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Neuroendocrine mechanisms of grief and bereavement: A systematic review and implications for future interventions

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Abstract

Bereavement is associated with many negative behavioural, psychological and physiological consequences and leads to an increased risk of mortality and morbidity. However, studies specifically examining neuroendocrine mechanisms of grief and bereavement have yet to be reviewed. This systematic review is a synthesis of the latest evidence in this field and aims to draw conclusions about the implications of neurobiological findings on the development of new interventions. PRISMA guidelines for systematic reviews were used to search for articles assessing neuroendocrine correlates of grief. Findings were qualitatively summarised. The National Heart, Lung, and Blood Institute Study Assessment Tool was used to assess the quality of the included studies. Out of 460 papers, 20 met the inclusion criteria. However, most were of fair quality only. As a neuroendocrine marker, the majority of the studies reported cortisol as the outcome measure and found elevated mean cortisol levels, flattened diurnal cortisol slopes and higher morning cortisol in bereaved subjects. Cortisol alterations were moderated by individual differences such as emotional reaction to grief, depressive symptoms, grief severity, closeness to the deceased and age or gender. Research on neuroendocrine mechanisms of grief is still in its early stages regarding grief measures and the use and timing of neuroendocrine assessments. Most of the studies focus on cortisol as outcome, and only limited data exist on other biomarkers such as oxytocin. Future research might consider assessing a broader range of neuroendocrine markers and use longitudinal designs with a focus on the psychobiological reactions to loss. Based on this, individually tailored psychosocial interventions, possibly in the palliative care context, might be developed to prevent prolonged grief disorder.

KEYWORDS

bereavement, cortisol, grief, hypothalamic-pituitary-adrenal-axis, oxytocin, stress, trauma

1 | INTRODUCTION

1.1 | Social loss and its consequences

The loss of a loved person is one of the most devastating experiences in life and is associated with psychological, behavioural and physiological changes in the surviving close persons. The term *loss* is referred to the actual loss event, whereas *grief* entails the subjective reactions that are associated with loss. Although grief also occurs after a separation, in this review, we focus exclusively on grief after an actual loss of a loved one through death. *Bereavement* is defined as the state of having suffered the loss of a loved one and entails the time after a loss during which grief is experienced.[1] Physiological reactions to bereavement include neuroendocrine, immunological and somatic changes.[2] Psychological consequences include insecurity, anxiety, aggression and depressive and (psycho-) somatic symptoms,[3] which result in a greater vulnerability to somatic or psychiatric problems, such as cardiovascular diseases[4] or clinical depression.[5,6] Some studies even associate loss with increased mortality among the survivors,[7-10] highlighting the massive effects of this experience. More recently, for example, systematic research revealed that social loss triggers the development of Takotsubo cardiomyopathy, or "Broken Heart Syndrome". This syndrome is a reversible, stress-induced cardiomyopathy that mimics acute myocardial infarction and occurs after intense emotional or physical stress.[11] Above this, patients with Takotsubo cardiomyopathy have a higher prevalence of neurological or psychiatric disorders than those with an acute coronary syndrome.[12]

The previously described non-pathological mourning process is an adaptive response and usually has no long-term negative effects.[13] If, however, grieving continues and symptoms occur, that are beyond typical grief, *Prolonged Grief Disorder* (PGD) or *Persistent Complex Bereavement Disorder* (PCBD) can be diagnosed. PGD is characterised by longing for and preoccupation with the deceased, along with emotional distress and significant functional impairments that persist beyond 6 months after the loss of a significant other.[14] Approximately 10%-20% of mourners develop PGD/PCBD.[15-18] The diagnosis has only recently been added to the latest versions of the International Classification of Diseases (ICD-XI, PGD) and the Diagnostic Manual for Psychiatric Disorders (DSM-5, PCBD),[19] and led to debate about the defining criteria and consequences.[7,16,20,21] The term *Complicated Grief* (CG), which was originally developed to distinguish grief from depression,[22] does not represent the official diagnosis but, instead, comprises a larger category with diagnostic disordered grief encompassing a smaller group.[23] This distinction has to be kept in mind when interpreting empirical studies on grief. In the following, we employ the original terms used in the studies in each case.

1.2 | Psychobiological models of pair bond formation and bond disruption

The death of a loved one goes along with several psychosocial consequences: loneliness, a disruption in daily routines, a substantial loss of coherence, impaired sleep, and, most centrally, being separated from the loved person. All of these factors individually have been associated with poor health outcomes. For example, loneliness enhances the risk of morbidity and mortality,[24] elevates cardiovascular activation,[25] leads to cortisol dysregulation[26-28] and is associated with a greater utilisation of health care institutions.[29] A lower level of sense of coherence is associated with increased burden in caregivers of patients with chronic illness.[30] Additionally, poor sleep quality is associated with blunted cortisol awakening responses.[31] As the above mentioned psychosocial consequences all come together in grieving survivors, it can be assumed that those neuroendocrine and psychological changes may be even more pronounced in those who suffer intensely from the loss.

In this context, attachment and attachment disruption theories give important indications towards a better understanding of grief and its role in physical and mental health. Sbarra and Hazan[32] postulated that understanding the functionality and cause of human adult attachment could give us deeper insights into human coregulation and biobehavioural reactions to loss. According to their model,[32] relationships function as interpersonal regulatory systems. Interpersonal regulation means that couples co-regulate their emotional and behavioural responses, which serves as an adaptive mechanism that is less effortful and more automatic than individually regulating them.[32,33] The disruption of a relationship ends these regulatory benefits and leads to stress-related grief responses (dysregulation). The main task in coping with loss would be to manage dysregulation by using behavioural, emotional or cognitive strategies (functional *self-regulation*), which then attenuate the physiological consequences. According to the model, the initial reaction to loss not only involves psychological, but also physiological changes accompanied by psychological reactions.[32] Therefore, it is important to know the associated biological mechanisms of grief to predict negative psychological changes and to prevent grieving persons from long-term negative effects such as PGD/PCBD.

On the neuroendocrine level, grief might be primarily associated with an unspecific neuroendocrine stress-reaction, especially hypothalamic-pituitary-adrenal (HPA) axis activity. HPA axis activation leads to the synthesis of corticotrophin-releasing hormones (CRH) and vasopressin (VP), stimulating the secretion of adrenocorticotrophic hormones (ACTH) into the peripheral circulation.[34] As a result, ACTH induces glucocorticoid (e.g., cortisol) release in the adrenal gland, leading to a negative-feedback inhibiting HPA axis activation in the brain.[34,35] Cortisol secretion normally reaches its peak 30-45 minutes after awakening (cortisol awakening response [CAR]), followed by a subsequent decline during the day and reaching its lowest point between midnight and 5.00 AM[36,37] Besides its stress-dependence, a healthy HPA axis function shows strong diurnal patterns, and deviations from the typical decline throughout the day provide valuable information regarding the role of the axis in disease processes. Cortisol can be measured in several ways. Basal urinary free cortisol is often used to interpret aggregated cortisol levels. Hair or nail samples indicate hormone secretion over weeks or even months.[36] Recent studies have started to examine the

circadian rhythm of cortisol by evaluating a strong CAR and daily pattern of pronounced cortisol decreases during the day as indicators of a highly functional feedback-sensitivity of the HPA axis.[37]

As an additional neuromodulator, oxytocin (OT) is a hypothalamic neuropeptide that, after secretion from the paraventricular nucleus of the hypothalamus (PVN) and supraoptic nucleus (SON), is stored in the posterior pituitary lobe[38] and released into the peripheral blood circuit and into central-nervous brain areas, as parts of the pain network and the reward-system,[39] OT interacts with the HPA axis system by accompanying its response to a given stressor and exerting stress-reducing effects, for example heart rate, blood pressure and cortisol level decrease.[40-42] OT plays an important role in the formation and maintenance of social relationships.[43,44] In turn, the OT system is also altered after the disruption of a relationship.[45]

1.3 | Neuroendocrine changes after social loss in animals

In the history of research on neurobiological changes after social loss in humans, researchers often relied on animal models of separation and loss. More specifically, they began to examine neurobiological factors of social loss in the prairie vole (*Microtus ochraster*), which serves as an animal model of human social loss. In these monogamous rodents, the loss of a companion is associated with the activation of the HPA axis with higher basal plasma corticosterone concentrations[46-48] and adrenal hypertrophy.[49]

Vole mothers show significant increases in the corticotrophin-releasing factor (CRF) mRNA expression in the PVN,[46] when separated from their pups. Interestingly, the stress response to separation can be reduced through the peripheral, subcutaneous application of OT.[50,51] The separation from an adult attachment figure in voles leads to decreased OT mRNA expression in the PVN[49] and increased density of OT-immunoreactive cells in the PVN and the SON. The latter has been interpreted as a consequence of a decreased release and limited OT receptor activity in reaction to loss.[48] Furthermore, OT fibres signalling to the NAcc show decreased activation after loss in voles.[44]

Translating these effects of OT to human attachment, one can assume that the OT system is also involved in social loss in human beings. Neuroendocrine mechanisms involving OT have already been discussed with relevance for different mental disorders.[52] Although they might only serve as one of many response domains after the death of a beloved person, they could be a key mediator in the relationship between grief and the development of psychiatric disorders such as PGD or PCBD.[32] Deviations from functional neuroendocrine stress responses have already shown to be involved in response to trauma[53-55] and could possibly serve as a prognostic indicator for the development of grief-related psychopathology. Furthermore, important implications could be derived regarding preventive psychosocial interventions before the death of the close person in order to enhance co-regulation, as well as the awareness of the upcoming relationship disruption.

