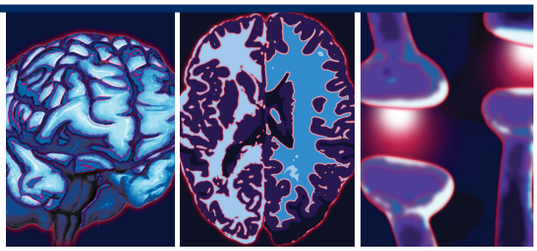
REVIEW



Baseline and acute changes in the HPA system in patients with anxiety disorders: the current state of research

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Practice points

- HPA system research in anxiety disorders has suffered from heterogeneous results due to confounding variables and a more profound picture addressing this issue is needed.
- After reviewing studies meeting certain criteria, distinct results with regard to HPA system functioning in different DSM-IV anxiety disorders were determined.
- It is conceivable that, to some extent, the differences between various entities might not only be due to the type of anxiety disorder, but could also be traced back to the load of mental stress (e.g., comorbid depression).
- Based on these findings, further research should control for comorbidity, with the investigation of a more tailored glucocorticoid-based pharmacotherapy in different anxiety disorders.

SUMMARY Research into the role of the HPA system in mental disorders has recently increased. It has been found that hormones involved in regulation of the HPA system play an important role in stress-related disorders. In the past, baseline alterations were mainly inspected in patients with anxiety disorders. In order to assess changes concerning the acute stress reaction in these subjects, many studies also applied stress protocols such as pharmacological or nonpharmacological challenges. This review aims to provide an overview of the results regarding HPA function in various anxiety disorders, such as panic disorder, generalized anxiety disorder, social anxiety disorder, specific phobia, obsessive-compulsive disorder and post-traumatic stress disorder. A PubMed-based literature search revealed 59 studies that met the inclusion criteria (i.e., double-blind randomized placebo-controlled trial; diagnosis based on DSM-III or -IV; and appropriate sample size – $n \geq 20$ in the verum group). Results are presented and integrated with regard to baseline HPA system activation and response to a challenge. Markers of interest reporting on HPA system functioning were cortisol and adrenocorticotropic hormone. In addition, suggested explanations regarding pathophysiological mechanisms underlying these findings are discussed. The majority of current data do not point to an alteration of the HPA system in anxiety disorders. There is some evidence for an association between the magnitude of mental stress and a change in cortisol levels. Nevertheless, pharmacotherapeutical interventions affecting stress hormones might be promising, not only in augmentation of psychotherapy in a specific phobia, but also for secondary prevention in post-traumatic stress disorder.

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The HPA system is regarded as one of the two main components of the stress system. It regulates adaptive responses to increased demand and maintains homeostasis after challenge. Corticotropin-releasing hormone (CRH) is an episodically released hormone that is secreted by the hypothalamus [1] and is mainly responsible for the regulation of the HPA system cascade. Under acute physical or psychological stress, CRH triggers the release of adrenocorticotropic hormone (ACTH) from the pituitary, which activates the release of glucocorticoids (e.g., cortisol) from the adrenal cortex [2]. Some studies suggest that dysregulation of the HPA system (i.e., hypo- or hyper-activity) plays a prominent role in both the vulnerability and maintenance of stress-related mental disorders [3–5], such as affective or anxiety disorders [2]. For example, Heim *et al.* demonstrated that neuroendocrine changes (i.e., persistent sensitization of the stress response and alterations in the HPA system), as a consequence of early-life stress, are associated with the risk of developing depression [6]. It was further suggested that so-called sensitive periods with heightened plasticity might have profound programming effects and are, therefore, considered to be central to the effects of early-life stress on depression risk [7]. Anxiety disorders also seem to be accompanied with basal and acute changes in the stress response; however, findings are often inconsistent. For example, regarding patients suffering from panic disorder (PD) or post-traumatic stress disorder (PTSD), some investigations found higher basal cortisol levels compared with healthy controls [8], but others did not [9–11]. This review presents and discusses selected studies performing either nonpharmacological or pharmacological challenge tests, or reporting on baseline alterations of the HPA system in patients with anxiety disorders. Distinct exclusion and inclusion criteria were applied in order to select those studies meeting a certain quality standard. By using this procedure, it was expected to improve the information value of HPA system research.

Methodology

A PubMed-based literature search was conducted in April 2012. As search terms, the following combinations were employed: each anxiety disorder according to DSM in combination with 'cortisol', 'ACTH', 'HPA axis', 'HPA system', 'challenge', 'yohimbine', meta-chlorophenylpiperazine ('mCPP'), 'caffeine', cholecystokinin-tetrapeptide ('CCK-4'), '5-HT2', 'lactate', 'CO_2', 'ipsapirone', Dexamethasone-Test ('DST') and Trier Social Stress Test ('TSST'), respectively. In the first selection process, only original research articles in English and dealing with human subjects were included. In a second step, all pharmacological and nonpharmacological challenge studies related to the HPA system were identified, as well as studies focusing on baseline measures of hormones associated with HPA system regulation. Furthermore, for final inclusion, studies were expected to meet the following criteria:

- With regard to pharmacological challenge paradigms, the study design should consist of a double-blind, randomized trial that is placebo controlled. When only baseline alterations were reported, follow-up designs or prospective studies were also accepted for inclusion. Concerning nonpharmacological challenges (e.g., TSST), a control group was considered necessary;
- The study sample should consist of adults diagnosed with an anxiety disorder (and also a control group);
- Diagnoses should be made according to either one of the following classification systems: DSM-III, DSM-III-R or DSM-IV;
- The size of the sample allocated to the verum group should be ≥20;
- Only original research articles reporting on empirical findings and published in peer-reviewed journals.

These strict criteria were chosen in order to ensure that this review comprises study results that are significant, due to appropriate design and sample size, and could, therefore, be regarded as interpretable.

