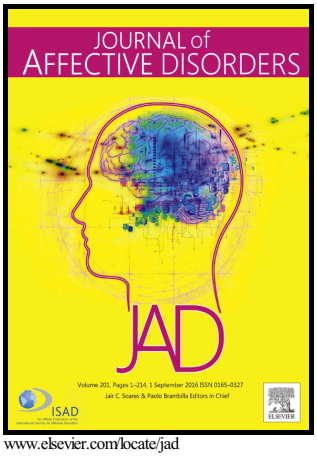

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Atypical Depression and Non-Atypical Depression: Is HPA Axis Function a Biomarker? A Systematic Review

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Abstract

Background

The link between the abnormalities of the Hypothalamic-pituitary-adrenal (HPA) axis and depression has been one of the most consistently reported findings in psychiatry. At the same time, multiple studies have demonstrated a stronger association between the increased activation of HPA-axis and melancholic, or endogenous depression subtype. This association has not been confirmed for the atypical subtype, and some researchers have suggested that as an antinomic depressive subtype, it may be associated with the opposite type, i.e. hypo-function, of the HPA-axis, similarly to PTSD. The purpose of this systematic review is to summarise existing studies addressing the abnormalities of the HPA-axis in melancholic and/or atypical depression.

Method

We conducted a systematic review in the literature by searching MEDLINE, PsycINFO, OvidSP and Embase databases until June 2017. The following search items were used: "hypothalamic-pituitary-adrenal" OR "HPA" OR "cortisol" OR "corticotropin releasing hormone" OR "corticotropin releasing factor" OR "glucocorticoid*" OR "adrenocorticotropic hormone" OR "ACTH" AND "atypical depression" OR "non-atypical depression" OR "melancholic depression" OR "non-melancholic depression" OR "endogenous depression" OR "endogenomorphic depression" OR "non-endogenous depression". Search limits were set to include papers in English or German language published in peer-reviewed journals at any period. All studies were scrutinized to determine the main methodological characteristics, and particularly possible sources of bias influencing the results reported.

Results

We selected 48 relevant studies. Detailed analysis of the methodologies used in the studies revealed significant variability especially regarding the samples' definition comparing the HPA axis activity of melancholic patients to atypical depression, including healthy controls. The results were subdivided into 4 sections: 1) 27 studies which compared melancholic OR endogenous depression vs. non-melancholic or non-endogenous depression or controls; 2) 9 studies which compared atypical depression or atypical traits vs. non-atypical depression or controls; 3) 7 studies which compared melancholic or endogenous and atypical depression subtypes and 4) 5 studies which used a longitudinal design, comparing the measures of

HPA-axis across two or more time points. While the majority of studies did confirm the association between melancholic depression and increased post-challenge cortisol levels, the association with increases in basal cortisol and basal ACTH were less consistent. Some studies, particularly those focusing on reversed vegetative symptoms, demonstrated a decrease in the activity of the HPA axis in atypical depression compared to controls, but the majority did not distinguish it from healthy controls.

Conclusions

In conclusion, our findings indicate that there is a difference in the activity of the HPA-axis between melancholic and atypical depressive subtypes. However, these are more likely explained by hypercortisolism in melancholia; and most often normal than decreased function in atypical depression. Further research should seek to distinguish a particular subtype of depression linked to HPA-axis abnormalities, based on symptom profile, with a focus on vegetative symptoms, neuroendocrine probes, and the history of adverse childhood events. New insights into the dichotomy addressed in this review might be obtained from genetic and epigenetic studies of HPA-axis related genes in both subtypes, with an emphasis on the presence of vegetative symptoms.

Keywords: hypothalamic-pituitary-adrenal axis, cortisol, atypical depression, melancholic depression, endogenous depression.

Introduction

Major depression is undoubtedly one of the major healthcare issues in the 21st century According to the latest WHO report on 23 February 2017, depression is now ranked as the single largest contributor of years lived with disability worldwide, and the major contributor to the global burden of disease. (WHO, 2017)

Stress response system abnormalities in Depression

The most robust and consistent finding in major depression so far has been its link to the abnormalities of the stress response system. The stress response system is a complex, multilevel mechanism largely dependent on feedback regulation. It relies on two main elements - the autonomic stress response which exerts immediate effects when the organism is faced with physiological or psychological stressors; and the impact on the hypothalamo-pituitary-adrenal (HPA) axis. A detailed account of the structure and physiology of both components of the stress response system is thoroughly given by Ulrich-Lai & Herman (2009) and is outside the scope of this review. However, to understand the nature of the abnormalities which will be discussed further, it is essential to highlight the key characteristics of the HPA-axis.

In response to stressful stimuli, the suppression of the subgenual prefrontal cortex and the activation of amygdala lead to the stimulation of the autonomic sympathetic

axis, and the HPA axis (Dioro et al. 1993; Phelps and LeDoux, 2015; Gold, 2015). The autonomic sympathetic axis is responsible for the most rapid response, and acts via the secretion of epinephrine by the adrenal glands; the HPA axis is activated minutes after the epinephrine surge, and represents a cascade of events starting with the secretion of the corticotropin releasing factor (CRF, also known as corticotropin-releasing hormone, or CRH) from the paraventricular nucleus of the hypothalamus into the portal circulation, which stimulates the synthesis and release of adrenocorticotropic hormone (ACTH) by the pituitary. The ACTH further stimulates the synthesis and release of the glucocorticoid hormone cortisol by the adrenal cortex. Glucocorticoids are known to exert a range of functions, such as promoting gluconeogenesis, catabolic and antianabolic activity, suppression of innate immunity in immune organs, insulin resistance and a prothrombotic state. The key role of glucocorticoids consists in maintaining homoeostasis in response to stress (Juruena, 2014).

The HPA-axis exerts its feedback through two major types of receptors: the glucocorticoid (GR) and the mineralocorticoid (MR) receptors. MRs have higher affinity to cortisol which results in their higher occupancy even at basal cortisol concentrations; at the same time, they are less specific and bind with both cortisol and aldosterone. GRs, on the other hand, bind more specifically to cortisol, yet respond to higher concentrations than MRs MR's seem to be the ones that regulate cortisol feedback during acute or normal stress. However, during severe or prolonged stress, GR's come into action (De Kloet et al.,1998).

The abnormalities of stress response system in affective disorders have been implied in several hundred studies (Stetler et al. 2013). However, accumulating evidence suggests that the presence, and type of, HPA-axis abnormalities may vary across various subtypes of depression (Gold et al. 2015; Porter and Gallagher 2006). Some studies have shown a robust association with HPA-axis overactivity with more severe or endogenomorphic forms of depression such as melancholic depression or psychotic depression (Nelson and Davis 1997).At the same time, in post-traumatic stress disorder, an enhanced HPA negative feedback was described (Yehuda et al. 1991). Some studies have hypothesised that atypical depression, unlike the melancholic subtype and similar to PTSD, is characterised by hypocortisolism and enhanced negative feedback. However, whether it is fair to claim there exists such a dichotomy, is not clear at the moment.

The aim of the current article is to review existing literature addressing the function of the HPA-axis in melancholic and atypical depressive subtypes. Evaluate whether: a) there is a significant difference between the two subtypes in terms of the activity of the HPA-axis; b) whether there is enough evidence to suggest that the HPA-axis is overactive in depression with melancholic features; c) whether there is sufficient evidence to indicate that the HPA-axis in depression with atypical features is hypoactive.

Melancholic vs atypical depression: a historical perspective on subtype definition and boundaries.

Considering the studies which have addressed HPA-axis abnormalities in either of, or both, melancholic and atypical subtypes, it is important to take into account that over the recent decades, approaches to identify them have been changing, and even today, appropriate criteria defining both subtypes remain a matter of debate.

A specifier introduced in DSM-III (1980), depression with melancholic features represents a subtype of depression clinically characterized by a distinct pattern of low mood, anhedonia, lack of reactivity to positive events, loss of appetite and weight, insomnia, loss of libido and diurnal mood variations. Although depression with «melancholic features» has been validated extensively (Shotte et al, 1997; Juruena et al 2011; Parker et al, 2015), there is still little agreement among researchers regarding the particular set of features that define it (Maes et al., 1992; Leventhal et al, 2005 ; Fink et al, 2007; Parker et al, 2013). Besides, the various diagnostic measures used for identifying melancholic depression have shown a considerable degree of inconsistency, as exemplified by the comparison of Research Diagnostic Criteria (RDC) endogenous depression definition, Newcastle scale endogenous depression definition, and DSM-III and DSM-IV diagnoses of melancholic depression (Rush et al., 1994 ; Coryell, 2007 Orsel et al., 2010).

Nevertheless, it is of high importance for this review to point out that the subsample identified by researchers as non-melancholic can be highly heterogeneous and represent various diagnostic entities, such as neurotic/reactive, atypical, non-differentiated depression, characterological depression (Fink and Taylor, 2007). Therefore, although atypical depression is indeed greatly antithetic to melancholic depression, it is not the same as non-melancholic depression, and for the purpose of

this review, unless specified as having features known to constitute the atypical subtype, non-melancholic subsamples of reviewed studies will not be not considered atypical.

Atypical depression had been recognized by some researchers as a depressive subtype since the 1960s. However, although addressed extensively in the context of differential responsiveness to pharmacological treatment, it had not been included in official DSM diagnostic criteria until 1994, following the formulation of Columbia atypical depression criteria (Quitkin et al. 1993). The validity of the atypical specifier has been demonstrated by some studies, that largely rests on data from psychopharmacological research - an approach introduced by Klein and according to which, the differential response to biological treatment represents different pathophysiology (Klein, 1989) and genetic-epidemiological studies which indicated that atypical depression subtype is genetically distinct from typical ones, which represents its etiological insularity (Kendler et al., 1996; Sullivan et al..1998). At the same time, the precise definition of «atypical depression» remains a matter of debate. According to DSM-IV diagnostic criteria ("atypical features" specifier), the disorder is primarily characterized by mood reactivity and 2 or more of the following symptoms as predominant features in patients with major depression or dysthymic disorder: overeating, oversleeping, "leaden paralysis," and interpersonal rejection sensitivity. The relevance of mood reactivity to the subtype, as well as its relationship with other symptoms, has been questioned. The studies cited above as providing evidence for the validity of the subtype, in fact, did not include the «mood reactivity» criteria. In a study by Posternak et al. (2001) mood reactivity did not show any association with any other psychopathological features of the proposed subtype. Similar results were reported by a few other researchers (Parker et al., 2002). A much more robust association was shown for the component of the symptom definition described as «reversed vegetative symptoms», namely hyperphagia and hypersomnia, and some of the studies selected for this review have focused on these characteristics rather than the DSM-defined specifier (Benazzi, 2002). In two studies of treatment response, Stewart et al. (2007) demonstrated a significant contribution of factors such as age of onset (early vs late) and chronicity (chronic or remitting course) to the correlation between atypical subtype and response to MAOI treatment.. Some authors proposed a reappraisal of the DSM criteria for an atypical subtype to reduce the number of symptoms besides mood reactivity to one, and to include the onset of dysphoria be-

fore 20 and chronic course as additional criteria. However, DSM-5 saw no amendments to the subtype criteria (Stewart et al. 2007).

Therefore, the definition of the atypical subtype appears even more vague than that of melancholic subtype, and data of all biological studies should be interpreted with account of the characteristics of the phenotype. Besides, in the majority of studies, as well as in the case with non-melancholic depression, the non-atypical descriptor does not necessarily identify melancholic depression, therefore, unless specified otherwise, non-atypical depression is considered as MDD not matching particular subtype criteria.

Methods

We conducted a systematic review of the literature by searching Ovid MEDLINE(R) Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid MEDLINE(R) Daily and Ovid MEDLINE(R) 1946 to Present, PsycINFO, Journals@Ovid Full Text and Embase databases using the Ovid platform.

