



# Associations of plasma testosterone with clinical manifestations in acute panic disorder

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## ABSTRACT

The probable implication of testosterone in the neurobiology of anxiety disorders, and particularly panic disorder (PD), is poorly studied. We explored for potential differences concerning testosterone (T) plasma levels and the ratio testosterone/cortisol (T/C) between medication-free, consecutively-referred patients with acute exacerbation of PD comorbid with agoraphobia (PDA) (N = 40; females = 24; age =  $31.4 \pm 7.1$  years) and healthy controls (N = 80; females = 48; matched for age). Moreover, we investigated for potential associations of T levels and T/C ratio with the severity of acute PDA psychopathology in the patients of the sample. Psychometric measures included panic attacks' number during last three weeks (PA-21days), the Agoraphobic Cognitions Questionnaire (ACQ) and the Hamilton Anxiety Rating Scale (HARS). Male patients –but not female ones– demonstrated significantly lower T levels compared to controls. Moreover, in male patients, a significant inverse association emerged between T/C ratio and PA-21days, so that lower T/C ratio is associated with significantly more panic attacks. On the contrary, female patients demonstrated significant positive associations: (a) between T levels and PDA-related pathological cognitions (ACQ); (b) between the T/C ratio and both PA-21days and anxiety symptoms' severity (HARS). The results of the study suggest that testosterone is significantly associated to the severity of clinical manifestations of acute panic disorder, although in a different fashion concerning the two genders.

## 1. Introduction

The biological basis of panic disorder (PD) remains largely unknown, despite the significant progress achieved during the last decades regarding this issue (see for a review: [Bandelow et al., 2017](#)).

Epidemiological data suggest that sex hormones may play a pivotal role in the neurobiology of PD, since its lifetime prevalence is more than double in women than in men ([McLean et al., 2011](#)). In men, testosterone is mainly produced by the Leydig cells of the testes and by the adrenal cortex as well. In women, testosterone is secreted by the adrenal zona fasciculata (25%) and the ovarian stroma (25%), each approximately 50 µg per day, with the remaining 50% being produced from circulating androstenedione. Furthermore in women, daily production rate ranges from 0.1 to 0.4 mg, while circulating levels range from 0.2 to 0.7 ng/ml ([Longcope, 1986](#); [Burger, 2002](#)). Testosterone

circulates both in its free form, and bound to protein including albumin and sex steroid hormone-binding globulin (SHBG), the levels of which are an important determinant of free testosterone concentration. As with the rest of the androgens, testosterone can be produced intracellularly from dehydroepiandrosterone sulphate (DHEAS) ([Burger, 2002](#)). Testosterone has an important role in the development of secondary sexual attributes, but is also of special interest regarding social-emotional behavior ([Eisenegger et al., 2011](#)).

Overall, animal studies and human studies in healthy subjects and women with polycystic ovary syndrome (PCOS) have produced various and often contradictory results concerning the association of testosterone to a range of manifestations of anxiety and fear. Thus, reductions in experimentally-induced fear after testosterone administration were reported in studies using animals, including heifers ([Boissy and Bouissou, 1994](#)), ewes ([Bouissou and Vandenheede, 1996](#)), male rats

**Abbreviations:** ACQ, agoraphobic cognitions questionnaire; C, cortisol; CO<sub>2</sub>, carbon dioxide; CRH, corticotropin-releasing hormone; DHEAS, dehydroepiandrosterone sulphate; DSM-5, diagnostic and statistical manual of mental disorders, 5th Edition, American Psychiatric Association; HARS, Hamilton anxiety rating scale; HPA-axis, hypothalamus-pituitary-adrenal axis; HPG-axis, hypothalamus-pituitary-gonadal axis; PA-21days, number of panic attacks during last 21 days; PCOS, polycystic ovary syndrome; PD, panic disorder; PDA, panic disorder comorbid with agoraphobia; SCID, structured clinical interview for DSM-IV (SCID); SHBG, steroid hormone-binding globulin; T, testosterone; T/C, testosterone to cortisol ratio

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(Frye and Seliga, 2001) and male house mice (*Mus musculus*) (Aikey et al., 2002). Likewise, testosterone administration in healthy humans reduced unconscious, but not conscious, fear to threatening stimuli (angry faces) (van Honk et al., 2005). On the other hand, females suffering from PCOS -in which elevated testosterone levels is the prominent feature- demonstrate symptoms of social and generalized anxiety and various phobias significantly more often than women without PCOS (Manson et al., 2008; Jedel et al., 2010; Dokras et al., 2012).

Regarding anxiety disorders as clinical entities, the evidence for a potential role of testosterone in their neurobiology is much more limited (Giltay et al., 2012). Concerning the potential association between panic disorder in particular and testosterone levels, only one study has as yet evaluated nocturnal urinary testosterone excretion in 16 medication-free male PD patients and 13 controls and found it to be similar between the two groups (Bandelow et al., 1997). However, the sample consisted only of males, who, furthermore, could be included in the procedure of the study even if they were medication-free for only one week. Moreover, some of them were undergoing psychotherapy, while it was not reported how many patients were clinically stable due to pharmacotherapy/psychotherapy or were suffering from acute panic symptoms. A subsequent community-based study found no association between salivary testosterone levels and the presence or not of PD, but patients received various medications from all categories of antidepressants and/or benzodiazepines from different physicians in the community (Giltay et al., 2012).

Thus, to the best of our knowledge, no study has as yet explored in a systematic way the potential association between clinical manifestations of panic disorder and plasma levels of testosterone in medication-free patients suffering from panic disorder, moreover in the acute phase of their disorder. This was exactly the aim of this study. More precisely, our first aim was to explore whether patients of both genders, suffering from acute panic disorder, moreover remaining medication-free for at least one month and not receiving any form of psychotherapy, would demonstrate significant differences from normal controls concerning testosterone plasma levels. The second aim of the study was to explore potential associations between testosterone plasma levels and severity of acute PD psychopathology in this sample of medication-free patients. In addition, since testosterone exerts an inhibitory effect on hypothalamus-pituitary-adrenal axis (HPA) axis (Handa et al., 1994; Roca et al., 2005; Rubinow et al., 2005), we used as a correlate to psychometric features the ratio testosterone to cortisol (T/C), which expresses the interaction of hypothalamus-pituitary-gonadal (HPG)-axis and HPA-axis (Williamson et al., 2005; Sollberger and Ehlert, 2016; Ludwig et al., 2018). As formulated by Mehta and Prasad (2015), testosterone and cortisol may jointly regulate social behaviors and traits, and T/C ratio has been studied in relation to dominance, social aggression, social threat, and risk-taking behavior (Terburg et al., 2009; Mehta and Josephs, 2010; Mehta et al., 2015).