We intended to exclude underpowered studies by requiring a sample size of at least 20 subjects in the verum group. The power to find significant differences is not only a function of sample size, but also of the size of the effects expected. However, in order to attain a desired power for a specified α-level (defined by convention as 0.05) and hypothesized effect size, a certain sample size is necessary, which was defined by Cohen [12]. We based the inclusion

criterion of at least 20 subjects in the verum group on Cohen's rule of thumb: according to Cohen, a statistical power of 0.80 is suggested to be a convention of general use. Even if large effects are expected and given an appropriate power of 0.80 and α-level of 0.05, a sample size of at least 26 is needed (in each group) in order to detect a large difference between two groups with a t-test for independent samples or analysis of variance. In order to conform to the required sample size of n = 26, we decided to expect a minimum sample size of n = 20. According to Cohen, even greater sample sizes would be preferable since the expected effect sizes would be small to medium instead of large (ergo even greater sample sizes would be desirable). Unfortunately, in most studies, only statistical test results were reported (including the p-value) instead of effect sizes such as Cohen's d, which would have been helpful to estimate the power of the study and the relevance of the results. Therefore, we were only able to report significant differences between the observed groups.

In terms of the selection process of the available literature, a multilevel mode was chosen. During the first step, we excluded studies that did not meet our criteria of enrolling at least 20 patients. Subsequently, trials that failed to have a study design as defined above were excluded. Finally, the diagnostic criteria determining the presence of an anxiety disorder were evaluated (Figure 1). After the selection procedure, studies performed in samples of patients with anxiety disorders, namely PD, generalized anxiety disorder (GAD), social anxiety disorder (SAD), specific phobia (SP), obsessive-compulsive disorder (OCD) and PTSD, were obtained.

Some of the selected studies might have assessed other variables apart from HPA system measures. Since the focus of this review is on the HPA system, only relevant results will be discussed. Furthermore, as cortisol and ACTH could be regarded as prominent indices reflecting HPA system functioning, the presentation of study results will be concentrated on these two markers. Differences regarding the method of cortisol sampling (i.e., blood, saliva and urine) were taken into account. In Tables 1–7, the various sampling methods are listed separately. It has been demonstrated that saliva represents a reliable and valid correlate of blood cortisol concentration [13,14].

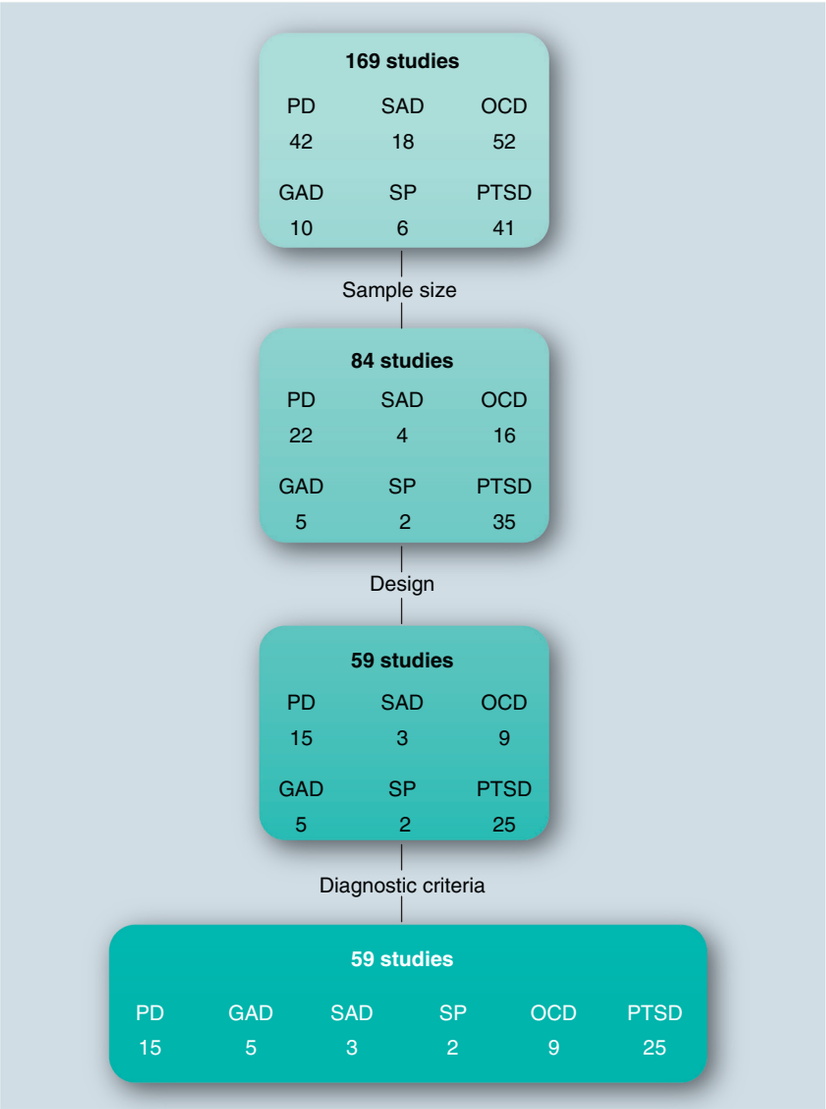

Figure 1. Selection process for the studies included.
GAD: General anxiety disorder; OCD: Obsessive-compulsive disorder; PD: Panic disorder; PTSD: Post-traumatic stress disorder; SAD: Social anxiety disorder; SP: Specific phobia.

Since there is growing evidence for dysregulation of the HPA system in the pathogenesis of depression [15], we also refer to the possible influence of comorbid major depressive disorder (MDD) in the reported findings. Therefore, included studies were additionally screened with regard to controlling for depression (i.e., comorbid MDD was excluded or considered in a subanalysis) in order to estimate its potential impact on cortisol alterations in anxiety disorders.

Finally, an overview of therapeutical implications regarding the use of glucocorticoids in anxiety disorders will be given.

Table 1. Studies investigating baseline alterations of cortisol in panic disorder.

