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Systematic review and meta-analysis of basal cortisol levels in Borderline Personality Disorder compared to non-psychiatric controls

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ABSTRACT

Objective: Borderline personality disorder (BPD) is a prevalent, complex, and serious mental disorder involving multiple symptoms and maladaptive behaviour. The underlying psychobiological mechanisms involved are not yet fully understood, but increasing evidence indicates that changes in hypothalamic-pituitary-adrenal stress axis (HPA) activity may contribute to BPD. Whilst various studies have demonstrated elevated levels of cortisol (the end-product of the HPA axis) in BPD sufferers, others have presented opposite findings. Inconsistent findings may be attributable to comorbidities, collection and measurement methods, gender, and sample size. Considering these discrepancies, the aim of this systematic review and meta-analysis was to assess available studies in the scientific literature examining basal/ baseline cortisol levels in patients diagnosed with borderline personality disorder compared to non-psychiatric controls.

Methods: A systematic literature review was conducted with descriptions of primary studies in addition to a meta-analysis of studies with a control group. Meta-analysis was performed using Comprehensive Meta-analysis software (CMA version 2). The effect size (Hedges' g) was calculated with random-effect model.

Results: A systematic literature search identified 16 studies that met the eligibility criteria from a total of 1076 unique records initially examined. Twelve studies (N = 546; 278 borderline personality disorder and 268 non-psychiatric controls) fulfilled the inclusion criteria for meta-analysis. The standardised mean difference (Hedges' g) of basal cortisol level between BPD and control groups was -0.32 (pooled data from 12 studies; 95% confidence interval -0.56 to -0.06, p = 0.01), indicating significantly lower mean cortisol level for the BPD group.

Conclusion: Cortisol as a biomarker of the HPA axis is an important and helpful measure in the study of stress disorders such as BPD. However, considerations of potential confounding factors must be considered.

1. Introduction

Borderline personality disorder (BPD) is a debilitating psychiatric illness that is common in both the general population and in clinical settings, with a life-time prevalence of 5.9% (Grant et al., 2008; Lenzenweger et al., 2007). BPD is characterised by a pervasive pattern of instability of interpersonal relationships, self-image and affects, and marked impulsivity beginning by early adulthood. These symptoms must persist and cause marked distress and or functional impairment within a variety of contexts, although symptoms often fluctuate markedly over short periods of time (Association, A.P. 2013). With a wide spectrum of symptoms that often overlap with other mental disorders, BPD is widely recognised as a complex and heterogeneous mental health syndrome, making both diagnosis and treatment difficult in many cases (Council, M.N.H.a.M.R. 2012).

1.1. Borderline personality disorder and HPA axis activity

The underlying psychobiological mechanisms hypothesised to be involved in the onset of BPD symptomatology is thought to be multifactorial including environmental, genetic, and neurobiological influences (Winsper et al., 2016). Acute stress and early life stress are considered an important feature for both the onset, and also the continuing prevalence and exacerbation of symptoms (Bourvis et al., 2017). Exposure to stress activates the hypothalamus pituitary adrenal (HPA) axis, the neuroendocrine system responsible for mediating the response of the body to physiological stress. Although healthy functioning of the HPA stress mechanism is important for neurodevelopment and to maintain stability and homeostasis, long-term, chronic activation may exceed an individual's allostatic load, thereby increasing the risk for developing a myriad of illnesses, including stress-related psychiatric disorders (Oken et al., 2015; Renoir et al., 2013). There is growing

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evidence that changes to the HPA axis may contribute to the aetiology of BPD (Wingenfeld et al., 2010).

1.2. Cortisol as the end-product to measure HPA axis activity

Glucocorticoids hormones (cortisol primarily in humans) are the end-product, effector hormones of the HPA axis neuroendocrine system, which bind two receptors expressed throughout the brain; glucocorticoid receptors (GR) and mineralocorticoid receptors (MR). Both receptors work in synergy to regulate the HPA axis, and regulate their own release via negative feedback via the central nervous system (Herman et al., 2012). Growing evidence indicates that cortisol levels have concentration dependent actions on neuronal viability and survival (Vyas et al., 2016), which have been suggested to contribute to psychopathological disease states (Zorn et al., 2017a). Cortisol levels have been demonstrated to dynamically increase with environmental and psychological stressors and as such represent the functional output of the HPA axis and are a widely used peripheral measure in psychiatric research (Herane Vives et al., 2015; Spencer and Deak, 2017).

1.3. Cortisol assessment: collection and measurement methods

Cortisol has been assayed by various methods using a range of substrates including blood, saliva, and urine that provide a sampling procedure that is simple, and relatively non-invasive (Levine et al., 2007). More recently, cortisol has been analysed using hair sampling, representing a longer, chronic level of cortisol (Staufenbiel et al., 2013).