The following search items were used:

"hypothalamic-pituitary-adrenal" OR "HPA" OR "cortisol" OR "corticotropin releasing hormone" OR "corticotropin releasing factor" OR "glucocorticoid*" OR "adrenocorticotropic hormone" OR "ACTH" AND "atypical depression" OR "non-atypical depression" OR "melancholic depression" OR "non-melancholic depression" OR "endogenous depression" OR "non-endogenous depressive» Search limits were set to include papers in English or German language, published in peer-reviewed journals at any period. Fig. (1) shows details of the search strategy.

The initial search yielded 9556 results in total, which was comprised of: 3192 results in the Embase database, 2029 in all MEDLINE databases, 2344 in PsycINFO database, and 1991 in Journals@ Ovid Full-Text database. Because the combined number of articles across all selected databases (9556) exceeded the 6000 limit allowing for deduplication, Further deduplication limited the number of articles to 3061.

Next, the filters «Human», «Adult» and «Depression» were applied, leaving 570 articles for title/abstract screen. For the full-text screen, we included original studies assessing the functional elements of the HPA-axis in samples including at least one of the following phenotypes: melancholic depression, endogenous depression, en-

dogenomorphic depression, atypical depression, their characteristic traits (such as «reversed neurovegetative symptoms»), and a comparison group.

After the title/abstract screen, 247 articles were retrieved for a full-text screen.

The criteria for inclusion in the review were:

a) The inclusion of adult patients diagnosed with major depressive episode according to DSM or ICD operational criteria;

b) An explicit description of criteria for melancholic and/or atypical depressive subtype identification in patients/ or precise description of symptom sets characteristic of melancholic/atypical subtype (e.g. reversed neurovegetative symptoms)

c) The evaluation of the levels of basal and/or post-challenge cortisol in the blood, saliva, urine or CSF, of basal and/or post-challenge ACTH in blood, and of basal and/or post-challenge CRF in blood or CSF.

Studies were excluded if:

a) They had no comparison group (e.g. studies in a small sample of melancholic patients before and after TMS, however, studies which compared the subtypes between each other without healthy controls, were included)

b) They addressed other neuroendocrine outcome measures (e.g. NE, Prolactin, Vasopressin) - unless they also assessed HPA-axis measures. Here it is important to acknowledge that the function of the HPA-axis is largely dependent on, and interrelated with, other endocrine factors and the immune system. However, given the focus of this review, measurements other than those directly related to the HPA-axis were not considered.

The details of the literature search have been structured using the Prisma Flowchart in Fig.1

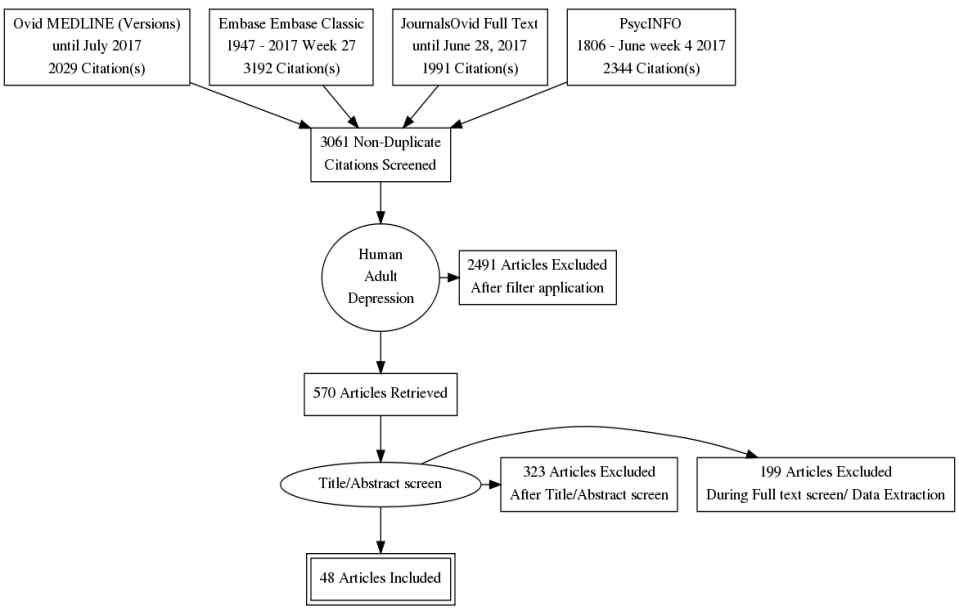

Fig.1 PRISMA Flow Diagram

Results

The final stage of the search process yielded 48 articles in general. The studies were subdivided into four groups:

Group 1. Studies (n=27) which compared «melancholic» (or other definitions) depression with depression not matching any of the definitions for melancholia, or a control group

Group 2. Studies (n=9) which compared «atypical» (or other definitions) depression with depressions not matching any of the definitions for atypicality, or a control group.

Group 3. Studies (n=7) which compared «melancholic» (or other definitions) depression with «atypical» (or other definitions) depression.

Group 4. Studies (n=5) which employed a longitudinal design.

The results for each section are summarized in Tables 1-4, respectively

Melancholic studies

The group comparing various definitions of melancholic depression with non-melancholic depression or controls included 27 studies. For clarity and simplicity, we suggest that within the group, these studies further be sub-grouped according to the measurement of HPA-axis activity used. However, since quite a few of the studies addressed more than one potential measure of HPA-axis activity, some of them may be mentioned several times both in further text and the tables.

The majority of studies assessing the function of the HPA-axis in melancholic depression have focused either on the rates of suppression following Dexamethasone Suppression Test (17 studies) or basal cortisol in either blood, CSF, or urine (16 studies). Those are followed by basal ACTH (3 studies), basal CRH (CRF) (2 studies), oCRH challenge (1 study), ACTH stimulation (1 study), fenfluramine challenge (2 studies), Dex/CRH (1 study).

Dexamethasone suppression test

Some of the earliest studies focusing on DST as a potential diagnostic test for melancholic depression were performed by B.Carroll et al. This review focuses on the 1981 study which lasted for 6 years and included 368 patients. In this study, the authors addressed not only the issues of the specificity and sensitivity of DST but also proposed the standard procedure, since the methodologies employed by various researchers vary markedly in the dose of administered dexamethasone, the time of measurement, as well as the threshold for «suppression». The authors concluded that the optimal balance between the measures of sensitivity, specificity and diagnostic confidence of DST for melancholic depression would be at the threshold of the non-suppression set at 5 μg/dL, the dose of dexamethasone set at 1 mg, and the measurements were taken at 4 pm and 11 pm. The latter had been demonstrated in their previous studies which indicated that the abnormalities of the HPA-axis are subtle in depression and therefore patients may still be capable of normal suppression in the morning hours but fail to do so later in the course of 24 hours post-challenge (Carroll et al., 1982, further confirmed by Rubin et al., 1987). Similar findings were also observed by Rubin et al., (1987), in a study which will be discussed further. In general, the sensitivity (defined here and further as the proportion of melancholic patients with abnormal DST results) across all values varied from 39% to 53%, the specificity (defined here and further as the percentage of melancholic subjects in whom normal results were observed) ranged from 85% to 97%, and the diagnostic confidence, i.e. the proportion of abnormal test results that were true-positive for melancholia, from 83% to 93 %. These results appear rather convincing, but an essential aspect of their findings is that for the definition of melancholia, they used their operational criteria.

Banki et al. (1986), apart from other hormonal challenges, investigated DST responses in female patients diagnosed with DSM-III defined melancholic depression, and reported that abnormal DST response was observed in 67% of melancholic patients, which significantly exceeded the rates for both other psychiatric disorders and controls ($p<0.05$). Evans et al. (1987), reported slightly higher rates of non-suppression for DSM-III melancholic depressed patients - 78%, however, it is of note that patients with psychotic depression demonstrated an even higher proportion of non-suppression (95%), which significantly exceeded that in the melancholic group even when non-suppression was defined as >10 μg/dl or >15 μg/dl.

The issue of the threshold for non-suppression was addressed in a study by Winokur et al. (1987). The authors looked at the extremities of the DST response: having tested 423 patients with a range of affective diagnoses, they split the results into two categories: strong suppressors defined as having post-dec cortisol levels below 1.5 µg/dl, and strong non-suppressors whose post-dec cortisol was > 6 µg/dl. The main results were that melancholic symptoms (defined by DSM-III criteria) were significantly associated with non-suppression (24% vs. 9% suppressors, p=0.01). At the same time, the diagnosis of secondary depression was significantly associated with suppression (38% vs. 19%, p=0.025). It is notable, however, that although there existed a significant association of non-suppression with melancholia, there were indeed only 24% of NS among all melancholic patients, which may indicate that the threshold of 6 mg/dl could be too high to yield sensitive results. (Winokur et al., 1987).

Contributing to the debate about the correct classification, or the ability of a certain definition of melancholia to identify non-suppressors, Peselow et al. (1992) demonstrated that patients who met both DSM-III criteria for melancholia and RDC criteria for endogenous depression, showed higher rates on non-suppression than those with «neither» subtype and controls, but not compared to those with «either of» the subtypes (Peselow et al., 1992). Paslakis et al. (2011) also used DSM-IV (SCID-validated) criteria for melancholia, and although they did demonstrate a significant difference in suppression levels between melancholic depressed patients and controls, both the mean effect size and the sensitivity of the test proved low (Paslakis et al., 2011). Interesting results, although in a very small number of patients (n=5 patients with "rapid improvement") were obtained by Barocka et al.,(1987). They showed that the clinical course of "rapid improvement» was significantly associated with normal suppression in the DST even in endogenous patients (p = 0.04). They suggested that by eliminating those patients who show a rapid improvement shortly after the test, the test sensitivity for endogenous depression could be increased by about 10%, while the specificity remains constant (Barocka et al., 1987).

Rubin et al. (1987) addressed the response to DST (with a threshold set at ≥ 3,5 µg/dL), in 40 patients with a definite RDC diagnosis of endogenous depression vs. 40 age-matched controls. Interestingly, even with this low a threshold, they only observed non-suppression in 15 of the 40 depressive patients, which, however, was significantly different from controls, yielding a sensitivity of 38% and a specificity of

88%. Their key observation was the melancholic patients who differed from each other of DST suppression rates were also different on some other HPA-related characteristics. At the same time, the suppressors did not differ from control subjects in any of the measures.

Related findings were reported by Amsterdam et al. (1989) who performed a complex study in two consecutive samples, employing a range of challenge tests. Tests other than DST will be discussed in the relevant section. Consistent with the results obtained by Rubin et al.,(1987) they found that the subgroup of melancholic patients who were DST non-suppressors demonstrated larger mean cortisol values for all of the response measurements compared to the other patient subgroups or healthy controls, and to all the other patient groups combined. Also, the melancholic DST-NS subgroup showed a skew toward larger overall cortisol response values, rather than the standard distribution of the healthy controls, indicating that this group of patients had members with particularly enhanced adrenocortical responsiveness. Besides, maximum cortisol response to ACTH was significantly decreased after treatment in the MEL/DST-NS group ($p=0.04$). Consistent with the conclusions made by Rubin et al., the authors inferred that the subgroup of DST-NS patients with melancholic features might represent a diagnostically homogeneous subpopulation who are more likely to demonstrate endocrine abnormalities at several sites within the HPA axis (Amsterdam et al. 1989).

However, there were studies that did not show a strong association between the diagnosis of endogenous depression and endogenicity. Hubain et al. (1996) performed DST in a large sample of 155 Newcastle Endogenous Depression Diagnostic Index (NEDDI) - defined endogenous patients vs. a similar number of non-endogenous patients. Authors failed to find any association, at a suppression threshold of 50 µg/l (i.e. 5 µg/dL). (Hubain et al., 1996). Berger et al. reported results both for the common ≥ 5 µg/dl threshold and for ≥ 8 µg/dl (a result that yielded 2.7% in a healthy sample). In their study, in three separate patients samples including the diagnosis of a) endogenous (+schizoaffective) depression, b) neurotic and situational depression and c) other psychiatric diagnoses, a positive DST failed to yield predictive value over 40-60%, indicating that only one of two patients with positive DST would suffer from endogenous depression. For subtyping, authors used three instruments: ICD-8, RDC and Newcastle Scale (Berger et al., 1984).

