UNIVERSITY OF CALIFORNIA, SAN DIEGO

Glucocorticoid mediated regulation of inflammation in human monocytes is associated with obesity and depressive mood

A Thesis submitted in partial satisfaction of the requirements for the degree

Master of Science

in

Biology

by

Tiefu Cheng

Committee in charge:

Suzi Hong, Chair
Kathleen A. French, Co-Chair
P. A. George Fortes

2014

The Thesis of Tiefu Cheng is approved and it is accepted in quality and form for publication on microfilm and electronically:

_____

_____
Co-Chair

_____
Chair

University of California, San Diego

2014

TABLE OF CONTENTS

Signature Page…… iii

Table of Contents……... iv

List of Figures……. v

List of Tables…….. vi

Acknowledgements…… vii

Abstract…….. viii

Introduction……... 1

Materials and Methods…….. 7

Results…… 13

Discussion……... 17

Figures and Tables…….. 23

References…….. 29

LIST OF FIGURES

Figure 1: Example flow cytometry outputs measuring % TNF+ monocytes from human blood after ex vivo stimulation with LPS…….. 24

Figure 2.1: Human monocyte LSP-stimulated TNF production from ex vivo treatment with three doses of cortisol…….……... 25

Figure 2.2: Inhibition of human monocyte TNF production by ex vivo cortisol……. 26

Figure 3: Human monocyte LPS-stimulated TNF production from ex vivo treatment with three doses of cortisol and glucocorticoid and mineralocorticoid receptor antagonists.…… 28

LIST OF TABLES

Table 1: Demographic characteristics……... 23

Table 2: Multiple regression results…….. 27

ACKNOWLEDGEMENTS

I would like to thank Dr. Suzi Hong for her tireless guidance and support for all my endeavors in the laboratory. Her commitment to my success and education has taught me the meaning of mentorship. I am also grateful for the teachings of Dr. Stoyan Dimitrov and all members of the Hong Lab and others in the Psychiatry department who made my work possible.

I am also thankful for Dr. Kathy French and Dr. George Fortes for being members of my committee.

ABSTRACT OF THE THESIS

Glucocorticoid mediated regulation of inflammation in human monocytes is associated with obesity and depressive mood

by

Tiefu Cheng

Master of Science in Biology

University of California, San Diego, 2014

Suzi Hong, Chair

Kathleen A. French, Co-Chair

Dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis is most significantly studied via glucocorticoid (GC) receptor desensitization and has been associated with depression, inflammation, and also obesity, while various bidirectional links seem to exist also between these variables. To understand better and characterize

the relationship between GC desensitization and these health variables, we recruited 36 average health participants and assayed their obesity via body mass index (BMI), depressive mood via Beck Depression Inventory (BDI-Ia), plasma cortisol via ELISA, and LPS stimulated monocyte TNF production via whole blood incubation with receptor agonists and antagonists for GC. Cortisol sensitivity was characterized as change in percent monocyte TNF production from baseline to inhibited conditions. The main findings were that % TNF+ monocytes without cortisol correlated with depressive mood (BDI-S: $r= -0.336$, $p= 0.045$), as did depressive mood with cortisol sensitivity after controlling for population demographics (BDI-S: $\beta= -0.289$, $p= 0.013$). BMI also independently correlated with cortisol sensitivity (BMI: $\beta= -0.273$, $p= 0.020$). With demographics, BMI, BDI-S, % TNF+ monocytes without cortisol in the final multiple regression model, only BDI-S ($\beta= -0.215$, $p= 0.074$) and % TNF+ monocytes ($\beta= 0.546$, $p= 0.000$) still predicted cortisol sensitivity. Secondary findings with antagonists saw that relative blocking effect of mifepristone increases with increasing concentration of cortisol inhibition, while not with spironolactone. Our results find a strong relationship between the triad of HPA dysregulation, depression, and obesity through the inflammatory potential of monocytes. This preclinical sample reinforces significance of the pathophysiological triad, though require further mechanistic exploration.

INTRODUCTION

The hypothalamic-pituitary-adrenal (HPA) axis is a complex set of direct influences and feedback interactions among the hypothalamus, the pituitary gland, and the adrenal glands. The HPA axis is a major part of the neuroendocrine system that controls reactions to stress and regulates many body processes. One of the final and key hormones released by the cortex of the adrenal gland of the HPA axis are glucocorticoids (cortisol in humans and corticosterone in rodents), which exert no shortage of regulatory and developmental functions essential for life. For example, glucocorticoids (GCs) regulate mood and psychological well-being, inhibit inflammation by modulating immune cell differentiation and cytokine production, and alter the metabolism of musculoskeletal tissue. Furthermore, chronic pathophysiological elevation or depression of GC levels can lead to conditions known as Cushing's or Addison's disease respectively, though GC dysregulation is also closely related to many other forms of abnormal health. The following introduction briefly identifies pathways of GC production and signaling while highlighting the significance of the role of GC sensitivity in the context of depression, inflammation, and obesity.

Glucocorticoid Biology & Pathology

Signaling for GC production begins in the central nervous system (CNS), where the paraventricular nucleus (PVN) of the hypothalamus secretes corticotropin-releasing hormone (CRH), which enters the hypophyseal portal circulation (Bellavance 2014).

CRH then stimulates the anterior pituitary gland to release adrenocorticotropic hormone (ACTH) into the peripheral circulation, leading to synthesis and release of GCs by cells in the zona fasciculata layer of the adrenal cortex into peripheral circulation (Kadmiel 2013). Outside of direct feedback inhibition by GCs, the suprachiasmatic nucleus (SCN) of the hypothalamus largely determines the physiological range of GCs present in basal conditions via control of normal circadian rhythms (Scheiermann 2013). CNS stressors of various sources also may activate the HPA axis via excitatory neural projections to the PVN (Bellavance 2014).

Abnormal GC fluctuations are seen in diseases associated with psychosocial stress, and chronic stress exposure may lead to HPA dysregulation via altered reactivity. This is seen in clinical depression, where the literature shows evidence of increased levels of cortisol, CRH, and size and activity of pituitary and adrenal glands in depressed subjects. Also characteristic of many with depression is decreased feedback inhibition of GC production by administration of the synthetic GC receptor agonist dexamethasone (Zunszain 2012). Down-regulation or desensitization of GC receptors is thought to mediate this non-suppression phenomenon, and the test is proposed by published meta-analysis as potentially having diagnostic value (Mokhtari 2013). When 24-hour diurnal cortisol rhythm is profiled via hourly plasma sampling, an even clearer difference is seen between depressed and control subjects at every hour (Paslakis 2011). Furthermore, in examining physiological responses to stress, some have found blunted decreased cortisol and CRH levels in depressed vs control individuals after administering controlled stressors (Burke 2005). However, studies using rodents find that stress induced GC elevation does not always correspond with behaviors characteristic of depression such as

anhedonia, helplessness, and social withdrawal (Sickmann 2014). Further considerations of HPA dysregulation looks to cellular and molecular GC signaling.