As a lipophilic molecule, the majority of circulating cortisol is bound to carrier proteins (cortisol binding globulin (CBG)), with only a small fraction existing in a soluble, free form that is bioactive. Salivary cortisol reflects the amount of cortisol that escapes such binding proteins and enters the salivary glands and saliva, said to be called 'bioavailable' (El-Farhan et al., 2017a). The concentration of cortisol in saliva accurately reflects the level in blood when compared to the amount of unbound cortisol in serum or plasma samples, whilst the total cortisol levels in blood usually varies due to different amounts of CBG found in blood. Notably, increased CBG is known to occur with estrogen excess, e.g. pregnancy and estrogen-containing oral contraceptives (OCP), resulting in higher concentrations of total cortisol (Šimůnková et al., 2008). Cortisol is excreted in urine in an unbound, bioavailable form and is therefore unaffected by fluctuations in CBG levels (El-Farhan et al., 2017b). Hair analysis provides the opportunity to determine cortisol exposure of the past. As hair grows at an average rate of 1 cm/ month, the 1 cm segment closest to the scalp is thought to represent cortisol levels during the most recent past month, with the following 1 cms' representing previous months (Meyer and Novak, 2012). This measure is not influenced by the circadian rhythm of the HPA-axis, nor by acute stress, and is therefore thought to represent a retrospective index of cumulative cortisol output (Staufenbiel et al., 2013; Wester and van Rossum, 2015). However, several limitations including hair characteristics (hair dye, and hair care routines), seasonal variation, and storage length have been documented to influence output and are potential confounders (Abell et al., 2016).

The time of sample collection is an important consideration for cortisol measurement as cortisol follows a circadian rhythm of secretion with low values upon first wakening, and peak values observed 30 min. after awakening, with a steady decline during the rest of the day. This peak can be measured using a minimum of three separate sampling times (using blood or saliva) upon waking (time of wakening, 30 min. after waking, 45 min. after waking), and is known as the cortisol awakening response or CAR (Sin et al., 2017). Majority of studies employ a 'minimal protocol' analysing one time point only (at either waking, peak, or evening measures), however this has potential implications for the reliability of measurement. For cross-sectional studies it is recommended that up to six consecutive days of samples are to be collected to accurately assess single time-points and that four measures at two consecutive weeks days are required to reliably measure the CAR response as a trait measure (Hellhammer et al., 2007).

HPA axis activity/ sensitivity is also measured via several measures. The dexamethasone suppression test (DST) has extensively been used to detect abnormal HPA axis activity with regards to negative feedback regulation. Various stressful stimuli can also be used to reliably induce stress in human research participants and detect reactivity of the HPA axis, including standardised psychosocial protocols, eg Tier social stress test (TSST), and physical stressors (electrical stimulation).

1.4. Cortisol levels in Borderline Personality Disorder

Whilst various studies have demonstrated elevated levels of cortisol in BPD patients, others have presented opposite findings. Inconsistent findings may be attributable to differences in sample size as well as variations in collection and measurement methods. Considering these discrepancies, the aim of this systematic review and meta-analysis was to assess available studies in the scientific literature examining basal / baseline cortisol levels in a BPD group compared to non-psychiatric controls. This review evaluated basal/ baseline measures, rather than HPA activity or cortisol stress reactivity measures, to broaden the scope of available data for comparison of cortisol levels in BPD.

2. Methods

This systematic review was performed according to the Preferred Reporting Items for Systematic review and Meta Analyses (PRISMA) guidelines (Moher et al., 2015).

2.1. Search strategy and selection criteria

Databases searched included Pubmed, and Web of science core collection for articles up until 01/05/2018, using the search terms ((Borderline personality disorder) AND ("cortisol" or "CAR" or "cortisol awakening response"). The search was limited to English, human studies, and original research articles only.

The lead author screened titles, abstracts, and methods for relevance based on selection criteria. Those articles deemed relevant was selected for further consideration (Fig. 1). The search was further limited to peer-reviewed articles with full text available and no grey literature was included in this search. All studies were identified using and imported into EndNote (version X7; Thomson Reuters), where all duplicates were removed.

Articles for qualitative review met the inclusion criteria if:

- Cross-sectional study or reported baseline data from longitudinal studies of cortisol concentrations comparing adult patients (aged > 18 years) who had BPD with non-psychiatric controls;
- Studies used well validated diagnostic criteria for BPD.
- Studies reported mean and SD of concentration levels or significance level between the two groups (BPD and non-psychiatric control).

Articles for meta-analysis met the inclusion criteria if:

- *All criteria above met, plus*
- Adequate data (direction of results reported).
- Pooled analyses, with no *a prior* subgrouping.
- No predicted means (based on modelling)

Studies were excluded if they:

- Included participants with comorbid psychotic disorder
- Included participants with severe medical illness, or hormonal - autoimmune or inflammatory disease; use of anti-inflammatory or immunomodulatory drugs;
- Were review articles