The potential association between testosterone levels and the presence of agoraphobia is not clear as yet and data are contradictory. For example, female -but not male- patients suffering from agoraphobia without PD demonstrated lower levels of testosterone, although no association was found in patients suffering from PD irrespective of the comorbidity with agoraphobia (Giltay et al., 2012). At any rate, for reasons of uniformity, we included only those PD patients whose agoraphobic-type avoidances were enough to meet the DSM-5 criteria for agoraphobia. Noteworthy, according to our previous experience, the large majority of patients who are referred from the Outpatient Clinic of our Department suffer from both diagnoses (e.g. Masdrakis et al., 2009, 2017).

## 2. Methods

### 2.1. Diagnostic procedures

The protocol of the study was approved by Hospital's Ethics-

Committee. All procedures followed were in accordance with the Helsinki Declaration (1975, revised 2008). All patients were consecutively referred to the Outpatient Clinic of Anxiety Disorders of our Department from the Outpatient Clinics of Emergency and General Psychiatry. All subjects gave written informed-consent following a comprehensive explanation of the procedure. Patients were informed that appropriate treatment and regular follow-up would start immediately after the end of the procedure of the study. Initial clinical evaluation of the patients, including a Structured Clinical Interview for DSM-IV (SCID) (First et al., 1998), was always performed by the first author, a psychiatrist. The definite diagnosis according to the DSM-5 criteria (American Psychiatric Association, 2013) was reached after discussion of the cases and common agreement by the clinicians participating in the study (VM, CP), both experienced clinical psychiatrists.

### 2.2. Inclusion and exclusion criteria of the study

#### 2.2.1. Inclusion criteria

DSM-5 Panic Disorder with comorbid Agoraphobia; current exacerbation of panic symptomatology; psychotropic medication-free for at least one month (three months for fluoxetine) prior to baseline evaluation.

#### 2.2.2. Exclusion criteria

Concurrent medical/psychiatric comorbidity; major medical/psychiatric (e.g. psychosis, bipolar disorders, recurrent major depression) disorders in the past; score > 10 in the Hamilton Depression Rating Scale (17-item) (Hamilton, 1960); currently undergoing any pharmacotherapy for psychiatric or other medical disorders, or psychotherapy; currently adopting any type of diet, or fasting; substance abuse disorder, except smoking; oral contraceptive use; pregnancy. Patients had to be declared healthy by an internist and a cardiologist and should have normal routine blood tests (including thyroid function-tests).

### 2.3. Subjects

Through the procedures described above, 40 (forty; females = 24) medication-free patients, consecutively referred from our Department's Outpatient Clinic were included in the study. All patients received a definite diagnosis of DSM-5 Panic Disorder with comorbid Agoraphobia, confirmed by a SCID-Interview (First et al., 1998) and, furthermore, fulfilled all inclusion/exclusion criteria of the study as specified above. None of the patients received any type of medication (exclusion criterion of the study). Control's sample included 80 (eighty; females = 48) healthy subjects, matched for age with patients, moreover not taking any type of medication. All female subjects (patients and controls) were normally menstruating.

### 2.4. Blood sampling and biochemical evaluations

In all cases, venous blood samples were collected between 08.00 and 10.00 h. Patients were previously instructed (in a written form) to abstain from coffee and any food/beverage/drug containing caffeine (cited in a list given to them) for at least 15 h, from alcohol for at least 24 h and from smoking from at least 3 h, before the procedure.

Blood was withdrawn in tubes with EDTA, plasma separated by centrifugation and kept in aliquots at  $-30^{\circ}\text{C}$  until estimation.

Cortisol and testosterone were measured using the RIA-kits of DIALsource Immunoassays SA, Belgium. For testosterone, standards for the calculation curve include concentrations of 0.11, 0.48, 1.55, 5.4, and 16.4 ng/ml. The manufacturer gives a detection limit of 0.05 ng/ml, and an intra-assay precision of 4.6% for low T levels. For the calculation of T in females we used for the calibration curve the three lower concentrations of standards. We calculated an intra-assay coefficient of variation for samples of the female population of  $4.1 \pm 2.4\%$  (range 0.5–8.9%). For testosterone in males, intra-assay coefficients of

variation were  $3.9 \pm 2.9\%$  (range 0.2–9.8%). For cortisol coefficients of variation were  $3.2 \pm 2.1\%$  (range 0.3–7.3%).

## 2.5. Psychometric evaluations

**(1) Number of panic attacks according to DSM-5 criteria (APA, 2013) during the last 21 days/three weeks (PA-21days)** (patient-rated). A brief definition of ‘panic attack’ was provided typewritten. Additionally, before its completion, a brief description of panic attack’s meaning was given to the patient using examples of her/his own experiences.

**(2) The Agoraphobic Cognitions Questionnaire (ACQ)** (Chambless et al., 1984), a self-rated, 14-item scale assessing thoughts of physical catastrophe due to anxiety symptoms or thoughts of mental, social, and/or behavioral disaster from loss of control. Items are rated on five-point scales ranging from 1–5. Total score ranges from 14 to 70.

The ACQ has been found to possess construct and discriminant validity, and its internal consistency coefficient (Cronbach alpha) was 0.80 (Chambless et al., 1984; Bouchard et al., 1997; Khawaja, 2003).

**(3) The Hamilton Anxiety Rating Scale (HARS)** (Hamilton, 1959), a clinician-rated, 14-item scale covering both psychic and somatic anxiety, with item-scores ranging from 0–4. Total score ranges from 0–56.

## 2.6. Statistical analysis

Comparisons were made using analysis of variance. Hormone levels of male and female patients were compared to same gender controls using analysis of variance with age and body mass index as covariates. Possible correlations of testosterone and T/C ratio to the scores of the psychometric evaluations HARS and ACQ were explored using Pearson correlation coefficient test. For variable PA-21days, which does not follow normal distribution, the nonparametric Spearman correlation coefficient test was used. Correlation coefficients were calculated separately for male and for female patients.