Study (year)	Sample			DSM			Cortisol			Results (patients vs HCs)			Ref.
	Patients	HCs	MDD	III	III-R	IV	Blood	Saliva	Urine	Lower	No difference	Higher	
Petrowski *et al.* (2012)	32	53[†]	Patients with comorbid MDD were excluded			X	X				X		[28]
Petrowski *et al.* (2010)	34	34	Patients with comorbid MDD were considered by subanalysis			X		X			X		[31]
Broocks *et al.* (2000)	40	12	No controlling for MDD			X	X				X		[9]
Germine *et al.* (1994)	27	22	No controlling for MDD		X		X				X		[27]
den Boer and Westenberg (1990)	20	20	No controlling for MDD		X		X				X		[26]
Kathol *et al.* (1989)	65[‡]	37	Patients with comorbid MDD were considered by subanalysis	X					X	PD–A–MDD > PD–MDD and PD–A > PD > HC*			[32]
Kathol *et al.* (1988)	96[§]	37	Patients with comorbid MDD were considered by subanalysis		X				X	MDD > PD–A–MDD > PD–MDD and PD–A > PD > HC*			[33]
Charney *et al.* (1987)	23	19	No controlling for MDD	X			X				X		[25]
Charney *et al.* (1987)	65	20	No controlling for MDD	X			X					X*	[30]
Charney *et al.* (1985)	21	17	No controlling for MDD	X			X				X		[29]

*$p < 0.05$.
[†]Including 32 HCs and 21 patients with MDD.
[‡]Including PD; PD and MDD; PD and A; and PD, A and MDD.
[§]Including PD; PD and MDD; PD and A; PD, A and MDD; and MDD.
A: Agoraphobia; HC: Healthy control; MDD: Major depressive disorder; PD: Panic disorder.

Results & the current state of research

The studies were allocated according to the anxiety disorder the patients of the sample were diagnosed with. In each section, the results were subdivided with regard to baseline alterations in cortisol/ACTH levels between patients suffering from anxiety disorders and healthy controls, or established challenge paradigms known to affect the HPA system [16–24]. Furthermore, trials concerning the use of anxiolytic medication and its concomitant influence on the parameters observed were included. These subdivisions were only used when data addressing them were included. In the case of more than two studies related to any of the topics above, the results will be presented in a table **(Tables 1–7)**. In studies with patients suffering from anxiety disorders that employed healthy subjects without any mental disorder as a control group, the term 'controls' is used. Trials using patients as control subjects will be indicated.

■ Panic disorder

The selection strategy revealed 15 studies that examined HPA system function in subjects diagnosed with PD.

Baseline alterations

While the majority of studies did not find significant differences between patients and healthy controls in basal levels of cortisol [25–29], Charney *et al.* observed a significant elevation of plasma cortisol levels in a large sample of subjects suffering from PD **(Table 1)** [30]. A study inspecting the cortisol awakening response using salivary cortisol (sC) did not find differences between PD patients and healthy subjects [31]. Another study by Petrowski *et al.* was identified as the only trial investigating ACTH in anxiety disorders [28]. A total of 32 patients suffering from PD according to DSM-IV showed significantly higher ACTH serum levels than patients with MDD. However, in comparison with healthy controls, the authors did not determine a statistically significant difference. Three studies also compared cortisol baseline measures in PD patients with and without MDD [31–33]. While one failed to observe differences between these groups, Kathol *et al.* were able to replicate an association between the occurrence of (comorbid) MDD and elevated urinary-free cortisol levels. Moreover, patients who suffered from both PD and agoraphobia showed a higher score in the Hamilton Depression Rating Scale

Table 2. Studies investigating pharmacological and nonpharmacological challenge paradigms in panic disorder.

Study (year)	Sample			DSM			Challenge	Cortisol		Results (patients vs HCs)			Ref.
	Patients	HCs	MDD	III	III-R	IV		Blood	Saliva	Lower	No difference	Higher	
Pharmacological challenges													
Petrowski *et al.* (2012)	32	53[†]	Patients with comorbid MDD were considered by subanalysis			X	CRH	X		X*[‡]			[28]
Schruers *et al.* (2002)	24	24	Patients with comorbid MDD were excluded			X	L-5-HTP		X		X		[34]
Broocks *et al.* (2000)	40	12	No controlling for MDD			X	mCPP/ ipsapirone	X			X		[9]
Germine *et al.* (1994)	27	22	No controlling for MDD		X		mCPP	X			X		[27]
den Boer and Westenberg (1990)	20	20	No controlling for MDD		X		L-5-HTP	X			X		[26]
Coryell *et al.* (1989)	82	38	Patients with comorbid MDD were considered by subanalysis		X		DST	X		PD–MDD > PD > HCs[¶]			[37]
Charney *et al.* (1987)	23	19	No controlling for MDD	X			mCPP	X			X		[25]
Charney *et al.* (1987)	65	20	No controlling for MDD	X			Yohimbine	X				X*	[30]
Charney *et al.* (1985)	21	17	No controlling for MDD	X			Caffeine	X			X		[29]
Coryell *et al.* (1985)	50	37[§]	No controlling for MDD		X		DST	X		PD = MDD[¶]			[36]
Nonpharmacological challenges													
Petrowski *et al.* (2010)	34	34	Patients with comorbid MDD were considered by subanalysis			X	TSST		X	X*			[31]

*$p < 0.05$.
[†]Including 32 HCs and 21 patients with MDD.
[‡]Compared with HCs and patients with MDD.
[§]Patients with MDD.
[¶]Number of nonsuppressors after DST.
CRH: Corticotropin-releasing hormone; DST: Dexamethasone-Test; HC: Healthy control; L-5-HTP: L-5-hydroxytryptophan; mCPP: Meta-chlorophenylpiperazine; MDD: Major depressive disorder; PD: Panic disorder; TSST: Trier Social Stress Test.

Table 3. Studies investigating baseline alterations of cortisol in generalized anxiety disorder.

Study (year)	Sample			DSM			Cortisol		Results (patients vs HCs)			Ref.
	Patients	HCs	MDD	III	III-R	IV	Blood	Saliva	Lower	No difference	Higher	
Lenze *et al.* (2011)	60	40	No controlling for MDD			X		X			X*	[40]
Mantella *et al.* (2008)	71	40	Patients with comorbid MDD were considered by subanalysis			X		X		GAD = GAD–MDD > HCs*		[39]
Pomara *et al.* (2005)	35	48	Patients with comorbid MDD were excluded	X			X			X		[41]

*p < 0.05.
HC: Healthy control; GAD: General anxiety disorder; MDD: Major depressive disorder.

(HADRS) as well as higher urinary-free cortisol basal levels compared with those with PD alone [32]. Current data suggest that the presence and severity of comorbid MDD might also be responsible for HPA system activation in PD and may provide an explanation for the unique finding of higher basal cortisol levels in PD patients [30].