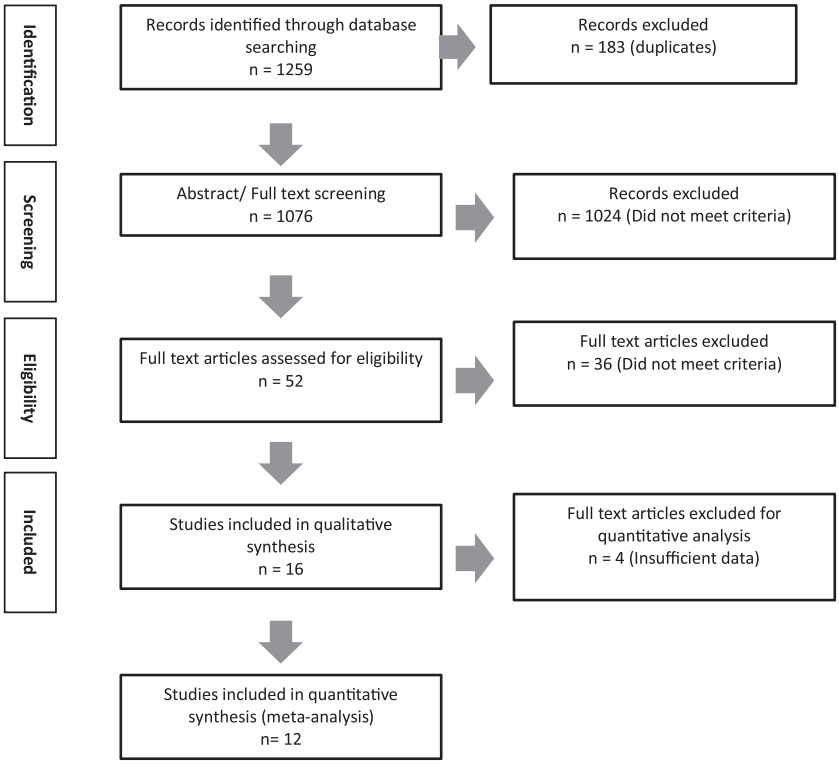


Fig. 1. PRISMA Flow diagram: Study selection.

- Were case studies

2.2. Statistical analyses

The difference of baseline cortisol as a continuous variable, between the BPD and control groups, was the primary analysis outcome. Sample means of peripheral blood, saliva, hair, or urine-assayed cortisol levels in the two groups across studies were measured within different units (nmol/l or ug/dl), therefore, we calculated the standardised mean difference, Hedges g for small samples, as the effect size. We used the random effect model treating the included studies as a random sample from a population of studies. The random model for meta-analysis assumes the effect size reported by each study is different from the population effect size by a random error, subsequently, the combined effect size is weighted by the inverse variance of the population. In studies in which mean basal cortisol standard deviations were not reported, we imputed values for meta-analysis using t statistic and p values. Heterogeneity between studies was anticipated to be present; a possible theoretical explanation of cortisol levels being sampled from different body fluids/sites. This, and the statistically significant overall effect size, led to the decision to conduct a subgroup analysis where effect size was moderated by sample type. Due to limited number of studies included, the moderator variable was coded for three categories: saliva (6 studies), blood (4 studies) and other (2 studies: 24 h urine and hair analyses). Publication bias were assessed by funnel plot and Egger's regression test. All analyses were written with CMA v.2.

3. Results

3.1. Data extraction

The characteristics extracted from each study were name of the first author; journal and publication year; number, sex, age; mean and SD of cortisol or significance level between patient and control group; time of day sample collected; biological sample medium; type of assay; intra/ inter assay variation, whether menstrual cycle was considered, comorbidities; whether patients were medication free (BPD patients were classified as medication free if they were not taking psychotropic medication at the time of the study).

A systematic literature search identified 16 studies that met criteria for qualitative analysis (Fig. 1), from a total of 1076 unique records initially examined. All studies investigated the function of HPA activity by measuring cortisol levels in BPD patients compared to non-psychiatric controls. Baseline comparisons were extracted from either cross-sectional studies or reported baseline data from longitudinal studies, including CAR and diurnal profiles of cortisol, dexamethasone suppression tests to investigate HPA activity, and reactivity designs using standardised psychosocial stress protocols (eg. TSST test). Various biological mediums were assessed and included saliva (6 studies; (Aleknaviciute et al., 2016; Jogems-Kosterman et al., 2007; Lieb et al., 2004; Nater et al., 2010; Rausch et al., 2015; Walter et al., 2008)) and blood (4 studies;(Bosch et al., 2012; Carrasco et al., 2007; Lange et al., 2005; Mazer et al., 2018; Paris et al., 2004)) and measurements of chronic stress; urine over 24 h (Wingenfeld et al., 2007) and hair analyses representing the past three months (Dettenborn et al., 2016). Characteristics of the qualitative studies included are described in Tables 1a and 1b.

The majority of studies reported details of known potential confounders of cortisol measurement including age, sex/ gender, comorbidity, excessive exercise, and medication. Majority controlled for time-of-day collection to control for diurnal activity of cortisol, although variable times of day across studies was noted (most likely due to the type of stress test, CAR, or basal assessment employed in the study). Several studies reported menstrual cycle phase, if regular or not, and/ or oral contraceptive use.

Of the 16 studies, five studies reported significantly lower levels of baseline cortisol levels in BPD compared to non-psychiatric controls. *Aleknaviciute* and authors (Aleknaviciute et al., 2016) were interested in subjective emotional responses and HPA physiological responses in women with BPD, cluster c personality disorder (CPD), and healthy controls. At baseline measures, BPD patients demonstrated significantly lower basal cortisol levels compared to both the (CPD) group and

Table 1a
Summary of studies meeting criteria for quantitative analysis (meta-analysis).