Particular attention should be paid to studies which focused not, or not only, on subtypes as a whole, but also on individual symptoms which reflect the more «biological» manifestations of depression. For instance, Miller and Nelson (1987) investigated the DST response in 95 depressed inpatients, 45 of them melancholic according to DSM-III criteria (although they also used RDC criteria which did not contribute much to the variance apart from a slight difference in p-values). However, they assessed the association of individual symptoms, not subtypes as a whole, with suppression rates. They demonstrated that with a threshold for non-suppression of 7 μg/dL (which is obviously higher than in general), the four factors most strongly associated with DST non- suppression were initial insomnia, loss of sexual interest, agitation, and weight loss. Factors associated significantly yet with a small effect size were also retardation and when cortisol levels were assessed as a continuous variable, ruminative thinking and midnight awakening. It is, nevertheless, notable that the four strongly associated factors were only shown to account for 24% of the variance. In a study by Berger et al., despite low predictive value observed, weight loss was shown to enhance the rate of abnormal DST results in psychiatric inpatients, regardless of their diagnostic classification (Berger et al., 1984).

Casper et al. (1987), also focused on neurovegetative symptoms of depression rather than defined subtypes. They assessed basal and post-DST cortisol levels in 28 patients diagnosed as having MDD and presenting with either marked weight loss, appetite loss, or both. Although both weight loss and appetite loss were significant predictors of elevated basal cortisol levels, non-suppression, defined as cortisol levels above or equal to 6 μg/dL, was only significantly associated with weight loss. Interestingly, as will be discussed in the relevant section, in another study comparing MDD patients with hypersomnia and/or increased appetite/ weight with MDD patients without these symptoms or controls did not reveal a strong association between reversed vegetative symptoms and HPA-axis function: the most significant association was demonstrated for DST non-suppression in MDD patients without hypersomnia or weight/appetite increase vs. the opposite or controls (Casper et. al., 1988). Another study focusing on individual symptoms was that by Maes et al. (1989), who distinguished between *vital* vs. *non-vital* symptom clusters instead of MDD subtypes, and they as well demonstrated that higher levels of cortisol post-DST were associated with sleep and appetite disturbances.

At the same time, Orsel et al. (2010) performed a cluster analysis and identified an endogenous cluster which differed from non- endogenous on the following characteristics: anorexia/weight loss; diurnal variation, depressed mood, loss of energy, early morning awakening, loss of interest, suicidal ideation, distinct quality of mood, cognitive disturbances, psychomotor disorders, psychotic symptoms, non-reactivity, feelings of guilt, and sleep disorder. However, of the 14 SCID-I items, only six factors - early morning awakening, distinct quality of mood, feelings of guilt, non-reactivity, suicidal ideation, and psychomotor disorders - were significant discriminators between the clusters. The clusters showed a high degree of correlation with DSM-IV melancholic and non-melancholic depression, respectively, although they were not a complete 100% match. The authors were able to demonstrate a significant difference in basal cortisol levels between endogenous and non- endogenous clusters, as well as in rates of DST suppression (≥ 3.5 μg/dl threshold), however, not in post-DST cortisol measured as a continuous variable. Moreover, rather unusually, in studies discussed above, only mood reactivity as a single symptom differed significantly between suppressors and non-suppressors (i.e. non-reactivity correlated with non-suppression; Orsel et al., 2010).

Finally, important observations regarding the role of phenotype in HPA-axis assessment in depression can be inferred from a study by Halbreich et al., 1989, who compared patients with RDC Endogenous Depression and DSM-III PTSD with RDC MDD-ED patients alone and controls. They demonstrated that the presence of a diagnosis of PTSD determined a significant difference in DST response in patients: all PTSD-ED patients proved to be suppressors (not different from controls – however, suppression threshold wasn't specified in the article, except for mean values of post-DST cortisol which were at mean of 3,72 in MDD-ED patients compared to 0,96 in PTSD-ED patients, p=0.01) and had lower basal plasma cortisol levels compared to MDD-ED patients (Halbreich et al., 1989).

Other challenge tests

Amsterdam (1989) attempted to assess several levels of the HPA axis and their abnormalities in hormonal response in depressive patients. In their paper, they referred to 2 studies which used ACTH as a hormonal challenge. The first one compared 16 patients with a depressive disorder (of which, 9 had melancholic features) and 11

healthy controls. They found no difference in cortisol levels at baseline between groups. However, after ACTH administration, the depressive group had a larger increase in cortisol concentrations than controls ($p<0,02$). Further, the authors tried to replicate these results in a larger sample of 72 depressed patients (of which 51 had melancholic features) compared to 34 healthy controls and were unable to find the same results. In contrast, they found that both at baseline and after ACTH administration, cortisol levels did not significantly differ between groups. The same article cites a study with ovine CRH challenge. They administered oCRH to 26 depressed patients (of which 14 had melancholic features) and compared to 11 healthy controls. They found no difference between groups in cortisol levels at baseline and after challenge; nonetheless, depressive patients as a group had lower ACTH response compared to controls. The effect was larger in patients with melancholic features (Joseph-Vanderpool et al 1991).

The study by Mitchell et al., (1990) tried a different challenge using the serotonin agonist Fenfluramine 60 mg but found no difference between groups of “endogenous” vs. “non-endogenous” depressive patients. The 30 patients in the study were evaluated with four different types of criteria (DSM-III, ICD 9, RDC, Newcastle criteria) to divide subtypes; however, they did not found differences in post-challenge cortisol levels between any of the groups.

Another study that used a similar compound, in this case, d-Fenfluramine 30 mg, also failed to show any significant difference. The study compared 23 depressive patients with 16 healthy controls and found that both groups had the same levels of cortisol after challenge (O'Keane & Dinan, 1991).

In 2011, Paslakis (Paslakis et al., 2011) compared 3 ways of assessing HPA axis and their relevance for detection of depression. One of those was the Dex/CRH test. They found that the test had low levels of sensibility (30,8%) and moderate specificity (78,8%) being the worst marker between the three compared (DST, 24h cortisol).

Basal measurements

Basal cortisol

The search identified 19 studies which compared the basal cortisol levels (either as part of further DST or separately) among patients diagnosed with melancholic depression and matched controls and/or non-melancholic depressed patients.

Rubin et al. (1987) demonstrated that the elevation of basal cortisol when measured continuously over 26 hours correlated most significantly with the DST status of the patients: i.e. patients that were non-suppressors had elevated 24-hour cortisol and nocturnal cortisol nadir compared to both controls and patients who were suppressors. This was shown in a sample of RDC-examined definite endogenous patients (Rubin et al., 1987).

Further evidence of the increase in basal cortisol levels between melancholic depressed patients and controls/non-melancholic patients comes from studies by Guechot et al. (1987), Wong et al. (2000), Paslakis et al. (2011), O'Keane and Dinan(1991). Guechot et al. performed a simple one-time salivary test of cortisol at 11 pm in patients diagnosed as «primary» depressive using DSM-III and Saint-Louis criteria, compared to both secondary depressives ($p<0.05$) and controls ($p<0.02$). They also reported high sensitivity (62,5%) and specificity (75% vs. secondary depressives, 90% vs. controls) rates of the test for the identification of primary depressive patients when the cut-off was set at 3.45 nmol/l (Guechot et al., 1987). A study by Wong et al. (2000), addressed multiple measures including basal plasma cortisol in patients with a DSM-II-R and RDC-defined melancholic subtype of depression and controls, concluded that mean 30-h plasma cortisol levels in depressed patients were significantly higher vs. controls. Paslakis et al. (2011), together with DST and Dex/CRH, which will be discussed, further, performed a comparison of Basal cortisol levels in melancholic patients defined using DSM-IV criteria vs. healthy controls. They confirmed that diurnal basal cortisol secretion as measured throughout 24 hours, is significantly higher than in controls, as measured both by cortisol profile graphs and area under the curve ($p=0.001$ and $p<0.01$, respectively). The maximum of variation was observed at 11.30 and 14.00. In this study, basal cortisol was the most sensitive marker for melancholic depression than DST, and at the time interval from 10.00 to 12.00 yielded results with optimal sensitivity (83.3%) and specificity (87.9%). O'Keane and Dinan (1991) study also reported elevated baseline cortisol in patients diagnosed with DSM-III-R major depression and Newcastle scale criteria for

endogenicity. They showed a significant increase in basal cortisol in patients compared to controls subjects. CORE measure was also used by Liu et al.(2016) in the definition of melancholic depression. They performed an analysis of 228 blood metabolic markers in 21 melancholic patients (as well as 58 patients matching criteria for «anxious depression» defined by one or more comorbid anxiety disorders on M.I.N.I, and 100 controls). One of the important outputs of this study was the confirmation that melancholic depression can represent a more biologically distinct subtype of depression. Regarding HPA-axis activity, increases in basal cortisol levels were significant for the melancholic group, as well as increases in other metabolites in the hormone biosynthesis pathway (androstenedione and corticosterone; Liu et al., 2016).

Michopoulos et al. (2008) studied whether elevated HPA-axis function was associated with executive dysfunction and memory deficits in melancholic (as defined by DSM-IV-TR criteria) depressed patients. They also reported no significant difference between plasma and salivary cortisol levels in melancholic vs. none- melancholic groups. The only significant correlation between cortisol values and CANTAB tests, either mnemonic or prefrontal, used for cognitive function assessment was an association between morning salivary cortisol and the ID/ED total errors.

In accordance, Mitchell et al. (1990) and Joyce et al. (2002) compared a range of basal measures in patients with melancholic depression defined by several criteria. Mitch-ell et al. compared such diagnostic systems as ICD-9, DSM-III, RDC and Newcastle Scale melancholic/endogenous phenotypes, while Joyce et al. (2002) compared DSM-IV based diagnose of melancholia with that focusing on CORE measures. In the second study, there was a significant correlation with basal cortisol: in male patients defined as melancholic by CORE criteria.

A few studies mentioned above about DST results also assessed the association of basal cortisol levels with particular symptoms. Casper et al. (1987) reported that basal plasma cortisol was significantly associated with weight and appetite loss; Kaestner et al. (2005) indicated that high baseline cortisol levels correlated with HAM-D severity and the presence of weight loss.

Basal ACTH

There were three studies which addressed basal ACTH levels, of which none indicated elevated plasma ACTH levels in melancholic patients.

Wong et al. (2000) who used both DSM-III-R and RDC criteria to define the melancholic subtype failed to find a significant association in the melancholic/endogenous group with elevated basal plasma ACTH; however, since they observed elevated cortisol levels, they also reported that plasma cortisol-to-ACTH ratio was significantly elevated in melancholic patients compared to controls. Similarly, Joyce et al. (2002) failed to show elevated ACTH in melancholic patients when applying either DSM-IV or CORE criteria. Finally, Gomez-Gil et al.(2010) got their negative results when applying the NEDDI criteria.

Basal CRF

There were two studies which reported basal CRF levels. Wong et al. found that melancholic patients did not differ from controls in their levels of basal CRF which was disproportional about elevated basal cortisol. Similarly, Joyce et al. (2002) failed to demonstrate elevated basal CRF using either DSM-IV or CORE definitions.

Atypical vs non-atypical or controls

We selected nine studies focusing on the atypical depressive subtype or its characteristic features. Among this group, we indicated: studies focusing on DST (n=4), studies assessing basal cortisol either in blood, urine, or the CSF (n=7), one study assessing basal ACTH levels (n=1), two studies using desipramine stimulation (n=2), a study using dextroamphetamine stimulation (n=1); a study using oCRH stimulation (n=1).

Dexamethasone Suppression Test (DST)

Of the four studies assessing dexamethasone suppression rates in atypical patients, only two used standard DSM-based criteria.