To date, a number of articles exist reviewing literature on the neuroendocrine mechanisms of grief, although they either exclusively focus on animal studies[44,45] or on prolonged grief in the context of only one neuroendocrine marker.[56] Therefore, the aim of the current work is to extend the existing literature by systematically reviewing studies investigating neuroendocrine mechanisms in the early stage of grief with potential predictive value for long-term pathological reactions to loss.

2 | MATERIALS AND METHODS

2.1 | Search strategy and eligibility criteria

A systematic literature review was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines.[57] A Boolean search was used to find the wide range of studies reporting neuroendocrine mechanisms of grief. The search terms were (grief OR bereavement OR bereaved OR "bond disruption" OR "social loss" OR "bond loss" OR sorrow OR mourning) AND (neuroendocrine OR endocrine OR neurobiol* OR psychobiol* OR psychophysiol* OR biomarker*). The initial search was performed in 22 March 2019 and updated on 23 April within four large databases including *Web of Science* (http://webofknowledge.com), *CINAHL* (https://www.ebscohost.com/nursing/products/cinahl-databases), *PubMed* (https://pubmed.ncbi.nlm.nih.gov) and *PsycINFO* (http://www.apa.org/psycinfo). The authors repeated the search on 13 November 2019 by adding more specific neuroendocrine words (oxytocin OR OXT OR OT OR cort* OR insulin OR prolactin OR endorphin OR catecholamin*) to find all the relevant articles concerning specific neuroendocrine changes after bereavement. Additionally, reference lists of relevant reviews, primary studies, and theoretical frameworks were searched for potential articles.[6,17,32,43-45,58-62] Two independent readers (DH and HM) screened the article abstracts and read the selected full-text articles in order to decide whether to include or exclude the articles according to predefined criteria. Non-consistent decisions were discussed until consensus was reached. The eligibility criteria for the studies were:

Inclusion criteria:

- Original study.
- Neuroendocrine markers (cortisol, epinephrine, norepinephrine, OT, insulin, prolactin, endorphin) investigated.
- Population: human adults (> 18 years) who lost a beloved person (partner, family member, close friend).
- Article available in English.

Exclusion criteria:

- Experimentally induced grief.
- Grief reactions did not occur as a result of death (eg, grief related to depression or post-traumatic stress disorder (PTSD); grief after divorce or break up).

- Article not available in English.
- No neuroendocrine markers assessed.
- Child or adolescent population (< 18 years).

2.2 | Data extraction

Relevant data of the incorporated studies, including publication date, study design, sample characteristics, grief assessment tools, neuroendocrine measure and results, were extracted for qualitative data analyses. Study quality was assessed independently by three authors (DH, ME and CAR) using the National Heart, Lung, and Blood Institute (NHLBI) Quality Assessment Tool for Observational Cohort and Cross-Sectional Studies.[63] This tool consists of 14 items (including 18 sub-items) assessing key issues of the study's internal validity; for example, population recruitment, statistical power considerations, assessment of exposure and outcome variables and consideration of confounding variables. The criteria can be met, not met, cannot be determined, are not applicable or not reported. The raters discussed their ratings to resolve discrepancies and come to a final decision. Studies were rated as "good", "fair" or "poor" to describe the risk of bias. A "good" quality rating indicates the least risk of bias. We decided to rate studies as "good" if they met more than 2/3 of the criteria. A "fair" rating indicates that the study shows higher risk of bias but not enough to invalidate results. Studies were rated as "fair if they met at least half of the criteria. A "poor" rating indicates high risk of bias that could significantly compromise the accuracy of the results. Studies were rated as "poor" if they met less than half of the criteria.

3 | RESULTS

In total, 677 papers were found during the systematic search (Figure 1), from which 469 articles remained after removing duplicates. After screening the abstracts, 39 articles remained for full-text eligibility search. Six articles were excluded because there was no full-text available online,[64-69] one study was excluded because it exclusively examined heart rate variability and cytokine production system,[70] one other study investigated receptor genes only[71] and five studies investigated early parental loss in childhood and

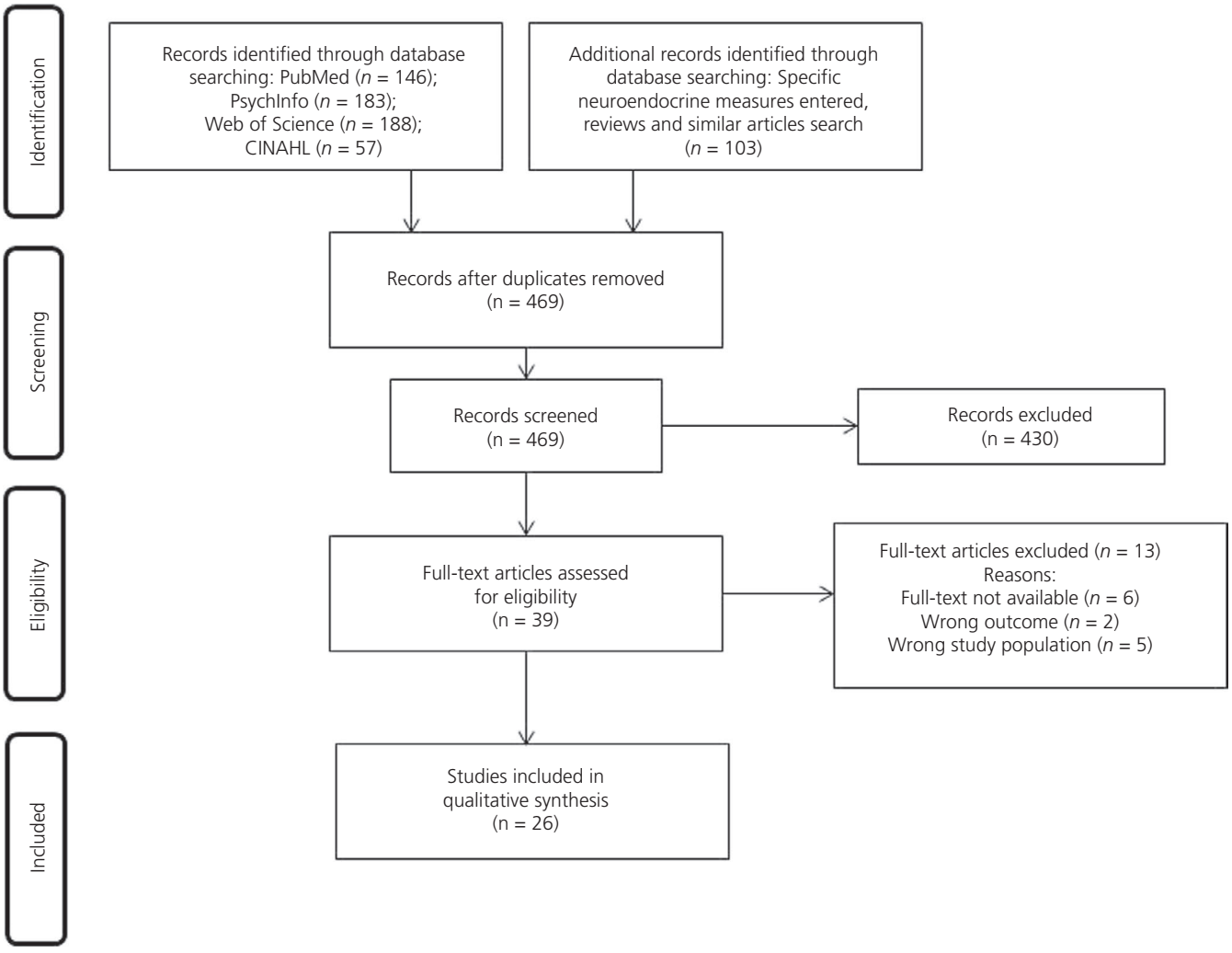

FIGURE 1 PRISMA flowchart on the study selection process

were therefore excluded.[72-76] The final sample consisted of 26 articles published between 1986 and 2019. According to the Quality assessment ratings, five studies showed good quality,[77-81] whereas 21 studies showed fair quality.[82-102] The results are shown in Table 1.