## 3. Results

### 3.1. Descriptive data of the patients and controls of the sample

The sample of the study ( $N = 120$  subjects) included 40 medication-free PDA patients (females = 24, 60%) and 80 healthy controls (females = 48; 60%) matched for age.

Patients’ mean age was 31.4 years ( $SD = 7.1$ ) and the mean duration of their illness was 57.7 months ( $SD = 62.5$ , median = 33.0). Nineteen patients (47.5% of the sample; 11 females) had undergone pharmacotherapy in the past, and seven patients (17.5% of sample; 5 females) had undergone psychotherapy in the past. Seventeen patients (42.5% of the sample; 10 females) reported family history for PD/PDA. Nineteen patients (47.5% of the sample; 11 females) were smokers.

From 24 female patients, 14 were in the follicular phase of the cycle when assessed and 10 in the luteal. Their testosterone levels were  $0.44 \pm 0.15$  and  $0.42 \pm 0.07$  ng/ml respectively, and the difference was not significant ( $F_{1, 22} = 0.19$ ,  $p = .67$ ).

Other descriptive data, including patients’ psychometric and both patients’ and controls’ hormone data are shown in Table 1.

### 3.2. Comparison between patients and controls regarding testosterone and cortisol plasma levels and the ratio T/C

The results of the comparison between patients and controls of each gender regarding testosterone and cortisol plasma levels and the ratio T/C are demonstrated in Table 1.

#### 3.2.1. Male subjects

With respect to male subjects, analysis revealed significantly lower testosterone plasma levels in PDA patients ( $N = 16$ ) compared to

controls ( $N = 32$ ). No significant differences emerged between patients and controls concerning age, cortisol plasma levels and the T/C ratio (Table 1).

#### 3.2.2. Female subjects

Regarding female subjects, no significant differences emerged between patients ( $N = 24$ ) and controls ( $N = 48$ ) concerning age, testosterone and cortisol plasma levels and the T/C ratio as well (Table 1).

It has to be noticed that testosterone levels of patients and controls of the study were within normal ranges, namely 0.2–0.7 ng/ml for females (Burger, 2002), and 2.64–9.16 ng/ml for males in the age range 19–39 years (Travison et al., 2017). However, male patients had lower T levels than controls (Table 1), with high frequencies (76%) of values below median value for controls.

### 3.3. Associations between testosterone plasma levels and T/C ratio and clinical manifestations of panic disorder

Variables followed normal distribution except PA-21days, which showed higher frequencies for low values. Using the nonparametric Spearman correlation coefficient test, the correlations of PA-21days to T/C ratio presented in Fig. 1 and Fig. 3 were both significant:  $R_s = -0.598$ ,  $p = 0.01$  for males, and  $R_s = 0.484$ ,  $p = 0.01$  for females.

Data regarding the correlations between testosterone plasma levels and the T/C ratio and the scores on the psychometric measures (HARS, ACQ, PA-21days) in the patients of the sample are shown in Table 2.

#### 3.3.1. Male patients

With respect to male PDA patients, a significant inverse association emerged between T/C ratio and number of panic attacks during last three weeks (Table 2 and Fig. 1).

#### 3.3.2. Female patients

Contrary to what was found in male patients, the female patients of the sample demonstrated significant positive associations between plasma testosterone levels and panic- and agoraphobia-related pathological cognitions as these are reflected in the ACQ psychometric measure (Table 2 and Fig. 2). Moreover, in the female patients of the sample, significant positive associations emerged between the T/C ratio and both the severity of anxiety symptoms (HARS) (Table 2) and the number of panic attacks during last three weeks (PA-21days) (Table 2 and Fig. 3).

### 3.4. Comparisons between male and female subjects regarding testosterone and cortisol plasma levels and the ratio T/C and between male and female patients regarding psychometric evaluations

Differences between male and female subjects were not significant for cortisol either for controls or for patients. Testosterone levels, as well as T/C ratio, were about ten times higher for males than for females in both controls and patients (Table 1). No significant differences between male and female patients were found for HARS (ANOVA,  $p = 0.34$ ), ACQ (ANOVA,  $p = 0.58$ ) or PA-21days (U-test,  $p = 0.30$ ).

## 4. Discussion

### 4.1. Summary of results

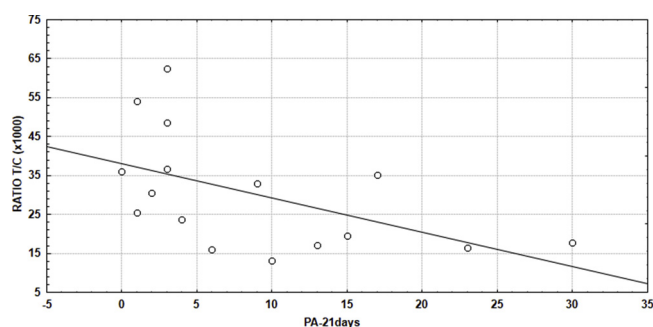
The first aim of the study was to explore potential differences regarding testosterone (T) plasma levels between medication-free patients suffering from acute exacerbation of DSM-5 panic disorder comorbid with agoraphobia (PDA) and normal controls. No such difference emerged with respect to female subjects. On the contrary, regarding male subjects, panic patients demonstrated significantly lower testosterone levels compared to normal controls. In both males and females, cortisol (C) and the ratio T/C were similar between patients and

**Table 1**

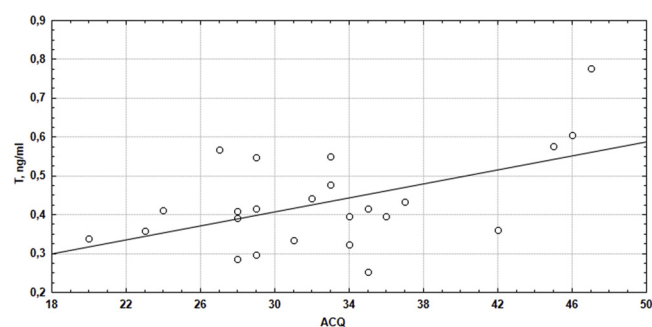
Descriptive data regarding sample's patients (N = 40) and controls (N = 80), and comparison of hormones' plasma levels and T/C ratio between the two groups for males and for females by analysis of variance with covariates age and BMI.