Glucocorticoid receptors

The lipophilic GCs readily diffuse across cell membranes to activate cytoplasmic GC receptors (GR), though dissociation of chaperone proteins like hsp90 from the activated ligand-receptor complex is still necessary before GR translocation into the nucleus for homodimerization. The dimer acts as a transcription factor and may bind to a variety of GC response elements in promoter regions of GC-responsive genes, but undimerized receptors may also interact with coactivator molecules in the nucleus (Barnes 2010). Via single-nucleotide polymorphisms (SNP) of key co-chaperones for GR such as *FKBP5*, GR sensitivity has been found to moderate the relationship between childhood physical abuse and adult depression (Appel 2011). Epigenetic control of *FKBP5*, namely allele specific methylation, has also been found to depend on exposure to early life stressors or trauma (Klengel 2013). When comparing hippocampal tissue samples from those that experienced childhood abuse with controls, a neuron-specific GR promoter (*NR3C1*) exhibited increased cytosine methylation, which was shown in a rat model to prevent the GR transcriptional factor NGFI-A from binding (McGowan 2009). Examining genetic variants of isoforms of GR, greater expression of a less active isoform results from the ER22/23EK SNP, which has been associated with risk for depression (van Rossum 2006, Kumsta 2008). The A3669G SNP increases mRNA stability of a dominant-negative GR-β isoform, which also decreases GC sensitivity (Silverman 2012).

Role in inflammation

Considering significantly altered HPA activity in depression and immunomodulatory role of GCs, there has also been interest in the interactions between inflammatory cytokines and GR sensitivity. Effects from multiple cytokines have been found in the HPA axis at multiple levels, from GC secretion to GR translocation and post-translational modifications (Zunszain 2012). For example, the proinflammatory cytokine interleukin(IL)-1 can activate mitogen-activated protein kinase (MAPK) kinase (MKK), regulating GR phosphorylation via activation of c-Jun amino-terminal kinase (JNK) or p38 MAPKs. Tumor necrosis factor (TNF) can also promote inflammation via activation of inhibitor κ-B (Iκ-B) kinase β (IKKβ), which phosphorylates Iκ-B to translocate nuclear factor κ-B (NFκ-B). Phosphorylation of GR prevents nuclear translocation, and NFκ-B prevents GR-DNA binding (Pace 2009). Importantly with IL-1, deletion of the IL-1 receptor in mice preserves GC sensitivity compared to wildtype mice when both were exposed to social disruption stressors, meaning that IL-1 may be necessary to development of GC resistance (Engler 2008). Also, evidence of chronic stress induced concomitant increase of inflammation with GC insensitivity is seen in humans, where peripheral blood monocytes of subjects under chronic stress show reduced expression of response elements for GC but increased for NFκ-B, a key pro-inflammatory transcription factor (Hayden 2006). These and other similar evidence suggests that inflammation is part of the same process as HPA hyperactivity and resulting insensitivity.

Role in obesity

GC sensitivity is also relevant in obesity, supported by data in obese children by similar approaches to depression such as dexamethasone non-suppression (Longui 2003) or decreased GC secretion feedback sensitivity in obese men (Mattson 2009). GC elevation reliably lead to changes reflected in metabolic diseases and obesity, where various methods of GC measurement are associated with higher abdominal obesity, impaired glucose tolerance, and blood lipid levels (Bose 2009). Review articles on stress related obesity seem to indicate that chronic exposure to cortisol favors accumulation of visceral fat, though systemic cortisol elevation is not required for obesity to occur (Chapman 2013). More importantly, increased expression of 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD-1) specifically in adipose tissue increases local availability of GC by converting inert cortisone to active cortisol in humans, which respectively have low and high affinity to GC receptors (Lee 2014). This process seems to facilitate weight gain in mice models of diet induced obesity (Liu 2008, Kershaw 2005). 11β-HSD-1 has also been shown to reduce inflammation in obesity (Chapman 2013). The exact mechanism through which GC induces adipocyte expansion is still unclear, though the previously mentioned GC pathway cross talk with inflammatory cytokines is of interest.

The present work aims to further clarify the importance of GC mediated inflammation in the context of depression and obesity. A previously established ex vivo model of stimulated tumor necrosis factor (TNF) production by peripheral blood monocytes (Dimitrov 2013) is modified for use to gauge cortisol suppression sensitivity as well as inflammatory potential of myeloid cells of study participants. Due to the affinity of

cortisol for both glucocorticoid and mineralocorticoid receptors (MR), pharmacological agonist and antagonists were used to differentiate cortisol effects on each receptor.

MATERIALS AND METHODS

Participants

The study sample consisted of otherwise healthy participants with normal to mildly elevated blood pressure (BP) from an ongoing parent prehypertension study at UCSD in the local community. All participants gave written informed consent and were compensated for time and travel. The protocol for recruitment and human subject treatment was approved by the UCSD Institutional Review Board.

Initial screening of participants via telephone interviews determined the absence of several exclusion criteria: diabetes, current or recent history (past 6 months) of smoking or substance abuse, history of cardiovascular disease (e.g. symptomatic coronary or cerebral vascular disease, arrhythmia, myocardial infarction, cardiomyopathy, congestive heart failure), history of bronchospastic pulmonary disease, inflammatory disorder or health conditions affecting immune function (e.g. vaccination, active infections, use of immunomodulatory medication, uncontrolled thyroid disease), psychosis, clinical depression, and blood pressure (BP) currently > 140/90 mmHg.

Testing Procedures

Blood samples were obtained between 8am and 10am for all participants after 12-hours of fasting via catheterization of an antecubital vein and collected in vacutainers (BD, Franklin Lakes, NJ) containing either heparin or ethylenediaminetetraacetic acid (EDTA) as an anticoagulant. Cellular assay was performed on whole blood aliquots from

heparin vacutainer within 2 to 3 hours of collection, and EDTA vacutainers were kept on ice until plasma was separated and aliquoted for storage in -80°C conditions in preparation for enzyme-linked immunosorbent assay (ELISA) for plasma free cortisol level. Average basal BPs and heart rates were calculated from six measurements using a Dinamap Compact BP® monitor (Critikon, Tempa, FL). To assess obesity, standard anthropometrics (i.e. height, weight, waist and hip circumference) were collected via conventional tape ruler and scale. Subsequently, Body Mass Index (BMI) was calculated by the formula; BMI = weight in kg/(height in m)^2. Depressive mood was measured via the Beck Depression Inventory (BDI-Ia), a comprehensive and clinically robust self-report 21-item questionnaire (Smarr 2011). Each question is scored from 0-3, summarily contributing to a BDI total score (BDI-T) that is subcategorized, depending on the specific symptoms assessed by each question, into cognitive (BDI-C) and somatic (BDI-S) components.

LPS-Stimulated Intracellular Monocyte TNF Production via Flow Cytometry

200 pg/mL of lipopolysaccharide (LPS) (E. coli 0111:B4, catalog # L4391, Sigma-Aldrich, St. Louis, MO) was applied to whole blood via incubation for 3.5 hours at 37°C with 5% CO_2. Brefeldin A (10 µg/mL, Sigma-Aldrich) was added during the last 3 hours of incubation to stop cytokine exocytosis.