3.1 | Mean cortisol level

Five studies examined the association between bereavement and mean cortisol levels. Jacobs et al[84] compared 56 bereaved with non-bereaved spouses, both 1 and 2 months after hospitalisation of their spouse. It was hypothesised that adults with rising separation anxiety and distress during bereavement would show higher cortisol levels than those with lower anxiety. They collected 24-hour urinary free cortisol on three separate days in the week before the second interview and averaged the daily values of cortisol. Participants with high separation anxiety showed higher cortisol levels than those whose anxiety level fell from 1 to 2 months after hospitalisation. There was no difference in cortisol levels between the bereaved and the anticipatory bereaved.[84] Irwin et al[92] assessed cortisol weekly over 1 to 2 months in 28 recently bereaved, anticipatory bereaved, or non-bereaved women. They found significantly higher cortisol levels in the bereaved compared to the other controls. Spratt and Denney[93] examined the effect of sudden child death on cortisol levels in 18 bereaved vs non-bereaved parents. They report no differences in cortisol levels between the two groups. One further study compared cortisol levels between 260 bereaved and 262 non-bereaved men and women at the same time as controlling for depressive symptoms. Levels were assessed at one time-point after the psychiatric interview. No significant differences between the two groups were found.[79] Minton et al[96] investigated changes in physiological stress 11, 12 and 13 months after loss in 47 widows. They compared mean morning and evening cortisol levels and hypothesised that during the first anniversary after their loss, physiological stress level would be the highest. However, no significant differences in cortisol levels were found. The authors suggest that the anniversary does not represent an immediate stressor, not being sufficiently salient to change neuroendocrine stress levels.[96] Andersen et al[97] investigated the psychological and physical health effects of repeated loss among university students after clustered peer deaths. Cortisol was measured via hair samples 3 months after the loss. A significant association of prior bereavement experiences with hair cortisol level was found, as well as a significant negative relationship between the number of bereavement experiences and cortisol levels. The latter finding is interpreted in the way that people with prior bereavement maintain average levels of cortisol across the extended period of loss, whereas those with no prior experience display dysregulated cortisol levels.[97]

3.2 | Morning/evening cortisol

Two studies investigated bereavement in the context of morning cortisol. Buckley et al[80] assessed morning cortisol in 62 bereaved and 50 non-bereaved men and women 2 weeks and 6 months post-loss by taking one sample each morning. They found significantly higher cortisol morning levels in the bereaved compared to the controls at both time-points.[80] The second study examined whether overnight basal urinary free cortisol 12 months after loss depended on gender, the emotional reaction to loss (emotional numbness) and circumstances of spousal bereavement (prolongation) which were assessed 6 months post-loss. It was hypothesised that longer forewarning of death ("How long before your spouse's death did you realise that s/he was going to die?") and higher emotional numbness would be associated with higher cortisol dysregulation (higher cortisol levels).[78] As expected, prolonged forewarning was significantly associated with elevated cortisol levels. During 6-12 months, cortisol levels increased in widowers and decreased in widows. Bereaved men with emotional numbness at time 1 had higher cortisol levels at time 2 compared to men without emotional numbness. This association was not found in women.[78]

3.3 | Diurnal patterns of cortisol

Four studies examined diurnal cortisol patterns. Ong et al[90] compared morning cortisol, CAR and cortisol slopes across the day between 22 bereaved and 22 non-bereaved adults. They hypothesised that affect moderated the relationship between bereavement and HPA axis dysfunction.[90] Significantly lower cortisol wake-up levels and flatter diurnal cortisol slopes were found in the bereaved compared to the non-bereaved adults. The results were also partly in line with the mediation hypothesis: pre- to post-loss changes in positive affect accounted for 29% of the effect of spousal loss on diurnal cortisol slopes.[90] Similar results were found in a small sample ($n = 12$) of study participants suffering from CG.[81] Only participants with CG showed flattened diurnal cortisol slopes, whereas participants experiencing normal grief did not. It was proposed that only individuals experiencing a prolonged reaction to loss might develop permanent HPA axis dysregulation.[81] By contrast, Holland et al[82] found dysregulated HPA axis function independent of grief level. They compared diurnal cortisol levels between 56 depressed controls and depressed bereaved men and women with or without elevated PGD symptoms. Significantly lower cortisol wake-up levels and flatter diurnal slopes were found in the depressed bereaved PGD group compared to the depressed controls. The differences in cortisol levels between the depressed bereaved with PGD and the depressed bereaved without PGD were not statistically significant. On a descriptive level, men and women who had lost a spouse showed greater cortisol dysregulation than those who lost someone else than their partner.[82] It was suggest that, according to the results, the loss of a loved one is predictive of more dysregulated cortisol, irrespective of one's level of PGD symptoms. Peréz et al[77] investigated diurnal cortisol levels both 2 years and 5 years post-loss in CG sufferers compared to normal grieving men and women and controls in a population-based sample of 2084 adults. Significantly lower morning cortisol and overall cortisol levels (represented by the area under the curve) were found in the CG group compared to the healthy grievers at time 1. No significant differences were found regarding the diurnal slope.

TABLE 1 Results of the qualitative systematic review grouped by outcome

Study	Study design	Sample characteristics		
		Grief types/groups	Loss relation (%)	N/n per group (% female)
Mean cortisol level				
Jacobs et al (1987)[84]	Longitudinal: two time-points: first interview 1 month after hospitalisation of partner (1), second interview 2 months after hospitalisation (2)	2 × 2 groups Bereaved vs anticipatory bereaved Rising separation anxiety vs declining separation anxiety from (1) to (2)	Spouse	*N* = 56 *n* (bereaved) = 40 *n* (anticipatory bereaved) = 16 (50)
Irwin et al (1988)[92]	Longitudinal: weekly assessment of cortisol in a 1-2 month period < 6 months post-loss	(1) Recently bereaved women vs (2) women with terminally ill husbands vs (3) women with healthy husbands	Spouse	*N* = 28 *n* (1) = 9 *n* (2) = 11 *n* (39 = 8 (100)
Spratt & Denney (1991)[93]	Longitudinal: four time-points: 2, 4, 6 and 8 months post-loss (sudden loss)	Suddenly bereaved (1) vs non-bereaved (matched to bereaved)	Child	*N* = 18 *n* (1) = 9 (66)
Andersen et al (2013)[97]	Cross-sectional	Undergraduate students who experienced repeated peer deaths	Friends classmate	*N* = 122 (61.48)
Cohen et al (2015)[79]	Cross-sectional (part of a larger biomarkers study): in average 1.04 years post-loss	Bereaved (1) vs non-bereaved (2)	NR	*N* = 529 *n* (1) = 260 (50)
Morning/evening cortisol				
Buckley et al (2009)[80]	Prospective controlled cohort study, longitudinal: two time-points: 2 weeks and 6 months post-loss	Bereaved (1) vs non-bereaved (2)	Spouse (94) Child (6)	*N* = 112 *n* (1) = 62 *n* (2) = 50 (66)
Minton et al (2009)[96]	Exploratory longitudinal correlational study 11, 12 and 13 months post-loss	Widows	Partner (100)	*N* = 47 (100)
Richard-son et al (2015)[78]	Prospective multi-wave study, longitudinal Three time-points (only 1 and 2 used in biomarker analysis): 6 months (1), 18 months (2) and 48 months (3) after the death	Bereavement vs no bereavement	Spouse	Widowers: n (1) = 64 n (2) = 61 controls: *n* = 1545 (only subsample used) (NR)

Age (mean/ range or SD)	Grief assessment	Dependent neuroendocrine measure	Results	QA rating
62.6 (NR)	SA	24-hour urinary free cortisol Assessment times: three separate days in the week before time-point (2)	Group, in which separation anxiety rose from (1) to (2) had sig. higher cortisol levels than group in which SA diminished or dropped sig. Higher cortisol levels were found both for the bereaved and the anticipatory bereaved subjects	Fair
52.2 (3.4)	None	Plasma cortisol	sig. Higher mean plasma cortisol levels in group (1) compared to group (3) Not significant: Plasma cortisol level in group (2) and (3)	Fair
(1) = 49 (38-61)	None	Plasma cortisol Assessment time: between 9.30 AM and noon) at the four defined time-points	No significant differences in plasma cortisol between the two groups	Fair
20.13 (1.14)	None Other variables: relationship to the deceased media exposure to deaths mental health history prior adverse experiences distress responses to peer deaths	Hair cortisol assessment times: once 3 months after the loss experience	Prior bereavement experiences (eg, death of friend or family member) are significantly associated with hair cortisol level sig. negative relationship between number of bereavement experiences and cortisol levels during the period of peer deaths single most important predictor of cortisol response is whether or not a student had previously experienced the loss of a friend or family member	
(1) = 54.27 (11.72) (2) = 53.23 (11.05)	One/two questions: Had someone close died since Project 1? - if yes, number of persons close to the participant who had died since the last interview	Urinary cortisol Assessment times: after interview during 2-day visit	No significant differences in urinary cortisol between the two groups [a]sig. association between number of bereavements and levels of cortisol	Good
(1) = 65.2 (33-84) (2) = 61.6 (36-87)	None	Plasma cortisol Assessment time: morning	sig. higher morning cortisol levels in group (1) compared to group (2) at time-point 1 and 2 Not significant: cortisol levels and depression Higher alcohol intake is associated with higher cortisol levels	Good
74.1 (6.3)	None	Morning and evening salivary cortisol (each averaged over 3 days) Assessment time: 45 minutes after awakening and 12 hours later three consecutive days	No significant differences in cortisol levels between months 11, 12 and 13	
Bereavement group: 70 (6.25) no bereavement group: NR	None	Overnight basal urinary free cortisol Assessment time: morning	Cortisol levels increased from (1) to (2) in widowed men and decreased in widowed women Prolonged forewarning as sig. predictor of cortisol levels Bereaved men who reported emotional numbness at (1) had higher cortisol levels at (2) compared to bereaved women	Good

(Continues)

TABLE 1 (Continued)