Challenge paradigms

In the selected studies, subjects underwent various pharmacological challenges to test for the hypothesis of serotonergic hypersensitivity in PD. mCPP, a serotonin receptor agonist (acting primarily on serotonin receptor subtype 5-HT1C), was administered in three studies and revealed inconsistent results, with two trials stating no statistically significant group differences with regard to serum cortisol in PD patients compared with controls [25,27]. In order to not only test the hypothesis of hypersensitivity of central 5-HT2C receptors (mCPP challenge), but also the proposed decreased responsiveness of 5-HT1A receptors, another study additionally administered ipsapirone, a selective 5-HT1A antagonist in patients with PD and/or agoraphobia, and controls [9]. A total of 55% of PD and/or agoraphobia patients experienced a panic attack after both challenge agents were administrated, while healthy subjects did not. Furthermore, they displayed a trend toward higher cortisol levels after mCPP and blunted cortisol secretion after ipsapirone in comparison with the control group.

Two other studies inspected the effect of L-5-hydroxytryptophan (L-5-HTP) administration on patients with PD, and found that L-5-HTP led to higher levels of plasma cortisol in PD patients and controls [26]. Schruers *et al.* also reported significantly increased sC levels in both groups after the challenge, while this effect was not observed after administration of the placebo [34]. Therefore, the results suggest that the sensitivity of 5-HT receptors does not appear to be changed in PD. A pharmacological challenge with yohimbine, an α-2-receptor agonist, was employed in a study with PD, and PD and/or agoraphobia patients, and healthy subjects. Yohimbine induced panic attacks in more than half (54%) of the patients and only 5% of the controls [30]. Patients who experienced panic symptoms displayed higher plasma cortisol levels than healthy controls. Baseline levels of cortisol were significantly higher in patients compared with the control group; however, there was no significant difference at baseline between patients who showed panic attacks and those who did not. In 1985, Charney *et al.* conducted

Table 4. Studies investigating baseline alterations of cortisol in obsessive-compulsive disorder.

Study (year)	Sample			DSM			Cortisol: blood	Results (patients vs HCs)			Ref.
	Patients	HCs	MDD	III	III-R	IV		Lower	No difference	Higher	
Charney *et al.* (1988)	42	42	No controlling for MDD	X			X		X		[50]
Hollander *et al.* (1998)	42	39†	Patients with comorbid MDD were excluded		X		X		X		[51]
de Leeuw and Westenberg (2008)	20	20	Patients with comorbid MDD were excluded			X	X		X		[49]
Monteleone *et al.* (1997)	20	20	Patients with comorbid MDD were excluded			X	X			X*	[52]

*p < 0.05.
†Including 21 patients with social anxiety disorder and 18 HCs.
HC: Healthy control; MDD: Major depressive disorder.

Table 5. Studies investigating pharmacological and nonpharmacological challenge paradigms in obsessive-compulsive disorder.

Study (year)	Sample			DSM			Challenge	Cortisol: blood	Results (patients vs HCs)			Ref.
	Patients	HCs	MDD	III	III-R	IV			Lower	No difference	Higher	
de Leeuw and Westenberg (2008)	20	20	Patients with comorbid MDD were excluded			X	mCPP	X		X		[49]
Khanna *et al.* (2001)	34	18	No controlling for MDD		X		mCPP	X	X*			[53]
Hollander *et al.* (1998)	42	39†	Patients with comorbid MDD were excluded		X		mCPP	X	X*‡			[51]
Monteleone *et al.* (1997)	20	20	Patients with comorbid MDD were excluded			X	Fenfluramine	X		X		[52]
Hollander *et al.* (1993)	42	21	Patients with comorbid MDD were excluded		X		mCPP	X		X		[55]
Hollander *et al.* (1993)	42	21	Patients with comorbid MDD were excluded		X		Fenfluramine	X		X		[55]
Hollander *et al.* (1992)	20	10	Patients with comorbid MDD were excluded		X		mCPP	X		X		[54]
Hollander *et al.* (1992)	20	10	Patients with comorbid MDD were excluded		X		Fenfluramine	X		X		[54]
Charney *et al.* (1988)	42	42	No controlling for MDD	X			mCPP	X		X		[50]
Charney *et al.* (1988)	42	42	No controlling for MDD	X			Tryptophan	X		X		[50]
Coryell *et al.* (1985)	20	82§	Patients with comorbid MDD were excluded		X		DST	X	PD > OCD¶			[36]

*$p < 0.05$.
†Including 21 patients with social anxiety disorder and 18 HCs.
‡Compared with patients with social anxiety disorder.
§Patients with PD.
¶Number of nonsuppressors after DST.
DST: Dexamethasone-Test; HC: Healthy control; mCPP: Meta-chlorophenylpiperazine; MDD: Major depressive disorder; OCD: Obsessive-compulsive disorder; PD: Panic disorder.

Table 6. Studies investigating baseline alterations of cortisol in post-traumatic stress disorder.

Study (year)	Sample			DSM			Cortisol			Results (patients vs HCs)			Ref.
	Patients	HCs	MDD	III	III-R	IV	Blood	Saliva	Urine	Lower	No difference	Higher	
Gola *et al.* (2012)	30	28	Patients with comorbid MDD were considered by subanalysis			X	X	X			X		[70]
Jovanovic *et al.* (2011)	30	60†	Patients with comorbid MDD were considered by subanalysis			X	X				X		[67]
Miller *et al.* (2011)	32	31†	Patients with comorbid MDD were considered by subanalysis			X		X			X		[68]
Muhtz *et al.* (2011)	25	25	Patients with comorbid MDD were considered by subanalysis			X	X	X			X		[69]
van Zuiden *et al.* (2011)	470‡		No controlling for MDD		X		X			X§			[77]
Vidović *et al.* (2011)	39	25	Patients with comorbid MDD were excluded			X	X					X*	[10]
Eckart *et al.* (2009)	24	19	Patients with comorbid MDD were considered by subanalysis			X		X			X		[72]
Yehuda *et al.* (2009)	28	22	Patients with comorbid MDD were considered by subanalysis			X			X	PTSD and PTSD–MDD < HCs*			[66]
Johnson *et al.* (2008)	32	20	Patients with comorbid MDD were considered by subanalysis					X		PTSD and PTSD–MDD > HCs*			[59]
Gill *et al.* (2008)	21	24	Patients with comorbid MDD were considered by subanalysis			X		X		PTSD and PTSD–MDD < HCs*			[63]
Metzger *et al.* (2008)	40	43	Patients with comorbid MDD were considered by subanalysis			X		X			X		[79]
Shalev *et al.* (2008)	31	124	Patients with comorbid MDD were considered by subanalysis			X	X	X	X		X		[74]
Simeon *et al.* (2007)	35	58	Patients with comorbid MDD were excluded			X			X		X		[71]
de Kloet *et al.* (2007)	23	24	Patients with comorbid MDD were considered by subanalysis			X	X	X		PTSD–MDD > PTSD and HCs*			[58]
Golier *et al.* (2007)	20	16	Patients with comorbid MDD were considered by subanalysis			X	X				X		[76]
Golier *et al.* (2006)	28	12	Patients with comorbid MDD were considered by subanalysis			X	X				X		[73]
Bierer *et al.* (2006)	32	10	No controlling for MDD			X			X	X*			[62]
Wessa *et al.* (2006)	29	15	Patients with comorbid MDD were considered by subanalysis			X		X		PTSD and PTSD–MDD < HCs*			[65]
Yehuda *et al.* (2005)	23	25	Patients with comorbid MDD were considered by subanalysis			X		X		PTSD and PTSD–MDD < HCs*			[61]
Bachmann *et al.* (2005)	75	33	Patients with comorbid MDD were excluded			X	X				X		[78]
Griffin *et al.* (2005)	42	14	Patients with comorbid MDD were considered by subanalysis			X	X			PTSD and PTSD–MDD < HCs*			[64]
Young *et al.* (2004)	29	16	Patients with comorbid MDD were considered by subanalysis			X		X			X		[75]
Yehuda *et al.* (1995)	22	15	Patients with comorbid MDD were excluded		X				X	X*			[60]