Resulting monocyte TNF production was measured via multiparametric flow cytometry using fluorochrome-conjugated antibodies. Flow cytometry technology

characterizes cells individually within a sample based on their fluorescence and light scattering properties. First, leukocytes were isolated via erythrocyte lysis with ammonium chloride solution, centrifugation (5-minute cycles of 500 x g), and PBS wash. Staining of surface markers for monocyte identification proceeded via 15-minute incubation with saturating concentrations of CD14/APC (Biolegend, San Diego, CA) and HLA-DR/PE (BD Biosciences, San Jose, CA). Cell fixation and permeabilization (Cytofix/Cytoperm Kit, BD Biosciences) preceded intracellular staining (30-minute incubation) with TNF/FITC (Biolegend). A dual-laser FACSCalibur (BD Biosciences) flow cytometer collected at minimum 10,000 cells per treatment condition for analysis by FlowJo software (Tree Star, Inc, Ashland, OR). Monocytes were distinguished from granulocytes and lymphocytes based on forward and side scatter characteristics as well as fluorescence indicating $CD14^{+/dim}HLA\text{-}DR^{+}$ phenotype, which allowed calculation of TNF producing monocytes ($CD14^{+/dim}HLA\text{-}DR^{+}TNF^{+}$) as a percentage ("% TNF^{+} monocytes") of total monocytes.

Cortisol and Antagonist Treatments

LPS-stimulated whole blood samples were treated with varying combinations of cortisol (catalog # H0888, Sigma-Aldrich), mifepristone (catalog # M8046, Sigma-Aldrich), and spironolactone (catalog # S3378, Sigma-Aldrich) as part of the stimulated incubation. Mifepristone and spironolactone are highly specific GR and MR antagonists respectively. Antagonists were added 15 minutes prior to addition of cortisol and LPS, which were added concurrently. Effective cortisol concentrations used were 1 µM (high-dose), 0.2 µM (moderate-dose), and 0.1 µM (low-dose), while antagonist concentrations

were 10 µM. Our pre-trial titration experiments showed that the concentration dependent nature of TNF production inhibition by cortisol disappears past 1 µM (high-dose), where no further inhibition occurred with higher concentrations of cortisol. To ensure saturation and complete block of either GR or MR, antagonists were diluted to a concentration of at least one order of magnitude greater than agonists. Combinations of either PBS or cortisol with either PBS, mifepristone, spironolactone, or both mifepristone and spironolactone were used, though antagonists were not used for samples from all participants.

Cortisol Sensitivity and Antagonist Efficacy

Cortisol sensitivity at all concentrations of stimulation was calculated with Equation 1, which finds the difference in % TNF^+ monocytes between inhibited and control conditions.

$$\Delta\ \%mono\ TNF_{Cort_x} = PBS - Cort_x \qquad (1)$$

PBS is % TNF^+ monocytes resulting from the control condition of LPS-stimulation without cortisol or antagonist treatments. *Cort* is the % TNF^+ monocytes resulting from concurrent cortisol and LPS-stimulation, and subscript *x* specifies the concentration of cortisol.

A similar index of change was used to quantify effect of antagonists, calculated with Equation 2.

$$\Delta\ \%mono\ TNF_{Blocker\&Cort_x} = Cort_x - Blocker\&Cort_x \qquad (2)$$

Blocker&Cort$_x$ indicates the % TNF$^+$ monocytes resulting from condition where antagonist was added concurrently with cortisol to block its inhibitory effects via either GR or MR.

Enzyme-Linked Immunosorbent Assay

Thawed plasma samples were assayed for concentrations of free cortisol with ELISA kit (Parameter^{TM}, R&D Systems, Minneapolis, MN). Manufacturer's protocols were followed.

Statistical Analysis

Calculations were performed using SPSS Statistical Software (v20.0) and Microsoft Excel. Descriptive data are presented as means ± SD. Normality of the data was determined by the Kolmogorov-Smirnov test. One-way ANOVA with Pairwise Comparisons were conducted to determine significant difference between groups. Linear Regression Correlations were performed by Pearson's test, and natural logarithm transformation of the data set was performed in cases where normality was absent to improve skewness and kurtosis. Multiple regression analyses were performed by controlling for other potential covariates to further determine significance of correlations found via bivariate linear regression. Multiple regression models were constructed step-wise, with general demographics (age, gender, systolic BP) being first and other predictors following as the second, and/or third steps. The results were considered

statistically significant if $P < 0.10$ due to the pilot nature of the research, and all tests were two-tailed. In case of missing data, cases were excluded pairwise.

RESULTS

Demographics

Demographics (Table 1) showed that on average, participants (n=36) were young to middle aged adults, with a high percentage of Caucasians, males, and obese individuals. When compared by BMI categories of less than 25 kg/m^2 (normal), between 25 kg/m^2 and 30 kg/m^2 (overweight), and above 30 kg/m^2 (obese), 52.8% of participants were obese. Depressive mood for this group was mostly under threshold for clinical referral (BDI-T score >11), which is expected because major depression diagnosis was an exclusion criterion. Average BP of the group fell within normotensive (<120/80 mmHg) to pre-hypertensive (>120/80 mmHg & <140/90 mmHg) range. Normality is seen in datasets of almost all variables with exception of BDI total and sub scores.

Study 1: TNF Production and Cortisol Sensitivity

Monocyte TNF production from treatment with cortisol or antagonists

Flow cytometry measurements showed that cortisol treatment of all doses significantly suppressed TNF production by monocytes, though the extent of inhibition differed between individuals regardless of the baseline TNF production levels without cortisol suppression (Figure 1).

Monocyte TNF expression was suppressed by cortisol in a reliable dose-response fashion, where the mean % TNF^+ monocytes decreased from 50.3± 12.8 % without cortisol to 44.8± 12.5 % at low-dose suppression, 37.1± 7.7 % at moderate, and 26.3±

9.1 % at high (Figure 2.1). The opposite trend was seen in cortisol sensitivity (Figure 2.2), where the mean calculated Δ %mono TNF increased from 6.0± 4.7 % at low-dose cortisol to 11.1± 8.8 % at moderate, to 24.1± 7.5 % at high.

LPS stimulated monocyte TNF production negatively correlates with depressive mood

Without the *ex vivo* cortisol treatment, % TNF^+ monocytes exhibit a negative correlation with BDI-T (r= -0.338, r^2= 0.114, p= 0.044) and BDI-S (r= -0.336, r^2= 0.113, p= 0.045) after natural log transformation of BDI scores. No correlations were found between the uninhibited % TNF production and the cognitive/affective depressive mood scores.