Levitan et al. (2002) evaluated DST response in 8 female patients with DSM-IV-defined atypical MDD vs. 11 healthy controls and demonstrated that atypical patients had higher rates of suppression vs. controls (91,9% suppression in atypical depressive patients vs. 78,3% in controls). However, it is notable that the authors used a

lower dose of dexamethasone than usually administered: they used both 0.25 and 0.5 mg dosages, and significant results were reported with the latter (Levitan et al., 2002).

Stewart et al. (2005) suggested stratifying patients into late/nonchronic atypical and early/chronic atypical subtypes. Patients with early/chronic atypical had significantly lower mean 3 h afternoon cortisol levels and 4:00 p.m. post- dexamethasone cortisol levels than compared to late/nonchronic atypical (Stewart et al., 2005). This indicates that the course of illness may also play an important role in the function of the HPA-axis and that it may also contribute to the heterogeneity of atypical depression. However, since the study had no control group, it is difficult to draw conclusions as to whether there is hypocortisolism in atypical patients compared to controls.

Casper et al., (1988) focused on somatic symptoms such as hypersomnia (n=23) and overeating (hyperphagia, n=22), looking at these two symptoms separately, with n=15 out of the 22 patients with hyperphagia also demonstrating weight gain, all measured by SADS. The groups were compared with MDD patients who exhibited neither of the symptoms and with matched controls. The study did not demonstrate any increase in DST response in hypersomnia/overeating patients. However, it is an important observation that patients with atypical features did not differ from controls. In patients presenting with hypersomnia, there were significantly higher rates of normal suppression than in «non-atypical» patients, and the latter was similar to controls.

Thase et al. (1989) compared a subgroup of bipolar depressed outpatients with «anergic» depression which they defined using own operational criteria as manifesting with «anergia» (score 2 on Hamilton scale item 13), «psychomotor retardation» (score of 2 or more on item 8), and «reversed vegetative symptoms» where weight gain was defined as an increase in weight of 2.2 kg or more, and hypersomnia as increase of 1 hour or more compared to normal sleep duration). The authors focused on identifying EEG disturbances and DST response in those patients compared to controls. Only 3 (13%) of the patients were non-suppressors, even considering the somewhat lower threshold for defining non-suppression (4mg/dl vs. the more common 5 mg/dl). Likewise, only 6 of the patients had baseline cortisol levels higher than 15 mg/dl. Authors also demonstrated that patients, and particularly 6 patients with hypercortisolism, had decreased REM latency values.

Other challenge tests

Two of the studies assessed the levels of cortisol following a challenge test with 75 mg desipramine.

Asnis et al. (1995), compared a group of 17 patients diagnosed as suffering from atypical depression to 55 patients not matching atypicality criteria. The criteria for atypicality were similar to those of DSM-IV, except only one of symptoms additional to mood reactivity (hypersomnia, hyperphagia, leaden paralysis, or rejection sensitivity) was obligatory for the diagnosis instead of two. It is striking how different the phenotypes of patients included in this study could be compared to those mentioned above. Patients were compared on their response to 75 mg of desipramine, which is a challenge test for noradrenergic function. Although basal cortisol levels did not differ significantly between groups, post-DMI cortisol was significantly higher in atypical group vs. non-atypical, which suggests that this group may have a less impaired noradrenergic system compared to MDD patients without atypical features.

McGinn et al. (1996) studied patients from the same cohort as Asnis et al.(1995). However, they stratified patients as having mood reactivity alone (n=29), having depression with atypical features as defined by mood reactivity plus one of the four atypical symptoms (n=33), and MDD not matching atypical criteria (n=52). The main conclusion of the study was that AD patients, similar to the previous study, had a significantly higher cortisol response to DMI.

Apart from DST, Stewart et al. used dextroamphetamine challenge in their sample. The difference in post-dextroamphetamine cortisol levels did not reach statistical significance although there was a trend for higher numbers in the early/chronic atypical group (Stewart et al. 2005).

Finally, Joseph-Vanderpool et al. (1991) examined HPA-axis function in patients with seasonal depression characterised by atypical features such as reverse vegetative symptoms. The authors used a challenge test with oCRH, which did not discriminate between the subjects and controls. ACTH response to oCRH was delayed and reduced in Seasonal Affective Disorders patients.

Baseline measures

Baseline cortisol levels were assessed in seven studies in the group, while basal ACTH was only mentioned in one study.

Both Asnis et al. (1995) and McGinn et al. (1996) who reported basal cortisol levels in ADDS-assessed atypical depressive patients showed no difference be-tween the patients and controls. Joseph-Vanderpool et al. (1991) reported a trend towards

lower basal cortisol in SAD patients vs. healthy controls which, however, was only significant at 22.00.

Studies that applied DSM-IV criteria showed slightly differing results. Anisman et al. (1999), compared 31 atypical MDD with 14 non-atypical MDD and 15 atypical dysthymic with 14 non-atypical dysthymic patients, assessing among other markers, on basal cortisol and ACTH levels, and demonstrated significantly decreased basal cortisol, but increased ACTH levels in atypical patients vs. controls.

Stewart et al. (2005) who also used DSM-IV criteria in their comparison of atypical depression with various courses demonstrated that Patients with early/chronic atypical had significantly lower mean 3 h afternoon cortisol levels (Stewart et al, 2005).

Finally, Casper et al. (1988) and Levitan et al.(1997) used reversed vegetative symptoms as criteria for atypicality. The former showed no differences in any of the measurements - i.e. plasma, CSF or urinary cortisol,and controls. At the same time, Levitan et al. (1997) demonstrated a significant negative correlation between the symptoms of hypersomnia and carbohydrate craving and basal cortisol values

Atypical depression vs melancholic depression

Studies directly comparing the function of the HPA-axis in melancholic vs. atypical depression are rather scarce. Practically, our search only yielded seven relevant articles. Of them, the majority (n=5) focused on baseline cortisol measures. There were two studies that assessed basal ACTH as well, one study that assessed DST; and one study that applied Dex/CRH test. The results of the studies are summarised in Table 3 below.

Challenge tests

The correlation between personality disorders, depression subtypes, and DST suppression rates was studied in 50 patients by Fountolakis et al.(2004). The authors reported the results of DST in 14 atypical patients and 16 melancholic patients defined by DSM-IV criteria. Other groups of patients included those with a «somatic syndrome» as defined by ICD-10 (n=32, partly overlapping with atypical and melancholic groups) and 9 patients without a clearly defined phenotype. The authors did

not observe any significant correlations between DST suppression rates with any of the phenotypes; however, the largest proportion of non-suppression was observed in the atypical group (42,85%), what goes against our previous described findings. They also demonstrated the accumulation of cluster B personality disorders in the atypical group, although that was not significant. The possible limitations of this study are a small sample size and, possibly, the lack of healthy control group (Fountolakis et al., 2004).

An elaborate study of stress reactivity patterns was performed by Heinzmann et al. (2014). This study primarily focused on mice divided into three phenotypes based on their stress response patterns; its second stage involved human partici-pants. Unlike previous studies, the main criterion of patient grouping was not their depression subtype, rather, patients were divided into high (hHR), intermediate (hIR) and low (hLR) responders according to their cortisol response in the Dex/CRH test. Although authors did not identify patients as having particular depressive subtypes, they applied HDRS subscale of non-atypical depression symptoms. Patients in the hLR group showed less sleep disturbance, less appetite loss and less weight loss than hHR patients. At the same time, hHR patients showed a strong trend towards higher 'agitation' scores and increased active stress-coping behaviour compared to hLR patients (Heinzmann et al. in 2014).

Basal cortisol and ACTH measures

The first study to compare the basal measures between the two subtypes was a study by Elizabeth Young et al. (2001). Authors investigated the whether cortisol secretion reflects a central CRF dysregulation or represents altered adrenal gland function. Over a period of 24 hours, ACTH and cortisol levels were assessed at 10-min intervals in a sample of 25 premenopausal women and 25 healthy controls. Regarding depression subtyping, compared patients meeting RDC criteria for endogenous depression (n=6) with patients meeting DSM-IV criteria for atypical depression. They found no significant differences in either mean 24-hour plasma cortisol or urinary cortisol secretion between groups, including between patients and controls, alt-

hough mean cortisol values tended to be higher in endogenous group vs. controls. Regarding ACTH, the only significant findings were those regarding basal ACTH, which was significantly increased in depressed patients in general vs. controls, and so was the AUC for basal cortisol. No other significant differences were identified. The obvious drawback of the study was essentially the small sample size which comprised only 6 and 7 patients in phenotypes of interest.

Brouwer et al.(2005), recruited a bigger total sample (n=113) of MDD patients. How-ever, the numbers of patients meeting DSM-IV criteria for atypical or melanchol-ic depression were relatively low (32 and 25 patients). The authors analyzed an ar-ray of endocrine measures including serum and urinary cortisol levels. In subtype analy-sis, only serum cortisol was significantly lower in atypical depressed patients vs. those not matching either subtype.

Karlovic et al. (2012) compared DSM-IV defined 23 melancholic depressed pa-tients, 23 atypical depressed patients and 18 healthy controls on the levels of serum cortisol (following a single morning blood test). The authors demonstrated a sig-nificant difference between melancholic and atypical subtypes in the levels of morn-ing cortisol, where the melancholic group showed an increased cortisol level while the atypical group was not different from controls.

Cizza et al. (2012), recruited 89 female patients from the POWER (Premeno-pausal, Osteoporosis, Women, Alendronate, Depression) having reported a depres-sive episode in the past 3 years. According to DSM-IV criteria, 51 patients had mel-ancholic depression, 16 presented with atypical features, and in 22, no subtype crite-ria were met. The 24-hour sampling of plasma ACTH and cortisol yielded no signifi-cant difference in 24-hour cortisol plasma cortisol between groups. However, plasma ACTH was significantly higher in the atypical subtype vs. the control group ($F (1, 83) = 4.01$, $p<0.05$) vs. controls. A group by time interaction demonstrated that ACTH was elevated in the atypical group only in the daytime, with greatest differences ob-served from 10 AM to 5 PM. Besides, after adjustment for total body fat, the mean 24-hour adjusted log leptin value was elevated in the melancholic subgroup, as compared with controls (Cizza et al. 2012).

The biggest patient sample analyzed so far was that recruited by Lamers et al., (2012), from the NESDA cohort. Authors compared some inflammatory, metabolic markers, saliva cortisol awakening curves, and diurnal cortisol slope in 111 chronic depressed patients with melancholic depression and 122 patients with atypical depression. However, their labels did not refer to DSM classifiers, rather to results of a latent class analysis performed using CIDI questionnaires, which showed no significant effect of the measure of mood reactivity or interpersonal sensitivity, but a robust effect of weight and sleep characteristics. In attempts to make the study more homogenous, the authors included only chronic severely depressed patients, as this category showed the most stable patterns of depressive symptoms. They demonstrated that the atypical subtype differed from the melancholic subtype on a whole range of symptoms, including area under the curve on the ground (AUCg) and diurnal cortsol slope measures (decreased in the atypical group) (Lamers et al., 2012).

Longitudinal studies

It is of note that due to a range of factors, and first of all the complexity of the tests, there is a substantial lack of studies assessing the function of the HPA axis across both phenotypes of interest longitudinally. Our search yielded only 5 articles which used a longitudinal design. The first study found with the search terms was that by R.G.Haskett et al. (2005), where the authors assessed the changes in the DST response across several weeks in hospitalized patients. This methodology was grounded in the previous observations that depressed patients who completed a DST on day 2 of hospitalization had a higher frequency of cortisol nonsuppression (71%) than depressed patients who were tested on days 3-6 (33%; Coccaro et al., 1984). The study demonstrated that although there was an overall decrease in the rates on non-suppression in both the patients with endogenous depression and control subjects (from 55 to 36%), this was mostly accounted for by the difference in the control group, while the sensitivity of the test for the endogenous depressive patients did not change significantly (Haskett et al., 1987).