Study	Journal	Group	n	Female (%)	Median age (Years)	Sample medium	Assay method
Aleknaviciut et al. (2016)	PNEC	BPD	26	100	29.2 (6.4)	Saliva	ELISA
		CTRL	35	100	28.6 (7.1)		
Carrasco et al. (2007)	British J Psych	BPD	32	59	30.6 (6.4)	Plasma	Not reported
		CTRL	18	61	29.7 (5.5)		
Dettenborn et al. (2016)	PNEC	BPD	18	100	25.6 (6.2)	Hair	LC–MS/MS
		CTRL	17	100	26.2 (5.7)		
Jogems-Kosterman et al. (2007)	J Psychiatr Res	BPD	22	100	33.2 (9.3)	Saliva	ECLIA
		CTRL	22	100	35.7 (8.8)		
Rausch et al. (2015)	PNEC	BPD	41	63	27.9 (1.3)	Saliva	CLIA
		CTRL	40	53	29.7 (1.5)		
Lieb et al. (2004)	J Psychiatr Res	BPD	23	100	28.5 (1.6)	Saliva	DELFIA
		CTRL	24	100	28.2 (1.4)		
Lange et al. (2005)	PNEC	BPD	21	100	31.7 (8.2)	Serum	CLIA
		CTRL	23	100	32.8 (8.4)		
Wingenfeld et al. (2007)	European Psych	BPD	21	100	28.1 (5.4)	24 hour urine	RIA
		CTRL	24	100	27.7 (6.9)		
Walter et al. (2008)	European Psych	BPD	9	76	18.7	Saliva	DELFIA
		CTRL	12	TOTAL	TOTAL		
Nater et al. (2010)	PNEC	BPD	15	100	32.7 (7.8)	Saliva	DELFIA
		CTRL	17	100	27.2 (6.2)		
Paris et al. (2004)	Psych Res	BPD	30	100	27.7 (7.4)	Plasma	RIA
		CTRL	21	100	29.0 (6.4)		
Mazer et al. (2018)	Behav Brain Research	BPD	20	100	28.4 (8.8)	Plasma	RIA
		CTRL	15	100	30.8 (7.1)		

Abbreviations: ELISA (enzyme-linked immunosorbent assay), RIA (Radioimmunoassay), LC–MS/MS (Liquid chromatography tandem mass spectrometry), CLIA (Chemiluminescence immunoassay), ECLIA (Competitive electrochemiluminescence immunoassay), DELFIA (Time-resolved fluorescence immunoassay), PNEC (psychoneuroendocrinology).

female healthy controls. *Carrasco* and authors (Carrasco et al., 2007) assessed HPA sensitivity in 32 non-medicated BPD without comorbid post-traumatic syndromes, and 18 healthy individuals using a modified dexamethasone suppression test (0.25 mg). Baseline cortisol levels in the patients were reported to be significantly lower than in the controls. The laboratory method conditions was strictly controlled with regards to environmental stress, including conditions of environmental stress, including 30–60 min of relaxed delay before sample 30–60 min of relaxed delay before sample extraction. The authors note that the results applied to a specific subgroup of patients selected by the presence of repetitive self-aggressive behaviours in the previous 6 months. *Nater et al* (Nater et al., 2010) investigated physiological responses to acute psychosocial stress in female only BPD patients compared to healthy controls. These reactivity measures included cortisol and alpha-amylase levels before, during, and after exposure to a standardised psychosocial stress protocol. Both cortisol and alpha-amylase were demonstrated to have significantly attenuated levels at baseline when compared to controls. With regards to cortisol measures, *Mazer et al* (Mazer et al., 2018) was interested in comparing morning plasma cortisol levels in both BPD and bipolar disorder (BP) compared to non-psychiatric controls. Post-hoc analysis of the three-group comparison demonstrated significantly reduced levels in both BPD and BP patient groups as compared to controls, however no differences between BPD and BP were observed. Interestingly, the control group showed the most varied levels of plasma cortisol. *Inoue et al* (Inoue et al., 2015) compared cortisol levels in four different experimental designs; before and after the implementation of two separate stress paradigms (psychosocial stress test and a physical stressor) in two cohorts; females and males. Results demonstrated that in the context of a psychosocial stress test (TSST), baseline cortisol measures were significantly decreased in female patients compared with matched controls. Due to *a prior* subgrouping of sex, these studies were not included in the quantitative analyses.

5 studies reported non-significant reductions in cortisol levels in BPD vs healthy controls including *Jogems-K et al (*Jogems-Kosterman et al., 2007*), Rauch et al (*Rausch et al., 2015*), Lange et al (*Lange et al., 2005*), and Walter et al (*Walter et al., 2008*). Inoue et al (*Inoue et al., 2015*)* reported non-significant lower levels in male BPD patients compared to matched controls, at baseline levels (of a physical stressor protocol).

In contrast, only one study reported a significantly increased level of baseline level of cortisol in total BPD patients compared to non-

Table 1b
Summary of additional studies meeting criteria for qualitative analysis (Systematic review).