	MALES			F/p	FEMALES			F/p
	CNTR	PDA			CNTR	PDA		
N	32	16			48	24		
HARS		21.9 ± 8.4				20.5 ± 7.2		
ACQ		31.5 ± 7.9				32.4 ± 7.0		
PA-21days		8.8 ± 8.7				4.3 ± 3.0		
AGE	32.9 ± 6.7	32.4 ± 7.9		0.06/.81	31.0 ± 6.8	30.5 ± 6.7		0.10/.75
AGE range	21 - 46	19 - 45			18 - 42	19 - 42		
BMI	25.2 ± 3.5	25.0 ± 2.7		0.03/.86	24.2 ± 3.7	21.4 ± 3.1		12.84/.001
CORT	167 ± 57	154 ± 68		0.52/.47	146 ± 67	147 ± 65		0.06/.81
T	5.11 ± 1.82	3.95 ± 1.16		5.98/.018	0.44 ± 0.15	0.43 ± 0.12		0.29/.59
T/C (x10 <sup>3</sup> )	33.6 ± 15.1	30.3 ± 14.7		0.48/.49	3.77 ± 2.38	3.68 ± 2.30		0.03/.87

Abbreviations: ACQ = Agoraphobic Cognitions Questionnaire; BMI = body mass index; CORT = cortisol (ng/ml); CNTR = healthy controls; HARS = Hamilton Anxiety Rating Scale; PA-21days = number of panic attacks during the last 21 days; PDA = patients with acute panic disorder comorbid with agoraphobia; T = testosterone (ng/ml); T/C = ratio testosterone/cortisol.



**Fig. 1.** Correlation between the number of panic attacks during last 21 days (PA-21days) and the testosterone/cortisol (T/C) ratio, in 16 medication-free male patients with acute exacerbation of panic disorder comorbid with agoraphobia ( $R_s = -0.598$ ,  $p = 0.01$ ).



**Fig. 2.** Correlation between the scores on the Agoraphobic Cognitions Questionnaire (ACQ) and the plasma levels of testosterone (T), in 24 medication-free female patients with acute exacerbation of panic disorder comorbid with agoraphobia ( $r = 0.523$ ,  $p = 0.009$ ).

**Table 2**

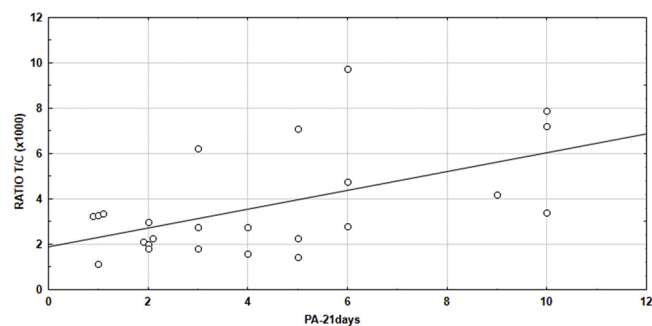
Correlations of testosterone plasma levels and the T/C ratio to the scores of the psychometric measures (HARS, ACQ, PA-21days) in male and female patients with acute panic disorder comorbid with agoraphobia. Pearson correlation coefficients for HARS and ACQ, and Spearman correlation coefficients for PA-21days.

	MALES (n = 16)		FEMALES (n = 24)	
	r	p	r	
T vs HARS	0.314	.24	0.081	.71
T vs ACQ	-0.108	.69	0.523	.009
T vs PA-21days	-0.456	.08	0.173	.42
T/C vs HARS	-0.274	.30	0.418	.04
T/C vs ACQ	-0.195	.47	0.295	.16
T/C vs PA-21days	-0.598	.01	0.484	.01

Abbreviations: ACQ = Agoraphobic Cognitions Questionnaire; C = cortisol (ng/ml); HARS = Hamilton Anxiety Rating Scale; PA-21days = number of panic attacks during the last 21 days; T = testosterone (ng/ml); T/C = ratio testosterone/cortisol.

controls.

The second aim of the study was to explore potential associations of plasma testosterone and T/C ratio with the severity of PDA psychopathology. Indeed, concerning male patients, an inverse association emerged between the T/C ratio and the number of panic attacks during the last three weeks, so that lower T/C ratio is associated with significantly more panic attacks. On the contrary, in the female patients of the sample, significant positive associations emerged between plasma testosterone levels and panic- and agoraphobia-related pathological cognitions, as these are reflected in the ACQ measure. Furthermore, in



**Fig. 3.** Correlation between the number of panic attacks during last 21 days (PA-21days) and the testosterone/cortisol (T/C) ratio, in 24 medication-free female patients, with acute exacerbation of panic disorder comorbid with agoraphobia ( $R_s = 0.484$ ,  $p = 0.01$ ).

female patients, significant positive associations were observed between the ratio T/C and both anxiety symptoms' severity (HARS) and the number of panic attacks during last three weeks.

#### 4.2. Potential neurobiological explanations of the results of the study

In his seminal 'false suffocation alarm' theory, Donald Klein hypothesized that the neurobiological mechanism underlying genuine panic attacks consists in the activation of PD patients' pathologically hypersensitive 'suffocation alarm system', an evolutionary adaptation with the specific function of detecting increased concentration-rate of carbon dioxide (CO<sub>2</sub>) and other exteroceptive and interoceptive cues indicating lack of useful air (Klein, 1993). This hypersensitivity of PD



patients to CO<sub>2</sub> accumulation –the most potent cue of asphyxia– has been well documented in numerous studies (see for a review: [Bandelow et al., 2017](#)).

In this respect, recent data suggest that testosterone attenuates the psychophysiological response of male rats to CO<sub>2</sub> administration, since rats' castration produced a 100% increase of their hyperventilatory response to 10% CO<sub>2</sub> ([Tenorio-Lopes et al., 2017](#)). This inhibitory influence of testosterone in “normal” rats regarding the emergence of respiratory panic-like manifestations was even more pronounced when considering that another group of male rats who had undergone neonatal maternal separation lost testosterone's protective effects against hypercapnic stimuli, since their castration did not alter their ventilatory response to CO<sub>2</sub> administration.