Calculated monocyte cortisol sensitivity negatively correlates with BMI and depressive mood

Δ %mono TNF at high and moderate dosages of cortisol inhibition correlated strongly with BDI-S (high-dose: r= -0.454, r^2= 0.206, p= 0.005; moderate: r= -0.594, r^2= 0.352, p= 0.005) and BDI-T (high-dose: r= -0.395, r^2= 0.156, p= 0.017; moderate: r= -0.421, r^2= 0.177, p= 0.056) after natural log transformation of calculated cortisol sensitivity at moderate-dose. Sensitivity also correlated to BMI at high- (r= -0.373, r^2= 0.139, p= 0.025) and low-dose cortisol (r= -0.373, r^2= 0.139, p= 0.027).

Further investigation utilized multiple regression models (Table 2) that controlled for demographic covariates (age, gender, and SBP) and physiological baselines variables, namely total cortisol and baseline % TNF+ monocytes (without cortisol). Total cortisol concentration was calculated by addition of plasma cortisol concentration with *ex vivo*

addition. The average level of plasma cortisol of the study participants was 87.3± 56.5 ng/mL (intra-assay coefficient of variability was 3.5%).

The results showed that BDI-T was independently associated with sensitivity at low-dose inhibition (β= -0.306, p= 0.096) and explained an additional 4.0% of the total variance compared to using only the covariates previously listed. BDI-S was independently associated with sensitivity at high-dose inhibition (β= -0.289, p= 0.013) and explained a greater difference of variance at 7.0%. BMI also predicted sensitivity at high-dose (β= -0.273, p= 0.020, ΔR^2= 0.063) and low-dose (β= -0.321, p= 0.085, ΔR^2= 0.085). However, when the model included both BMI and BDI-S, only BDI-S still independently predicted sensitivity at high-dose (β= -0.215, p= 0.074, ΔR^2= 0.025). Age, baseline %TNF remained significant predictors in all final models, while SBP and gender were in some.

Study 2: Pharmacologic Receptor Antagonism

Receptor antagonism prevents cortisol inhibition

Figure 3 shows various recovery of monocyte TNF production due to addition of receptor antagonists at various dosages of cortisol inhibition. Significant pairwise differences between % TNF^+ monocytes were found between cortisol and cortisol with blockers at all concentrations. Minor effects were also observed of cortisol receptor antagonists in blocking of TNF inhibition by endogenous (plasma) cortisol, though the differences were statistically insignificant. With increasing cortisol concentration, the relative antagonistic efficacy of mifepristone and spironolactone changed. Spironolactone recovers 4.0± 3.8% of % TNF^+ monocytes relative to that of just cortisol at 0.1 μM. This

figure changes to 4.9± 4.3% at 0.2 μM cortisol and 2.4± 5.1% at 1 μM cortisol. However, mifepristone recovers from 4.8± 4.2% to 12.2± 5.8% then 23.6± 7.7% as concentration of cortisol increases.

DISCUSSION

This sample of participants represent a population of middle aged adults of generally average health without major illnesses with the exception of being overweight or obese. When exposed to LPS *ex vivo*, monocytes of those with greater depressive mood produced less inflammatory cytokine TNF. In response to inhibition with added cortisol, participants' monocyte production of TNF was inhibited by cortisol in a dose dependent manner. The responsiveness of monocytes to cortisol inhibition is most diminished in more obese and more depressed individuals after controlling for demographic covariates.

TNF production has been positively correlated with depression previously, as well as production of other inflammatory cytokines such as IL-6, transforming growth factor-1, and interferon-gamma (Kim 2007). More recent literature characterizing differential monocytic response to LPS in depressed patients also suggest the usefulness of monocyte reactivity as a marker of depression (Lisi 2013). Previous reports of positive association between inflammatory cytokine production and clinical depression relied on plasma measurements, while our intracellular results showed that preclinical levels of depression correlated with decreased TNF production in monocytes after LPS stimulation. The mechanism underlying this association may be an important step in bridging the relationship between depression and cortisol sensitivity.

Via inhibition of monocyte TNF, GC sensitivity in monocytes was found to also correlate negatively to depressive mood. These results are consistent with previous

reports that depression or depressive symptoms associated with other affective psychiatric conditions reliably correlate with GC insensitivity in immune cells. For example, low sensitivity to dexamethasone suppressed T-cell proliferation has been prospectively associated with high depressive symptoms in deployed military personnel (van Zuiden 2012) and multiple sclerosis patients (Fischer 2012). However, TNF is rarely measured as an outcome variable for GC sensitivity, and no reports were found where specifically monocytic TNF was measured following cortisol inhibition. Via endogenous hormones at levels that replicate physiological values as well as relatively brief whole blood stimulation of 3 hours instead of 24 hours, our ex vivo environment highly mimic the in vivo environment of the human body while preventing unnecessarily invasive methods. Furthermore, we find that even normal individuals with heightened depressive mood experience GC desensitization at an immune modulatory level. While our correlative data cannot suggest causation, if relationship between depressive mood and LPS stimulated TNF production without additional suppression is considered, it seems likely that depressive mood is driving GC desensitization at the monocyte level. Since individuals with higher depressive mood may also experience GC desensitization due to overall overproduction of cortisol, it follows that their innate immune response, thus monocyte inflammatory potential, would be suppressed. Though plausible, the results with this particular group failed to show associations between depressive mood and plasma cortisol levels. Inter-subject variations in circadian rhythms may reduce the reliability of single time-point sampling of plasma cortisol in predicting dysregulation that were found to coincide with depression. This hypothesis is supported by an animal study that find hypersecretion of cortisol to be dependent on the stress induced

entrainment of circadian rhythm of a mild chronic stress model of depression (Christiansen 2012).

The link between GC sensitivity and obesity is not well explored specifically via immune cells, though the role of GC in development of adiposity has been reviewed at length (Hryhorczuk 2013, Lee 2014). Viewed in the context of HPA dysregulation, depression, and inflammation, obesity may also contribute as a significant source of inflammatory chemicals that drive cytokine-induced depressive symptoms. For example, in rodent model of high-fat feeding, genetic markers of neuroinflammation including IL-6, TNF, and NFκ-B were found to have upregulated in the hypothalamus, activating and promoting infiltration of microglia and astrocytes (Thaler 2012). Though depression wasn't particularly examined, other studies find that central induction of inflammatory cytokines, TNF especially, induced depressive mood or sickness behavior in rodents (Goshen 2008), while targeted deletion of TNF receptors protects against depression in stress tasks compared with controls (Simen 2006). Increased adipocytes in obesity introduce a number of cytokines (adipokines) that have inflammatory potential or may otherwise contribute to chronic, low-grade inflammation. These adipokines include adiponectin, leptin, though infiltrated macrophages may be responsible for secretion of classical cytokines like TNF and IL6 from visceral adipose (Techernof 2013). It can be seen that monocyte GC insensitivity may also contribute to increased TNF secretion potential in those with higher adiposity. Specifically for macrophages resident in adipose tissue, GC insensitivity may be part of the mechanism contributing to increased inflammatory cytokine production.

While a mechanistic understanding of the obesity, depression, and GC sensitivity mediated inflammation triad remain elusive, our final multiple regression model results indicate the relatively greater significance of depressive mood in GC sensitivity.