In 1997, Steiger et al. performed a study assessing the levels of basal plasma cortisol in 12 endogenous depressive patients diagnosed using RDC criteria on admission and post-treatment. They demonstrated that in ED patients, plasma cortisol was significantly elevated on admission compared to healthy controls, and that it was

also significantly reduced after treatment, indicating that elevation was specific to acute endogenous depression.

A study by Kaestner et al (2005) also employed a longitudinal design - pa-tients were assessed on admission (t1) and after treatment, in remission (t2). The study focused on assessing a range of factors, basal including basal cortisol and ba-sal ACTH, in unmedicated, acutely depressed melancholic patients (n=37) compared with 37 controls. They demonstrated that on admission, both cortisol and ACTH were elevated in melancholic patients compared to controls, but not when compared with non-melancholic patients. Plasma ACTH was still increased in melancholic patients in remission compared to controls. At the same time, cortisol was elevated in acutely depressed melancholic patients only, but not in remitted ones (Kaestner et al., 2.005).

Pintor et al, 2013, measured ACTH and cortisol response using synthetic hu-man CRF challenge in relapsing, non-relapsing and partially relapsing patients with DSM- IV melancholic depression over a follow-up period of two years. In terms of the overall comparison of depressed and healthy groups, significant differences were observed for post-CRF ACTH, cortisol levels and for area under cortisol curve bet-ween healthy controls and the three groups of melancholic patients.

Finally, the only article assessing the longitudinal course of HPA-axis abnormali-ties in atypical depression is that by Geracioti et al., 1992, and represents a case study of a female patient diagnosed with atypical depression across 6 months. The results obtained by the authors correspond with the notion that atypical depression has a different pattern of abnormalities: the patient was eucortisolemic in an acute phase of depression, and further cortisol levels showed a negative correlation with a deterioration of depressive symptoms.

Discussion

The key problem in diagnosis is the fact that elaborate classification systems that ex-ist today are solely based on subjective descriptions of symptoms. Such detailed phenomenology includes the description of multiple clinical subtypes; however, there

is no biological feature that distinguishes one subtype from another. Integrative approaches to understanding complex health issues can transcend disciplinary and knowledge boundaries and provide opportunities to view phenomena from diverse perspectives. A future diagnostic criteria system in which aetiology and pathophysiology are essential in diagnostic decision-making would bring psychiatry closer to other specialities of medicine (Juruena et al. 2007). Thus, the heterogeneity of clinical conditions encompassed under the concept of major depression seems to be one of the limiters of these advances. Therefore, the identification of distinct subtypes of depression may allow advances in these areas by allowing the identification of more homogeneous groups of patients, both in clinical aspects and in those related to the aetiology and pathophysiology of the disorder presented. This depends on many factors like severity and type of depression, genotype, and history of exposure to stress, temperament, and probably resilience (Mello et al., 2007)

The analysis of the articles focusing on the differences in the function of the HPA-axis depending on depressive subtype has revealed a range of sufficient pitfalls in research methodologies. In this sense, although the concept of a melancholic depression subtype, equivalent to the concepts of endogenous or psychotic depression, has a long history of psychiatry and is well defined (Sullivan et al., 2002, Baumeister & Parker, 2012). The atypical subtype, in turn, encompasses a heterogeneous group of patients and has only recently been introduced into the DSM-IV as a specifier. Moreover, although the literature has given extensive support to the validity of depression with atypical features as distinct from melancholia and depression without atypical or melancholic features, there is still a certain degree of disagreement among researchers about which particular symptoms constitute this specifier, and whether such factors as mood instability and interpersonal sensitivity have the same weight in the dichotomy as biological reversed vegetative symptoms. Thus, the lack of well-defined diagnostic criteria to characterize these subtypes of depression is reflected in the diversity of nomenclatures used in the literature to define these subtypes.

In this sense, in this systematic review, we find a significant variation in the terms used in the articles to define the melancholic and atypical subtypes. Besides the variation in definition, a major complication is presented by the variation in approaches to challenging tests (e.g. dosage, time of response measurement and threshold used to defining non-suppression), and the variety of classifications used in order to define

«melancholic» or «atypical» subtypes complicate the task of arriving at a steady conclusion.

Recently we have published a systematic review comparing the neuropsychological performance of melancholic patients to non-melancholic depressive patients), including atypical depressives, and healthy controls (Bosaipo et al. 2017). In this study, the findings suggest that melancholic may have a distinct and impaired cognitive performance compared to non-melancholic depressive patients on tasks involving verbal and visual memory, executive function, maintained attention and span, as well as psychomotor speed, this last mainly when cognitive load is raised (Bosaipo et al. 2017).

Besides, although the literature is increasingly demonstrating distinct differences in clinical, biological, anatomical and response to treatment characteristics be-tween these two subtypes of depression, this debate is still ongoing. In this sense, the HPA axis play a vital role in the distinction between these subtypes, since stressful life events play a major role in the pathogenesis and onset of depressive episodes (Kendler et al., 2002). According to some authors, stress could lead to the onset of the first depressive episode in genetically vulnerable individuals, making them even more sensitive to stress in a fast forwarding fashion, compatible with the kindling hypothesis by Post (1992). With this, the individual would need less stress to trigger new crises, and it would become more vulnerable to the reprint of new depressive episodes before different, sometimes milder, stressors (Post, 1992). Also, adverse experiences in early life have been associated with significant increases in the risk of developing depression in adulthood, particularly in response to additional stressors (Tofoli et al. 2011; Cohen et al., 2001; Juruena, 2014). Thus, as the HPA axis is activated in response to stressors, changes in the functioning of this axis, at any level of its components, and its regulations may play a pivotal etiological role in the onset of depressive disorders (Holsboer, 2000; Tyrka et al., 2008).

Among the studies included in this systematic review that evaluated patients with melancholic depression, most (n=17) studies focused on DST response. Of them, the vast majority did indicate significantly elevated degrees of non-suppression in melancholic patients.

Nevertheless, there have been two studies, which did not demonstrate this significant association. The one Hubain et al. (1996), who performed DST in a large sample of 155 Newcastle Endogenous Depression Diagnostic Index (NEDDI) - de-fined

endogenous patients vs. a similar number of non-endogenous patients, in fact, did initially show was a statistically significant difference in the dexamethasone suppression test response at 1600 h, but when the effects of age and severity of depression were controlled, those differences disappeared. In a study by Berger et al.(1984), the majority of comparison groups were other psychiatric patients, which somewhat complicates drawing conclusions about the melancholic-nonmelancholic dichotomy, however, this study showed the importance of biological symptoms such as weight loss as a factor in non-suppression, confirming the notion that research may need to focus more on the vegetative symptoms of subtypes of interest.

The inconsistence of results may partly be influenced by different dexamethasone doses and suppression thresholds that were used. However, our review has demonstrated that the most dramatic differences lie between studies that used different approaches to defining melancholic depression. So, when RDC was used as a definition scale, those having a diagnosis of endogenous depression showed higher non-suppression rates than those with probable endogeneicity. Patients with DSM-defined melancholic features tended to show higher non-suppression in melancholia, too. It is also of note that strong support of elevated post-dex cortisol in melancholic patients comes from the studies which either focused on particular symptoms which are characteristic of the melancholic subtype or considered patients were meeting more than one diagnostic scale criteria (e.g. both DSM-IV and RDC) or used their operational criteria of endogeneicity. Another suggestion made by a few authors is that depression characterised both by melancholic features and DST-non-suppression is, in fact, a distinct form of depression. This inference stems from the observations that melancholic patients who are non-suppressors exhibit higher basal cortisol levels as well compared to melancholic patients normally responding to DST. Also, this increase seems to be associated mainly with melancholic depression with psychotic symptoms (Contreras et al., 2007).

Evidence of elevated basal cortisol and basal ACTH in melancholic patients is much less consistent. Approximately half of the selected studies failed to demonstrate differing levels of cortisol in melancholic patients compared to non-melancholic ones or controls. This may be due to differences in methodology, or differences in the diagnosis of melancholia (e.g. RDC definite endogenous criteria showed a more consistent association than DSM-III melancholic criteria). Regarding ACTH, none of the studies showed alteration in this measurement compared to controls.

However, when studies of melancholia focused on particular biological symptoms such as weight loss and appetite loss/insomnia or used their operational criteria for endogeneity mainly focusing on vital symptoms, they reported significant increases in basal cortisol. This means that there may be a stronger association with biological symptoms rather than subtypes as a whole.

When atypical studies were evaluated, importantly, the majority of those, re-regardless of the outcome measures, did not show a difference between atypical patients and control subjects, although there was a significant difference between atypical and melancholic patients. There were indeed studies (Levitan et al., 2002; Anisman et al., 1999), which showed significantly decreased post-DST and basal cortisol in atypical patients. However, it is important to consider that while Levitan et al. (2002) compared atypical patients to healthy controls and their results indeed may suggest hypoactive HPA-axis; Anisman et al. (1999) compared patients to non-atypical depressed patients, which rather indicates the difference with another subtype.

The studies directly comparing the function of the HPA-axis between melancholic and atypical patient groups are scarce and difficult for analysis since their methodologies vary largely. In particular, of the 7 studies comparing HPA axis functioning between patients with melancholic and atypical depression, only 2 used challenge tests (Fountoulakis et al., 2004; Heizmann et al., 2014.). Among them, only the one by Heinzmann et al.,(2014) despite a very different design from the rest of DST studies observed in the whole review, showed a significant difference between sub-types,

There was no consensus in the studies assessing basal cortisol, while Brouwer et al. (2005) and Lamers et al. (2012) did show decreased cortisol levels in atypical patients vs. controls and also underline the difference be-tween melancholic and atypical groups), others only indicated that they were not different between atypical and controls. Notably, the design employed by Lamers et al. (2012) showed no significant effect of the measure of mood reactivity or interpersonal sensitivity, but a robust effect of weight and sleep characteristics. At the same time, the authors only recruited severely depressed atypical patients, which may also have contributed to the strength of association.

This systematic review also considered a separate group including only longitudinal studies. Although these studies are just a few and are also different in methodological aspects, it could be suggested that in melancholic depression, elevated HPA-axis

function is a state rather than a trait characteristic, i.e. that remit-ted patients have lower basal and post-challenge cortisol levels compared to acutely depressed patients. At the same time, the case study - and the only longitudinal study of the atypical subtype - showed a negative correlation between the severity of depressive symptoms and cortisol levels, thus supporting the idea of the dichotomy.

Thus, although data in the literature seem to confirm that there are distinct patterns of HPA axis functioning between the melancholic and atypical depression subtypes, further studies with refining and homogeneous methodology are needed to characterize this pattern better. These may be attributed to methodological differences, including varying challenges and doses and non-suppression thresholds, varying availability of cortisol in urine, blood, saliva, or CSF. However, mainly the heterogeneity of clinical conditions assessing the same endophenotype and incorporated under the concept of major depression seems to be one of the limiters of these advances.

Novel advances in the methodology may shed light on the dichotomy in a more precise manner. In particular, there are currently no published studies evaluating hair and nail cortisol levels between the subtypes, but a few are underway. Be-sides, speaking of challenge tests, among the articles included in this review, the majority used the Dexamethasone Suppression Test. However, although the Dexamethasone Suppression Test remains widely used and widely studied as a biological marker in psychiatry, this test has some limitations because of the pharmacokinetic and pharmacodynamic characteristics of Dexamethasone that are very different from cortisol. Unlike cortisol, Dexamethasone has low affinity to MR receptors. Therefore, these studies allow us to investigate only the functioning of GR receptors in the subtypes of depression (Pariante et al., 2002; Juruena et al. 2006). Future studies might use different challenges, like MR antagonists, such as Spironolactone, MR agonists, such as Fludrocortisone, the Prednisolone suppression test, which appears to bind to MR as well as GR. MR function, and perhaps more important, MR/GR ratio, remains understudied in depressed populations, and it seems like an interesting prospect in this area (Juruena et al. 2013).

