their co-players had been real people.

2.4. Dictator and ultimatum game (sharing and punishment behavior)

Participants played three rounds of the dictator game (“sharing”) and six rounds of the ultimatum game (“punishment behavior”). Beforehand, participants read written instructions on the games and completed test questions to ensure that they had understood the rules of the games. The instructions stated that they would play each of the games with one of their Cyberball co-players, i.e., a real person, whom they would not see or meet in person. All players were represented by the same pictograms and names (e.g., “Player A”) as in the Cyberball game. Participants were debriefed at the end of the experiment that there were no co-players and that all ultimatum game offers were pre-programmed. We programmed and ran the games using Presentation® (Neurobehavioral Systems 2003–2018). The instructions can be found in the supplement S1.

In the three rounds of the dictator game participants started each round with 30 monetary units and had to decide which amount from 0 to 30 they wanted to share with the other player. Participants had five seconds to type their elected amount into the blank field on the screen. If participants took longer than five seconds, they received a message that they were too slow and that the round was not counted. The other player had no active role and automatically received the amount that the participant had allocated to them.

In the six rounds of the ultimatum game participants received fictional offers from their co-player. In each round, the co-player made an offer, distributing 40 monetary units between herself and the participant (in fact this was computer-generated). Participants had to decide whether to accept or reject each offer by pressing a green (accept)

or red (reject) key on the keyboard. In randomized order, the pre-programmed offers were 10 % of the total 40 monetary units (the co-player keeps 36 units and the participant receives 4 units; 36:4), 15 % (34:6), 25 % (30:10), 30 % (18:12), 40 % (14:16), or 45 % (12:18). If participants rejected an offer, both players received no compensation. If participants took longer than three seconds to decide, they received a message that they were too slow and that the round was not counted. Offers less than 20–30 % of the stake are generally perceived to be unfair, while offers of 40–50 % or more of the stake are perceived to be fair (Camerer, 2003).

At the end of the game, the gained monetary units from both games were added together and participants received monetary compensation accordingly (number of units multiplied by 0.2 Euros).

2.5. Assessment of hormones

We collected saliva to measure testosterone using SaliCap devices (1.5 ml polypropylene tubes; IBL, Hamburg, Germany) at baseline (0), after Cyberball (+20), and after the dictator and ultimatum games and MET (+35). Participants were instructed to salivate through a straw into the tube filling up at least half of the tube's volume. Participants were not allowed to drink, eat or chew gum until 30 minutes before saliva collection. Until biochemical analyses at the Neurobiology Laboratory of the Department of Psychiatry, Charité – Universitätsmedizin Berlin, samples were stored at −80 °C and were allowed to completely thaw at 4 °C prior to analysis. Testosterone levels were determined using a competitive immunoassay (IBL/TECAN, Hamburg, Germany) following the manufacturer's recommendations. The limit of detection was 2.1 pg /ml, the precision parameters (coefficient of variation; CV) for medium concentrations at 50 pg/ml were determined to average below 5 % CV for intra- and 10 % CV for inter-assay variance.

2.6. Statistical analyses

We log-transformed testosterone values, as they were not normally distributed. We analyzed log-transformed testosterone values using repeated measures ANOVAs with the within-subjects factor time (0 min, +20 min, +35 min) and between-subjects factors group and condition.

To measure sharing, we summed up the amount of shared monetary units of all valid rounds of the dictator game and calculated mean percentage scores. We conducted an ANOVA with group and condition as independent variables and with percentage of shared money as the dependent variable. To analyze punishment behavior, we conducted a binary logistic regression with repeated measurement using generalized linear mixed model (GLMM) to examine whether group, condition or offer type helped to explain the answers to the six different offers. The target variable in the GLMM was offer acceptance with a binomial distribution (reject or accept) and a logit link function. The fixed effects (predictors) included group (BPD vs. HC), condition (overinclusion vs. exclusion), type of offer (10 %, 15 %, 25 %, 30 %, 40 %, 45 %) and interactions between these effects. Random effects were participant ID's.