The findings of the study by [Tenorio-Lopes et al. \(2017\)](#), in conjunction with Klein's ‘false suffocation alarm’ theory (1993), might provide a plausible biologically-oriented explanation regarding the finding of this study of an inverse association between testosterone levels and panic symptoms' severity in male PD patients. More precisely, lower testosterone levels may further deteriorate the already pathological hypersensitivity of patients' suffocation alarm system, thus resulting in the emergence of panic symptoms as a response to stimuli falsely perceived as signaling asphyxia.

A mutually inhibitory functional connection exists between the hypothalamic-pituitary-adrenal (HPA) and hypothalamic-pituitary-gonadal (HPG) axes. In this context, testosterone inhibits the stress-induced activation of the HPA-axis at the level of the hypothalamus ([Viau, 2002](#); [Williamson et al., 2005](#); [Ludwig et al., 2018](#)). Regarding healthy humans who underwent pharmacological (CRH, corticotrophin releasing hormone) and physiological (exercise) stressors in the context of induced gonadal suppression with leuprolide acetate, men showed increased stimulated ACTH and cortisol compared with women ([Roca et al., 2005](#)). Moreover, in healthy men testosterone regulates CRH-stimulated HPA-axis activity, but its divergent effects on ACTH and cortisol suggests a peripheral (adrenal) locus for the suppressive effects on cortisol ([Rubinow et al., 2005](#)). With regard to panic disorder, although the actual association between human panic attacks and HPA-axis activation is still discussed (e.g. [Sinha et al., 1999](#); [van Duinen et al., 2007](#); [Masdrakis et al., 2015](#)), nevertheless HPA-axis seems overall to be implicated in the pathophysiology of PD ([Bandelow et al., 2017](#)). Consequently, still another potential explanation of the findings of this study may be that PD patients with lower testosterone levels are less ‘protected’ regarding HPA-axis activation during daily exposure to an array of potentially panicogenic interoceptive or exteroceptive cues.

Another prominent biological theory ([Gorman et al., 2000](#); [Dresler et al., 2013](#)) attributes panic emergence to the pathologic function of a ‘fear circuit’ involving –among other neural structures– the amygdala, hippocampus, and prefrontal cortex. In this respect, previous data suggest that in healthy humans, increased/greater testosterone is associated with increased amygdala reactivity and decreased amygdala-prefrontal cortex connectivity ([van Wingen et al., 2010](#); [Volman et al., 2011](#); [Spielberg et al., 2015](#)). Subsequently, given that increased amygdala activity is broadly associated with anxiety disorders, one would expect testosterone to be linked to greater anxiety and panic symptoms, which was not the case with the male patients of our sample. However, numerous previous animal studies suggest reductions in fear after treatment with testosterone (e.g. [Boissy and Bouissou, 1994](#); [Bouissou and Vandenheede, 1996](#); [Frye and Seliga, 2001](#); [Aikey et al., 2002](#)). Likewise, testosterone administration in healthy humans reduced unconscious fear to threatening stimuli (angry faces) ([van Honk et al., 2005](#)). A potential explanation regarding this discrepancy –i.e. testosterone increases amygdala reactivity, but animal and human (healthy subjects) studies suggest fear-reducing properties of testosterone– may lay in the above-mentioned mutually inhibitory functional connection between the HPA and the HPG axes, and, therefore, in the mutually antagonistic properties of the hormones cortisol and testosterone ([Viau, 2002](#); [Roca et al., 2005](#); [Rubinow et al., 2005](#); [Williamson](#)

[et al., 2005](#); [Ludwig et al., 2018](#)). Of note, heightened levels of the steroid hormone cortisol exaggerate and sustain fearfulness by facilitating corticotropin-releasing hormone (CRH) gene expression at the amygdala ([Corodimas et al., 1994](#); [Schulkin et al., 1998](#)). Another potential explanation comes from most recent data in healthy humans which suggest that in the context of acute threat, testosterone administration reduces the bottom-up coupling between a sub-cortical ‘threat system’ (central-medial amygdala-hypothalamus-periaqueductal gray) and the left lateral orbitofrontal cortex and therefore facilitates goal-directed action, as opposed to reductions in top-down control from the medial orbitofrontal cortex which underlie risk-taking and social aggression ([Heany et al., 2018](#)). Finally, it has been suggested that in humans, panic is a distinct biological phenomenon when compared to both fear and anxiety ([Klein, 1993, 2002](#)).

In sum, data from previous studies in humans and animals suggest that testosterone exerts action on numerous neural structures implicated in the neurobiology of PD, including the neural system(s) detecting asphyxia cues, the amygdala and related structures of the ‘fear circuit’ and the HPA-axis. However, this study cannot delineate the exact biological mechanism(s) underlying the association between testosterone levels and panic symptomatology observed in the female patients of our sample.

#### 4.3. Potential explanations regarding the positive association between testosterone levels and panic manifestations in the female patients of the sample

Contrary to male patients, the female PDA patients of the sample demonstrated a significant positive association between testosterone levels and panic manifestations. Previous research data which might explain this finding come from animal studies exploring how estrogens –all of which are synthesized from androgens, especially testosterone and androstenedione, by the enzyme aromatase ([Frye and Walf, 2004](#))– affect the amygdala and from human studies of female patients suffering from clinical conditions with pathologically increased testosterone levels.

More precisely, animal studies suggest that estrogens exert action on the amygdala, but whether they trigger or decrease anxiety-like behaviors is disputed. Thus, injection of estradiol into the amygdala of ovariectomized female rats decreased anxiety-like behavior in elevated plus maze and other tests ([Frye and Walf, 2004](#)). On the contrary, silencing estrogen receptor-alpha in the posterior medial amygdala of intact female rats decreased anxiety-like behavior in a light-dark box test, suggesting that estrogens' action on the amygdala may trigger anxiety ([Spiteri et al., 2010](#)). It is not clear, as yet, under which conditions estrogens' action on the amygdala triggers or attenuates anxiety manifestations and researchers speculate that different nuclei or different receptor subtypes may mediate the opposite effects ([McHenry et al., 2014](#)).