*$p < 0.05$.
†Traumatized patients without PTSD.
‡Combat veterans.
§No association between cortisol awakening response and symptom severity.
HC: Healthy control; MDD: Major depressive disorder; PTSD: Post-traumatic stress disorder.

a classical challenge paradigm using caffeine and failed to detect significant differences between PD patients and control subjects [29]. One study performed a challenge with CRH after DST pretreatment. Results revealed a significant attenuation of the cortisol response pattern but no differences in post-DST ACTH levels were found when compared with a mixed control group [13].

In response to a psychosocial stressor, the TSST [35], patients diagnosed with PD displayed a distinct cortisol nonresponsiveness compared with healthy subjects, who showed increased sC levels in response to the stressor [31].

Coryell *et al.* tested, in a series of studies including up to 80 PD patients with and without agoraphobia according to DSM-III, the effect of the DST as a marker of HPA system disturbances. They demonstrated that before, during and after the 8-week treatment with alprazolam, rates of nonsupressors in response to dexamethasone were at first comparable between patients suffering from PD and depressed patients, treatment led to a rise in these rates, but differences between the groups remained the same [36]. This finding was replicated and it was further demonstrated that, at baseline, PD patients were approximately twice as likely as healthy controls to escape suppression. This effect was more pronounced and significant on a statistical level after the treatment [37]. In a subsequent study, Coryell and Noyes found that a sample of 56 PD patients benefited from alprazolam treatment independent of DST results (e.g., HPA system hyperactivity) [38].

Trials controlling for comorbid depression found the occurrence of MDD to be associated with both a lower cortisol response and a higher rate of nonsuppressors after pharmacological or nonpharmacological challenges [29,31,37]. However, Coryell *et al.* found the same number of nonsuppressors after DST in patients suffering from PD or MDD [36]. Although a direct comparison of the severity of MMD is hindered by the use of different diagnostic tools, these findings also point to a potential influence of depression on HPA system functioning in PD.

In summary, baseline alterations in PD remain unclear, with studies presenting evidence for and against HPA system dysregulations. While comorbid depression might affect HPA system regulation in PD, results of pharmacological challenge paradigms do not support the hypothesized supersensitivity of 5-HT receptors as a main factor in HPA system activation. For details of these studies, see **Table 2**.

■ Generalized anxiety disorder

With regard to GAD, the literature search revealed five studies that met the inclusion criteria.

Baseline alterations

Regarding diurnal profiles of cortisol, Mantella *et al.* demonstrated that patients diagnosed with GAD displayed increased sC levels and higher peak cortisol compared with control subjects [39]. Furthermore, severity of GAD symptoms was associated with sC secretion. However, findings concerning baseline alterations are inconsistent with studies attempting to replicate these results [40] and studies that did not detect group differences [41]. Considering comorbid MDD, one trial failed to detect an influence of depression on the extent of cortisol release in GAD [39]. This finding was supported by the results of Lenze *et al.* Although they did not control for the presence or the magnitude of MDD, the GAD sample investigated showed a comparable severity of depression and significantly elevated basal cortisol levels compared with healthy controls [40]. However, due to the small number of studies addressing this topic, these conclusions should be considered preliminary.

Challenge paradigms

In another trial, cortisol levels of 30 GAD patients and 30 controls were comparable in the suppressor and nonsuppressor groups after administration of 1-mg dexamethasone [42]. While comorbid depression was excluded, all GAD patients became suppressors after being treated with cognitive behavioral therapy. However, plasma dexamethasone levels of the initial nonsupressor remained lower than in patients originally classified as supressors.

Anxiolytic interventions

Investigations of acute stress responses in GAD patients mainly employed anxiolytic interventions. It was shown that a 12-week treatment with escitalopram led to a significant decrease in peak and total sC secretion in GAD patients compared with the patients who received placebo [40]. Additionally, results of a consecutive study revealed that alterations in sC were significantly associated with

changes in immediate and delayed memory (i.e., improvement in memory was linked to sC reduction but only in selective serotonin reuptake inhibitor-treated patients, not in the placebo group) [43]. Pomara *et al.* did not find baseline differences between GAD patients and healthy controls; however, in the patient group, diazepam reduced plasma cortisol levels in an acute administration [41]. The reductions were more pronounced in the elderly (aged 65–70 years).

Overall, the proposed basal hyperactivity of the HPA system in GAD is not clearly evident. Based on the scarce evidence, definite conclusions cannot be drawn. However, treatment with escitalopram and diazepam revealed reduced cortisol responses in these patients and, therefore, a reduction of a potentially increased HPA system functioning.

■ Social anxiety disorder

Of the selected studies, only three investigated HPA system dysregulation in SAD.