3. Results

3.1. Demographic variables

There were no group differences in age, years of education, intake of hormonal contraception, menstrual cycle phases or number of participants reporting "no regular cycle" including participants using hormonal contraception. Sixteen participants with BPD and 16 HC used hormonal contraception. In both groups, 15 participants used Combination Oral Contraceptives (COCs) containing estrogen and progesterone. In both groups one participant each used a Progestin-only Pill (POP) containing only progesterone. Because healthy individuals were matched to patients with BPD on these variables, no group differences had been expected. There were more smokers and BMI was higher in the BPD group. Results are listed in Table 1. Fifty patients with BPD reported intake of at least one psychotropic substance, while 48 patients were free of psychotropic medication. Posttraumatic stress disorder was the most frequent comorbid diagnosis determined in patients with BPD, $n = 25$. Forty-nine individuals with BPD and 48 HC were in the exclusion condition, and 49 individuals with BPD and 50 HC in the overinclusion condition. For further details on medication and comorbid diagnoses, see supplement S2.

3.2. Manipulation check

Participants reported greater need threat and ostracism intensity after exclusion than after overinclusion, both $ps < .001$, which indicates a successful manipulation. Detailed results on the NTQ and the mood questionnaire are listed in supplement S3 and (Graumann et al., 2023).

3.3. Testosterone

We analyzed testosterone values for 95 patients with BPD and 98 HC. For three participants with BPD, all testosterone values were missing, because of measurement errors (e.g., the collected amount of saliva was too small for detection). Additionally, for three individuals with BPD, testosterone values for one of the three measurement time points (T1, T2, T3) each was missing. For one HC testosterone values of T2 and T3 were messing. We calculated an overall mean testosterone score from the values of the three time points including those participants with at least one valid measured variable. Individuals with BPD had significantly higher overall mean testosterone values than HC, $t(191) = 3.40$, $p < .001$. Testosterone values at each time point significantly correlated with testosterone values at the other two time points in both patients with BPD and HC, all $ps < .001$. Descriptive statistics of raw testosterone values as well results of the correlational analyses between these values are described in Table 2 for the BPD and HC group each.

In the BPD group we found significant positive correlations between raw baseline testosterone levels (T1) and borderline symptom severity ($r(190) = .28$, $p = .006$), and between baseline testosterone and depression symptom severity ($r(189) = .34$, $p = .001$). Because there was too little variance in symptom scores, we did not analyze correlations in HC.

To analyze the effect of group (BPD vs. HC) and Cyberball condition (overinclusion vs. exclusion) on testosterone levels over time, a repeated measures (rm) ANOVA was calculated for participants with valid saliva samples of all three measurement time points, resulting in $N = 92$ individuals with BPD and $N = 97$ HC. The analysis revealed a main effect of group ($F(1, 185) = 7.59$, $p = .006$, $\eta^2 = 0.04$), showing that female

Table 1
Sample Characteristics and Psychopathology Questionnaire Data.

Variable	BPD $n = 98$	HC $n = 98$	Statistics
Age (*mean/SD*)	27.78 (7.23)	27.93 (6.89)	$t(194) = -0.15$, $p = .880$
Years of school education (*mean/SD*)	11.70 (1.21)	11.96 (1.00)	$t(194) = -1.61$, $p = .110$
Hormonal contraception (*y/n*)	16/82	16/82	$\chi^2(1) = 0.00$, $p = 1.00$
Smokers (*y/n*)	42/56	14/84	$\chi^2(1) = 19.6$, $p < .001$
Body-Mass-Index (*mean/SD*)	22.84 (3.23)	21.91 (2.46)	$t(193) = 2.34$, $p = .020$
Cycle phase (*follicular / luteal / no natural cycle*)	26/50/20	30/50/18	$\chi^2(2) = 0.37$, $p = .831$
BDI-II (*mean/SD*)	27.21 (12.17)	1.74 (2.4)	$t(193) = 20.31$, $p < .001$
BSL−23 (*mean/SD*)	1.96 (.88)	0.08 (.12)	$t(194) = 20.98$, $p < .001$

Note. BPD = borderline personality disorder; HC = healthy controls; n = sample size; *SD* = standard deviation; y = yes; n = no; BDI-II = Beck Depression Inventory; BSL-23 = Borderline Symptom List- short version.