In general, this review has provided a rather convincing support for the presence of a difference in HPA-axis activity between the two subtypes, melancholic and atypical depression, regardless of the classification. However, it is much more difficult to conclude whether atypicality is associated with hypofunctional HPA-axis and en-

hanced negative feedback (such as implied in PTSD which was confirmed in a study by Halbreich et al..1989), or simply is not different from controls. It may be that the severity and the course of atypical depression, as well as the presence of particular vegetative symptoms (hypersomnia, weight gain as opposed to interpersonal sensitivity), are stronger predictors of decreased basal and post-challenge cortisol levels. However, this is yet to be established in studies employing a more unified efficient methodology.

The assessment of other factors potentially interfering with the dichotomy is outside the scope of this review. However, it should be noted that in the same studies which showed conflicting results regarding hypocortisolism in atypical depression, there was a much stronger association with elevated inflammatory factors (Lamers et al., 2012). This has driven a novel appraisal of the two subtypes, suggesting that while "typical"(or melancholic) depression has core pathophysiological features of overactive HPA-axis, what we call "atypical" depression may rather be comprehended as immuno-metabolic depression (Penninx et al., 2016). The precise interaction of potentially decreased activity of the HPA-axis with immune and metabolic abnormalities in the atypical subtype remains to be investigated.

CONCLUSION

The correct definition of depression subtypes remains a cornerstone in biological research in affective disorders. The evaluation of study results is dramatically hampered by the variation of definitions, and there is very little consistency between research groups in what they name "endogenous" or "melancholic" depression. Our review confirmed the presence of different HPA axis function between Melancholic and Atypical Depression, and a trend towards a more robust association with biological, or vegetative symptoms, or reverse vegetative symptoms, respectively. Patients with Melancholic depression are associated with increased cortisol levels, both baseline and post different challenges. Moreover, the research data also suggest a reduction of inhibitory feedback in patients with melancholic depression, demonstrated by increased cortisol concentrations and the number of non-suppressive patients following HPA axis challenge, mainly dexamethasone. Whether the difference between melancholic and atypical subtypes is better explained by the true hyperactive HPA-axis in the latter or a rather normal function.

Future studies might need to focus on evaluating the symptom profiles in patients with definite HPA-axis abnormalities to identify symptom constellations that are strongly associated with neuroendocrine variations rather than rely on phenomenologically defined subtypes. Moreover homogenize samples and methods, assessing more naturalistic measures, like salivary, hair or nails cortisol levels. Further insights into the dichotomy addressed in this review might be obtained from genetic and epigenetic studies of HPA-axis related genes in both subtypes, with an emphasis on the presence of vegetative symptoms.

Disclosures

Dr MF Juruena is a Consultant Psychiatrist at University of Sao Paulo and Honorary Consultant at South London and Maudsley NHS Foundation Trust (SLaM-NHS UK). Professor AH Young is the Director of the Centre for Affective Disorders and is supported by the National Institute for Health Research (NIHR); Biomedical Research Centre (BRC) at SLaM-NHS UK and King's College London. The views expressed are those of the authors and not necessarily those of the NHS, the NIHR, or the Department of Health. Dr MB and BA are a MSc student at Masters in Affective Disorders, KCL and has no conflicts of interest to declare. Only the authors were involved in the study design and preparation of this report.

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Conflicts of Interest:
MF Juruena has within the last year received honoraria for speaking from GSK, Lundbeck and Pfizer. AH Young received honoraria for lectures and advisory boards for all major pharmaceutical companies with drugs used in affective and related disorders. Investigator-initiated studies from AZ, Eli Lilly and Lundbeck. Dr MB and BA has no conflicts of interest to declare.

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TABLE 1: Melancholic depressed studies

Type of measurement	Author, year	Dose (For challenge tests)	Measurement time (s)	Threshold (for challenge tests)	Subtype definition	№ of subjects	Comparison group	Results
Dexamethasone Suppression Test (DST)	**Carroll et al., 1981a**	Dose 1:1 mg (n= 183) Dose 2: 2 mg (n= 185)	8 am 4 pm 11 pm	3, 4, 5, and 6 µg/dL compared	Endogenous - RDC Endogenous - Clinical assessment (matching RDC in 98% cases)	n = 47	non-endogenous: n = 42 (of them n = 32 neurotic depression)	Sensitivity for melancholic depression: 39% to 53% Specificity: 85% to 97% Diagnostic confidence: 83% to 93%

	Banki et al. 1986	1 mg	8 am 3 pm	50 ng/dL	DSM-III melancho-lic MDD	n = 21	DSM-III melancholic MDD n = 15 healthy con-trols n = 20 schiz-ophrenia n = 11 alco-hol depend-ence n = 13 ad-justment dis-order	67% NS in the Mel group vs 26% in the schizophre-nia+adjustme nt disorder group
	Evans et al., 1986	1 mg	4 pm 11 pm	5 µg/dl	DSM-III melancho-lic MDD	n = 23	n = 23 non-melancholic MDD n = 19 psy-chotic MDD	Highest rate of non-suppression in psychotic pa-tients (95% vs 78% in MEL group at 5 µg/dl, p<0.001); Higher non-suppression rates in MEL depression vs non-MEL (48%, p<0.02)
	Mil-ler et al., 1987	1 mg	4 pm 11 pm	7 µg/dl	DSM-III+ Endoge-nous - RDC Individual symp-toms: Yale De-pression Inventory	n = 45 MDD + melancho lia	n = 39 MDD + 5 psycho-tic MDD + 3 bipolar depression + 3 schizoaf-fective	**DST non-suppression correlated with:** **both melan-cholic and endogenous subtype;** **insomnia; agitation;** **loss of sexual interest;** **weight loss**

	Rubin et al., 1987	1 mg	7 am 3 pm 11 pm	3,5 µg/dl	RDC «definite» endogenous	n = 40	n = 40 healthy subjects	**38% NS in the endogenous group vs 12% in controls**
	Casper et al., 1987	1 mg	8.30 am 4 pm 10 pm	6 µg/dl	Loss of appetite: items 12 on the Hamilton scale (30), 32 on Vibes (31), HSCL-90, item 19 (32), and SADS-C item 228 (28). Weight loss: continuous severity measures from 1lb or more	n = 38 MDD patients with: weight loss and/or appetite loss	n = 42 MDD patients without weight/appetite loss n = 80 control subjects	Depression with weight/appetite loss associated with increased basal and post-Dex cortisol at all time points compared to MDD without weight/appetite loss
	Winokur et al., 1987	**1 mg**	**8 am and/or 4 pm**	"Strong suppressors": cortisol equal to or lower than 1.5 µg/dl, n = 163 High non-suppressors: (cortisol equal to or greater	DSM-III melancholic MDD	n = 423 MDD patients	no healthy controls	Melancholia significantly associated with high-degree non-suppression (24% vs 9%, p=0.01).

			than 6 µg/dl, n=164)				
Berger et al., 1984	1,5 mg or 1 mg	4 pm 11 pm	5 mg/dl or 8 mg/dl	RDC/ICD/ Newcastle Scale	SAMPLE 2: n = 45 MDD, of them n = 20 endogenous SAMPLE 3: n = 93 psychiatric patients, of them n = 41 endogenous MDD, n = 52 other diagnoses SAMPLE 4: n = 93, of them n = 19 endogenous MDD n = 74 patients	SAMPLE 1: n = 75 healthy subjects, of them n = 24 DST 1 mg n = 51 DST 1,5 mg SAMPLE 5: n = 24 fasting patients	Control subjects: 12% NS at 5 mg/dl 2,7% NS at 8 mg/dl SAMPLE 2: no significant differences in suppression rates, however, **Higher NS rates among patients with weight loss (p< 0.001)** SAMPLE 3: 38,6% in ED vs 7,4% in non-ED (5 mg/dl) 29,5% vs 8,7% (5 mg/dl) SAMPLE 4: Higher NS rates in neurotic depression vs ED at both thresholds

					with other diagnoses		
Barocka et al., 1987	1 mg	7 am 4 pm	5 µg/dl	ICD-10 Endogenous depression	n=26	n = 22 neurotic depression +adjustment disorder	77% non-suppression in endogenous group vs 23% in non-endogenous
Amsterdam et al., 1989	1 mg	4 pm	5 µg/dl	DSM-III melancholic MDD	n = 51	n = 21 non-melancholic MDD n = 37 healthy controls	Larger mean cortisol values for all response values in melancholic non-suppressors vs all other groups and healthy controls

	Halbreich et al., 1989	1 mg	4 pm 11 pm	not specified	DSM-III PTSD RDC MDD-endogenous depression	n = 14 PTSD+MDD-ED n = 23 MDD-ED	n = 21 healthy controls	No difference between PTSD patients with comorbid ED and controls; not a single case of non-suppression in the PTSD-ED group. Lower basal cortisol and higher dex suppression rates in PTSD-ED vs MDD-ED
	Paslakis et al., 2009	1,5 mg	2 pm 3 pm	non-specified (assessed as a continuous measure)	DSM-IV (SCID-IV)	n = 26	n = 33 healthy controls	Higher post-Dex cortisol on MEL patients (28.24 ng/ml vs 12.1 ng/ml, p=0.02)

	Maes M. et al., 1989	1 mg	8 am	Non-specified (assessed as a continuous measure)	Psychopathological correlates: A. 14 SCID items B. Clustering: 1.«biological» cluster: increments in FT, residual cortisol, and ACTH, and by decrements in basal TSH, L-TRP, and L-TRP ratio (all $p < 0.001$) 2.«non-biological» cluster» C. Vital (6 symptoms) vs Nonvital (7 symptoms) syndrome as validated in previous studies by Maes et al., 1990 (Pt 1)	n = 96 patients assessed for individual symptoms/presence of «vital» cluster; n = 33 «vital»	for the vital/nonvital distinction: n = 53 «nonvital»	DST non-supression significantly associated with symptoms of anorexia, insomnia and early morning awakening;

	Peselow E.D. et al., 1992	1 mg	8-9 am + 4-5 pm	5 μg/dl	RDC definite endogenous subtype; DSM III melancholic subtype	1) Meeting both RDC and DSM- III criteria for melancholia: n = 42 2) Meeting either RDC or DSM-III criteria for melancholia: n - 20	MDD meeting neither RDC nor DSM-III criteria for endogeneicity/melancholi a: n = 43 Healthy subjects: n - 29	Morning post-DST plasma cortisol; Afternoon post-DST plasma cortisol and Frequency of abnormal DST significantly elevated in «both» vs «neither» subtype and in «both» vs controls
	Hubain et al., 1996	1 mg	4 pm 11pm	50 ng/dl	Newcastle E ndogenous Depression Diagnostic Index (NEDDI): >= 6 for endogenous <6 for non-endogenous	n = 155 MDD endogenous	n = 155 MDD non-endogenous	Cortisol post-DST significantly elevated in ED only at 16.00 ($p<0.01$), but not at 23.00 Controlled for age and severity, post-DST 4 pm cortisol no longer significantly associated with ED
	Valdivieso et al., 1996	1 mg	8 am 4 pm 11 pm	≥ 138 nmol/l. l	DSM-III melancholic depression	n = 18 melancholic MDD	n = 29 non-melancholic MDD n = 20 healthy volunteers	Higher levels of DST non-suppression in the MEL group ($p=0.004$)

	Orsel, S. et al., 2010	1 mg	8 am	3.5 g/dl	DSM-III melancholic depression + cluster analysis which identified «endogenous» and «non-endogenous» subtype Discriminators: •early morning awakening • distinct quality of mood • feelings of guilt, non-reactivity • suicidal ideation •psychomotor disorders	n = 38 (DSM-III criteria) n = 40 «endogenous» cluster (included both DSM mel and non-mel patients)	DSM-III criteria: n = 40 nonmelancholic MDD, incl. n = 27 «simple» MDD n = 4 BD1 n = 2 Dysthymia n = 5 Depressive disorder NOS n = 2 Adjustment disorder «Non-endogenous» cluster: (included 8 mel patients) n = 38	Only non-reactivity differed significantly between non-suppressors and suppressors ($p<0.01$)