Baseline alterations

Uhde *et al.* examined 24-h urinary-free cortisol excretion on 2 consecutive days in 64 patients with SAD diagnosed according to DSM-III [44]. Compared with healthy controls, there were no differences in urinary-free cortisol levels between the groups, which led to the conclusion that hyperactivity of the HPA system is not present in SAD. Based on DSM-IV, van Veen *et al.* investigated a sample of 43 patients suffering from SAD and 43 matched controls without any mental disorder [45]. Again, the groups did not differ regarding sC levels before they were challenged by the DST. In both studies, MDD was excluded using standardized and established questionnaires.

Challenge paradigms

Results of a challenge with DST revealed no group differences in cortisol secretion between SAD and healthy controls [45]. Moreover, Uhde *et al.* did not find a correlation between symptom severity and cortisol levels (baseline and after challenge) [44]. A trial in which various challenges were applied that yielded at three different neurobiological systems, such as fenfluramine (serotonergic system), levodopa (dopaminergic system) and clonidine (noradrenergic system), resulted in an elevated cortisol response to fenfluramine in

Table 7. Studies investigating pharmacological and nonpharmacological challenge paradigms in post-traumatic stress disorder.

Study (year)	Sample			DSM			Challenge	Cortisol		Degree of suppression after DST	Ref.
	Patients	HCs	MDD	III	III-R	IV		Blood	Saliva		
Pharmacological challenges											
Jovanovic *et al.* (2011)	27	44[†]	Patients with comorbid MDD were considered by subanalysis			X	DST	X		PTSD = TCs	[67]
Miller *et al.* (2011)	32	31[†]	Patients with comorbid MDD were considered by subanalysis			X	HCS		X	PTSD = TCs	[68]
Metzger *et al.* (2008)	40	43	Patients with comorbid MDD were considered by subanalysis			X	DST		X	PTSD = HCs	[79]
Simeon *et al.* (2007)	35	58	Patients with comorbid MDD were excluded			X	DST	X		PTSD = HCs	[71]
de Kloet *et al.* (2007)	23	24	Patients with comorbid MDD were considered by subanalysis			X	DST	X	X	PTSD = PTSD–MDD > HCs*	[58]
Golier *et al.* (2006)	28	12	Patients with comorbid MDD were considered by subanalysis			X	DST	X		PTSD > PTSD–MDD and HCs*	[73]
Griffin *et al.* (2005)	42	14	Patients with comorbid MDD were considered by subanalysis			X	DST	X		PTSD > PTSD–MDD and HCs*	[64]
Bachmann *et al.* (2005)	75	33	Patients with comorbid MDD were excluded			X	DST	X		PTSD = HCs	[78]
Yehuda *et al.* (2004)	34	17	Patients with comorbid MDD were considered by subanalysis			X	DST	X		PTSD > PTSD–MDD and HCs*	[80]
Yehuda *et al.* (2002)	34	26[†]	Patients with comorbid MDD were considered by subanalysis			X	DST	X	X	PTSD > PTSD–MDD and HCs > MDD*	[81]
Nonpharmacological challenges											
Simeon *et al.* (2007)	35	58	Patients with comorbid MDD were excluded			X	TSST	X		PTSD = HCs	[71]

*$p < 0.05$.
[†]Traumatized subjects without PTSD.
[‡]Including seven patients with MDD.
HC: Healthy control; HCS: Hydrocortisone; DST: Dexamethasone-Test; MDD: Major depressive disorder; PTSD: Post-traumatic stress disorder; TC: Traumatized control; TSST: Trier Social Stress Test.

21 DSM-III-diagnosed SAD patients without any comorbidity [46]. Since group differences in response to the two other challenge agents were lacking, the authors suggested an increased sensitivity only with regard to the serotonergic system in SAD.

Due to a small number of studies addressing this topic, the conclusions have to be seen as preliminary. However, results of the studies meeting our selection criteria revealed that baseline dysregulations of the HPA system are not common in SAD, while studies on acute stress responses suggested a hypersensitivity of the serontonergic system.

■ Specific phobia

With regard to the relationship between SP and HPA activity, data are scarce. Only two studies on SP fulfilled the criteria to be discussed in this review. Both trials diagnosed the mental condition using DSM-IV and included more than 40 patients.

Baseline alterations

Brand *et al.* assessed subjects suffering from a specific form of claustrophobia (a protective mask phobia) and demonstrated that SP subjects displayed a higher cortisol awakening response compared with controls [47].

Challenge paradigms

Furthermore, Brand *et al.* assessed sC levels before and after subjects with protective mask phobia underwent a 2-day intensive course of exposure therapy and found increased cortisol secretion before and after the course [47]. However, the sC levels of the SP group decreased significantly from pre- to post-assessment. After exposure treatment all subjects were able to wear the mask. Elevated levels of sC in SP patients in response to a stressor were further illustrated by a study that exposed women with a phobia of spiders to phobic and nonphobic pictures [48]. It was shown that during phobic stimulation, the phobics displayed elevated cortisol responses compared with controls and reported higher subjective arousal. While the first study did not control for comorbid depression [47], Knopf and Pössel excluded all other Axis I disorders other than SP [48].

Although the number of studies investigating HPA system measures in SP is small and data are not reliable, the current results point in the direction of an existing acute stress response (i.e., sC levels rise in response to a stressor or phobic stimuli, while basal HPA activity is comparable to healthy subjects).

■ Obsessive-compulsive disorder

Nine studies were identified that examined HPA system functioning in OCD.

Baseline alterations

Several studies suggested no differences in baseline cortisol between OCD patients and healthy controls [49–51]. Three out of four studies excluded patients with MDD from further analysis and only Monteleone *et al.* reported elevated plasma cortisol secretion in OCD in comparison with healthy subjects [52]. For details of these studies, see **Table 4**.