Table 2
Descriptive Statistics and Correlations of Testosterone Levels Over Time (T1-T3) in BPD and HC.

Group	Variable	*n*	*M*	*SD*	Pearson Correlation *r*		
					T1	T2	T3
BPD	Testosterone T1 (0 min)	94	30.05	27.70	-	.697**	.652**
	Testosterone T2 (+20 min)	93	28.57	20.72	.697**	-	.831**
	Testosterone T3 (+35 min)	93	30.64	20.43	.652**	.831**	-
	Mean Testosterone (T1-T3)	95	29.61	20.65			
HC	Testosterone T1 (0 min)	98	21.79	16.91	-	.600**	.672**
	Testosterone T2 (+20 min)	97	19.89	17.57	.600**	-	.735**
	Testosterone T3 (+35 min)	97	20.64	17.37	.672**	.735**	-
	Mean Testosterone (T1-T3)	98	20.75	15.21			

Note. BPD = Borderline personality disorder; HC = Healthy controls; *n* = sample size; *M* = mean; *SD* = standard deviation; Pearson correlation coefficients (*r*) indicate the relationships between testosterone levels measured at three time points: T1 (0 minutes), T2 (+20 minutes), and T3 (+35 minutes); ** = statistical significance at $p < .001$. mean testosterone = average testosterone level across three time points.

patients with BPD displayed higher testosterone levels than HC across time. Additionally, testosterone values did not significantly change over time ($F(2, 370) = 0.84$, $p = .434$, $\eta^2 = 0.01$) and there was no main effect of condition ($F(1, 185) = 0.00$, $p = .988$, $\eta^2 = 0.00$) or any significant interactions between time, group and condition, all *ps* >.05. Raw testosterone levels are across groups and time are depicted in Fig. 1.

Because there were significantly more smokers in the BPD group than in the HC group and testosterone levels have been shown to be elevated among smokers (Zhao et al., 2016), we added smoking status as a covariate into our analysis. We also calculated mean testosterone scores for the following four groups: BPD smokers, BPD non-smokers, HC smokers, HC non-smokers, the distributions are visualized in Fig. 2. Because the group of participants with BPD had significantly higher BMI than HC and this may impact testosterone levels as well

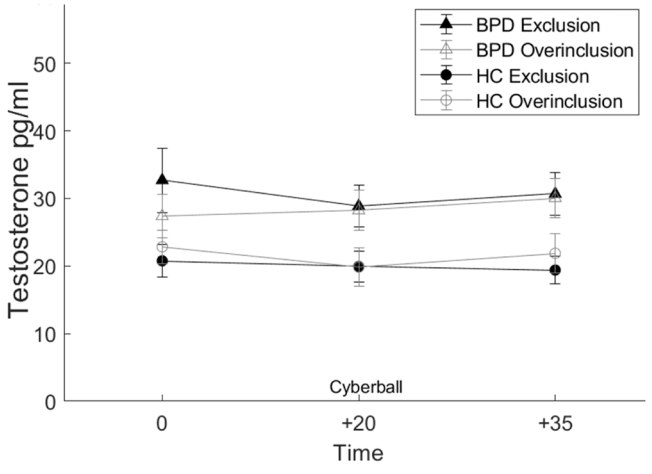

Fig. 1. *Raw Testosterone Values Before and After Cyberball Overinclusion and Exclusion in Individuals with Borderline Personality Disorder (BPD) and Healthy Controls (HC). Note.* There was a significant difference between groups in testosterone levels, patients with BPD displayed higher levels than HCs across time ($p = .006$). Error bars represent standard errors.