Other challenge tests

d Fenfluramine	**O'Keane, 1991.**	30mg	Baseline at 8:30; After challenge every 60 minutes, for 5 hours	Quantitative, comparison between groups	DSM III criteria	23 DMS III R criteria	16 healthy control subjects	High baseline cortisol levels were correlated with severity of depres-

								sion (p<0,01) and weight loss (p<0,01). No difference between groups after challenge
d Fenfluramine	**Mitchell, 1990**	60mg	8 am (3 baseline measures 20 min, apart); after that challenge and 5 hourly measures	Quantitative, comparison between groups	Varied according to used criteria: DSM III RDC , ICD-9, Newcastle scale	16 DSM III 18 RDC 15 ICD-9 7 Newcastle scale	14/12/15/23 for each group	No significant cortisol differences between groups
ACTH	**Amsterdam, 1989**	250ug (ACTH)	8:30 3 baseline measures; after ACTH 30,60,90,120,180,240 min	Quantitative comparison	HDRS	1st: 16 (9 with melancholic features); 2nd: 72 (51 with melancholic features, 21 without)	1st: 11 healthy controls 2nd: 37 healthy controls	1st: No difference between groups at baseline. Larger increases in cortisol in the MDD group after ACTH (p<0,02); 2nd: No

							difference at baseline; no statistically significant differences between groups, but a trend to higher cortisol levels in the melancholic group (P=0,31).
oCRH	**Amsterdam, 1989**	1,0ug/kg	8:30. 3 (every 15 minutes) baseline measures for ACTH and cortisol levels; after oCRH:0, 30,60,90,120,180, 240 min	HDRS	26 (14 melancholic features)	11 healthy controls	No difference in cortisol levels after challenge; depressive patients had lower ACTH response compared to controls. (p<0,05) The effect was larger in melancholic features (p<0,04)

Dex/CRH test	**Paslakis, 2010**	100ug hCRH	3 pm of day 2 (1 day after DST)	Quantitative comparison	HDRS 21	26 moderate to severe melancholic depression	33 healthy controls	Low specificity and low sensibility for the CRH/Dex test (78.8% and 30,8% respectively)

Basal Measures

Type of measurement	Authors, year	Time/source of measurement	Subtype definition	Sample size	Control group	Results
Basal cortisol levels	**Rubin et al., 1987**	26-hour cortisol curve: Blood sampling every 30 minutes over 26 hours urinary free cortisol	RDC «definite» endogenous	n = 40	n = 40 healthy subjects	DST NS vs Supressors: - elevated 24-hour cortisol (11.4 vs 8.3 nmol/L, p<0.01) - elevated nocturnal nadir (3.1 vs 1.8 nmol/L, p< 0.04) Only a moderate correlation between basal serum and UFC cortisol

	Casper et al., 1987	Morning plasma cortisol obtained at 8:30 a.m. on days 9, 10, 12. Evening plasma cortisol level drawn at 10 p.m. on Day 12. CSF cortisol sample - lumbar puncture at 9 a.m. on Day 11. A 24-hour urine predexamethasone collection assayed for urinary free cortisol (UFC) completed at 10:35 p.m. on Day 12.	Loss of appetite: items 12 on the Hamilton scale (30), 32 on Vibes (31), HSCL-90, item 19 (32), and SADS-C item 228 (28). Weight loss: continuous severity measures from 1lb or more	n = 38 MDD patients with: weight loss and/or appetite loss	n = 42 MDD patients without weight/appetite loss n = 80 control subjects	Basal plasma cortisol significantly associated with weight loss and appetite loss
	Guechot et al., 1987	baseline salivary cortisol 11 pm saliva cortisol	DSM-III Saint-Louis criteria for primary endogenous/seconda ry depression	DSM-III Saint-Louis criteria for primary endogenous/seconda ry depression	n = 40 «secondary depressive» n = 20 «non-depressive»	Higher saliva cortisol in endogenous depressives vs secondary ($p<0.05$)/ non-depressives ($p< 0.02$)
	Amsterdam et al., 1989	basal morning plasma cortisol (8.30 am)	DSM-III melancholic MDD	n = 26 (14 melancholic features)	n = 11 healthy controls	No difference in basal cortisol concentration between melancholic and non-melancholic groups

	Halbreich et al., 1989	basal plasma cortisol at 11 pm	DSM-III PTSD RDC MDD-ED	n = 14 PTSD+MDD-ED n = 23 MDD-ED	n = 21 healthy controls	Lower basal cortisol in PTSD-ED vs MDD-ED
	Wong et al., 2000	basal plasma cortisol began 9.00-10.00 am every 30 min for 30 hours	DSM-III-R RDC	n = 10	n = 14 healthy controls	Elevated basal cortisol in MEL patients vs controls ($p<0.02$)
	Michopoulos et al., 2008	basal morning cortisol levels Salivary test to assess cortisol levels (three daily samples: morning, 08.00 a.m. [CS1]; noon, 16.00 p.m. [CS2] and night, 23.00 p.m)	DSM-IV (SCID-IV)	n = 20	n = 20 non-mel n = 20 healthy controls	No significant elevation in MEL group
	Paslakis et al., 2009	24-hour basal plasma cortisol	DSM-IV (SCID-IV)	n = 26	n = 33 healthy controls	Basal cortisol significantly elevated in MEL group
	Marquez-Deak et al., 2007	Basal plasma cortisol 8.00 am	DSM-IV (SCID-IV)	n = 28 female melancholic MDD	n = 41 healthy controls n = 18 non-melancholic MDD	No significant differences indicated between groups
	Mulder et al., 2003	Basal plasma cortisol at 09.00 h, blood drawn at 30-min intervals over 3 h 30 min	DSM-III-R	n = 39 melancholic MDD	n = 69 non-melancholic MDD, n = 20 control subjects	No significant differences indicated between groups

	Joyce, P.R. et al., 2001	Basal plasma cortisol **(13.00 - 15.00 at 15-min intervals)**	DSM-IV melancholic MDD vs CORE checklist	n = 86 melancholic patients n = 32 «severely melancholic» patients CORE definition: 116 «broadly defined» melancholic patients; 39 «narrowly defined» melancholic patients	n = 77 non-melancholic patients	No differences on any parameters between DSM-IV-defined groups CORE definition: Basal cortisol increased only in male patients in combined broad+narrow melancholic vs non-melancholic groups (p = 0.016)
	O'Keane & Dinan, 1991	Basal plasma cortisol Single measure at 8.30 before d,l-fenfluramine test	DSM-III-R, Newcastle scale	n = 23	n = 16 healthy subjects	Elevated in endogenous patients vs controls (t= 3,56; df=37, p=0.0001) High baseline cortisol correlated with HAM-D severity (r=0.97, p<0.001) and weight loss (r=0.85, p<0.001).

	Mitchell P. et al., 1990	Baseline plasma cortisol	Four different definitions of endogenicity/melancholy compared: • ICD-9 • DSM-III • RDC • Newcastle scale	n = 15 (ICD-9) n = 16 (DSM-III) n = 18 (RDC) n = 7 (Newcastle scale)	n = 15 non-endogenous (ICD-9) n = 14 non-endogenous (DSM-III) n = 12 non-endogenous (RDC) n = 23 non-endogenous (Newcastle scale)	No significant differences in baseline cortisol in any classification
	Valdiviezo et al., 1996	Baseline plasma cortisol at midnight	DSM-III melancholic depression	n = 18 melancholic MDD	n = 29 non-melancholic MDD n = 20 healthy volunteers	No differences between depressed patients and controls
	Orsel, S. et al., 2010	Basal plasma cortisol 8 am	DSM-III melancholic depression+cluster analysis which identified«endogenous» and «non-endogenous» subtype Discriminators: • early morning awakening • distinct quality of mood • feelings of guilt, non-reactivity • suicidal ideation • psychomotor disorders	n = 38 (DSM-III criteria) n = 40 «endogenous» cluster (included both DSM mel and non-mel patients)	DSM-III criteria: n = 40 non-melancholic MDD, incl. n = 27 «simple» MDD n = 4 BD1 n = 2 Dysthymia n = 5 Depressive disorder NOS n = 2 Adjustment disorder «Non-endogenous» cluster: (included 8 mel patients) n = 38	Significantly elevated basal cortisol in the «ëndogenous» cluster

	Liu et al., 2016	cortisol as part of 228 metabolites	The CORE scale for melancholic depression; Anxious depression defined as number of comorbid anxiety disorders on the M.I.N.I. International Neuropsychiatric Interview > 0	n = 21	n = 58 anxious depression n = 100 healthy controls	Increased basal cortisol in melancholia vs healthy controls;
Basal ACTH measures	**Wong et al.**	24-hour basal plasma ACTH began 9.00-10.00 am every 30 min for 30 hours	DSM-III-R, RDC (not specified which used for diagnosis of melancholia)	n = 10	n = 14 healthy controls	No difference in plasma ACTH between patients and controls
	Joyce, P.R. et al., 2001	basal afternoon plasma ACTH (13.00 - 15.00 at 15-min intervals)	DSM-IV melancholic MDD vs CORE checklist	n = 86 melancholic patients n = 32 «severely melancholic» patients CORE definition: 116 «broadly defined» melancholic patients; 39 «narrowly defined» melancholic patients	n = 77 non-melancholic patients	No differences between groups independent of subtype
	Gomez-Gil et al.	basal morning plasma ACTH	Newcastle Endogenous Depression Diagnostic Index (NEDDI): >= 7 for endogenous	n = 14	n = 15	No difference in baseline ACTH between groups

CRF	**Wong et al., 2000**	Basal **CSF CRF** - CSF sampling began at 09:00–10:00 a.m. and lasted for 30 hours	DSM-III-R, RDC (not specified which used for diagnosis of melancholia)	n = 10	n = 14 healthy controls	No difference in baseline CRF between groups
	Joyce et al., 2001	Basal afternoon plasma **CRF** **(13.00 - 15.00 at 15-min intervals)**	DSM-IV melancholic MDD vs CORE checklist	n = 86 melancholic patients n = 32 «severely melancholic» patients CORE definition: 116 «broadly defined» melancholic patients; 39 «narrowly defined» melancholic patients	n = 77 non-melancholic patients	No differences in basal plasma CRF between groups independent of subtype

Table 2 Atypical vs. non-atypical or controls

Type of measurement	Author, year	Dose (For challenge tests)	Measurement time(s)	Threshold (for challenge tests)	Subtype definition	№ of subjects	Comparison group	Results
Dexamethasone suppression test (DST)	**Casper et al., 1988**	1 mg	8.30 am 4 pm 10 pm	5 µg/dl	Hypersomnia/Increased appetite diagnosed with SADS	Sample 1: n = 23 MDD + hypersomnia Sample 2: n = 22 MDD + incr.appetite	n = 23 depressed patients without appetite/weight increase or hypersomnia n = 22 healthy subjects	**In patients with hypersomnia only**: Significantly higher levels of morning suppression vs non-atypical depressed patients ($P < 0.04$). No difference between hypersomnia patients and controls. **In patients with weight gain only:** no significant difference between patients and controls **In patients with both appetite/weight gain and**

							hyper-somnia DST non-suppres-sion simi-lar to con-trols
Thase et al., 1989	1 mg	4 pm	5 µg/dl	Own opera-tional criteria of anergia: 1) definite anergia (score 2 on HADRS-13) 2) psychomoto r retardation (score 2 or more on HADRS-13) 3) at least one of two associated re-versed neu-rovegetative features (weight gain 2.2 kg or more, hypersomnia as +1 hour of extra sleep)	n = 23 «anergi c bipolar» patients	n = 26 healthy subjects	13% DST nonsup-pression rate (3/26 patients)
Levi-tan et al., 2002	0.25/0 .5 mg	8 am 3 am	percent change scores	DSM-IV atyp-ical depression	n = 8 female patients	n = 11 healthy subjects	91,9% suppres-sion in atypical depressive patients vs 78,3% in

								controls;
	Stewart et al., 2005	1 mg	4 pm	5 µg/dl	DSM-IV atypical depression	n = 84 chronic atypical patients, of them 31 early-onset, 53 late-onset; 61 chronic (dysthymia)	no healthy controls (comparison between atypical groups)	Lower mean. post- dexamethasone cortisol levels in early/chronic atypical vs late/nonchronic atypical patients
Desipramine challenge test	**Asnis et al., 1992**	75 mg	9 am: cortisol levels every 15 min for 1 hour; Imipramine vs placebo: blood test for 2 hours, (every 15 min for cortisol levels; every 30 min for desipramine levels)	N/A (measured as a continuous variable)	ADDS	n = 33	n = 81 non-atypical MDD	Significantly higher post-desipramine cortisol (blunted response) in atypical group vs non-atypical