In examining covariates, it was found that age is significantly negatively correlated with cortisol sensitivity in monocytes, suggesting impact of senescence on HPA regulation of inflammation. Previous review article by Bauer (2005) finds that immunosenescence has been associated with changes similarly seen in chronic stress or GC treatment. For example, lymphocytes from older subjects are less sensitive to in vitro GC treatments as measured via proliferation. Overall dysregulation of HPA axis is also seen in the elderly, where dexamethasone induced suppression of ACTH and GC production is reduced (Hatzinger 2011). Gender, though less significant in the final multiple regression model, was also found to correlate with low-dose cortisol sensitivity. The direction is opposite of what was described previously in monocytes, where IL-6 and TNF production is higher and cortisol sensitivity lower in men (Wirtz 2004). As hypertension has been well observed in hypercortisolemia, blood pressure is also a known correlate with HPA dysregulation.

Due to use of a more physiologically relevant agonist, attention was given to distinguish the receptor through which cortisol exerted its anti-inflammatory effects. The significance of MR in inflammation is apparent, though the available literature shows a lack of agreement for the directionality of the relationship. GR and MR share 94% in their DNA binding domains and could be expected to perform similar immunosuppressive functions in regulating expression. Animal research shows mineralocorticoid treatment induces monocyte and macrophage adhesion in vascular,

cardiac, and kidney tissues via increased tissue expression of inflammatory cytokines such as IL-6 and MCP-1. In macrophage MR knockout mice, adhesion and infiltration still occur following mineralocorticoid administration, though inflammation-induced tissue damage is reduced (Rickard 2009). However, macrophage expression of MR is suppressed by LPS antigen challenge (Barish 2005), and MR mediated low-dose GC inhibition of macrophages attenuate immune activation (Lim 2007). Our *ex vivo* results show that blocking MR receptors via spironolactone decreased the ability of GCs to suppress monocyte TNF production. This suggests that human monocyte MRs mediate suppression of inflammatory cytokine production through various concentrations of GCs representative of physiological stress conditions, though with high levels of GCs the effect of MR immunosuppression diminishes.

While MR and GR share 57% ligand binding homology (Arriza 1987), the affinity of GC is much higher for MR than for GR. Thus, GC signal transduction occurs significantly through MR throughout normal circadian fluctuations of HPA activity, with MR saturated during circadian peaks or stressful events. However, level of GR binding is more graduated, increasing much more during stress (Zunszain 2011). It follows then to reason that effects of abnormal GC elevation may functionally result more via GR rather than MR. Chosen concentrations of GC in our ex vivo experiments simulated the extracellular environment during stress, producing results that indicate the dose-dependent importance of GR activity with increasing GC levels through the high end of the human physiological spectrum. It can be concluded that correlations found between obesity and depressive mood with sensitivity measured via high-dose cortisol

immunosuppression was likely due to greater binding with and activation of GR in monocytes.

Effect sizes for significant correlations ranged between 10-20%, indicating small associations. However, sources of uncontrollable variance are likely to reduce the measured effect size (Cohen 1988). For example, the metric used to assess depressive mood is a self-report questionnaire that rely on accuracy of self-assessment of study participants, which is subject to a degree of bias. Associations found between continuous behavioral variables and biomarkers in similar exploratory studies have been shown to also have small effect sizes with ranges similar to ours (Laake 2014, Kuebler 2013). However, use of flow cytometry to analyze nuclear factor inhibitory effects on protein synthesis for correlation with BDI score has not been performed previously. Individual differences for the intermediates of the GR and MR signaling pathways likely contributed to a greater variance in the proposed models of association.

Consideration should be given in reproducing these results in clinical populations of depression as well as further characterizing leukocyte response by measuring activity markers of different cells, such as T lymphocytes, as well as other inflammatory cytokines and chemokines. Also, blood sampling of subjects should ideally be done at multiple times during the day to address GC circadian rhythms. To analyze further the endocrine activity of visceral adipose, adipokine levels should also be measured as part of the next steps.

FIGURES AND TABLES

Table 1. *Demographic characteristics.* Values reported are in format of sample mean (standard deviation), and range where applicable. Percentages are rounded to integers.

Participants (N)	36
Sex (% Male, Female)	61, 39
Age (years)	38 (12), 20 – 59
Race (% White, African American, Asian, Other)	47, 25, 17, 6
BMI (kg/m^2)	31 (6.8), 18.8 – 45.2
BMI category (%)	22, 25, 53
BDI (score)	5.4 (6.4), 0 – 26
Systolic Blood Pressure (mm Hg)	123.9 (13.9), 98 – 152
Diastolic Blood Pressure (mm Hg)	73 (8.1), 57 – 89.2