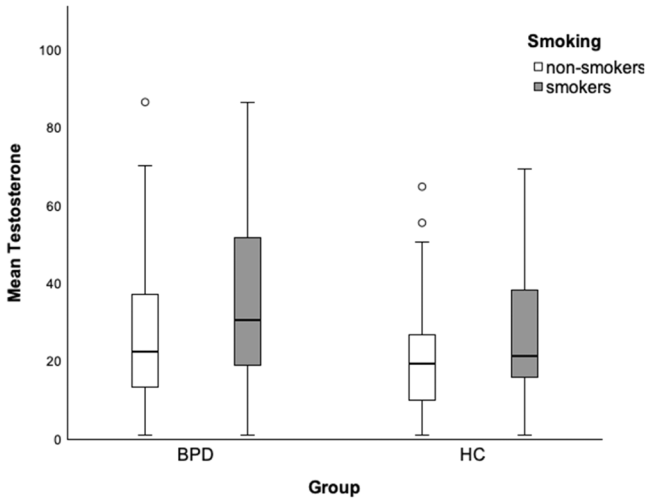

Fig. 2. *Mean Testosterone Levels in Smokers and Non-Smokers across groups (BPD and HC).*

(Zhao et al., 2023), BMI was also included as a covariate into the analysis. After including these variables into the analysis, we found no significant between-subjects effect for BMI, smoking or Cyberball condition, all *ps* >.05, but a significant group effect ($F(1, 184) = 4.23$, $p = .041$, $\eta^2 = 0.02$).

3.4. Dictator and ultimatum game (sharing and punishment behavior)

We analyzed sharing and punishment data in 94 individuals with BPD and 97 HC. Data was missing for four patients with BPD and one HC due to computer errors.

3.4.1. Dictator game (Sharing)

In a one-way ANOVA, we found a significant group effect for percentage of shared money ($F(1187) = 6.19$, $p = .014$, $\eta^2 = .03$). Regardless of condition, individuals with BPD shared more money than HC. There were no condition or condition × group interaction effects, both *ps* >.05. Results are shown in Fig. 3a.

We found significant correlations between percentage of shared money and log-transformed testosterone values after Cyberball ($r(185) = .21$, $p = .003$) and after all tasks ($r(185) = .20$, $p = .005$).

3.4.2. Ultimatum game (Punishment)

We used GLMM to examine whether group, condition or offer type helped to explain the answers to each of the six different offers (accept or reject choices). In the binomial logistic regression model, 1086 observations were included. The model was evaluated using two information criteria, Akaike Corrected (6135.21) and Bayesian (6140.18), which are based on the −2 log likelihood (6133.21). Smaller values of the information criteria indicate better model fit. The model correctly classified an overall 90.8 % of rejections and acceptance, including 66.8 % of rejections and 97.4 % of acceptances.

Our model revealed a significant effect of offer type ($F(5, 1062) = 26.25$, $p = .000$). There were no main effects of condition ($F(1, 1062) = .00$, $p = .965$) or group ($F(1, 1062) = .00$, $p = .974$). Furthermore, there was a significant condition × offer type interaction ($F(5, 1062) = 2.52$, $p = .028$). There were no other significant interactions, all *ps* >.05. As displayed in Table 3, the odds of rejection were significantly higher for the 10 % offer, the 15 % offer and the 25 % offer than for the 45 % offer, all *ps* <.01. Simple contrasts with sequential Sidak adjusted significance level ($\alpha = .05$) showed a significant difference in rejection rates between overinclusion vs. exclusion for 30 % offers ($t(1062) = 2.48$, $p = .013$). Participants in the overinclusion rejected the 30 % offers more frequently than those in the exclusion condition. There were no