Challenge paradigms

Two studies demonstrated that challenges with mCPP led to a significantly attenuated serum cortisol response in comparison with healthy controls [53] or to SAD controls [51], while other studies did not detect differences between the groups [49,50,54]. Charney *et al.* further explored tryptophan as a challenge agent in OCD and did not find group differences in the cortisol response [50]. In two studies, fenfluramine, a 5-HT release/reuptake blocker, was employed and results revealed that cortisol secretion was comparable in both groups [54,55]. However, Monteleone *et al.* found evidence for a gender effect (i.e., elevated cortisol levels were observed in healthy females, but not in men) [52]. Performance of the DST demonstrated that only a few OCD patients display an abnormal escape from dexamethasone suppression and post-dexamethasone cortisol values were found to be significantly lower when compared with those of PD patients [56]. Again, the majority of the studies investigating HPA system functioning in OCD via challenge paradigms did not include patients with comorbid MDD and failed to detect differences in cortisol response compared with healthy controls. Performing a DST challenge, Coryell *et al.* found a lower number of nonsuppressors in OCD patients compared with subjects suffering from PD [36]. This finding might be explained by a more pronounced comorbid depressive symptomatology in PD patients reflected by mean HADRS score of 18 (vs a mean HADRS score of 4 in patients with OCD). For details of these studies, see **Table 5**.

Anxiolytic interventions

In a sample of 44 oral clomipramine-resistant patients, Mathew *et al.* conducted a challenge with clomipramine, which was administered intravenously over 14 days, and found that lower cortisol levels on day 1 were predictive of treatment response on day 14 [57].

In summary, most of the reviewed studies presented no evidence for baseline alterations of HPA system function in OCD. The conducted challenge trials did not lead to definite evidence for HPA disturbances in the acute stress response.

■ Post-traumatic stress disorder

A total of 25 studies inspected HPA system functioning in PTSD.

Baseline alterations

Only a minority of results point to elevated cortisol levels in samples of patients suffering from PTSD and some showed inconsistencies [10,58,59]. For example, changes over time were investigated in Croatian War veterans with PTSD; who displayed elevated plasma cortisol levels at first, while at a second assessment after approximately 5 years this effect was no longer detectable [10]. While seven studies observed an association between PTSD and a decrease in basal levels of cortisol [60–66], other trials did not find group differences in baseline cortisol between PTSD patients and traumatized or healthy controls [67–76]. Another study reported a lack of association between cortisol awakening response and PTSD symptoms after military deployment in Afghanistan [77]. Most of the studies did not reveal a substantial influence of MDD on cortisol measures. Only de Kloet *et al.* showed that veterans who suffered from both PTSD and MDD displayed higher basal levels than those without comorbid MDD [58]. For details of these studies, see **Table 6**.

Challenge paradigms

A total of 11 studies, on the basis of DSM-IV, employed a DST, pharmacological or non-pharmacological challenge in large samples (20 patients or more) of patients suffering from PTSD. In five studies, performance of the DST did not lead to group differences in cortisol or ACTH in plasma [67,71,78] or in sC [69,79] between subjects with PTSD and controls, while other data demonstrated a higher extent of suppression in PTSD patients compared with healthy controls without any history of trauma [64,73,80,81]. However, Jovanovic *et al.* demonstrated that a strongly pronounced fear-potentiated startle in PTSD patients was reduced after dexamethasone [67]. A study employing the TSST did not find differences in cortisol reactivity to psychosocial stress; however, patients with PTSD showed a significant inverse relationship between dissociation severity and cortisol reactivity [71]. Another challenge revealed that administration of hydrocortisone led to an acute increase in sC and suppressed the extent of fear-potentiated startle in veterans with and without PTSD [68]. In contrast to the findings regarding basal levels of cortisol, comorbid depression might have an impact on HPA system functioning in PTSD after DST. In studies demonstrating that PTSD was associated with hyporeagibility of the HPA system, comorbid MDD led to more attenuated suppression of cortisol after challenging **(Table 7)**. Furthermore, when a control group with MDD only was included, these subjects showed the highest degree of nonsupression compared with patients with PTSD as well as healthy controls [81].

Anxiolytic interventions

One study by Olff *et al.* found a significant increase in cortisol in PTSD patients responding to cognitive behavioral therapy, while nonresponders showed a decrease in cortisol and dehydroepiandrosterone [82].

No significant alterations or hyporeagibility in HPA system activity was observed in PTSD, as represented by basal levels of cortisol. However, a small number of different challenge procedures led to mixed results and points to the potential impact of comorbid MDD on HPA system functioning.

Conclusion & future perspective

During the last three decades, numerous studies have investigated HPA system functioning in anxiety disorders. Although some authors also investigated parameters such as growth hormone, prolactin and norepinephrine, cortisol and ACTH were generally considered to be the main representatives of the fear-related endocrinological response. Against this background, previous results concerning alterations in basal levels of cortisol and ACTH, as well as variations of the HPA system reagibility in response to established pharmacological and nonpharmacological challenges in DSM-defined anxiety disorders, remained heterogeneous.

In a placebo-controlled trial with a sample of OCD patients, a challenge with mCPP was followed by an amplified cortisol response in patients but not in healthy subjects [83]. Again, consecutive studies using a comparable challenge paradigm within the same psychiatric condition failed to replicate this observation [50,53]. These inconsistent findings might be due to inhomogeneity concerning diagnostic criteria and study design, as well as a distinct variance regarding the sample size within the available studies. Given the intention to sustain the informative value of former investigations in this field, only the results of trials that met defined criteria concerning the classification system, study design and number of included patients were used. In addition to baseline values of cortisol and ACTH, we considered further aspects of HPA system functioning such as the DST challenge and pharmacological challenge paradigms, as well as anxiolytic interventions, with a focus on changes in stress hormones.

The results of this review revealed no indication for a significant alteration of the basal cortisol levels in patients suffering from PD, SAD, SP or OCD. In GAD, preliminary results point to higher sC compared with controls. Although most of the studies addressing PTSD failed to observe any differences, a distinct number of trials found reduced cortisol concentrations in blood, urine and saliva.

The use of the DST also provided no clear evidence for differences in the degree of HPA system suppression in OCD, SAD or PTSD. Furthermore, the majority of trials failed to find hypo- or hyper-sensitivity of the stress hormone system in response to established pharmacological challenge paradigms in PD and OCD. If differences were detected, results often turned out to be directly opposed [9,28]. In SAD, SP and PTSD, fenfluramine or a disorder-specific nonpharmacological challenge led to a higher cortisol response compared with healthy control subjects. Moreover, results in GAD or OCD suggested that therapeutic interventions are followed by changes in cortisol levels and baseline cortisol levels might have the potential to predict treatment response [40,41,57]. Again, these findings are only based on a maximum of two studies and need replication by further investigations. Our results also suggest that the impact of depression on HPA system activity in anxiety disorders might be different between the disorders. While MDD seems to have no substantial influence in OCD, alterations of HPA functioning in patients with PD and PTSD might be explained to some extent by the presence and severity of comorbid depression. Few studies on GAD, SAD and SP are available and conclusions cannot be drawn at this point.