Study	Journal	Group	n	Female (%)	Median age (Years)	Sample medium	Assay method
Carvalho Fernando et al. (2012)	PNEC	BPD	24	96	26.9 (6.0)	Saliva	RIA
		CTRL	41	68	33.4 (10.4)		
Carvalho Fernando et al. (2013)	PNEC	BPD	32	100	27.9 (8.9)	Saliva	RIA
		CTRL	32	100	29.5 (9.1)		
Kahl et al. (2006)	Biol Psychiatry	BPD	12	100	26.3 (5.1)	Serum	RIA
		CTRL	12	100	25.6 (3.9)		
Inoue et al. (2015)	Psych Res	BPD	72	46	23.9 (1.8)	Saliva	ELISA
		CTRL	377	39	24.95 (3.5)		

Abbreviations: ELISA (enzyme-linked immunosorbent assay), RIA (Radioimmunoassay), LC–MS/MS (Liquid chromatography tandem mass spectrometry), CLIA (Chemiluminescence immunoassay), ECLIA (Competitive electrochemiluminescence immunoassay), DELFIA (Time-resolved fluorescence immunoassay), PNEC (psychoneuroendocrinology).

psychiatric controls. *Fernando et al* (Carvalho Fernando et al., 2012) investigated basal cortisol release and HPA feedback sensitivity after a 0.5 mg dexamethasone suppression test in BPD, MDD, and control samples, over the course of a day (4 time points). Cortisol concentrations showed a significant diurnal variation over time in both patient groups compared with controls, and higher overall cortisol levels over the course of the day compared to controls. Post-hoc analyses of each time point revealed significantly higher cortisol levels for BPD patients than controls at the first time point taken (7:30am). Of note, when sex is studied in separate cohorts, seen in the *Inoue et al* (Inoue et al., 2015) study, cortisol levels were demonstrated to be significantly higher when compared to matched controls.

4 studies *Dettenborn et al (*Dettenborn et al., 2016*), Lieb et al (*Lieb et al., 2004*), Wingenfeld et al (*Wingenfeld et al., 2007*), Kahl et al (*Kahl et al., 2006*))*, reported non-significantly elevated levels in BPD patients when compared to healthy controls, including the two studies investigating chronic, longer levels of cortisol levels in hair *(Dettenborn at el)* and 24 h urine output (*Wingenfeld et al))*. With respect to baseline measures, the study by *Paris et al* (Paris et al., 2004) had no change in absolute cortisol levels, and no significant changes were reported in the *Fernando et al* (Carvalho Fernando et al., 2013) study, nor direction of change.

12 studies fulfilled the inclusion criteria for meta-analysis (Fig. 1); 4 studies were excluded due to inadequate data. Characteristics of the quantitative studies included are described in Table 1a. Results from the meta-analyses are shown in Fig. 3. Three studies' effect sizes were statistically significant. The effect sizes between the two groups ranged from -0.88 to 0.39, with a significant pooled effect size (-0·31[-0·56 to -0·06], p = 0.014), indicating lower mean cortisol level for the BPD group.

The significant mean difference of basal cortisol between the two groups was confined to the subgroups in which cortisol samples were obtained from saliva or blood; the effect sizes were (-0.48 [-0.80 to -0.16], p = 0.004) for blood, and (-0.39 [-0.72 to -0.04], p = 0.03) for saliva, indicating significantly lower mean cortisol level for the BPD group for both sample types (Fig. 4). There was no significant mean difference in the subgroup in which cortisol level was assayed from a hair or urine samples (0.34 [-0.09 to 0.78], p = 0.12).

We found heterogeneity within studies (I^2 = 53.0) that suggest the effect size across the studies included, was not constant. However, no heterogeneity was found in the subgroups (for blood sample (I^2 = 25.2), for saliva sample (I^2 = 43.3) and for the "other" sample type (I^2 =0.00). No indication for publication bias based on the analysis of the funnel plot (Fig. 2) and Egger's regression test (t = 0.41, p = 0.68) was observed.

4. Discussion

To our knowledge, this is the first meta-analysis of cortisol basal/ baseline levels in BPD. A funnel plot of standardised error by Hedges's g and sensitivity analyses (when all studies removed, no change in result) confirmed no publication bias. Statistical heterogeneity was moderate (53%) which may reflect clinical/ laboratory heterogeneity, including gender, comorbidity, biological sample type, collection and measurement methods, and sample size. The pooled evidence suggest cortisol basal/ baseline levels in BPD are reduced compared to non-psychiatric controls, with a reported effect size of 0.31 SMD. This result suggests an impaired functioning of the HPA axis in BPD, which could be hypothesised as a maladaptation to stressors.

Cortisol reactivity has been implicated in several stress-related psychopathologies including major depressive disorder (MDD) and posttraumatic stress disorder (PTSD). Neuroendocrine studies have consistently demonstrated that MDD is associated with higher basal cortisol levels, reduced HPA feedback activity, and progressive desensitisation of the HPA axis, whereas PTSD report lower basal cortisol activity, enhanced HPA feedback function and a progressive sensitisation of the HPA axis (Handwerger, 2009; Morris et al., 2012). Whilst the precise mechanism behind these differences is not yet understood, evidence supporting epigenetic modifications, in particular of allelic variants of the glucocorticoid receptor, may be responsible for driving these opposing outcomes (Morris et al., 2012; Palma-Gudiel et al., 2015). The results of the current meta-analysis suggest BPD may parallel PTSD in over-active HPA feedback function, with reports of lowered basal/ baseline levels compared to non-psychiatric controls. This is interestingly considering the current debate to consider the use of the diagnostic category 'complex posttraumatic stress disorder' (cPTSD) as detailed in the forthcoming ICD-11 classification system rather than the diagnostic classification of a cluster b personality disorder, to decrease stigma and provide a needed trauma informed approach for BPD treatment (Kulkarni, 2017). In line with this, *Aleknaviciute et al (*Aleknaviciute et al., 2016*)* demonstrated that despite reporting similar subjective experience to a psychosocial test, BPD patients showed a distinct cortisol endocrine response compared to cluster C personality disorder (CPD) patients. Whilst BPD patients showed a blunted cortisol response, CPD patients contrastingly demonstrated a heightened cortisol response, providing further support that BPD be classified as a stress-related psychopathology.