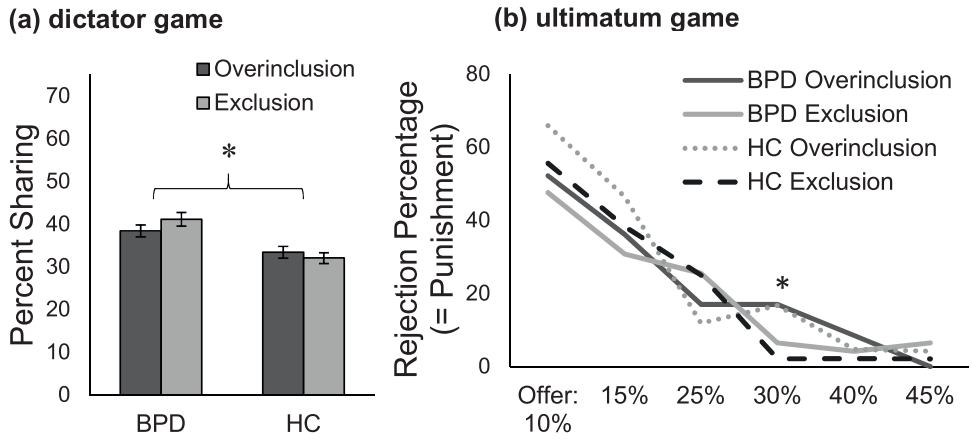

Fig. 3. *Dictator and Ultimatum Game Results – (a) Percentage of Shared Money and (b) Percentage of Offer Rejections in Patients with Borderline Personality Disorder (BPD) and Healthy Controls (HC) Across Cyberball Conditions. Note.* (a) Patients with BPD shared more money than HC (p =.014 (*)); error bars represent standard errors. (b) lower offers were rejected more frequently than higher offers across groups and Cyberball conditions; participants in the overinclusion condition rejected the 30 % offer significantly more than those in the exclusion condition (p =.013 (*)).

Table 3
Ultimatum Game Offer Rejections Across Different Offers, Groups, and Conditions.

Model Term	Coefficient	Std. Error	t	Sig.	Odds ratio (OR)	OR 95 % CI
Intercept	−4.25	1.05	−4.07	.000	0.01	[0.00, 0.11]
BPD	1.28	1.23	1.04	.301	3.59	[0.32, 40.37]
HC	0[b]	.	.	.	.	.
Overinclusion	0.65	1.30	0.50	.617	1.92	[0.15, 24.78]
Exclusion	0[b]	.	.	.	.	.
Offer 10 %	4.56	1.08	4.21	.000	95.93	[11.43, 804.91]
Offer 15 %	3.70	1.08	3.43	.001	40.24	[4.84, 334.83]
Offer 20 %	3.03	1.09	3.77	.006	20.70	[2.42, 177.41]
Offer 30 %	0.001	1.44	0.00	1.00	1.00	[0.06, 17.02]
Offer 40 %	0.06	1.45	0.04	.968	1.06	[0.06, 18.08]
Offer 45 %	0[b]	.	.	.	.	.

Note. [b] This coefficient is set to zero because it is redundant; BPD = borderline personality disorder; HC = healthy controls; CI = confidence interval

significant differences for any other offer types. Results are pictured in 3b, regression coefficients and statistics are reported in Table 3. We did not find any significant correlations between testosterone values and punishment scores for each offer type.

4. Discussion

Female patients with BPD displayed higher levels of salivary testosterone than healthy controls before and after both Cyberball conditions as well as overall mean testosterone levels across all time points. Furthermore, participants with BPD shared more money with their co-players than controls. There were no differences in punishment between groups. Cyberball condition did not significantly affect testosterone levels or prosocial behavior across groups. We found an interaction effect of Cyberball condition on punishment of marginally unfair offers. Those in the overinclusion condition punished more 30 % offers than those in the exclusion condition.

4.1. Elevated testosterone levels in patients with BPD

We were able to confirm previous findings of elevated testosterone levels in females with BPD (Bertsch et al., 2018; Bonfig et al., 2022; Dettenborn et al., 2016; Rausch et al., 2015; Roepke et al., 2010) and extended them to a comparatively large sample with a matched control group. These results were not explained by higher BMI or higher percentage of smokers in the BPD group. We found that current symptom load positively correlated with higher salivary testosterone levels. Our results suggest that heightened testosterone levels might contribute to BPD pathology, however no causal relationships can be drawn from these cross-sectional data.