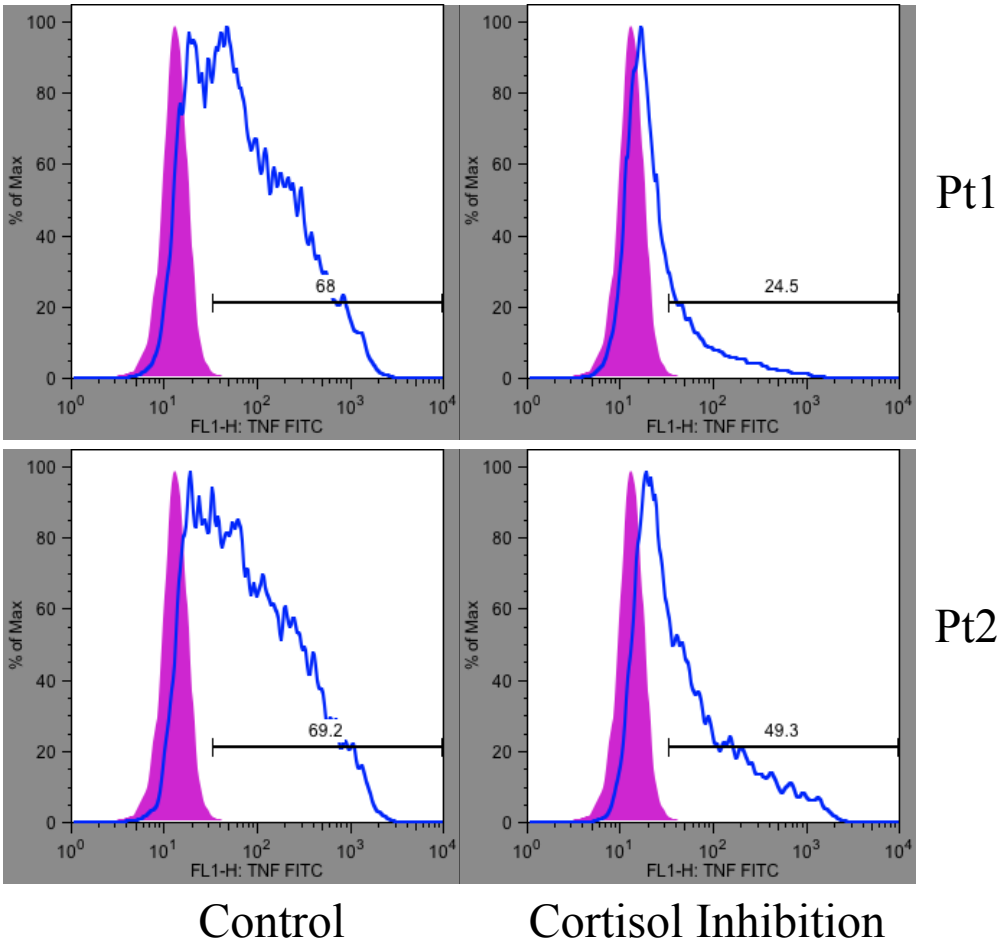

Figure 1. *Example flow cytometry outputs measuring % TNF$^+$ monocytes from human blood after ex vivo stimulation with LPS*. Monocyte count on the vertical axes is shown against fluorescence intensity of FITC-conjugated intracellular TNF antibody on the horizontal axes. Purple area indicates unstimulated control sample, while area under the blue line indicates LPS stimulated sample. The difference between area under the blue line and purple area is calculated as a percentage of total monocytes, termed % TNF$^+$ monocytes. This value for each sample is shown above each bar (e.g. 68 for panel a and 24.5 for panel b). Panels (a) and (b) are output data from 1 subject that show treatment without and with cortisol, respectively. Panels (c) and (d) are similar data from another subject.

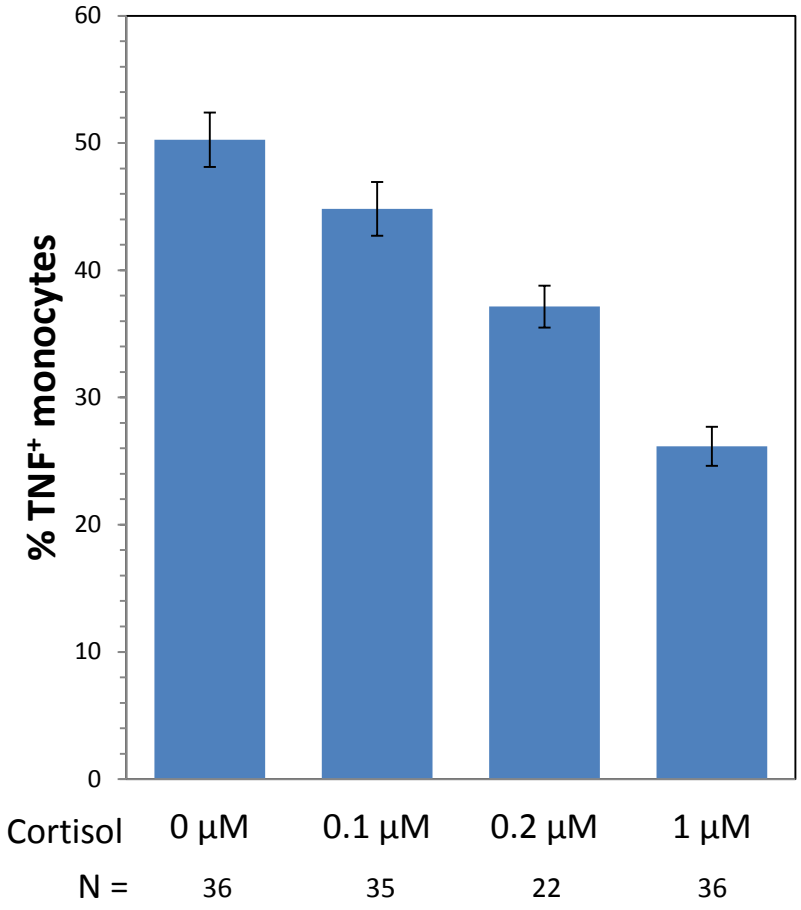

Figure 2.1. *Human monocyte LSP-stimulated TNF production from ex vivo treatment with three doses of cortisol.* Mean values are reported with standard error bars. ANOVA results indicate differences between all groups are significant at $P < 0.10$.

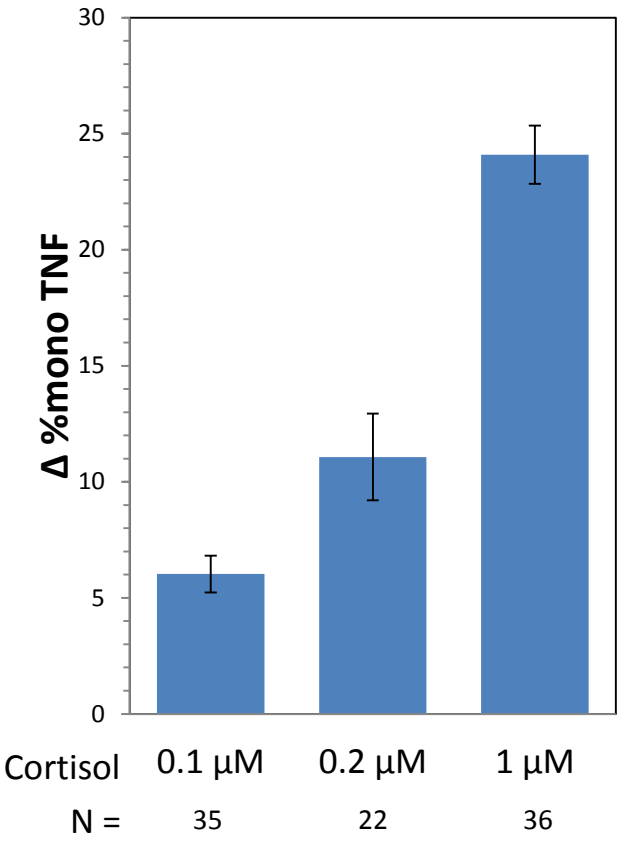

Figure 2.2. *Inhibition of human monocyte TNF production by ex vivo cortisol*. Δ %mono TNF is calculated as the difference between monocyte TNF production in control and cortisol stimulation. Mean values are reported with standard error bars. ANOVA results indicate differences between all groups are significant at $P < 0.10$.

Table 2. *Multiple regression results with Δ %mono TNF at 1 μM cortisol as Outcome Variable*. Two models were built, each having age, gender, and systolic blood pressure as the 1st step and total cortisol (plasma + *ex vivo* addition) and baseline % TNF+ monocytes (LPS only without cortisol) as the 2nd step. The first model has either obesity (1.1) or depressive index (1.2) as the 3rd step. The second model builds upon the first model, with both obesity and depressive mood in the 4th step. Statistics were performed with natural log transformed BDI values. Significance was determined at $P < 0.10$.