Study	Study design	Sample characteristics		
		Grief types/groups	Loss relation (%)	N/n per group (% female)
Cortisol diurnal pattern				
Ong et al (2012)[90]	Cross-sectional: in average 17.5 months post-loss	Bereaved (1) vs non-bereaved (2)	Spouse	*N* = 44 n (1) = 22 (86)
O'Connor et al (2012)[81]	Cross-sectional: up to five years post-loss	CG vs NG	Mother (NR) Sister (NR)	*N* = 24 *n* (CG) = 12 (100)
Holland et al (2014)[82]	Cross-sectional: in average 3.1 years post-loss	(1) Depressed nonbereaved vs (2) depressed bereaved without elevated PGD vs (3) depressed bereaved with elevated PGD symptoms	Spouse/ partner (33) Parent (16.7) Sibling (12.5) child (4.2)	*N* = 56 *n* (1) = 32 *n* (2) = 15 *n* (3) = 9 (60.7)
Peréz et al (2017)[77]	Population-based cohort study (1) two years post-loss (2) between two and five years post-loss	CG vs NG vs no grief	Partner (NR) Child (NR) Parent (NR) Brother/ sister (NR) Others (NR)	*N* = 2084 *n* (NG) = 131 *n* (CG) = 31 *n* (no grief) = 1922 (55)

Age (mean/ range or SD)	Grief assessment	Dependent neuroendocrine measure	Results	QA rating
65.8 (48-80)	None	Salivary cortisol Assessment times: three to 6 months after questionnaire assessment on four successive days awakening, 30 minutes after awakening, before lunch, at bed- time	Sig. lower average wakeup levels of salivary cortisol in group (1) compared to group (2) sig. flatter diurnal cortisol slope curve among group (1) compared to group (2) Not significant: effect of spousal loss on CAR response Pre- to post-loss changes in positive emotion accounted for 29% of the effect of spousal loss on diurnal cortisol slopes *mediating effect of positive emotion, even if controlling for confounding factors	Fair
CG = 42.67 (10.54) NG = 46.91 (9.32)	Interview for Complicated Grie	Salivary cortisol Assessment times (Diurnal pattern): waking, 45 minutes post waking, 4.00 PM and 9.00 PM	sig. slope differences between CG and NG groups: diurnal slope of the CG group was lower in the morning and higher in the evening --> flatter slope Sig. lower cortisol level 45 minutes post-wake in CG compared to NG Sig. higher cortisol levels at 4.00 PM in CG compared to NG	Good
69.9 (7.6)	Prolonged Grief Disorder Scale (PG-13)	Salivary cortisol Assessment times: awakening - 5.00 PM - 9.00 PM Two consecutive days (combined for analysis) log-transformed values as independent variable	Sig. lower levels of log-cortisol levels at wake and flatter diurnal slopes in group (3) compared to group (1) Not significant: differences between group (2) and (3), although descriptively flatter profile in group (3) compared to group (2) Bereavement independently of its strength is associated with dysregulated cortisol levels Subsidiary analysis: Those who most recently lost a spouse showed sig. greater cortisol dysregulation (higher log-levels at wake and flatter slope) than those who lost someone else than the partner Not significant: continuous PG did not predict log-cortisol	Fair
64.9 (5.5)	Inventory of Complicated Grief (ICG), Dutch version	Salivary cortisol Assessment times: awakening - 30 minutes after awakening - 5.00 PM - bedtime	(1) Sig. lower levels of morning cortisol in CG vs NG Sig. lower overall diurnal cortisol levels (AUCg) in CG vs NG Sig. lower levels of morning cortisol in CG vs control Not significant: slope difference between CG and NG Not significant: cortisol differences between NG and controls (2) [a] Sig. AUCg and cortisol morning response differences between CG (2-5 years) and CG (< 2 years) [a] Sig. higher scores in ICG are associated withlower morning cortisol	Good

(Continues)

TABLE 1 (Continued)

Study	Study design	Sample characteristics		
		Grief types/groups	Loss relation (%)	N/n per group (% female)
Cortisol:DHEAS ratio				
Khanfer et al (2011)[89]	Cross-sectional: within 2 months post-loss	Bereaved (1) vs non-bereaved (2)	Close family member (NR) Friend (NR)	*N* = 48 *n* (bereaved) = 24 *n* (non-bereaved) = 24 (67)
Vitlic et al (2014)[85]	Cross-sectional 2 x 2 design	Young bereaved (1) vs young non-bereaved (2) vs old bereaved (3) vs old non-bereaved (4)	Spouse (65 for (1) and 9.5 for (2)) Close relative (35 for (1) and 91.5 for (2))	*N* = 93 n (1) = 31.8 n (2) = 20 n (3) = 26 n (4) = 26 (58)
DST/CRH stimulation test				
Roy et al (1988)[86]	Cross-sectional: CRH stimulation test	Bereavement complicated with depression (1) vs uncomplicated bereavement (2) vs depressed controls (3) vs healthy controls (4) Sample (3) and (4) are used from earlier study	Spouse (25) 1st degree relative (75)	*N* = 92 *n* (1) = 9 *n* (2) = 19 *n* (3) = 30 *n* (4) = 34 (41)
Petitto et al (1992)[102]	Cross-sectional: assessment of adults with affective disorder who had experienced loss earlier in life DST	Patients with affective disorder Early loss (<= 19 years) vs late loss (> 20 years) Only first loss examined	Mother (20) Father (60) Sibling (20)	*N* = 45 *n* (early loss) = 22 *n* (late loss) = 23 (58)
Gerra et al (2003)[88]	Longitudinal: 3 time-points: 10 days (1), 40 days (2) and 6 months after stress-full life event DST administered	Bereaved vs controls	Parent (57) Son (14) Spouse (29)	*N* = 28 n (bereaved) = 14 n (control) = 14

Age (mean/ range or SD)	Grief assessment	Dependent neuroendocrine measure	Results	QA rating
73 (5.3)	None	Blood cortisol Cortisol:DHEAS ratio	[a] Sig. higher cortisol:DHEAS ratio in group (1) compared to group (2) Not significant: Differences in cortisol level between group (1) and (2), although higher mean values in group (1)	Fair
(1) = 31.8 (9.03) (2) = 31.7 (8.41) (3) = 71.3 (5.79) (4) = 72.6 (5.72)	Core Bereavement Items (CBI) IES	Venous blood samples, Cortisol, DHEAS, cortisol:DHEAS-ratio	Sig. lower DHEAS, higher cortisol and higher cortisol:DHEAS ratio in (3) compared to (4) No significant differences in these outcomes between (1) and (2) Those with higher CBI - scores showed higher cortisol:DHEAS ratios Those with higher social support reported lower cortsiol:DHEAS ratios	Fair
(1) = 47.6 (14) (2) = 41.5 (13.7) (3) = 42.3 (13.1) (4) = 29.4 (5.1)	DSM-III assessment of complicated vs non-complicated bereavement (with vs without depression) Texas Inventory of Grief (128)	Plasma ACTH and cortisol after CRH administration (1-μg/kg) Assessment times: 30 minutes, 50 minutes, 60 minutes, 75 minutes, 105 min135 min and 165 minutes after injection of needle	Sig. higher basal cortisol levels in group (1) compared to group (2) and (4) No significant differences in ACTH-levels Sig. smaller ACTH responses to CRH in group (1) compared to group (2) and (4) Sig. greater cortisol responses to CRH in group (1) compared to groups (2) - (4) No significant differences in ACTH responses to CRH between groups (1) and (2)	Fair
44.7 (14.1)	None	Blood cortisol Assessment times: 4.00 PM and 11.00 PM 1 day after dexamethasone application 11.00 PM day before)	Among the affective disorder patients of the early loss group, younger age at first loss significantly[a] correlated with higher 4.00 PM cortisol levels First loss as strongest predictor for HPA axis functioning Late loss predicts higher cortisol levels at 11.00 PM	Fair
38 (17-75)	None Degree of stress (Social Adjustment Scale)	Blood cortisol blood ACTH DST Assessment of blood samples: between 9.00 and 11.00 PM at times (1), (2) and (3)	Sig. higher cortisol plasma levels after DST in time (1) compared to time (2) and (3) [a] Sig. higher cortisol plasma levels after DST in time (1) in bereaved group compared to control Sig. higher mean basal ACTH concentrations in bereaved subjects in time (1) compared to (2) and (3) Sig. higher mean basal ACTH concentrations in bereaved subjects compared to controls in time (1) Sig. higher plasma cortisol concentrations in response to dexamethasone in high responders compared to low responders in the bereavement group Sig. correlations between HRSD and cortisol levels at time (1)	Fair

(Continues)

TABLE 1 (Continued)

Study	Study design	Sample characteristics		
		Grief types/groups	Loss relation (%)	N/n per group (% female)
Pfeffer et al (2009)[91]	Longitudinal: two time-points: one after study entry and one within 6 months after entry	(1) Bereaved (as a result of a traumatic event - terror attack at 09/11/2001) vs (2) non-bereaved	Spouse	*N* = 45 *n* (1) = 23 (96)
Catecholamines				
Jacobs et al (1986)[83]	Cross-sectional: 2 months after hospitalisation/death of the partner	Bereaved (1) vs anticipatory bereaved (2)	Spouse	*N* = 59 *n* (1) = 39 *n* (2) = 20 (51)
Jacobs et al (1997)[87]	Longitudinal: six time-points after hospitalisation over the course of 25-months follow-up: 1st time-point (1): directly after intake 2nd time-point (2): 1 month after intake 3rd time-point (3): 2 months after intake 4th time-point (4): between 2 and 13 months after intake 5th time-point (5): 13 months after intake 6th time-point (6): 25 months after intake 2nd time-point: baseline symptom assessment 3rd time-point: defensive and neuroendocrine assessment 5th and 6th time-point: outcome assessment	Bereaved/anticipatory bereaved	Spouse	*N* = 67 (50)
Insulin				
Cankaya et al (2009)[98]	Cross-sectional (part of a larger investigation of stress, individual differences, and health in a middle-aged and older primary care sample)	Sudden unexpected loss (linear or ordinal) Natural vs unnatural death	NR	*N* = 75 (100)

