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ORIGINAL ARTICLE

Depressive symptoms are associated with higher morning plasma cortisol in primary care subjects

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Abstract

BACKGROUND: Cortisol dysregulation has a potential role in depression.
AIM AND METHODS: We evaluated depressive symptoms using the Hamilton Rating Scale for Depression in 48 primary care subjects without history of previous or current depression and its association with cortisol dysregulation (morning plasma cortisol, 24-hour urinary free cortisol and cortisol metabolites). Presence of metabolic syndrome and inflammatory parameters were also assessed.
RESULTS: Hamilton Rating Scale for Depression correlated significantly with morning cortisol, but not with urinary free cortisol or metabolites. A significant increase in morning cortisol by Hamilton groups (asymptomatic ≤8; mild to moderate: 9–18; moderate to severe: ≥19) was observed even when adjusted by age/gender. We observed no association of depressive symptoms with metabolic or inflammatory parameters.
CONCLUSIONS: Depressive symptoms in primary care subjects not consulting for their mood are associated with higher morning plasma cortisol, but not urinary cortisol or its metabolites. These observations suggest that systemic hypercortisolism and related metabolic disorders are not observed in mild/initial states of depressive disorders.

Abbreviations:

HPA	- Hypothalamic-Pituitary-Adrenal
CAR	- Cortisol awakening response
UFC	- Urinary free cortisol
HPLC-MS/MS	- High performance liquid chromatography-tandem mass spectrometry
hs-CRP	- High sensitive-C reactive protein
RIA	- Radioimmunoassay
HOMA-IR	- Homeostasis model assessment – Insulin resistance
MetS	- Metabolic syndrome
IDF	- International diabetes federation
NHLBI	- National Heart, Lung and Blood Institute
AHA	- American Heart Association
IAS	- International Atherosclerosis Society
IASO	- International Association for the Study of Obesity
BMI	- Body Mass Index
ACTH	- Adrenocorticotropic hormone

INTRODUCTION

Depression is highly prevalent and associated with significant functional disability and economic burden worldwide (Harvey *et al.* 2011). In the United States, up to 20% of adults have had a depressive episode during their life and in Chile the estimated prevalence of depressive disorder is 11.3% in the general population (Hirshfeld 2012; Vicente *et al.* 2002).

Depression is often under recognized in primary care settings (Culpepper *et al.* 2008). This is important, as these patients may be less likely to adhere to medical treatments and recommendations of lifestyle changes (Ziegelstein *et al.* 2000). Furthermore, this condition not only increases the likelihood of having a chronic disease, but could also worsen its course and long term outcome (Ang *et al.* 2005). Depressive symptoms are associated with an increased stress response, that is, a chronic dysregulation in the Hypothalamic-Pituitary-Adrenal (HPA) axis (Holsboer 1995). Over the years, this increased stress response may explain the higher prevalence of diabetes, stroke, heart disease and metabolic syndrome observed in these patients (Garcia-Rizo *et al.* 2016; McEwen 2017).

Dysregulation of HPA axis may include circadian rhythm alterations (e.g. with high morning or midnight cortisol), increased daily cortisol production (e.g. high urinary cortisol) and/or inappropriate feedback suppression (e.g. abnormal dexamethasone suppression test [DST]). Recent studies have shown a correlation between depressive disorders in non-severe cases with morning plasma cortisol, also when measured as cortisol awakening response (CAR) (Cubala & Landowski 2014; Mangold 2011). Thus, morning cortisol values evaluate hypothalamus-pituitary-adrenal (HPA) axis activation, which has been shown to be dysregulated with mood disorders (Pruessner 1997). Milder states of hypercortisolism, may be unrecognized by just measuring urinary free cortisol, and urinary cortisol metabolites such as tetrahydrocortisol [THF], allo-tetrahydrocortisol [5α-THF] and tetrahydrocortisone [THE] have been associated with metabolic syndrome and insulin resistance by our group (Baudrand *et al.* 2011; Baudrand *et al.* 2014) and in depressed female inpatients (Romer *et al.* 2009). Later, others (Zhai *et al.* 2015) found similar elevations in cortisol metabolites only in overt depressed men.(Baudrand, 2014 #6)

However, published studies regarding cortisol dysregulation in patients with mild depressive symptoms or minor depression in primary care settings are scarce and have methodological limitations, mainly due to the lack of a several tests to measure cortisol dysregulation or non-generalizable samples because of selection bias by gender (Romer *et al.* 2009; Zhai *et al.* 2015). These patients might have cortisol dysregulation as well, which could be associated with long term morbidity.