It remains important to consider whether alterations in testosterone levels are specific to BPD or might also be observed in other disorders involving impulsivity or affective dysregulation, such as attention-deficit/hyperactivity disorder (ADHD) or major depressive disorder (MDD). A meta-analysis indicated a small positive correlation between endogenous testosterone and risk-taking behaviors, including impulsivity, in both males and females, suggesting that testosterone might be linked to broader impulsivity traits (Kurath and Mata, 2018). However, findings related to ADHD are inconsistent. While some studies suggest a relationship between testosterone and ADHD, others have failed to establish robust causal effects of bioavailable testosterone on ADHD in both sexes (Dinkelbach et al., 2024). This is supported by findings by showing no association between testosterone levels and ADHD in adulthood, despite elevated symptoms in females with polycystic ovary syndrome (PCOS), who tend to have higher testosterone levels (Hergüner et al., 2015). Both males and females with MDD, in contrast, tend to exhibit lower testosterone levels than healthy controls (Zito et al., 2023). Given these mixed results across disorders, our findings of elevated testosterone in BPD might reflect disorder-specific mechanisms, particularly given the correlation with symptom load in our sample.

Our results may suggest impaired functioning of the HPG axis, which is the main source of testosterone production in females (Burger, 2002). The HPG axis is influenced by early life experiences, such as chronic early life stress or stressful events in adulthood (Toufexis et al., 2014). The development of BPD is frequently associated with such adverse early life experiences (Yuan et al., 2023), which were also reported by our BPD sample. However, it remains unclear whether elevated testosterone is a cause or a result of a BPD. Higher smoking rates or other unhealthy behaviors may elevate testosterone levels. To establish causal relationships, future studies could adopt longitudinal designs that track testosterone levels from adolescence onward, allowing researchers to observe

whether elevated testosterone precedes the development of BPD symptoms. Additionally, randomized controlled trials (RCTs) in which testosterone levels are experimentally manipulated could help assess whether changes in testosterone directly impact BPD-related behaviors, such as impulsivity or aggression.

The findings of elevated testosterone levels are of clinical relevance, as higher testosterone levels in females are associated with negative health outcomes including obesity or breast cancer (Roepke et al., 2010; Ruth et al., 2020). Also, there is evidence that higher testosterone levels in mothers with BPD are associated with more negative child interactions (Bonfig et al., 2022).

4.2. Prosocial behavior in patients with BPD

In the ultimatum game, patients with BPD equally often punished unfair offers like HC after inclusion and exclusion, which is in line with results of several previous studies (De Panfilis et al., 2019; Jeung et al., 2020; Wischniewski and Brüne, 2013). In other studies, individuals with borderline personality (features) rejected less offers than HC and accepted even unfair offers (Polgár et al., 2014) or rejected more fair offers (De Panfilis et al., 2019; Thielmann et al., 2014). In the present sample of female patients with BPD we could not find evidence for less prosocial behavior in terms of aggressive punishment behavior. Instead, participants with BPD displayed even higher prosocial behavior than HC by actively sharing more money in the dictator game after both Cyberball conditions. This is in contrast to other studies, where individuals with borderline personality (features) shared equal amounts of money with coplayers as healthy individuals (Hepp et al., 2018; Jeung et al., 2020). These divergent outcomes might be due to variations in experimental methodology including the number of rounds of the game. Unlike one-shot games used in prior studies, our multiple-round dictator game allowed for repeated interactions, potentially influencing participants' reciprocal and self-regulatory behaviors (Nitschke et al., 2022). Instead of acting out of altruistic motives, it might be possible that individuals with BPD shared more money in the dictator game in order to prevent rejection by their co-players. Together with the finding of elevated testosterone levels and the positive association between testosterone and sharing, this might be interpreted along the status-seeking hypothesis of testosterone, which proposes that testosterone promotes social status-seeking (Cheng and Kornienko, 2020; Eisenegger et al., 2010). Due to higher levels of testosterone, individuals with BPD might have shared more money in the dictator game to secure their social standing. These results are in contrast to the previous assumption that higher levels of testosterone relate to aggressive „fight-or-flight" behavior.

4.3. Social exclusion, testosterone and prosocial behavior

Cyberball condition did not affect testosterone levels across groups, which is in line with findings of previous studies in healthy individuals (Radke et al., 2018). While there are no comparable testosterone results in female patients with BPD, several studies found no changes in cortisol in response to Cyberball (Gaffey and Wirth, 2014; Seidel et al., 2013) which was also the case in the present sample (Graumann et al., 2023) (see supplement S4). We conclude that Cyberball does not induce a neuroendocrine response on the hypothalamic-pituitary-adrenal axis or on the HPG axis. Our findings regarding testosterone parallel the previously observed dissociation between tasks found for cortisol. While social stress induced with the Trier Social Stress Test (TSST) (Kirschbaum et al., 1993) resulted in an increase in both cortisol and testosterone (Deuter et al., 2021; Duesenberg et al., 2019), the social stressor solely targeting social exclusion increased neither in patients with BPD and HC.