Data from humans come from the study of women with polycystic ovary syndrome (PCOS), where serum testosterone levels are increased. Thus, in a community-based study comparing PCOS patients and controls, no significant differences emerged between the two groups concerning ‘panic attacks’. However, significantly more PCOS women (almost half of them) reported panic- and agoraphobia-like ‘phobias’, including feelings of unreasonable fear in specific situations, such as buses, grocery stores, crowds, feeling enclosed and being alone ([Jedel et al., 2010](#)).

Moreover, a strong positive association between testosterone and panic/agoraphobia symptoms was suggested by the rare case of a 46-year old woman with a 2 cm Leydig-cell tumor within the right ovary ([Nardo et al., 2005](#)). At the time of the first referral, the patient demonstrated markedly raised serum testosterone levels (28.3 nmol/l), severe hyperandrogenism and associated complex medical history. Of note, random cortisol, androstenedione, 17-hydroxyprogesterone, DHEAS and SHBG concentrations were all within the normal range.

Furthermore, the patient suffered ‘anxiety disorder with agoraphobia’ -most probably corresponding to a diagnosis of PDA- severe enough to delay the surgical treatment for three years, during which she was house-bound. Tumor’s surgical removal significantly improved the symptoms of virilization and normalized the pre-operative markedly increased serum testosterone levels. Most importantly, PDA symptomatology completely remitted and pharmacotherapy was no longer needed.

In sum, previous data suggest a positive association between testosterone levels and agoraphobia-like fears -but not panic attacks- in females with PCOS, while the case-report of a woman suffering from a rare Leydig-cell ovary tumor suggested that pathologically increased testosterone levels may be associated with full-blown PDA which abates after surgical treatment and subsequent normalization of testosterone levels. However, our study is the first to provide direct evidence of a significant positive association between plasma testosterone levels and severity of panic and agoraphobic symptoms in female patients specifically suffering from acute PDA.

#### 4.4. Clinical implications and limitations of the study and future research

Our results have different clinical implications for the two genders. Previous research data provide evidence that treatment with testosterone drastically reduces depressive symptomatology in hypogonadal depressive patients (Burris et al., 1992; Zarrouf et al., 2009; Pope et al., 2010). No related data exists, as yet, concerning the potential usefulness of testosterone administration in male patients suffering from panic disorder with agoraphobia. Since lower plasma testosterone levels were associated with more severe panic symptoms, therefore the results of this study provide support to the notion that testosterone administration might alleviate panic symptoms, especially in males with low testosterone levels. However, this remains a hypothesis in need of systematic research in the future. Our results do not support the same for female PDA patients. Nevertheless, they highlight the importance of dealing with PCOS or other clinical conditions (e.g. specific gynecological tumors as in the above-mentioned case-report) in females, which are characterized by pathologically increased testosterone levels.

The main limitation of the study is its modest sample of patients. Nevertheless, we must stress that, tracing PDA patients who meet the inclusion/exclusion criteria of this study –especially remaining medication-free for at least one month while suffering from exacerbation of panic symptoms, furthermore not meeting diagnostic criteria for any other mental disorder- is not an easy task in clinical practice. Furthermore, a limitation of the study could be that testosterone levels, especially for females, were measured by radioimmunoassay and not by more sensitive methods like liquid chromatography / mass spectrometry. However, the results of the study are based on comparisons between patients and controls, or on correlations of hormone levels to psychometric correlates. They are not expected to be different if hormone levels are measured by another method that gives systematically higher or lower levels. Additional limitations may be considered to be the cross-sectional data and the measurements of testosterone and cortisol levels in only one blood sample for patients and controls. At any rate, future studies in larger samples of patients, who might undergo more extensive hormone testing (e.g. SHBG, free hormone levels, estrogen) are needed in order to replicate and expand the results of the present study, addressing its limitations.

## 5. Conclusion

This study is the first to provide indications that testosterone plasma levels are significantly lower in male patients –but not in female ones- suffering from acute exacerbation of panic disorder comorbid with agoraphobia compared to healthy controls. What is more, in male patients lower T/C ratio is significantly associated with more severe PDA

symptomatology. On the contrary, in female patients a significant positive association emerged between both testosterone levels and the T/C ratio with the severity of PDA symptoms. Future studies are needed to replicate our findings in larger patients’ samples and to explore their potential therapeutic implications in clinical practice.

## Declarations of interest

None of the authors report any conflict of interest.