The aim of this pilot study was to determine the association of depressive symptoms and cortisol dysregulation in a primary care sample of patients without previous diagnosis or treatment for depression. Cortisol dysregulation was assessed by three different strategies: two well-known cortisol studies (morning plasma cortisol and urinary free cortisol (UFC) and a new and potentially more sensitive method such as urinary cortisol metabolites (THF, 5α-THF and THE) in both genders. A secondary outcome was to evaluate the association of metabolic parameters with both depressive symptoms and cortisol measurements.

SUBJECTS AND METHODS

Individuals included in this study were Hispanic adults recruited from primary care centers in Santiago, Chile as previously described (Baudrand *et al.* 2014). We excluded subjects with a previous diagnosis or treatment of depression. Of note, classic cortisol studies in depression have been mainly conducted in psychiatric outpatient clinics. The study was approved by our Institutional Review Board for Human Studies and an informed consent was provided according to the guidelines of the Declaration of Helsinki.

We excluded all subjects with use of steroid medication in the last 3 months, severe organ failure, alcohol abuse or depression or any mood disorder as these conditions could modify cortisol production and metabolism. We also excluded total plasma cortisol in women using estrogens (contraceptive or supplementation), but not urinary free cortisol or other free metabolites, due to the known effects on cortisol binding globulin.

Clinical assessments

Semi-structured interviews with patients and family members, and medical records provided socio-demographic data and clinical details of illness history. Patients were assessed clinically by trained psychiatrists and rated with the 17-item Hamilton Rating Scale for Depression [HDRS], assessed in primary analysis as a continuous variable (Hamilton 1960; Ramos-Brieva *et al.* 1986). We also categorized depression symptoms in three groups described as no symptoms (Hamilton <8

points), mild to moderate symptoms (Hamilton 9–18 points) or moderate to severe symptoms (≥19 points), to evaluate our results in a more clinical analysis (Hamilton 1960; Ramos-Brieva *et al.* 1986).

In our protocol to minimize cortisol variability, all participants visited our outpatient clinic on a non-working day (Saturday) after a 12-h fasting period and all blood samples were obtained between 08:00 to 09:00 am. Plasma cortisol was measured by immunoassay using Roche Modular EP170 automated analyzer (Roche Diagnostics GmbH, Manheim) as previously described (Baudrand *et al.* 2011). All participants were instructed to collect complete urine samples over 24 h. In the case of females their collection was not performed during menstruation. The participants were asked to discard their first morning urine void (07:00 h) and to collect all the urine voided over the following 24 h up to and including the first morning urine void (07:00 h) the next day. Upon collection of the bottles, research assistants verified the collection procedure and measured the volume of the 24 h urine sample. To ensure that the collection of urine was complete we also measured creatinine by kinetic colorimetric Jaffe method in automated chemistry analyzer (Roche/Hitachi, Kobe, Japan). The coefficient of variation was 2.3% for 80.6 mg/dl and 4.0% for 240.9 mg/dl and the sensitivity was 4.2 mg/dl. These measurements were carried out in our clinical laboratory under the control of College of American Pathologist (CAP nº 3000101-01). UFC, free cortisone (UFE) and free cortisol metabolites (tetrahydrocortisol [THF], allo-tetrahydrocortisol [5α-THF] and tetrahydrocortisone [THE]) were measured by high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) as previously reported by our group (Allende *et al.* 2014). The 24h urinary sample was collected the day before the blood sample was drawn. Enzymatic activity was evaluated by calculation of ratios between the substrate and product, as previously described by Romer (Romer *et al.* 2009). Serum lipid profile, serum fasting glucose, total serum adiponectin, serum leptin and high sensitive-C reactive protein (hs-CRP) were measured as previously described (Baudrand *et al.* 2011). We evaluated insulin by radioimmunoassay (RIA) and Homeostasis model assessment – Insulin resistance (HOMA-IR) by the Oxford University HOMA Calculator 2.2® as previously described (Baudrand *et al.* 2014). Participants were categorized as having metabolic syndrome (MetS) or hypertension according to the Joint Scientific Statement of the IDF, NHLBI, AHA, World Heart Federation, IAS and IASO (Alberti *et al.* 2009).

Statistical analysis

Continuous variables are presented as mean ± SD and categorical variables as percentage of the total sample. Normality of the parameters was assessed using normality (Q-Q) plot. Correlations between continuous variables were evaluated using Pearson´s correlation test.