Excluding aspects of diagnostic criteria, study design and sample size, inconsistency of results may be explained by an association between HPA system alterations and the load of anxiety-induced stress. A positive correlation between the symptom severity as well as the number of psychiatric comorbidities and cortisol levels was found in patients suffering from GAD and PD, respectively [33,39]. Together with results regarding comorbid depression, these observations point to a certain threshold that might lead to HPA system activation if sufficient symptomatology is present. Beside the aspect of cumulative mental stress, the duration of illness may provide another potential explanation for different findings in HPA system functioning. It is conceivable that chronicity and the type of stress experience (tonic vs phasic; e.g., GAD vs SP) also have an impact on the magnitude of HPA system alterations. As an implication of these assumptions, new studies addressing this topic should include measures of symptom severity, symptom duration and a distinct type of anxiety disorder, as well as further mental stress, within an appropriate sample size.

As mentioned above, the use of antidepressants and benzodiazepines was followed by a normalization of formerly altered cortisol levels [40,41]. The anxiolytic properties of escitalopram and diazepam are widely evident. Selective serotonin reuptake inhibitors, such as escitalopram or paroxetine, are considered to be the current first-line medication in the treatment of DSM-IV anxiety disorders [84–86]. However, the observation of a decrease in cortisol levels after successful selective serotonin reuptake inhibitor treatment might also be caused by a close neurobiological relationship between the serotonergic transmission and the HPA system, rather than an indirect effect via a reduction of symptom severity [39].

A link between clinical improvement and recovery of HPA system functioning was recognized in studies that investigated the effect of an antagonist of CRF-1. After several trials demonstrated a significant anxiolytic activity of CRF-1 antagonists in animal models [87], two studies investigated two CRF-1 antagonists

(R121919, and CP 316 and 311) in samples of patients suffering from MDD. While one study failed to show a difference between verum and placebo [88], Zobel *et al.* revealed a significant improvement in depression as well as concomitant anxiety symptoms induced by R121919 [89]. Drawing a comparison between these studies, the sample of Zobel *et al.* had a more severe depressive symptomatology as well as distinct symptoms of anxiety [89]. These findings may be consistent with the proposition of a certain threshold of mental stress necessary to activate the HPA system in psychiatric disorders.

Some trials inspected glucocorticoids, not only as factors increasing vulnerability for symptom development, but also as a potential pharmacological treatment tool in anxiety disorders. Soravia *et al.* investigated the effect of an acute administration of cortisone prior to a disorder-specific stressor in a mixed sample of 60 patients suffering from SAD or spider phobia [90]. The stimulus-induced fear was significantly attenuated in both groups compared with placebo and this effect was maintained during re-exposure a few days later. Furthermore, there was a negative correlation between symptom severity and stress-induced cortisol levels in placebo-treated SAD patients, suggesting a protective effect of endogenous cortisol [90]. Blunted HPA system plasticity, as a risk factor for symptom development, was also observed in (pre)clinical trials regarding PTSD [91,92]. Subsequently, a number of trails aimed to investigate the effect of glucocorticoids in secondary prevention of PTSD. In animal models, as well as in humans, an acute administration of cortisol or hydrocortisone immediately after traumatization was associated with significantly lower rates of PTSD compared with placebo [93,94]. These observations might be due to the effect of cortisol on different domains of cognition such as integration of autobiographical memory and the retrieval of fear-related memory [95,96], and might provide further evidence for hypocortisolism as a potential risk factor for the development of PTSD.

During the last decade, a growing number of trials have investigated the role of genetic factors as a causal mechanism of altered neurobiology in the pathogenesis of DSM-IV anxiety disorders. Against this background, molecular techniques such as linkage and association analysis have come to the forefront of current research. Therefore, association studies that compare single-locus alleles or genotype frequencies in candidate genes hypothesized to be causally related to the phenotype represent a core area in this field. While studies in GAD, OCD and SAD mainly focus on genes related to the serotonergic and dopaminergic transmission, some trials in PD and PTSD have also investigated an association between a potential susceptibility locus in genes associated with the HPA system functioning and the observed phenotype. Two studies failed to reveal an association between a polymorphism in the *CRHR-1* promotor region and the occurrence of PD [97,98], but Unschuld *et al.* found an association between two haplotypes of the *CRHR-2* promotor region and symptom severity in female patients suffering from PD [99]. In PTSD, studies have found polymorphisms of *FKBP5*, a gene involved in the activity of the glucocorticoidreceptor, interacted with childhood abuse and adversity, to predict severity of adult PTSD symptoms [100,101]. Results regarding genetic variants of *GCCG* remained heterogeneous. While Bachmann *et al.* did not find an association between polymorphism in the *GCCG* and combat-related PTSD [78], another study observed significantly higher PTSD scores in homozygote carriers of a certain allele of *GCCG* within a sample of patients undergoing intensive care unit treatment [102]. Although a more detailed discussion of genetic research and further aspects of altered neurobiology in anxiety disorders is outside the the scope of this review, these findings underline the inhomogeneity of current results on HPA system alteration in anxiety disorders, as well as the need for further research in this field.

The aim of this review was to provide an overview and a summary of the current state of research on HPA system activity in anxiety disorders. Since the findings in the literature are still very heterogeneous, we tried to filter relevant findings by applying distinct selection criteria. In this context, existing results concerning HPA system functioning in anxiety disorders do not point to an alteration in general. When considering limitations, such as the small number of available studies, it is probable that changes in stress hormones might be a result of the severity of mental stress and the kind/type of anxiety disorder (i.e., GAD or PTSD). Current findings also suggest that changes in stress hormones

(e.g., hypocortisolism) may represent a risk factor for symptom development in PTSD and different derivatives of glucocorticoids may be promising for the acute treatment or prevention of anxiety disorders. Although there is some evidence for genetic polymorphisms affecting HPA system functioning in PD and PTSD, at this point of research it appears difficult to establish a direct association between clinical findings and basic neurobiological research, or to generate hypotheses concerning potential pathogenesis models of anxiety disorders. A more detailed overview regarding potential explanations and models, as well as a verification of our findings of HPA system activity in studies with an appropriate design (as explained above), is warranted.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

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