4.1. Influence of biological sampling

Comparisons of effect size in the sub-group meta-analysis confirm both saliva, and blood sample mediums have significant reductions in cortisol levels in BPD, consistent with overall pooled studies. Heterogeneity of the pooled blood samples (25%) compared to saliva (43%) suggests blood sampling may better explain random error. Interestingly, 'other' sampling mediums including hair (*Dettenborn et al)* and 24-hour urine *(Wigenfeld et al)* reported cortisol levels in the opposite direction (increased cortisol levels). While more studies are required to confirm a statistical increase, this may reflect differences in basal levels compared to longer, more chronic readings of cortisol which consider the total temporal dynamics of cortisol levels over time.

4.2. Influence of sex and sex hormones

Mounting evidence suggests there are substantial sex differences in HPA activity and cortisol response to stress in healthy individuals, and is therefore important to account for when investigating cortisol reactivity in psychiatric patients (Zorn et al., 2017b). The HPA stress response in females is characterised by a larger, more sustained secretion of ACTH and cortisol, suggesting enhanced activity and reduced negative feedback (Rj and McGivern, 2017) (Toufexis et al., 2014). Although the mechanisms are yet to be understood, estradiol has been shown to enhance HPA activity (Toufexis et al., 2014), whilst testosterone appears to have an inhibitory effect by acting upon the hypothalamus (Toufexis et al., 2014; Viau, 2002) The *dual-hormone hypothesis* postulates that basal cortisol and testosterone interact, rather than act independently, to influence behavioural systems implicated in traits such as empathy, and aggression, and that these interactions are sex dependent (Zilioli et al., 2015). In addition, there is evidence that both GR and MR are less sensitive to cortisol modulation in females than males, suggesting reduced feedback by autoregulation of the receptors (Herman et al., 2016). In line with this, clear association between menstrual cycle phase and cortisol basal measurement has been shown. Women in the luteal phase of the menstrual cycle have a similar cortisol response as men, whereas in the follicular phase, and menopause, attenuation of cortisol production has been shown (Kirschbaum et al., 1999). Moreover, high levels of CBG due to oral contraceptive use results in high total cortisol levels, but free cortisol levels are usually unaltered in states of increased CBG) (Šimůnková et al., 2008). Of note, *Inoue et al* (Inoue et al., 2015) demonstrated clear sex differences in cortisol levels in BPD; whilst significant attenuated cortisol measurements in the female BPD population was demonstrated, the male

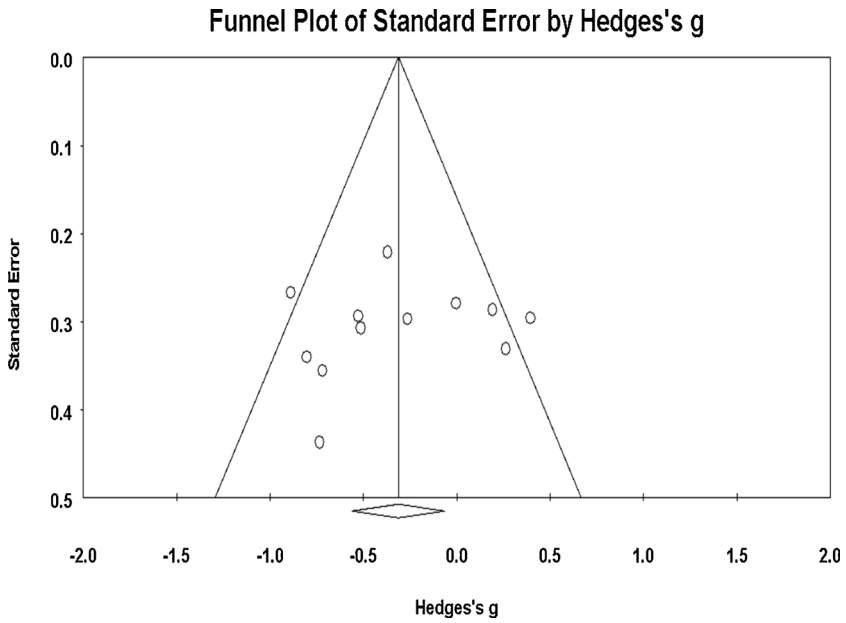


Fig. 2. Funnel plot of the distribution of Hedges g for the studies included.

population demonstrated significantly higher levels, at baseline and reactivity. Additionally, *Rauch 2015 (*Rausch et al., 2015*)*, investigated gender specific differences in cortisol levels and demonstrated female only elevated cortisol awakening responses, although no baseline significant measures were obtained. This was after controlling for potential confounding variables such as menstrual cycle, body mass index (BMI), and smoking status

Majority of studies analysed within the meta-analysis either excluded males, or were more weighted toward females, most likely reflecting the higher prevalence of females to males within the clinical population. Therefore, like many psychiatric disorders, the pooled systematic review and meta-analysis result supports sex differences in cortisol basal/baseline levels in BPD.