In addition, Hamilton Rating Scale for Depression, as a continuous variable, was analyzed with the explanatory variable adjusted by covariates (age and gender), presenting the results as partial correlation coefficients. Analysis for interactions and normality of residuals for the regression were also performed.

As a sensitivity analysis, and for clinical usefulness, we performed a linear regression model with different outcomes of interest using three groups of Hamilton score as an ordinal predictor. In the multivariate analysis, we included age and gender as covariates for their known effect on cortisol and the results are presented as β coefficients.

Since some variables were not normally distributed, a bootstrapping procedure with 1000 iterations was

Tab. 1. Subjects demographic and biochemical characteristics categorized by Hamilton score.

48 subjects	No depressive symptoms (Group 1: Hamilton score <8) 42%	Mild to moderate (Group 2: Hamilton score 8–18) 42%	Moderate to severe symptoms (Group 3: Hamilton score ≥19) 16%	*p*-value
Age (years)	40.0±11.1	46.5±14.3	56.9±11.3	0.002
Female (%)	45%	80%	87%	0.01
Body Mass Index (kg/m^2)	29.9±3.8	30.9±6.4	31.23±4.3	0.493
Hypertension (%)	70%	45%	88%	0.841
Metabolic Syndrome (%)	65%	40%	75%	0.922
HOMA-IR[a]	1.2±0.6	1.1±0.7	1.3±0.4	0.698
Plasma cortisol (nmol/L)	9.8±0.70	12.1±1.1	16.5±2.4	0.003
UFC[b] (nmol/24 h)	19.8±7.9	26.75±16.1	18.03±10.1	0.736
Cortisol metabolites (mg/24 h)	86.03±23.34	107.51±44.66	88.92±40.80	0.433
hs-CRP[c]	3.61±3.41	3.42±3.01	3.0±2.59	0.648

a) HOMA = Homeostasis model assessment; b) UFC = Urinary free cortisol; c) hs-CRP = high sensivity- C reactive protein. *p*-value represents *p* trend for ordinal groups, bold values denote statistical significance.

applied to all analyses. Differences were considered statistically significant at p-value <0.05 of two sides. All analyses were performed using STATA 13 [StataCorp. 2013. Stata Statistical Software: Release 13. College Station, TX: StataCorp LP.] and SPSS 21 [IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.] statistical packages.

RESULTS

Characteristics of participants

A total of 48 adults were included in our analysis. Participants characteristics are described in Table 1.

Univariate analysis of Hamilton score

When analyzing all subjects (n=48), we observed a significant correlation between Hamilton score with age (r=+0.28, p=0.03) and higher scores with female gender (p=0.04). We observed no correlations between Hamilton score with anthropometric variables such as weight, BMI or waist circumference. When evaluating HPA axis with the three different cortisol measurements, we observed a significant correlation between Hamilton score and morning plasmatic cortisol (r=+0.47, p<0.001, Figure 1), but no correlation regarding Hamilton score with UFC (p=0.32) or cortisol metabolites (p=0.72), adjusted by urinary creatinine. In addition, we explore the correlation of Hamilton score with metabolic and inflammatory parameters like insulin levels, HOMA-IR and hs-CRP, that were not significant.

Multivariate analysis of Hamilton score

We observed an association between morning plasma cortisol and HDRS score, even when adjusted by age and gender [partial correlation coefficient = +0.46, p<0.001]. We did not observe an association between HDRS score and adjusted UFC (partial correlation coefficient = +0.51, p=0.736) or cortisol metabolites (partial correlation coefficient = +0.18, p=0.433. A sensitivity analysis exploring HDRS score showed no significant association with urinary cortisol/cortisone ratio.

Characteristics of participants categorized by Hamilton score (Clinical scenario)

Of the 48 recruited participants, 20 (42%) had no depressive symptoms (Group 1, Hamilton score ≤8), 20 (42%) had mild to moderate symptoms (Group 2, Hamilton score 9–18) and 8 (16%) had moderate to severe symptoms (Group 3, Hamilton ≥19).

When analyzing the 3 Hamilton groups as an ordinal variable in a linear regression model we observed a significant increase in morning plasma cortisol by groups (β=2.74 μg/dL, p-value for trend = 0.003), and a significant difference between Group 3 with moderate to severe symptoms when compared to Group 1 with no symptoms (β= 5.79 μg/dL, p=0.01). We again observed no changes in UFC or cortisol metabolites when analyzed by groups as an ordinal variable or when compared by groups.