Model	Sig. predictors	β-coefficient	t	R^2	R^2_{adj}	ΔR^2	ΔF
1.1	BMI	-.273	-2.464	.701	.639	.063	6.070
	Age	-.308	-2.832				
	Baseline TNF	.602	5.339				
1.2	BDI-S	-.289	-2.649	.709	.649	.070	7.015
	Gender	-.190	-1.830				
	Age	-.258	-2.465				
	SBP	-.210	-1.934				
	Baseline TNF	.573	5.017				
2	BDI-S	-.215	-1.854	.734	.667	.025	2.611
	Age	-.296	-2.828				
	Baseline TNF	.546	4.861				

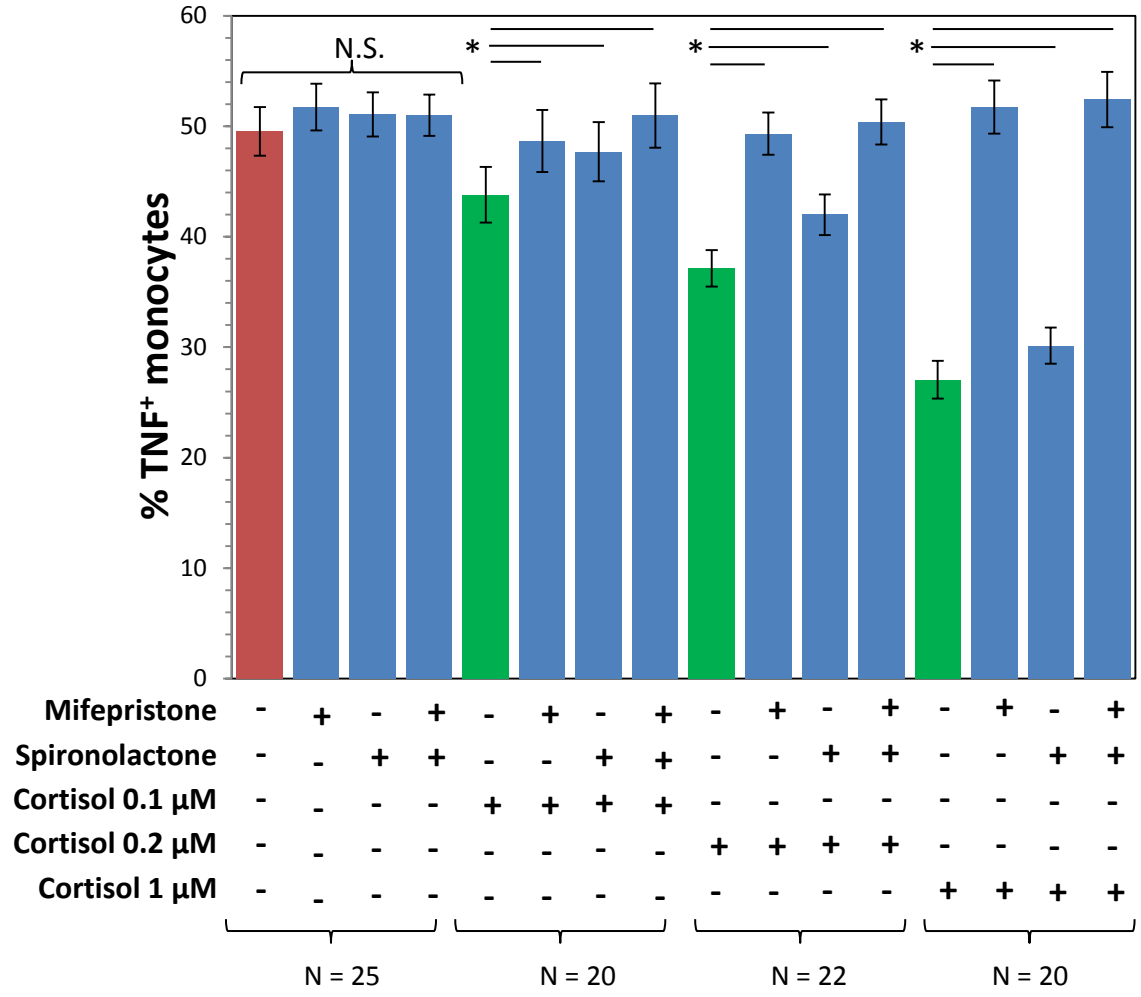

Figure 3. *Human monocyte LPS-stimulated TNF production from ex vivo treatment with three doses of cortisol and glucocorticoid and mineralocorticoid receptor antagonists.* Red bar identifies production without any ex vivo treatments. Green bars identify conditions where samples were only treated with cortisol. Blue bars identify conditions where samples contain blockers. Mean values are reported with standard error bars. Differences between cortisol groups and their respective antagonist co-treatment conditions were compared by ANOVA, and significance (*) was determined at $P < 0.10$.

REFERENCES

Appel K, Schwahn C, Mahler J, Schulz A, Spitzer C, Fenske K, Stender J, Barnow S, John U, Teumer A, Biffar R, Nauck M, Völzke H, Freyberger HJ, Grabe HJ. Moderation of adult depression by a polymorphism in the FKBP5 gene and childhood physical abuse in the general population. Neuropsychopharmacology. 2011 Sep;36(10):1982-91. doi: 10.1038/npp.2011.81. Epub 2011 Jun 8.

Arriza JL, Weinberger C, Cerelli G, Glaser TM, Handelin BL, Housman DE, Evans RM. Cloning of human mineralocorticoid receptor complementary DNA: structural and functional kinship with the glucocorticoid receptor. Science. 1987 Jul 17;237(4812):268-75.

Barish GD, Downes M, Alaynick WA, Yu RT, Ocampo CB, Bookout AL, Mangelsdorf DJ, Evans RM. A Nuclear Receptor Atlas: macrophage activation. Mol Endocrinol. 2005 Oct;19(10):2466-77. Epub 2005 Jul 28.

Barnes PJ. Mechanisms and resistance in glucocorticoid control of inflammation. J Steroid Biochem Mol Biol. 2010 May 31;120(2-3):76-85. doi: 10.1016/j.jsbmb.2010.02.018. Epub 2010 Feb 25. Review.

Bauer ME. Stress, glucocorticoids and ageing of the immune system. Stress. 2005 Mar;8(1):69-83. Review.

Bellavance MA, Rivest S. The HPA - Immune Axis and the Immunomodulatory Actions of Glucocorticoids in the Brain. Front Immunol. 2014;5:136. Review.

Bose M, Oliván B, Laferrère B. Stress and obesity: the role of the hypothalamic-pituitary-adrenal axis in metabolic disease. Curr Opin Endocrinol Diabetes Obes. 2009 Oct;16(5):340-6. doi: 10.1097/MED.0b013e32832fa137. Review.

Burke HM, Davis MC, Otte C, Mohr DC. Depression and cortisol responses to psychological stress: a meta-analysis. Psychoneuroendocrinology. 2005 Oct;30(9):846-56. Review.

Chapman K, Holmes M, Seckl J. 11β-hydroxysteroid dehydrogenases: intracellular gate-keepers of tissue glucocorticoid action. Physiol Rev. 2013 Jul;93(3):1139-206. doi: 10.1152/physrev.00020.2012. Review.