Age (mean/ range or SD)	Grief assessment	Dependent neuroendocrine measure	Results	QA rating
(1) = 41.79 (6.52) (2) = 41.12 (6.46)	None	Basal and post-dexamethasone cortisol Assessment times: 30 minutes after awakening, 7.00 PM , 4.00 PM , 9.00 PM on four consecutive days Dexamethasone administration: on day 3 in the evening	Sig. higher AM - cortisol in group (1) compared to group (2) PM - cortisol tended to be higher in group (1) compared to group (2) Sig. less afternoon cortisol suppression in group (1) compared to group (2) Not significant: group differences in cortisol suppression during AM assessment Sig. higher PM cortisol suppression in bereaved with accompanied PTSD compared to bereaved without any psychiatric disorder	Fair
61.9 (NR)	Emotional Distress associated with loss	24-hour urinary catecholamines (epinephrine and norepinephrine) Assessment times: three successive day	Higher outputs of catechloamines in (1) compared to (2) - not in a range associated with adrenal medullary disease No significant difference in norepinephrine or epinephrine in (1) compared to (2) Sig. negative correlation between norepinephrine and depression score	Fair
62 (0.9)	Unresolved grief/separation distress (as outcome variable)	As predictors: mean 24-hour urinary free cortisol at time-point (3) Mean 24-hour urinary free epinephrine at time-point (3) three samples	No significant difference between bereaved and non-bereaved in neuroendocrine functioning No sig. correlations between neuroendocrine measures and separation distress, depression, anxiety and demoralisation High mean cortisol predicted better self-rated health at time (5) High mean epinephrine predicted higher hopelessnes/helplessness scores at time (5) Mean cortisol was inversely correlated with symptoms of hopelessness and helplessness at time (6) Mean epinephrine was positively correlated with symptoms of hopelessness/helplessness at time (6)	Fair
52.07 (9.67)	None Traumatic Life Events Scale 1) Lifetime history of any sudden unexpected loss 2) Number of lifetime sudden losses 3) Type of sudden loss	IGF-1 assessed in blood assessment times: between late morning and late afternoon after the interview	Sig. lower IGF-1 levels in women who had experienced a sudden unexpected loss compared to women without a history of sudden loss Number of sudden losses is significantly associated with IFG-1 levels: the greatest decrease in IGF-1 was shown in the group with the most losses (> 5 sudden losses) No significant differences between those who lost someone as a result of an unnatural event vs a natural event	

(Continues)

TABLE 1 (Continued)

Study	Study design	Sample characteristics		
		Grief types/groups	Loss relation (%)	N/n per group (% female)
Oxytocin				
Bui et al (2019)[99]	Cross-sectional pilot study loss occurred at least 6 months prior to the study	Bereaved with primary diagnosis of CG (1) vs Major Depressive Disorder (MDD) (2) vs bereaved controls (3)	Parent (25.6 in (1), 40 in (2), 42.9 in (3)) Spouse (41 in (1), 12 in (2), 8.6 in (3)) Other (33.3 in (1), 48 in (2), 48.6 in (3))	*N* = 139 *n* (1) = 47 (70.21) *n* (2) = 46 (69.57) *n* (3) = 46 (69.6)
Prolactin				
Lane et al (1987)[100]	Cross-sectional bereaved sample, 8 weeks after death of the spouse	Widows/widowers with low (1), moderate (2), or high (3) developmental level of object representation (DLOR)	Spouse	*N* = 26 (46)
Intervention studies				
Theorell et al (1987)[101]	Intervention study (activation program)	Anticipatory bereaved and bereaved men and women who are about to lose/who lost a close relative (1) Activation programme Group (2) Comparison Group	Close female relatives (wives, sibling or child-ren)	*N* = 72 (100) *n* (1) = 36 *n* (2) = 36
Goodkin et al (1998)[94]	Randomised, controlled intervention study; longitudinal: Baseline (before intervention), 10 weeks (right after intervention), 6 months follow-up 10 months bereavement support group vs standard care control about 6 months post-loss	Bereaved HIV + homosexual men (1) vs bereaved HIV- homosexual men (2)	Close friend (NR) Partner (NR)	*N* = 119 *n* (1) = 45 (0)
O'Connor et al (2013)[95]	Part of a larger randomised clinical trial. longitudinal: Pre-Post Complicated Grief Intervention mean time post-loss = 87 months	Complicated Grief (continuously measured)	Close friend (NR) Spouse (NR) Parent (NR) Child (NR) Sibling (NR)	*N* = 16 (88)

Abbreviations: AUCg, area under the curve with respect to the ground and the slope; NR, not reported; QA, Quality Assessment.

ACTH, adrenocorticotrophic hormone; CAR, cortisol awakening response; CBI, Core Bereavement Items; CG, complicated grief; CRH, corticotrophin-releasing hormone; DHEAS, dehydroepiandrostheron-sulphate; DSM-III, Diagnostic and Statistical Manual of Mental Disorders III; DLOR, developmental levels of the survivors' object representation; DST, dexamethasone suppression test; HRSD, Hamilton Rating Scale for Depression; ICG, Inventory of Complicated Grief; IES, Impact Event Scale; IGF, insulin-like growth factor; MDD, major depressive disorder; NG, normal grief; OT, oxytocin; PG, prolonged grief; PGD, Prolonged Grief Disorder; PRL, prolactin; PTSD, post-traumatic stress disorder; SA, separation anxiety. sig.,significant.

Age (mean/ range or SD)	Grief assessment	Dependent neuroendocrine measure	Results	QA rating
(1) = 49.49 (12.87) (2) = 49.33 (13.27) (3) = 48.64 (12.7)	Inventory of Complicated Grief (ICG) Structured Clinical Interview for Complicated Grief (SCI-CG)[99]	Overall plasma levels of OT, measured through one simple blood collection	Sig. higher plasma OT levels for group (1) compared to group (2) No significant OT differences between (1) and (3) ICG symptom severity explained only 2% of the variation in plasma OT levels Secondary analysis: a primary or probable CG diagnosis is positively associated with plasma OT levels	
58.9 (26.4)	None DLOR (high vs moderate vs low)	Serum prolactin assessment times: before and after semistructured interview pre-to post interview prolactin change	Sig. larger mean PRL change in women compared to men Sig. negative correlation between PRL change and DLOR in women Sig. positive correlation between PRL change and DLOR in men	
(1) = 51 (24-77) (2) = 52 (21-77)	None Others: Depression Anxiety Mental Exhaustion	Serum prolactin serum cortisol assessment times: during treatment period before death 1 month after death 2 months after death	Increasing degree of mental exhaustion during the treatment period is associated with increasing cortisol levels and decreasing prolactin levels Sig. increased cortisol levels 1 month after death compared to the last observation before death Sig. lower prolactin levels during treatment in the activation programme compared to group (2) no significant differences in cortisol or prolactin levels from 1 to 2 months after death	
38.3 (9.5)	None	Plasma cortisol at all three time-points	sig. decrease of plasma cortisol levels in the intervention group compared to the control group Group (1) intervention subjects showed a decrease in cortisol levels from time 1 to time 3, whereas group 2 intervention subjects showed an increase in cortisol levels from time 1 to time 3 Sig. effect of intervention on cortisol levels (time 1 and 3 included) when controlling for baseline cortisol levels	Fair
64 (4.3)	Inventory of Complicated Grief (ICG)	Blood catecholamines: epinephrine, norepinephrine, dopamine Assessment times: up to 4 weeks before first therapy session between 10.00 AM and 3.30 PM	[a] Sig. prediction of post-treatment ICG score by pre-treatment epinephrine Not significant: pre-treatment dopamine and epinephrine in predicting post-treatment CG score	Fair

At time 2, significant higher cortisol morning responses and overall cortisol responses were found in the CG group compared to the same group at time 1. Furthermore, higher scores in grief severity were associated with lower morning cortisol levels.[77]

3.4 | Cortisol:DHEAS ratio

Two studies investigated the association between bereavement and the cortisol:dehydroepiandrostheron-sulphate (DHEAS) ratio. DHEAS is a sulfated steroid-hormone that is associated with HPA axis activity. By contrast to cortisol, which has immunosuppressive effects, DHEAS enhances the immune response. Studies have shown that DHEAS can buffer the suppressive effects of cortisol on neutrophil function.[89] Additionally, an increased cortisol:DHEAS ratio, which represents an imbalance between those biomarkers, appears to be a contributing factor to the process of age-related immunosenescence. Khanfer et al[89] hypothesised that ageing and stress had an additive and deleterious effect on immunity and that bereaved older adults should have higher cortisol:DHEAS ratios than non-bereaved older adults. They used the cortisol:DHEAS ratio as an indicator of neutrophil function and assessed cortisol levels in bereaved and non-bereaved older adults. Although cortisol levels were slightly higher in the bereaved group, a higher cortisol:DHEAS ratio was found in the bereaved compared to the non-bereaved subjects.[89] Vitlic et al[85] compared cortisol:DHEAS ratios between younger and older bereaved vs non-bereaved adults and found significant lower DHEAS, higher cortisol and higher cortisol:DHEAS ratios in the older bereaved compared to the older non-bereaved. These differences were not shown in the young groups.[85] Although the younger bereaved showed higher psychological effects of loss than the older subjects, these changes were not reflected in neuroendocrine outcomes. Finally, those with stronger grief symptoms showed higher cortisol:DHEAS ratios, whereas those with higher levels of social support showed lower ratios.[85]

3.5 | Dexamethasone suppression test (DST)/CRH stimulation test

The DST is applied to assess HPA axis feedback sensitivity.[103] By applying the corticosteroide dexamethasone, which mimics the effects of cortisol, cortisol release should be suppressed in healthy individuals. Non-suppression is considered an indicator of hypercortisolism. The CRH stimulation test was designed to test HPA axis dysregulation by stimulating the ACTH response.[103] After the administration of CRH, a rapid rise in ACTH and cortisol is expected, followed by a gradual decrease.