Further, we analyzed by groups including in the model age and gender as covariates, since both variables were also associated with higher morning plasma cortisol. Consistently, we observed a strongly significant increase between groups in adjusted morning plasma cortisol (β=3.85 μg/dL, p-value for trend = 0.004). In addition, we observed a significant difference comparing Group 3 with moderate to severe symptoms to Group 1 with no symptoms (β=7.83 μg/dL, p=0.007) and Group 2 with mild-moderate symptoms to Group 1 (β=3.45 μg/dL, p=0.01) when controlling for the effect of age and gender. As previous analyses, we observed no significant changes of UFC or cortisol metabolites when comparing groups categorized by Hamilton score in this multivariate model.

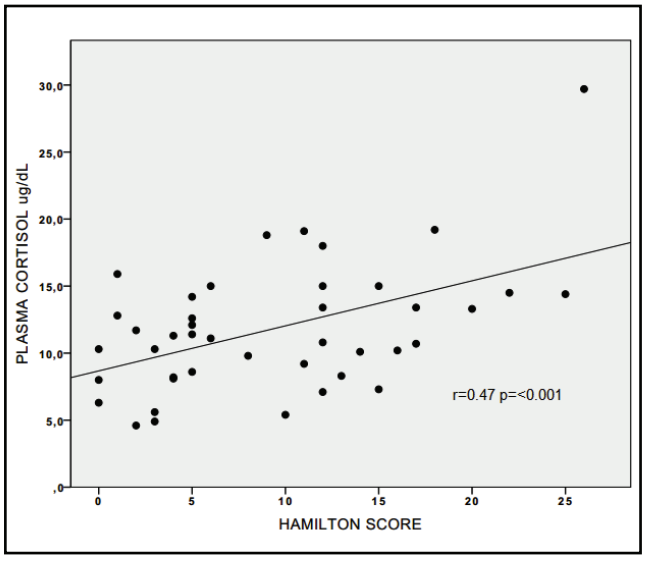

Fig. 1. Linear regression of Hamilton score for depressive symptoms and morning plasmatic cortisol in primary care subjects.

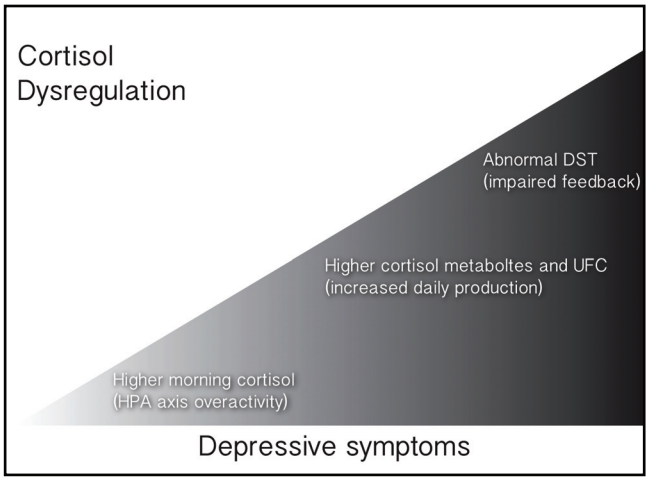

Fig. 2. Schematic figure of hypothetic cortisol dysregulation according to symptom severity/chronicity of depression. Potential mechanisms are shown in parenthesis. HPA = Hypothalamic-Pituitary-Adrenal axis; UFC = Urinary free cortisol; DST = Dexamethasone suppression test.

DISCUSSION

The purpose of our study was to determine the association of depressive symptoms in a primary care setting, which subjects not consulting for mood symptoms nor with prior story of depression, with cortisol dysregulation assessed by three different strategies: morning plasmatic cortisol, urinary cortisol and cortisol metabolites. Our results showed a significant direct correlation between Hamilton score and morning plasmatic cortisol, even when adjusting by known confounders. Despite cortisol metabolites are a sensitive measure to assess cortisol production, our study suggests that hypercortisolism and metabolic disorders are NOT observed in early/mild stage of depression.