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## References

- Aikey, J.L., Nyby, J.G., Anmuth, D.M., James, P.J., 2002. Testosterone rapidly reduces anxiety in male house mice (*Mus musculus*). *Horm. Behav.* 42, 448–460.
- American Psychiatric Association, 2013. *Diagnostic and Statistical Manual of Mental Disorders*, 5<sup>th</sup> ed. American Psychiatric Association, Arlington VA, Washington DC.
- Bandelow, B., Sengos, G., Wedekind, D., Huether, G., Pilz, J., Broocks, A., Hajak, G., Ruther, E., 1997. Urinary excretion of cortisol, norepinephrine, testosterone, and melatonin in panic disorder. *Pharmacopsychiatry* 30, 113–117.
- Bandelow, B., Baldwin, D., Abelli, M., Bolea-Alamanac, B., Bourin, M., Chamberlain, S.R., Cinosi, E., Davies, S., Domschke, K., Fineberg, N., Grunblatt, E., Jarema, M., Kim, Y.K., Maron, E., Masdrakis, V., Mikova, O., Nutt, D., Pallanti, S., Pini, S., Strohle, A., Thibaut, F., Vaghi, M.M., Won, E., Wedekind, D., Wichniak, A., Woolley, J., Zwanzger, P., Riederer, P., 2017. Biological markers for anxiety disorders, OCD and PTSD –a consensus statement- Part II: neurochemistry, neurophysiology and neurocognition. *World J. Biol. Psychiatry* 18, 162–214.
- Boissy, A., Bouissou, M.F., 1994. Effects of androgen treatment on behavioral and physiological responses of heifers to fear eliciting situations. *Horm. Behav.* 28, 66–83.
- Bouchard, S., Pelletier, M.-H., Gauthier, J., Cote, G., Laberge, B., 1997. The assessment of panic using self-report: A comprehensive survey of validated instruments. *J. Anxiety Disord.* 11, 89–111.
- Bouissou, M.F., Vandenheede, M., 1996. Long-term effects of androgen treatment on fear reactions in ewes. *Horm. Behav.* 30, 93–99.
- Burger, H.G., 2002. Androgen production in women. *Fertil. Steril.* 77 (Suppl. 4), S3–S5.
- Burris, A.S., Banks, S.M., Carter, C.S., Davidson, J.M., Sherins, R.J., 1992. A long-term, prospective study of the physiologic and behavioral effects of hormone replacement in untreated hypogonadal men. *J. Androl.* 13, 297–304.
- Chambless, D.L., Caputo, G.C., Bright, P., Gallacher, R., 1984. Assessment of fear of fear in agoraphobics: the body sensations questionnaire and the agoraphobic cognitions questionnaire. *J. Consult. Clin. Psychol.* 52, 1090–1097.
- Corodimas, K.P., LeDoux, J.E., Gold, P.W., Schulkin, J., 1994. Corticosterone potentiation of conditioned fear in rats. *Ann. N. Y. Acad. Sci.* 746, 392–393.
- Dokras, A., Clifton, S., Futterweit, W., Wild, R., 2012. Increased prevalence of anxiety symptoms in women with polycystic ovary syndrome: systematic review and meta-analysis. *Fertil. Steril.* 97, 225–230 e2.
- Dresler, T., Guhn, A., Tupak, S.V., Ehls, A.C., Herrmann, M.J., Fallgatter, A.J., Deckert, J., Domschke, K., 2013. Revise the revised? New dimensions of the neuroanatomical hypothesis of panic disorder. *J. Neural Transm. Vienna (Vienna)* 120, 3–29.
- Eisenegger, C., Haushofer, J., Fehr, E., 2011. The role of testosterone in social interaction. *Trends Cogn. Sci. (Regul. Ed.)* 15, 263–271.
- First, M.B., Spitzer, R.L., Gibbon, M., Williams, J.B., 1998. *Structured Clinical Interview for DSM-IV Axis I Disorders, Patient Edition (SCID-I/P, Version 2.0, 8/98 Revision)*. Biometric Research Department. New York State Psychiatric Institute, New York.
- Frye, C.A., Seliga, A.M., 2001. Testosterone increases analgesia, anxiolysis, and cognitive performance of male rats. *Cogn. Affect. Behav. Neurosci.* 1, 371–381.
- Frye, C.A., Wolf, A.A., 2004. Estrogen and/or progesterone administered systematically or to the amygdala can have anxiety-, fear-, and pain-reducing effects in ovariectomized rats. *Behav. Neurosci.* 118, 306–313.
- Giltay, E.J., Enter, D., Zitman, F.G., Penninx, B.W.J.H., van Pelt, J., Spinhoven, P., Roelofs, K., 2012. Salivary testosterone: associations with depression, anxiety disorders, and antidepressant use in a large cohort study. *J. Psychosom. Res.* 72, 205–213.
- Gorman, J.M., Kent, J.M., Sullivan, G.M., Coplan, J.D., 2000. Neuroanatomical hypothesis of panic disorder, revised. *Am. J. Psychiatry* 157, 493–505.
- Hamilton, M., 1959. The assessment of anxiety states by rating. *Br. J. Med. Psychol.* 32, 50–55.
- Hamilton, M., 1960. A rating scale for depression. *J. Neurol. Neurosurg. Psychiatry* 23, 56–62.
- Handa, R.J., Nunley, K.M., Lorens, S.A., Louie, J.P., McGivern, R.F., Bollnow, M.R., 1994.