4.3. Influence of co-morbidity,heterogeneity, and early life stress

Changes in HPA axis functionality in BPD also appears to be moderated by comorbid symptomatology. As considered above, PTSD and MDD are highly prevalent co-morbidities of BPD, and have demonstrated opposing findings regarding HPA activity (Wichmann et al., 2017; Zorn et al., 2017b). *Lang et al (*Lange et al., 2005*)* investigated HPA activity in BPD patients with and without comorbid PTSD compared to controls. Although basal levels were not significantly different comparing the three groups, BPD patients with comorbid PTSD showed increased feedback sensitivity compared to BPD patients without comorbid PTSD. In addition, *Jogems-K et al* (Jogems-Kosterman et al., 2007) found no significant differences in cortisol levels comparing BPD patient group as a whole with controls, but found significant increases in cortisol in those patients diagnosed with comorbid PTSD and a history of childhood abuse compared to controls In contrast, the reduced

Study name	Hedges's g	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Aleknaviciute.2016	-0.885	0.268	0.072	-1.410	-0.360	-3.305	0.001
Carrasco.2007	-0.524	0.295	0.087	-1.101	0.054	-1.777	0.076
Dettenborn.2016	0.266	0.332	0.110	-0.384	0.917	0.803	0.422
Lieb.2004	0.196	0.288	0.083	-0.368	0.759	0.680	0.496
Rauch.2015	-0.366	0.222	0.049	-0.801	0.069	-1.650	0.099
Jogems-K.2006	-0.510	0.308	0.095	-1.114	0.094	-1.656	0.098
Lange.2005	-0.261	0.298	0.089	-0.845	0.322	-0.877	0.380
Wingenfeld.2007	0.398	0.297	0.088	-0.183	0.979	1.343	0.179
Walter.2008	-0.732	0.438	0.192	-1.591	0.127	-1.671	0.095
Nater.2010	-0.715	0.357	0.127	-1.414	-0.016	-2.005	0.045
Paris.2004	0.000	0.280	0.078	-0.549	0.549	0.000	1.000
Mazer.2018	-0.801	0.341	0.116	-1.469	-0.132	-2.346	0.019
	-0.312	0.126	0.016	-0.560	-0.064	-2.467	0.014

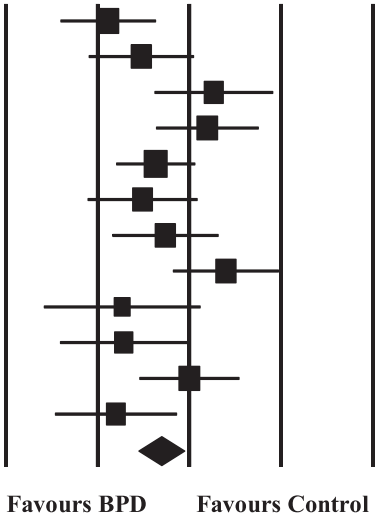
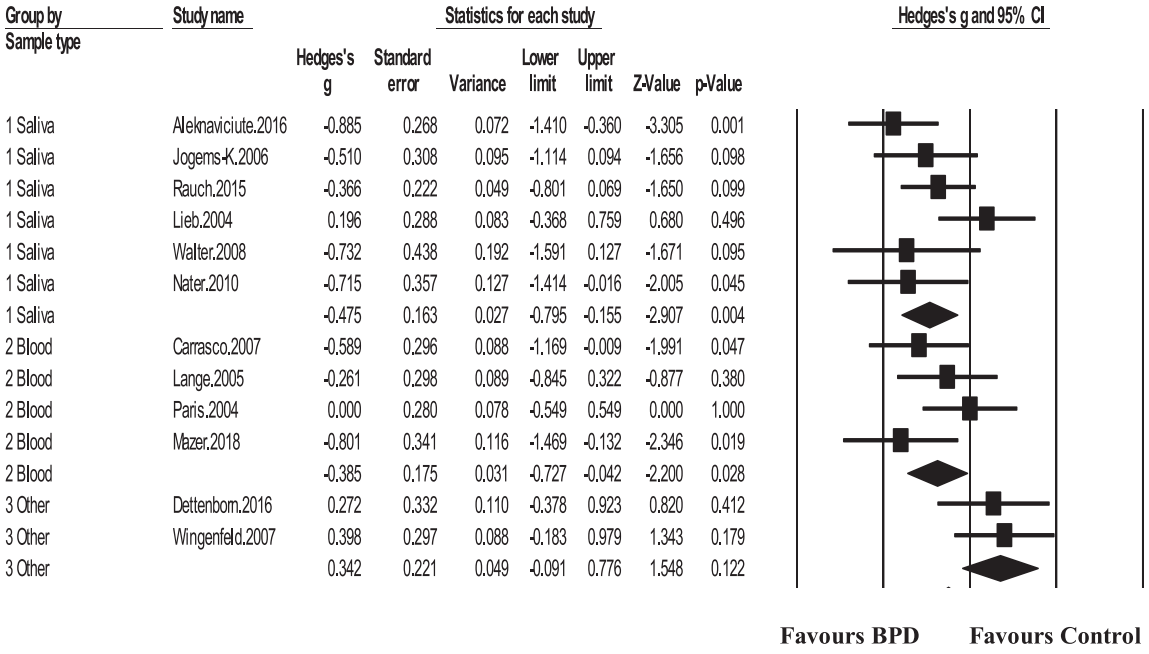


Fig. 3. Forest plot of cortisol measurement in Borderline personality disorder compared to non-psychiatric controls, overall studies.