To date, large evidence exists between HPA axis dysregulation and mood disorders. Also, there is compelling evidence that hypercortisolism has negative effects in the central nervous system, cognition and mood. For example, patients with Cushing's syndrome, have multiple psychiatric manifestations, including severe depression and anxiety (Cohen 1980; Sonino & Fava 2001), and mifepristone (glucocorticoid receptor inhibitor), has been shown to improve these symptoms (Kling *et al.* 2009). However, the wide range of results regarding cortisol and depression could be interpreted as a consequence of the differences in methodology to measure cortisol, the different stages and classifications of mood disorders and difficulties in the diagnosis and screening of major depression. For example, early studies have correlated severe depressive symptoms such as psychotic and melancholic depression with an elevated 24-hour urinary cortisol, suggesting hypercortisolism or a pseudo-cushing state (Nelson & Davis 1997). Multiple studies have also associated non suppression to dexamethasone test, suggesting impaired central feedback, with psychotic depression and other severe symptoms of mood disorders (Arana *et al.* 1985; Carroll 1968; 1982), and also worse response to treatment and more prevalence of clinical relapses (Evans & Nemeroff 1983; Ribeiro *et al.* 1993; Schatzberg *et al.* 1984). Further, others (Nemeroff *et al.* 1984) have proposed that the inappropriate cortisol suppression by dexamethasone may be a predictor of suicide. These studies suggest that the type of dysregulation of cortisol secretion and feedback may be different depending on the severity of depression.

Morning cortisol represents HPA axis activation and is related to the ability to have a dynamic response and is considered by some authors a physiologic response to different stressful situations. Higher morning cortisol have been found elevated in depression and proposed as a biomarker of impaired mental condition of healthy children (Shibuya *et al.* 2014). Elevated morning plasmatic cortisol could reflect the delay of awakening cortisol to return to normal values and/or an overactivated HPA axis. Our results support the association of morning dysregulation of HPA axis with mood symptoms in an initial clinical scenario even when adjusted by gender and age, and has been described also by other authors (Cubala & Landowski 2014). As figure 1 shows, there is direct association between depressive symptoms (assessed by Hamilton scores) and morning plasma cortisol concentrations.

On the other hand, we didn't found correlation between UFC or cortisol metabolites (a more sensitive proxy for cortisol production) and Hamilton score or severity of depression. Consistently, mild mood symptoms are not associated with metabolic or inflammatory markers as described in severe cases of depression, suggesting these findings are related to severity rather than type of measurement. Of note, Römer (Romer *et al.* 2009) reports increase in cortisol metabolites in overt depressive female inpatients, that represent a more severe phenotype in the spectrum of depression. In our group only 16% had moderate symptoms, with no truly overt cases, which probably have a lesser degree of cortisol metabolism dysregulation.

We hypothesize that a progressive spectrum of HPA axis dysregulation might be observed with mood disorders over time, and our patients (no prior depression, currently not consulting for mood symptoms, studied on non-working days) only reflect a mild stage of depression with increased morning cortisol. In a second stage of depression, where severity and chronicity may dysregulate cortisol, daily production could be increased (and not only in the morning peak), and this hypercortisolism could be confirmed with elevated UFC or cortisol metabolites**,** consistent with a pseudo Cushing state classically described in chronic depression (Pecori Giraldi 2015; Poór *et al.* 2004). Finally, a third stage could be psychotic depression that has been associated not only with hypercortisolism but also abnormal DST, suggesting the additive presence of an impaired negative feedback of ACTH (Rihmer *et al.* 1984). Figure 2 summarizes our hypothesis.

Strengths of our study are: A) Primary care subjects with no previous or current depression, with a complete endocrine evaluation decreasing the chance of selection bias or undiagnosed Cushing syndrome. B) First study to assess cortisol metabolites in non-overt cases of depression in both genders, as available studies described clinically depressed female inpatients or males (Romer *et al.* 2009; Zhai *et al.* 2015), which may represent a selection bias by gender and/or severity of depression. C) HPA axis was evaluated by three different strategies: morning plasma cortisol, urinary cortisol and cortisol metabolites by LC-MS/MS. To the best of our knowledge there are scarce studies analyzing cortisol metabolites in patients with previously undetected diagnosis of depression that are not consulting for their symptoms (thus probably representing mild or initial cases).

This study has limitations; is cross sectional and a pilot study with not an impressive sample size. Also, morning ACTH levels were not available. Prospective

studies are needed to validate our hypothesis of progression of cortisol dysregulation regarding the severity of depression, specifically using cortisol metabolites in a bigger sample size.

In summary, our data indicate a correlation between morning plasmatic cortisol and depressive symptoms in primary care subjects, without a previous or current diagnosis of depression. However, cortisol metabolites are not sensitive enough to detect early/mild cases, suggesting that hypercortisolism and metabolic disorders are not present in this stage of depression.

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