Four studies investigated DST/CRH results in bereaved individuals. Roy et al[86] applied the CRH stimulation test in bereaved men and women with or without depression and hypothesised that depressed bereaved would show similar reactions to CRH stimulation as depressed non-bereaved. The non-depressed women appear to have "normally" blunted responses to CRH stimulation, which may reflect their normal reaction to the negative feedback of hypercortisolism that is often found in depressive patients.[86] ACTH and cortisol were assessed in 92 participants after receiving the DST. Higher cortisol and lower ACTH levels were found in the depressed bereaved compared to the non-depressed bereaved and the healthy controls.[86] Petitto et al[102] examined the relationship between loss experience and HPA axis function in subjects with an affective disorder. They compared cortisol levels after DST in 45 men and women who had a loss experience at the age of 17 years or earlier with those who had a loss experience at the age of 18 years or later and included major depressive disorder as a control variable. Depressed men and women showed lower cortisol levels than the non-depressed. Among the affective disorder patients of the early loss group, younger age at first loss significantly correlated with higher afternoon cortisol levels. Furthermore, in the afternoon, men in the early loss group showed significantly higher cortisol levels than women. Late loss significantly predicted higher cortisol levels in the morning.[102] Gerra et al[88] compared ACTH, cortisol levels and immune markers after DST in 28 bereaved vs non-bereaved men and women 10 days, 40 days and 6 months after loss. They found higher cortisol levels in the bereaved, compared to the non-bereaved. ACTH levels were significantly higher in the bereaved group at time-point 1 only. Interestingly, cortisol and ACTH levels were highest in the early stage of bereavement. Furthermore, the effect of temperament was investigated: they found non-suppression of dexamethasone in subjects with high depression and harm avoidance compared to subjects with low depression and harm avoidance 6 months after bereavement.[88] Pfeffer et al[91] examined basal and post-DST cortisol in 23 traumatically bereaved participants over two time-points following the 9/11 terror attacks. Bereaved spouses showed higher morning basal cortisol and less afternoon post-dexamethasone suppression than non-bereaved subjects. Additionally, bereaved subjects with PTSD showed significantly greater afternoon post-dexamethasone suppression than bereaved subjects without PTSD, indicating higher glucocorticoid receptor sensitivity in the bereaved with PTSD.[91]

3.6 | Catecholamines

Two studies examined the association between bereavement and catecholamines as outcomes of sympathetic adrenal medullary function (SAM). Jacobs et al[83] investigated 24-hour urinary free epinephrine and norepinephrine on three successive days in 59 bereaved and anticipatory-bereaved subjects and found higher catecholamine outputs in the bereaved compared to the anticipatory bereaved; however, these differences were not significant. Norepinephrine was inversely correlated with depression scores and positively correlated with age. The latter finding is in line with past research showing that the SAM system in older adults adapts more slowly to stress.[83] Jacobs et al[87] examined the predictive effect of adrenal function on depression, anxiety, hopelessness, or unresolved grief. They assessed 24-hour urinary cortisol, epinephrine

and norepinephrine in bereaved and anticipatory-bereaved individuals. The neuroendocrine markers were assessed three times at time-point 3 (2 months after hospitalisation), at which 63% of the subjects were widowed. The psychological variables were assessed at time-points 2, 3, 5 and 6 (1, 2, 13 and 25 months after intake.) The neuroendocrine markers did not differ between the two groups. Epinephrine and cortisol only predicted hopelessness at time-point 5 in the bereaved subjects, although they did not predict any other psychological outcomes. Additionally, higher mean cortisol levels (average of the three assessments) at time-point 3 predicted better self-rated health at time-point 5. Mean cortisol measures were inversely correlated and mean epinephrine levels were positively correlated with hopelessness scores at time-point 6. The results indicate that adrenal function may serve as a mediator between social loss and health-related outcomes.[87]

3.7 | Insulin

Cankaya et al[98] investigated associations of interleukin (IL)-6 and insulin-like growth factor (IGF)-1 with the sudden death of a loved one in 75 females in an urban primary care setting. IGF-1 is posited as a protective factor in ageing-related diseases and is negatively correlated with immune markers such as IL-6. It was hypothesised that a prolonged exposure to stress and a sudden death would result in greater insulin changes than shorter exposure and a less sudden death. Significantly lower IGF-1 levels were found in women who had experienced a sudden unexpected loss compared to women without a history of sudden loss. The number of sudden losses was significantly associated with IGF-1 levels, meaning that the greatest decrease in IGF-1 was shown in the group with the most losses.

3.8 | Oxytocin

Bui et al[99] investigated peripheral plasma OT levels in men and women with CG. They compared a single assessment of OT levels of participants with a primary CG diagnosis to participants suffering from depression as primary diagnosis and bereaved control participants with no comorbid diagnosis. They found significantly higher OT levels in the CG group compared to the depressed group. There were no significant differences between the CG group and the group of non-pathological grief.[99] Secondary analyses revealed that a primary or probable CG diagnosis was positively associated with plasma OT levels.

3.9 | Prolactin

Lane et al[104] investigated sex differences in prolactin (PRL) changes during mourning in 26 spouses. Amongst others, PRL plays a role in the stimulation of maternal care, acts as an endogenous anxiolytic agent and regulates oxytocin neurones.[104] They assessed serum PRL before and after a semi-structured interview. The aim was to examine sex differences in the association between the developmental levels of the survivors' object representation (DLOR). The DLOR represents the verbal description of a person and the level of cognitive complexity of that description.[100] The results show a significant larger mean PRL change in women compared to men. A negative correlation between PRL change and DLOR was found in women, whereas a positive correlation was found in men.[100]

3.10 | Effects of bereavement interventions on neuroendocrine stress markers

Three studies examined the effects of bereavement interventions on stress-related neuroendocrine markers. In the first study, the effect of an activation programme on plasma cortisol and prolactin levels was examined in 72 close female relatives of cancer patients.[101] Plasma cortisol and prolactin, as well as anxiety, depression and mental exhaustion, were assessed during the intervention, right before the death of the relative and 1 and 2 months after loss. The results show that an increasing degree of mental exhaustion during the treatment period is significantly associated with increasing cortisol levels and decreasing prolactin levels. Furthermore, significantly higher cortisol levels were found 1 month after death compared to the last assessment before death. Also, lower prolactin levels during treatment were found in the activation group compared to the control group.[101] In the second study, the effects of a short-term bereavement support group intervention with 119 widowed men infected with HIV on immune variables and cortisol levels were assessed.[94] Recently bereaved HIV seropositive (HIV+) and HIV seronegative (HIV–) men were randomly assigned to either a bereavement support group intervention or a standard care group. Plasma cortisol was assessed pre, post and at 6-month follow-up. Significantly lower cortisol levels were found in the intervention group compared to the control group 6 months after the intervention. HIV + men in the intervention group showed significant decreases in cortisol levels from pre-assessment to follow-up, whereas HIV– men in the intervention group showed increased levels of cortisol within the same time-period.[94] The third study assessed predictive effects of catecholamines as moderators of a bereavement intervention and CG treatment outcomes after bereavement.[95] Sixteen bereaved individuals provided information on the Inventory of Complicated Grief (ICG) pre- and post-psychotherapy and blood epinephrine, norepinephrine and dopamine were assessed 4 weeks before the intervention. The post-treatment ICG-score was significantly predicted by pre-treatment epinephrine levels. SAM activity and autonomous function in the participants showed impaired CG outcomes after therapy.[95]

3.11 | Summarized results of good-quality studies

In summary, the results of "good quality" studies suggest the following neuroendocrine changes after the loss of a loved one:

- The more deaths of loved ones someone experiences, the higher his/her the cortisol levels.[77]
- Morning cortisol levels are significantly higher in bereaved compared to non-bereaved 2 weeks and 6 months after bereavement.[80]
- The longer the forewarning of someone's death, the higher the cortisol levels after bereavement.[80]
- Bereaved men suffering from emotional numbness 6 months after loss show higher cortisol levels 12 months after death compared to bereaved men who do not suffer from emotional numbness.[78]
- Compared to non-pathologically grieving subjects, people with CG show flattened diurnal cortisol slopes, suggesting that HPA axis dysregulation is more pronounced in prolonged grief.[79] People with CG show significantly lower morning and overall cortisol levels compared to non-pathological grievers 2 years after loss.[77]
- People with CG show significant higher cortisol morning responses 5 years after loss compared to two years after loss.[77]
- Higher scores in grief severity 5 years after loss are associated with lower morning cortisol levels.[77]