*Other includes hair, 24 hours urine analyses.

Fig. 4. Forest plot of cortisol measurement in Borderline personality disorder compared to non-psychiatric controls, sub-grouped by biological sample type.

cortisol levels demonstrated in the BPD patient group compared to controls and CPD patients in the *Aleknaviciute et al* study (Aleknaviciute et al., 2016) remained even after performing a sensitivity analysis excluding any patient with co-morbid PTSD, suggesting results were not explained by PTSD co-morbidity.

In diagnosed BPD patients, reduced feedback sensitivity was found in those patients who presented with a low number of PTSD symptoms, while findings in patients presenting with a high number of PTSD symptoms did not differ from those in controls (Wingenfeld et al., 2008). The same authors showed that depressive symptoms were positively correlated to cortisol levels (Wingenfeld et al., 2008). Such results have led to the hypothesis that the BPD population may contain at least two subgroups of different BPD patients with different endocrine patterns; where patients who present with predominately trauma associated PTSD symptomatology demonstrate significantly decreased cortisol levels and an increase in HPA axis suppression, whist increased cortisol, and decrease in HPA axis suppression in BPD patients predominately presenting with affective dysregulation and depressive symptoms (Wingenfeld et al., 2010).

BPD is often clinically misdiagnosed as bipolar disorder due to many overlapping clinical features. A recent meta-analysis reported that bipolar disorder was associated with significantly increased levels of basal cortisol levels when compared to controls (Belvederi Murri et al., 2016), however *Mazer* et al *(*Mazer et al., 2018*)* demonstrated that both BPD and bipolar disorder had lower plasma cortisol levels when compared to non-psychiatric controls. Intriguingly, correlation analyses revealed the HPA axis activity were distinguishable between the two diagnostic groups when taking into account reports of early life stress; bipolar disorder patients demonstrated lower plasma cortisol as the severity of sexual abuse increased, in contrast to the opposite pattern in BPD. In addition, a negative association was identified between emotional neglect and physical neglect, and cortisol levels only in BPD patients. BPD patients reporting higher overall levels of childhood trauma defined by the Childhood Trauma Questionnaire also demonstrated lower overall cortisol levels in the study of *Aleknaviciute et al (*Aleknaviciute et al., 2016*)*. Early life stress as an environmental contributing factor to the onset of BPD is widely accepted, and the influence of early life adversity on cortisol levels has been supported by a recent meta-analysis (Bunea et al., 2017). A transition in cortisol activity from hypersecretion of cortisol activity from adversities *before* the age of 11, and hyposecretion *after* the age of 11, also emphasises timing as an important factor in the development of the neuroendocrine axis (Bosch et al., 2012). This highlights the importance of considering type and timing of early life stress within the context of stress neurobiology in BPD, as different modalities and neurodevelopmental timing of such adversity, could correspond to biologically unique subtypes.

To further add to the complexity of investigating cortisol levels and HPA activity in BPD, distinct symptom domains of BPD including dissociation (Koopman et al., 2003) and aggression (Yu and Shi, 2009), have been shown to correlate with cortisol levels. It therefore may be useful to consider cortisol, and other neuroendocrine factors in relation to symptom domains, and to make comparisons between other psychiatric disorders rather than solely non-psychiatric controls in future studies. This pooled sample is clinically representative however, since it includes co-morbidities which are prevalent with BPD diagnosis.

Residual unexplained heterogeneity could be related to trauma background, alcohol consumption, reactivity to acute transient stress, body mass index, genetics, or a combination of all these factors. It is also important to acknowledge that basal/ baseline measurements don't reflect information about temporal dynamics of HPA axis reactivity/ activity, nor chronic accumulation of HPA activity, and therefore more experiments are required to assess this, alongside future meta-analyses. This review evaluated single basal/ baseline measures, rather than longitudinal (ie CAR assessment), HPA activity, or cortisol stress reactivity measures, to broaden the scope of available data for comparison of cortisol levels in BPD. A limitation of this study design is that baseline cortisol measures extracted from cross-sectional and longitudinal experiments, and before varying induced stressor protocols, have been pooled and analysed collectively.

5. Summary and conclusion

In summary, the results of this systematic review and meta-analyses represent pooled data investigating baseline cortisol levels in BPD, vs

non-psychiatric controls, demonstrating lower cortisol levels in BPD. Cortisol as a biomarker of the HPA axis is an important and helpful measure in the study of stress disorders such as BPD. However, considerations of potential confounding factors must be considered.

Conflict of interest

The authors declare they have no conflict of interest

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