- Androgen regulation of adrenocorticotropin and corticosterone secretion in the male rat following novelty and foot shock stressors. *Physiol. Behav.* 55, 117–124.
- Heany, S.J., Bethlehem, R.A.I., van Honk, J., Bos, P.A., Stein, D.J., Terburg, D., 2018. Effects of testosterone administration on threat and escape anticipation in the orbitofrontal cortex. *Psychoneuroendocrinology* 96, 42–51.
- Jedel, E., Waern, M., Gustafson, D., Landen, M., Eriksson, E., Holm, G., Nilsson, L., Lind, A.K., Janson, P.O., Stener-Victorin, E., 2010. Anxiety and depression symptoms in women with polycystic ovary syndrome compared with controls matched for body mass index. *Hum. Reprod.* 25, 450–456.
- Khawaja, N.G., 2003. Revisiting the factor structure of the agoraphobic cognitions questionnaire and body sensations questionnaire: a confirmatory factor analysis study. *J. Psychopathol. Behav. Assess.* 25, 57–63.
- Klein, D.F., 1993. False suffocation alarms, spontaneous panics, and related conditions. An integrative hypothesis. *Arch. Gen. Psychiatry* 50, 306–317.
- Klein, D.F., 2002. Historical aspects of anxiety. *Dialogues Clin. Neurosci.* 4, 295–304.
- Longcope, C., 1986. Adrenal and gonadal androgen secretion in normal females. *Clin. Endocrinol. Metab.* 15, 213–227.
- Ludwig, B., Roy, B., Dwivedi, Y., 2018. Role of HPA and the HPG axis interaction in testosterone-mediated learned helpless behavior. *Mol. Neurobiol.* <https://doi.org/10.1007/s12035-018-1085-x>. [Epub ahead of print].
- Manson, M., Holte, J., Landin-Wilhelmsen, K., Dahlgren, E., Johansson, A., Landen, M., 2008. Women with polycystic ovary syndrome are often depressed or anxious – a case-control study. *Psychoneuroendocrinology* 33, 1132–1138.
- Masdrakis, V.G., Markianos, M., Vaidakis, N., Papakostas, Y.G., Oulis, P., 2009. Caffeine challenge and breath-holding duration in patients with panic disorder. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 33, 41–44.
- Masdrakis, V.G., Markianos, M., Oulis, P., 2015. Lack of specific association between panicogenic properties of caffeine and HPA-axis activation. A placebo-controlled study of caffeine challenge in patients with panic disorder. *Psychiatry Res.* 229, 75–81.
- Masdrakis, V.G., Papageorgiou, C., Markianos, M., 2017. Associations of plasma leptin to clinical manifestations in reproductive aged female patients with panic disorder. *Psychiatry Res.* 255, 161–166.
- McHenry, J., Carrier, N., Hull, E., Kabbaj, M., 2014. Sex differences in anxiety and depression: role of testosterone. *Front. Neuroendocrinol.* 35, 42–57.
- McLean, C.P., Asnaani, A., Litz, B.T., Hofmann, S.G., 2011. Gender differences in anxiety disorders: prevalence, course of illness, comorbidity and burden of illness. *J. Psychiatry Res.* 45, 1027–1035.
- Mehta, P.H., Josephs, R.A., 2010. Testosterone and cortisol jointly regulate dominance: evidence for a dual-hormone hypothesis. *Horm. Behav.* 58, 898–906.
- Mehta, P.H., Prasad, S., 2015. The dual-hormone hypothesis: a brief review and future research agenda. *Curr. Opin. Behav. Sci.* 3, 163–168.
- Mehta, P.H., Welker, K.M., Zilioli, S., Carré, J.M., 2015. Testosterone and cortisol jointly modulate risk-taking. *Psychoneuroendocrinology* 56, 88–99.
- Nardo, L.G., Ray, D.W., Laing, I., Williams, C., McVey, R.J., Seif, M.W., 2005. Ovarian Leydig cell tumor in a peri-menopausal woman with severe hyperandrogenism and virilization. *Gynecol. Endocrinol.* 21, 238–241.
- Pope, H.G. Jr., Amiaz, R., Brennan, B.P., Orr, G., Weiser, M., Kelly, J.F., Kanayama, G., Siegel, A., Hudson, J.I., Seidman, S.N., 2010. Parallel-group placebo-controlled trial of testosterone gel in men with major depressive disorder displaying an incomplete response to standard antidepressant treatment. *J. Clin. Psychopharmacol.* 30, 126–134.
- Roca, C.A., Schmidt, P.J., Deuster, P.A., Danaceau, M.A., Altemus, M., Putnam, K., Chrousos, G.P., Nieman, L.K., Rubinow, D.R., 2005. Sex-related differences in stimulated hypothalamic-pituitary-adrenal axis during induced gonadal suppression. *J. Clin. Endocrinol. Metab.* 90, 4224–4231.
- Rubinow, D.R., Roca, C.A., Schmidt, P.J., Danaceau, M.A., Putnam, K., Cizza, G., Chrousos, G., Nieman, L., 2005. Testosterone suppression of CRH-stimulated cortisol in men. *Neuropsychopharmacology* 30, 1906–1912.
- Sinha, S.S., Coplan, J.D., Pine, D.S., Martinez, J.A., Klein, D.F., Gorman, J.M., 1999. Panic induced by carbon dioxide inhalation and lack of hypothalamic pituitary-adrenal axis activation. *Psychiatry Res.* 86, 93–98.
- Sollberger, S., Ehler, U., 2016. How to use and interpret hormone ratios. *Psychoneuroendocrinology* 63, 385–397.
- Schulkin, J., Gold, P.W., McEwen, B.S., 1998. Induction of corticotropin-releasing hormone gene expression by glucocorticoids: implication for understanding the states of fear and anxiety and allostatic load. *Psychoneuroendocrinology* 23, 219–243.
- Spielberg, J.M., Forbes, E.E., Ladouceur, C.D., Worthman, C.M., Olino, T.M., Ryan, N.D., Dahl, R.E., 2015. Pubertal testosterone influences threat-related amygdala-orbitofrontal cortex coupling. *SCAN* 10, 408–415.
- Spiteri, T., Musatov, S., Ogawa, S., Ribeiro, A., Pfaff, D.W., Agmo, A., 2010. The role of the estrogen receptor alpha in the medial amygdala and ventromedial nucleus of the hypothalamus in social recognition, anxiety and aggression. *Behav. Brain Res.* 210, 211–220.
- Tenorio-Lopes, L., Henry, M.S., Marques, D., Tremblay, M.E., Drolet, G., Bretzner, F., Kinkead, R., 2017. Neonatal maternal separation opposes the facilitatory effect of castration on the respiratory response to hypercapnia of the adult male rat: evidence for the involvement of the medial amygdala. *J. Neuroendocrinol.* <https://doi.org/10.1111/jne.12550>. In press.
- Terburg, D., Morgan, B., van Honk, J., 2009. The testosterone-cortisol ratio: a hormonal marker for proneness to social aggression. *Int. J. Law Psychiatry* 32, 216–223.
- Travis, T.G., Vesper, H.W., Orwoll, E., Wu, F., Kaufman, J.M., Wang, Y., Lapauw, B., Fiers, T., Matsumoto, A.M., Bhasin, S., 2017. Harmonized reference ranges for circulating testosterone levels in men of four cohort studies in the United States and Europe. *J. Clin. Endocrinol. Metab.* 102, 1161–1173.
- van Duinen, M.A., Schruers, K.R.J., Maes, M., Griez, E.J.L., 2007. CO<sub>2</sub> challenge induced HPA axis activation in panic. *Int. J. Neuropsychopharmacol.* 10, 797–804.
- van Honk, J., Peper, J.S., Schutter, J.L.G., 2005. Testosterone reduces unconscious fear but not consciously experienced anxiety: implications for the disorders of fear and anxiety. *Biol. Psychiatry* 58, 218–225.
- van Wingen, G., Mattern, C., Verkes, R.J., Buitelaar, J., Fernandez, G., 2010. Testosterone reduces amygdala-orbitofrontal cortex coupling. *Psychoneuroendocrinology* 35, 105–113.
- Via, V., 2002. Functional cross-talk between the hypothalamic-pituitary-gonadal and adrenal axes. *J. Neuroendocrinol.* 14, 506–513.
- Volman, I., Toni, I., Verhagen, L., Roelofs, K., 2011. Endogenous testosterone modulates prefrontal-amygdala connectivity during social emotional behavior. *Cereb. Cortex* 21, 2282–2290.
- Williamson, M., Bingham, B., Via, V., 2005. Central organization of androgen-sensitive pathways to the hypothalamic-pituitary-adrenal axis: Implications for individual differences in responses to homeostatic threat and predisposition to disease. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 29, 1239–1248.
- Zarrouf, F.A., Artz, S., Griffith, J., Sirbu, C., Komor, M., 2009. Testosterone and depression: systematic review and meta-analysis. *J. Psychiatr. Pract.* 15, 289–305.