4 | DISCUSSION

The loss of a loved one can be associated with neuroendocrine alterations and dysfunction both in the early and late stage of bereavement. This systematic review summarises original articles examining neuroendocrine correlates of social loss. Of the original studies included in this review, most focused on HPA axis (eg, cortisol) or SAM-related hormones (epinephrine, norepinephrine) as primary outcomes. These studies not only suggest elevated mean cortisol levels and flattened diurnal cortisol slopes, but also increased epinephrine and norepinephrine levels after social loss. In general, flattened diurnal cortisol slopes have been associated with negative health outcomes in different study populations.[104] Individuals suffering from CG show flattened diurnal cortisol slopes[70,77] as well as lower morning and mean cortisol levels,[77] than those showing non-complicated grief. Furthermore, both closeness to the deceased[82] and grief severity[79] play an important role in the development of neuroendocrine dysregulations. The closer the relationship and the more or enduring the subjective impairment is articulated, the more endocrine dysregulation is pronounced. Particularly, higher grief levels and lower social support are associated with higher cortisol levels.[85] In addition, specific stressors, as well as psychological and demographic factors, partially account for HPA axis alterations. For example, increases in separation anxiety in the course of bereavement were associated with higher levels of cortisol[84] and having a longer forewarning before death lead to higher cortisol levels than experiencing an unexpected loss.[78] Sudden, unexpected losses, as well as a rising number of losses, are associated with lower insulin levels, showing that those context variables influence health-reducing neuroendocrine alterations after bereavement.[98] A positive affect was inversely correlated with cortisol levels,[90] whereas rising emotional numbness in men during the course of bereavement enhanced cortisol levels,[78] suggesting again that psychological variables are important when examining neuroendocrine changes after loss. Regarding gender differences, one study revealed that men showed decreasing cortisol levels, whereas women showed increasing cortisol levels during the course of bereavement.[78] Older men and women showed stronger alterations in their neuroendocrine stress responses than younger cohorts,[85,89] indicating that high age may have an additive effect on loss consequences. Furthermore, changes in stress-related alterations were shown, especially in the early stage of bereavement, although there is inter-individual variability.[88] In the latter study, however, almost no direct correlations between psychological and biochemical reactions were found. Neuroendocrine alterations were not only found directly after bereavement, but also months after loss experience.[97] Interesting results evolved with regard to psychiatric diseases: especially depressive symptoms were associated with higher cortisol levels[86,102] and higher cortisol non-suppression[88] in bereaved subjects. Furthermore, individuals suffering from PTSD after a traumatic loss showed higher cortisol levels than those with no trauma-related psychiatric diagnosis.[80] The same study suggests that trauma-related psychopathology may foster a prolonged neuroendocrine response to social loss up to 8 years after the event. One study investigated OT as a biomarker of grief and found higher OT levels in people suffering from CG.[99] Regarding prolactin changes, women have higher prolactin levels than men after having been interviewed about the deceased partner. Interestingly, women who have a more complex insight into the deceased person also show higher prolactin levels, whereas the opposite association is observed in men.[100]

In summary, these studies suggest that not only bereavement by itself, but also bereavement-associated psychopathology in particular is associated with stress-related neuroendocrine alterations. This is in line with research on trauma,[53,54] loneliness[26-28,105] and disrupted attachment,[44] which can all be individual psychosocial aspects of bereavement and separately serve as causal factors of stress-related neuroendocrine dysregulation. Bereavement can have health-impairing and fatal consequences for the surviving individual[4,6,9,106] and, as a consequence, interventions have been designed and evaluated to buffer these effects. The available studies on the effects of these interventions found the intervention to reduce cortisol levels.[94,95] Furthermore, epinephrine levels predicted psychopathology-related treatment outcomes suggesting that pre-treatment stress levels moderated the effectiveness of the intervention.[95] In line with this, catecholamines were correlated with helplessness and hopelessness in the course of bereavement,[87] and thus can serve as endocrine markers of subjective burden and treatment effects. An intervention before the partners' death was found to elevate cortisol levels and reduce prolactin levels, especially right before death.[101] The latter finding is consistent with the hypothesis that grief is activated by an intervention and that the active mourning may have a prophylactic value to the relative's grief reaction.[101]

The results indicate that, even years after loss, bereavement might be associated with neuroendocrine changes. These changes are

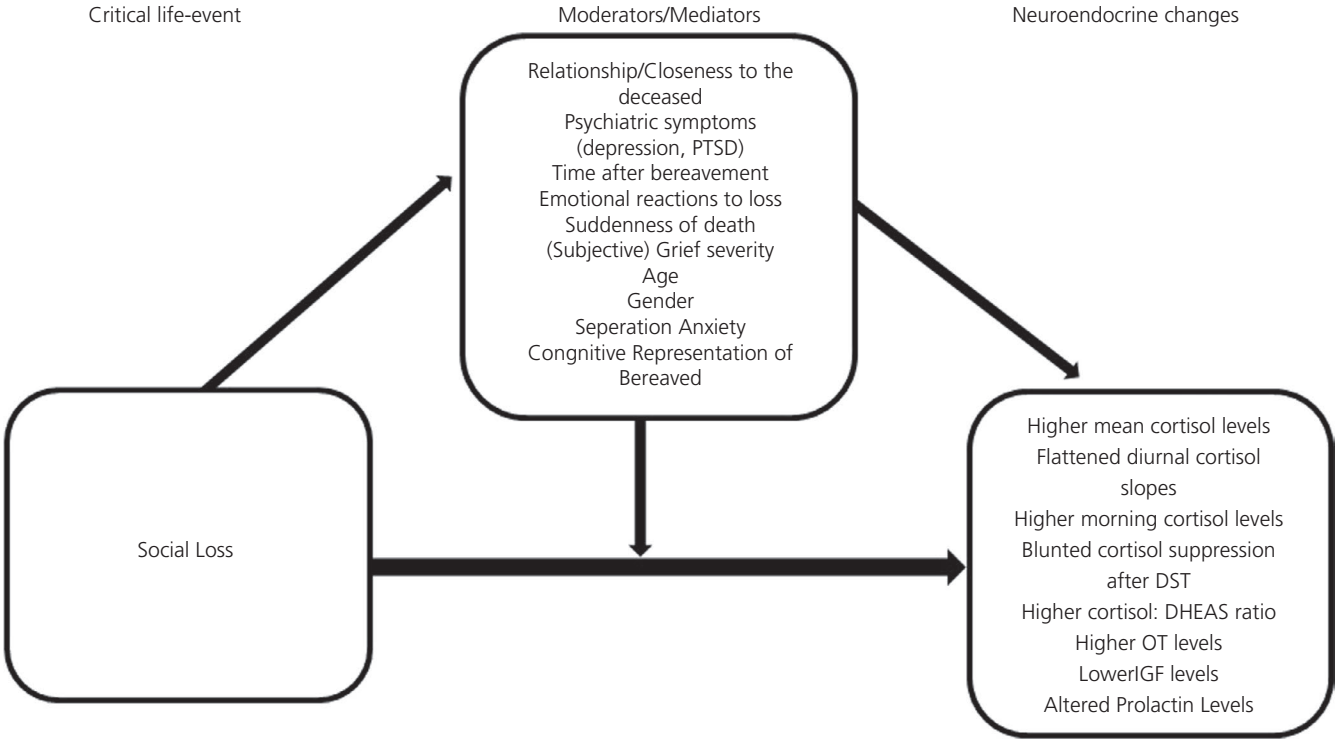

FIGURE 2 Model with summarised results (including potential moderators/mediators) of the studies investigating neuroendocrine mechanisms of grief. DHEAS, dehydroepiandrostheron-sulphate; DST, dexamethasone suppression test; IGF, insulin-like growth factor; OT, oxytocin; PTSD, post-traumatic stress disorder

moderated by grief severity, psychiatric state and psychological reactions to loss, as well as age and gender. On a psychobiological level, neuroendocrine responses may serve as moderators between the loss-event and long-term psychological outcomes (Figure 2). However, because of the methodological difficulties and contradictory results of the studies, these conclusions must be treated with caution.

For example, Ong et al[90] found that prolonged forewarning of death was associated with higher cortisol levels. They argue that a longer duration of care is associated with more stressful experiences, and thus leads to stronger physiological stress reactions.[90] Research on the development of PGD/PCBD shows that suddenness of death is a risk factor,[107] which initially appears to contradict the findings of Ong et al[90]. In this context, it is important to note that PGD/PCBD symptoms are not only characterised by physiological distortions such as elevated cortisol levels, but also by emotional and behavioural symptoms such as intense yearning, longing or emotional pain.[108] The contradictory findings may indicate that there are moderator variables between instant physiological reactions and the development of PGD/PCBD that foster the maintenance of an abnormally high cortisol level. It is not clear, yet, whether high cortisol levels or flattened diurnal slopes are risk factors of the development of PGD/PCBD. So far, only correlative conclusions can be drawn about HPA axis dysfunction and PGD/PCBD. To obtain a better understanding of what role HPA axis function may play in PGD/PCBD, it would be essential to measure cortisol levels in regular intervals over a longer time span after bereavement at the same time as measuring moderator variables.