Furthermore, social exclusion did not affect prosocial behavior in terms of sharing and punishment. Cyberball condition only affected punishment of marginally unfair offers (30 % of the stake), which were rejected more after overinclusion than after exclusion across groups. After overinclusion it might be safer to reject offers, because of a better social standing in the group. This effect might only become evident for marginally unfair offers, but not for clearly fair or unfair offers. The absence of strong effects of social exclusion on prosocial behavior may be due to its schematic appearance. Cyberball lacks face-to-face contact and does not include exclusion by real people, as is the case in the TSST or in real life.

4.4. Strengths and limitations

Strengths of the study include that we tested a relatively large sample of patients with BPD and compared results to a tightly matched control group. Second, we investigated the effects of psychosocial stress, i.e., social exclusion, without the confound of an endocrine reaction. The study's limitations include that our sample was solely female and highly educated, which might not allow for generalization to other groups. Patients with BPD had slightly higher Body Mass Index (BMI) than HC, which might have influenced testosterone levels. However, when BMI was included in the analysis, these differences did not account for our results. Testosterone values were missing for some participants, because they had difficulty collecting the required amount of saliva. In the future, it should be ensured that enough saliva is collected for each participant by using chewing film to help produce more saliva. Furthermore, the paradigms that were used do not reflect real-life situations. Other variants of Cyberball using partial exclusion are more realistic and might induce stronger hormonal and behavioral reactions. In addition, when interpreting results from economic decision-making games, variability in methodology has to be considered, including number of rounds or amount of money at stake. We did not assess motivation to share in the economic exchange games, which could have provided insight into the patients' reasons for sharing more money and should also be investigated in future studies.

5. Conclusion

The finding of higher salivary testosterone in female participants with BPD suggests that testosterone may play a role in the neurobiology of the disorder. Our results add to the growing body of evidence that BPD involves complex interactions between genetic, hormonal, and neurochemical factors. Our finding that patients with BPD shared more money in an economic exchange game challenges the hypothesis of a "fight-or-flight" response to stress. Instead of exhibiting aggressive behavior in response to stress, individuals with BPD may act in a more dominant, but prosocial way to secure social their social standing in ambiguous social situations.

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Author contributions

KW, OTW, CO and SR conceptualized the study. JHR performed the biochemical analyses. LG, EK, ABC and JS collected data and recruited patients. LG, ABC, and KW designed the methodology. EK and CED programmed software and implemented computer code. Formal analysis and data curation were done by LG and JS. LG wrote the original draft. All authors contributed, reviewed, and commented on the final draft. All authors approved the submitted version. Funding was acquired by KW.

CRediT authorship contribution statement

Jill Schell: Writing – review & editing, Investigation, Formal analysis, Data curation. **Julian Hellmann-Regen:** Writing – review &

editing, Resources, Conceptualization. **Christian Eric Deuter:** Writing – review & editing, Software. **Oliver T. Wolf:** Writing – review & editing, Conceptualization. **Eugenia Kulakova:** Writing – review & editing, Software, Investigation. **An Bin Cho:** Writing – review & editing, Conceptualization. **Livia Graumann:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Christian Otte:** Writing – review & editing, Conceptualization. **Katja Wingenfeld:** Writing – review & editing, Methodology, Funding acquisition. **Stefan Roepke:** Writing – review & editing, Conceptualization.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the first author used ChatGPT in order to improve language and readability. After using this tool, the author reviewed and edited the content as needed and takes full responsibility for the content of the publication.

Declaration of Competing Interest

None of the authors of the manuscript entitled elevated testosterone and prosocial behavior in female patients with borderline personality disorder independent of social exclusion have any conflicts of interest.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.psyneuen.2024.107232.

Data Availability

The raw data will be made available by the authors upon request.

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