	McGinn et al., 1995	75 mg	9 am: cortisol levels every 15 min for 1 hour; Imipramine vs placebo: blood test for 2 hours, (every 15 minutes for cortisol levels; every 30 min for desipramine levels)	N/A (measured as a continuous variable)	ADDS	n = 17 atypical depressives (AD)	n = 19 mood reactivity (MR) depressives, n = 36 non-MR/AD MDD	Significantly blunted response to DMI in AD compared to MR and controls
Dextroamphetamine stimulation test	**Stewart et al., 2005**	0.15 mg/kg	Cortisol levesl taken each 30 min, from 1pm to 4pm; After that Dextro stimulation over 45 seconds, and blood collected every 15 min for 90min	After 30 minutes of Dextro infusion, cortisol levels <1,5µg/dL were considered abnormal	DSM-IV	n = 84	no HC group	No significant differences between groups (early/chronic atypical vs late/nonchronic atypical)

oCRH stimulation test	**Josephvanderpool, J.R. et al., 1991**	100 *ng*	Cortisol levels taken 15 minutes before challenge (9am); then at the time, 5, 10, 15, 30, 60, 90 and 120 minutes later	Quantitative between groups	DSM-III-R SAD with reverse vegetative symptoms/ Major depression with seasonal pattern	n = 10 SAD patients	n = 13 healthy controls	ACTH and cortisol responses to oCRH significantly blunted in untreated SAD vs controls; Delayed timing of the ACTH peak vs controls

Table 2 Atypical vs non-atypical or controls. Basal cortisol and ACTH levels

Type of measurement	Authors, year	Time/source of measurement	Subtype definition	Sample size	Control group	Results
Basal cortisol	**Levitan at al., 1997**	baseline plasma cortisol at 8am	DSM-III for Bulimia Nervosa HDRS-29 to assess reversed neurovegetative symptoms	n = 16 «bulimia nervosa» with atypical features	n = 14 healthy controls	Strong negative correlation for hypersomnia and basal cortisol levels; for «carbohydrate craving» and basal cortisol levels

Anisman et al., 1999	baseline plasma cortisol at 7am, and each 10 minutes until 9:30am	ADDS DSM-III/IV, HAM-D-29	n = 31 atypical depressed patients, n = 15 atypical dysthymic patients,	n = 14 non-atypical depressed patients; n = 14 non-atypical dysthymic patients	Decreased basal cortisol in atypical depressed subjects vs controls
Casper et al., 1988	Basal plasma cortisol 8 am on days 9,10,12 (further averaged) Basal CSF cortisol: 8-8.30 am Basal urinary cortisol: 24-h specimen	Hypersomnia/ Increased appetite diagnosed with SADS	Sample 1: n = 23 MDD + hypersomnia Sample 2: n = 22 MDD + incr.appetite	n = 23 depressed patients without appetite/weight increase or hypersomnia n = 22 healthy subjects	1.Morning plasma cortisol higher in MDD without H or AI vs controls, no difference otherwise 2.Urinary free cortisol: higher in all MDD groups vs controls 3.No significant differences in CSF cortisol
Levitan et al., 2002	basal plasma cortisol (11 pm)	DSM-IV atypical depression	n = 8	n = 11	Lower basal cortisol in subjects vs controls
Stewart et al., 2005	basal plasma cortisol 3-hour afternoon cortisol curve	DSM-IV atypical depression	n = 84	no HC group	Lower mean 3 h afternoon cortisol levels (N=21) in early/chronic atypical vs late/nonchronic atypical patients
Asnis et al., 1992	basal morning plasma cortisol	ADDS	n=33	n=81	No difference between atypical and non-atypical de-

						pression (reported as "no effect for group on baseline cortisol levels")
	McGinn et al., 1995	basal morning plasma cortisol	ADDS	n = 17	n = 19 MR patients n = 19 non-MR/AD-MDD patients	No difference in baseline cortisol between any groups
	Joseph-Vanderpool, J.R. et al., 1991	basal plasma cortisol (24-hour curve)	DSM-III-R	n = 10	n =13	Basal cortisol levels NS lower in patients vs controls except at 22.00 when basal cortisol in patients was significantly lower vs controls (46.59 ± 27.59 nmol/L *vs.* 137.95 ± 71.73 nmol/L; p = 0.02)
Basal ACTH	**Anisman et al., 1999**	Basal ACTH at 7am, and each 10 minutes until 9:30am	ADDS DSM-III/IV, HAM-D-29	n = 31 MDD n = 15 dysthymia	n = 14 non-atyp MDD; n = 14 non-atyp dysthymia	Increased basal ACTH in atypical depressed subjects vs controls

TABLE 3 ATYPICAL DEPRESSION VS. MELANCHOLIC DEPRESSION. BASAL CORTISOL. BASAL ACTH, DST, DEX/CRH

Type of measurement	Authors, year	Measurement time	Definition of melancholic subtype/ sample size	Definition of atypical subtype/ sample size	Comparison group	Results
	Young et al., 2001	24-hour plasma cortisol: 9 am-9 am; 10-min intervals 24-hour urinary cortisol	RDC definite endogenous; n=6	DSM-IV criteria; n=7	n = 25 neither subtype MDD	A trend towards elevated cortisol in definite endogenous patients; normal cortisol in atypical patients. No significant differences.
Basal cortisol	**Brouwer et al., 2005**	Morning basal serum cortisol (before 10am) 24-h urinary cortisol	DSM-IV n = 32	DSM-IV n = 25	n = 56 MDD neither subtype n = 113 control subjects	Serum cortisol lower in AD vs neither subtype MDD only
	Karlovic et al., 2012	Baseline morning serum cortisol between 8am and 9am	DSM-IV n = 32	DSM-IV n = 23	n = 18	Serum cortisol significantly higher in MDD-M vs MDD-A, and in MD vs control Similar in AD and controls.

Type of measurement	Authors, year	Measurement time	Definition of melancholic subtype/ sample size	Definition of atypical subtype/ sample size	Comparison group	Results
	Lamers et al., 2012	Saliva cortisol awakening curves (CAR 30, 45 and 60 minutes later. Additional sample at 11am. Diurnal cortisol slope	CIDI + previous latent class analysis (own operational criteria) of patients with persistent chronic depression n = 122	CIDI + previous latent class analysis (own operational criteria) of patients with persistent chronic depression n = 111	n = 543 healthy controls	Lower AUCg, lower diurnal cortisol slope in atypical vs melancholic patients and vs controls
	Cizza et al., 2012	24-hour serum cortisol, hourly, starting at 8am.	DSM-IV n = 53	DSM-IV n = 16	n = 22 neither subtype MDD n = 44 healthy controls	No variation in serum cortisol levels
Basal ACTH	**Young et al., 2001**	24-hour plasma ACTH 9 am-9 am; 10-min intervals	RDC n = 6	DSM-IV n = 7	n = 25 neither subtype MDD	No difference between groups; a trend toward increased ACTH in endogenous patients vs controls.
	Cizza et al., 2012	24-hour serum cortisol hourly, starting at 8am.	DSM-IV n = 53	DSM-IV n = 16	n = 22 neither subtype MDD n = 44 healthy	Significant increase in serum ACTH in atypical

Type of measurement	Authors, year	Measurement time	Definition of melancholic subtype/ sample size	Definition of atypical subtype/ sample size	Comparison group	Results
					controls	group vs controls
DST	**Fountolakis et al., 2004**	1 mg	4 pm 11 pm	5 μg/dl	DSM-IV/ICD-10 n = 16	DSM-IV/ICD-10 n = 14
DST/CRH	**Heinzmann et al., 2014**	Dex: 0.05 mg/kg - 2 mg/kg CRH dose: 0.15 mg/kg.	11.30 pm	AUC measurement	Based on AUC response to Dex/CRH, patients (n=657) were divided into: hHR: 219 hLR:219 hIR: 219	n/a

TABLE 4 **LONGITUDINAL STUDIES**

Au-thors, year	Type of measurement (incl.dose and timing	Design	Subtype + sample size	Subtype definition	Comparison group	Results

Haskett et al., 1987	Dexame-thasone Sup-pression Test (1 mg; 4 pm-11 pm, 5 ng/dL)	DST performed on admission (day 1-2) and after 7-12 days	Endoge-nous Depression	RDC "definite endogenous" depression	Non-endogenous MDD pa-tients, incl."probabl e endoge-nous"	Non-suppression rates signifi-cantly lower at second DST overall and in nonpendoge-nous patients, Non-suppression rates did not significantly decrease in the ED group
Geraci-oti et al., 1992	Basal plasma cortisol (biweekly tests at 7.30 am)	6-month follow-up	Atypical Depression	Clinical assessment (the presence of reversed neurovegeta-tive signs)	n/a	Eucortisolemic in acute de-pression (on admission) Negative corre-lation between mood ratings and serum cor-tisol (r = - 0.36, p = 0.002)
Steiger et al., 1997	Basal plasma cortisol (from 23.00 until 07.00)	Time 1. On admission Time 2. Post-treatment	Endoge-nous De-pression	RDC	n = 25 nor-mal controls	Basal cortisol significantly elevated in ED vs controls; in ED on admis-sion vs ED post-treatment
Pintor et al. 2013	Plasma ACTH levels following 100 μg hCRF stimulation test	Patients followed-up for 2 years af-ter hCRF stimula-tion	melan-cholic pa-tients n = 62	DSM-IV melancholic MDD, con-firmed by MES, NEDDI	n = 23 healthy subjects	No differences between re-lapsed, non-relapsed or partially re-lapsed groups; Significant

	Plasma cortisol levels following100 µg hCRF stimulation test	NAUCC stratified at three levels — <150; 150 – 350 and >350 µg/ml/min				differences between patient groups and controls on both ACTH and cortisol significant difference only between complete relapse groups and controls
Kaestner et al., 2005	• Basal plasma ACTH (8 am) • Basal plasma cortisol (8 am)	Time 1. On admission Time 2. Post-treatment	Endogenous Depression n = 21	DSM-IV + Newcastle Endogenicity Scale 6 points or more	n = 16 non-melancholic patients n = 37 healthy controls	Plasma ACTH increased in both acute and remitted MEL patients vs controls. Elevated plasma cortisol in acute melancholic vs control groups only Non-MEL patients not different from control on either measure

HIGHLIGHTS

- Different depressive subtype classification criteria may influence severity and treatment.
- Depressive subtype is an important factor influencing Hypothalamus-Pituitary-Adrenal (HPA) axis activity
- Melancholic patients has increased post-challenge cortisol levels than Atypical Depressive patients
- Studies focusing on reversed vegetative symptoms, demonstrated a decrease in the activity of the HPA axis in atypical depressives compared to controls, but the majority did not distinguish it from healthy controls.
- The correct definition of depression subtypes remains a cornerstone in biological research in affective disorders.
- Future studies should consider epigenetic studies of HPA-axis related to both subtypes, with an emphasis on vegetative symptom and standardized methodologies.