There are further inconsistent study results regarding morning and mean cortisol. Buckley et al[80] found significantly higher morning cortisol levels in the bereaved, whereas Perez et al[77] found lower morning cortisol levels in people with CG. This discrepancy might be because of the different methods and timeframes investigated. Cortisol levels might be differentially affected depending on the time since loss. Furthermore, Buckley et al[80] compared grieving with non-grieving people, whereas Perez et al[77] compared people with CG and non-CG. Additionally, two studies found significantly elevated cortisol levels in bereaved and anticipatory bereaved,[86,92] whereas two other studies did not.[79,93] One reason for the conflicting results could be their methodological diversity, using different measurements of cortisol, different time-frames and different measurement foci. Furthermore, the study populations were somehow different. For example, Spratt and Denney[93] only examined suddenly bereaved parents, whereas Jacobs et al.[87] and Irwin[92] had participants with a longer history of end-of-life care.

4.1 | Limitations

The studies included in this systematic review reveal some limitations – one of them is the overall small sample size, which limits the statistical power of the results. Although 21 studies were of fair-quality and only five studies were rated as high-quality studies, the results must be interpreted with caution. For one thing, most of the studies considered the loss event as the exposure

variable without assessing continuous levels of subjective grief. According to some results, grief severity and subjective appraisal of loss have a stronger influence on neuroendocrine reactions than the loss experience itself.[77,78,84,85,90] Based on this, grief levels should be measured continuously to enhance the validity of the study. Furthermore, many studies took place years after the loss event. Potential confounding factors could have occurred within this time frame, which makes it difficult to disentangle the effect of bereavement from other factors influencing long-term endocrine changes. Another important fact is that many studies do not report whether the survivors made use of social support or psychosocial bereavement interventions, although social support can buffer the loss reaction. In the studies underlying this review, grief severities, as well as neuroendocrine outcomes, were assessed differently, which hampers their comparability or calculation of effect sizes using meta-analytic methods. More importantly and as a result of the unpredictability of death, neuroendocrine markers were not assessed before the loss of a loved person, and thus provide limited information on stable predictors only.

Despite the limitations mentioned, the findings of elevated cortisol and flattened diurnal slopes are relatively reliable, which suggests that they seem to be robust.

It has to be noted that, so far, only cortisol has been examined more extensively and research on other neuroendocrine measures, such as OT, ACTH or catecholamines, is still scarce.[99] The only study examining OT in the context of bereavement[97] has some methodological flaws, as the authors measured OT in the periphery. The assessment of peripheral OT levels is criticised because of its lack of association with central-nervous OT levels,[109] and ongoing methodological discussion about the reliable measurement of OT in the periphery even.[110] These points make it is difficult to draw reliable conclusions, especially with respect to neuroendocrine grief reactions in the human central nervous system. Based on the findings of animal studies on social loss as well as human studies with healthy couples, however, it can be assumed that the painful experience of a close person's death might also involve the OT system. The rewarding, stimulating effect of a well-functioning relationship is eliminated and the OT system remains under-stimulated.[111] This under-stimulation could in the long run even be related to the symptoms of PGD/PCBD. Additionally, studies investigating neural correlates of social loss indicate grief-related altered activation in brain areas such as the NAcc[112] and the ACC,[113] which are associated with the reward-system and high OT receptor densities. However, endogenous OT mechanisms in the central nervous system cannot be measured in the human living brain so far, which limits the possibilities to test for direct involvement of OT in the grieving process. Therefore,[44] animal models can be helpful to better understand those mechanisms. Moreover, human and animal studies can complement each other in a meaningful way because their methods lead to context-dependent results: experimental settings are artificial and may lead to different reactions than real-life events.[45] Above this, animal models cannot give us sufficient insights into the psychological reactions to loss. On the other hand, so far, the highly individual human grief-reaction cannot be investigated in a standard procedure or related to specific neuroendocrine changes in the living brain. Despite the lack of transferability, animal research can give us important hints as to where to start in human research and what hypotheses to establish. Therefore, the combination of knowledge from animal and human research can provide a broader picture on this complex topic.

Finally, some limitations should be mentioned regarding the recruitment of bereaved individuals. First, bereaved people are in an altered state of mind, with some describing numbness and a genuine loss of interest in daily matters. They might be difficult to reach with broad recruitment tools. For those who are, the reason for participating might be the hope for psychological support, meaning a rather vulnerable and selective subgroup might agree to participate. As to working with a bereaved sample, it can be challenging to maintain the motivation of the participants to remain in the study. Also, because the time of death most often occurs unforeseen, there usually are no individual baseline measures before the loss. To obtain pre-bereavement measurements, participants should be recruited before the death of their close one, treating them with the highest sensibility and psychological supervision.

4.2 | Future research and implications for psychosocial interventions

To help establish a comprehensive model of the neuroendocrine factors underlying the psychobiological reactions to social loss, in addition to the neuroendocrine stress response, future research can benefit from a focus on further and interacting neuroendocrine systems. Animal research on social loss suggests that, for example, the OT system interacts with the HPA axis and might be involved during grief reactions. Both CRH and OT have been shown to interact with the dopamine) system, which regulates reward and is involved in depressive disorders and addiction. In both animals and humans, dopamine appears to play a role in the formation of a romantic relationship; for example, reward-associated brain regions are highly activated in association with positive attachment interactions,[114-116] and it is assumed that this system could also be affected after loss by remaining under-stimulated.[111] Indeed, human studies already indicate activations in brain-regions with high OT and dopamine receptor density.[91,92] In line with this, withdrawal from drug abuse has been associated with similar activation patterns compared to separation from a partner.[46,111]

In addition, longitudinal studies assessing subjective and neuroendocrine markers before and after loss could minimise confounding inter-individual variations and thereby improve statistical power and long-term predictive power. Although necessarily in such studies, loss would always be predicted by a lethal illness, thereby limiting the range of different possible events to trigger grief.

Initial studies investigating neuroendocrine alterations after a bereavement intervention show promising effects and suggest that, beside subjective measures, neuroendocrine and stress-related outcomes can serve as meaningful indicators of treatment success.[94,95,101]

In this context, it is important to keep in mind that subjective and objective measures often diverge in research on stress, and thus it is important to reveal the differences between these measurements. This leads to the next step of exploring the reasons why these differences occur and what they mean for treatment success. However, to better interpret the meaning and importance of neuroendocrine measures for therapeutic success, more research is necessary. Furthermore, the assessment of neuroendocrine measures is associated with some hurdles. For example, the assessment of blood, urine or saliva samples is time-consuming and may be a reason for grieving participants not to take part in a study. One possibility to address this issue and to enable data on aggregated cortisol levels to be collected over an extended time period of weeks to months is the use of hair samples to quantify, for example, cortisol secretion. Ecological momentary assessment could be used to reduce the participants' burden of being torn out of their every-day life. Regardless of discussing in what way neuroendocrine measures serve as an indicator for treatment success, the Research Domain Criteria (RDoC) initiative of the National Institute of Mental Health explicitly recommends the inclusion of objective measures into interventional studies.[117] All in all, more research is necessary, investigating potential factors that may influence the efficacy of bereavement interventions with different populations, varying age groups and social background So far, only one early study has investigated the effects of a psychosocial intervention before the separation experience with the aim of *preventing* grief reactions and associated neuroendocrine alterations after loss. As far as we know, the anticipation of losing someone close may already lead to neuroendocrine changes,[84] which highlights the need for early strategies preventing neuroendocrine dysfunction and buffering the negative effects of social loss. It is known from studies on healthy couples' interventions that positive psychosocial interventions together with the partner or a family member activates the reward system and exerts stress-buffering effects.[118,119] This leads to the assumption that the described stress and under-stimulation reaction to social loss can be influenced by appropriate interventions and specific death and bereavement management programmes.[6,46] Psychosocial interventions before the loss of the partner might strengthen the bond, reduce stress levels, affect the endogenous OT release and buffer grief-related stress-reactions, preventing long-term negative health effects such as the development of CG or other psychiatric problems. According to the dual process model of coping,[120] oscillations between thoughts about the lost attachment figure on the one hand and evaluating a future without the lost loved one on the other hand are considered important factors of an adaptive grief coping process. In this context, interventions that help to strengthen the bond, and which make unresolved issues a subject of discussion, might foster a healthy coping process and therefore affect neuroendocrine as well as psychological health changes after the loss. Although there is no study investigating the effects of pre-death interventions on neuroendocrine reactions such as OT signalling, initial studies show that psychosocial interventions before loss are able to improve the well-being of the participants.[121,122] However, this hypothesis needs further investigation and additional research is necessary to understand whether mechanisms such as OT signalling contribute to the efficacy of such treatments. Furthermore, it is important to consider inter-individual differences when deciding on whether to implement an intervention or not.

In summary, neuroendocrine correlates of anticipatory grief and grief after social loss could help us identify individual needs and serve as tools to evaluate not only impairment, but also treatment success. In the long run, this knowledge might allow the development of specific interventions that improve stress-related responses in the survivors and thereby their health.

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CONFLICT OF INTERESTS

The authors declare that they have no conflicts of interest.

AUTHOR CONTRIBUTIONS

DH, ME, CAR and BD defined the literature search criteria. DH conducted the literature search and summarised the findings. DH, ME and CAR rated the internal validity of the studies by the National Heart, Lung, and Blood Institute Study Assessment Tool. DH, CAR, ME and MW wrote the paper. BD reviewed the manuscript and gave critical advice.

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