Chapman KE, Coutinho AE, Zhang Z, Kipari T, Savill JS, Seckl JR. Changing glucocorticoid action: 11β-hydroxysteroid dehydrogenase type 1 in acute and chronic inflammation. J Steroid Biochem Mol Biol. 2013 Sep;137:82-92. doi: 10.1016/j.jsbmb.2013.02.002. Epub 2013 Feb 19. Review.

Christiansen S, Bouzinova EV, Palme R, Wiborg O. Circadian activity of the hypothalamic-pituitary-adrenal axis is differentially affected in the rat chronic mild stress model of depression. Stress. 2012 Nov;15(6):647-57. doi: 10.3109/10253890.2011.654370. Epub 2012 Feb 23.

Cohen J. Statistical power analysis for the behavioral sciences. 2nd edn. Hillsdale, NJ: Lawrence Erlbaum Associates; 1988.

Dimitrov S, Shaikh F, Pruitt C, Green M, Wilson K, Beg N, Hong S. Differential TNF production by monocyte subsets under physical stress: blunted mobilization of proinflammatory monocytes in prehypertensive individuals. Brain Behav Immun. 2013 Jan;27(1):101-8. doi: 10.1016/j.bbi.2012.10.003. Epub 2012 Oct 6.

Engler H, Bailey MT, Engler A, Stiner-Jones LM, Quan N, Sheridan JF. Interleukin-1 receptor type 1-deficient mice fail to develop social stress-associated glucocorticoid resistance in the spleen. Psychoneuroendocrinology. 2008 Jan;33(1):108-17. Epub 2007 Nov 26.

Fischer A, Otte C, Krieger T, Nicholls RA, Krüger S, Ziegler KJ, Schulz KH, Heesen C, Gold SM. Decreased hydrocortisone sensitivity of T cell function in multiple sclerosis-associated major depression. Psychoneuroendocrinology. 2012 Oct;37(10):1712-8. doi: 10.1016/j.psyneuen.2012.03.001. Epub 2012 Mar 27.

Goshen I, Kreisel T, Ben-Menachem-Zidon O, Licht T, Weidenfeld J, Ben-Hur T, Yirmiya R. Brain interleukin-1 mediates chronic stress-induced depression in mice via adrenocortical activation and hippocampal neurogenesis suppression. Mol Psychiatry. 2008 Jul;13(7):717-28. Epub 2007 Aug 14.

Hamer M, Steptoe A. Cortisol responses to mental stress and incident hypertension in healthy men and women. J Clin Endocrinol Metab. 2012 Jan;97(1):E29-34. doi: 10.1210/jc.2011-2132. Epub 2011 Oct 26.

Hatzinger M, Brand S, Herzig N, Holsboer-Trachsler E. In healthy young and elderly adults, hypothalamic-pituitary-adrenocortical axis reactivity (HPA AR) varies with increasing pharmacological challenge and with age, but not with gender. J Psychiatr Res. 2011 Oct;45(10):1373-80. doi: 10.1016/j.jpsychires.2011.05.006. Epub 2011 Jun 8.

Hayden MS, West AP, Ghosh S. NF-kappaB and the immune response. Oncogene. 2006 Oct 30;25(51):6758-80. Review.

Henley DE, Lightman SL. New insights into corticosteroid-binding globulin and glucocorticoid delivery. Neuroscience. 2011 Apr 28;180:1-8. doi: 10.1016/j.neuroscience.2011.02.053. Epub 2011 Mar 1. Review.

Hryhorczuk C, Sharma S, Fulton SE. Metabolic disturbances connecting obesity and depression. Front Neurosci. 2013 Oct 7;7:177. doi: 10.3389/fnins.2013.00177. Review.

Kadmiel M, Cidlowski JA. Glucocorticoid receptor signaling in health and disease. Trends Pharmacol Sci. 2013 Sep;34(9):518-30. doi: 10.1016/j.tips.2013.07.003. Epub 2013 Aug 14. Review.

Kershaw EE, Morton NM, Dhillon H, Ramage L, Seckl JR, Flier JS. Adipocyte-specific glucocorticoid inactivation protects against diet-induced obesity. Diabetes. 2005 Apr;54(4):1023-31.

Kim YK, Na KS, Shin KH, Jung HY, Choi SH, Kim JB. Cytokine imbalance in the pathophysiology of major depressive disorder. Prog Neuropsychopharmacol Biol Psychiatry. 2007 Jun 30;31(5):1044-53. Epub 2007 Mar 13.

Klengel T, Mehta D, Anacker C, Rex-Haffner M, Pruessner JC, Pariante CM, Pace TW, Mercer KB, Mayberg HS, Bradley B, Nemeroff CB, Holsboer F, Heim CM, Ressler KJ, Rein T, Binder EB. Allele-specific FKBP5 DNA demethylation mediates gene-childhood trauma interactions. Nat Neurosci. 2013 Jan;16(1):33-41. doi: 10.1038/nn.3275. Epub 2012 Dec 2.

Kuebler U, Ehlert U, Zuccarella C, Sakai M, Stemmer A, Wirtz PH. An in vitro method to investigate the microbicidal potential of human macrophages for use in psychosomatic research. Psychosom Med. 2013 Nov-Dec;75(9):841-8. doi: 10.1097/PSY.0000000000000008. Epub 2013 Nov 1.

Kumsta R, Entringer S, Koper JW, van Rossum EF, Hellhammer DH, Wüst S. Glucocorticoid receptor gene polymorphisms and glucocorticoid sensitivity of subdermal blood vessels and leukocytes. Biol Psychol. 2008 Oct;79(2):179-84. doi: 10.1016/j.biopsycho.2008.04.007. Epub 2008 Apr 16.

Laake JP, Stahl D, Amiel SA, Petrak F, Sherwood RA, Pickup JC, Ismail K. The Association Between Depressive Symptoms and Systemic Inflammation in People With Type 2 Diabetes: Findings From the South London Diabetes Study. Diabetes Care. 2014 May 19.

Lee MJ, Pramyothin P, Karastergiou K, Fried SK. Deconstructing the roles of glucocorticoids in adipose tissue biology and the development of central obesity. Biochim Biophys Acta. 2014 Mar;1842(3):473-81. doi: 10.1016/j.bbadis.2013.05.029. Epub 2013 Jun 2. Review.

Lim HY, Müller N, Herold MJ, van den Brandt J, Reichardt HM. Glucocorticoids exert opposing effects on macrophage function dependent on their concentration. Immunology. 2007 Sep;122(1):47-53. Epub 2007 Apr 23.

Lin HY, Muller YA, Hammond GL. Molecular and structural basis of steroid hormone binding and release from corticosteroid-binding globulin. Mol Cell Endocrinol. 2010 Mar 5;316(1):3-12. doi: 10.1016/j.mce.2009.06.015. Epub 2009 Jul 28. Review.

Lisi L, Camardese G, Treglia M, Tringali G, Carrozza C, Janiri L, Dello Russo C, Navarra P. Monocytes from depressed patients display an altered pattern of response to endotoxin challenge. PLoS One. 2013;8(1):e52585. doi: 10.1371/journal.pone.0052585. Epub 2013 Jan 3.

Liu Y, Nakagawa Y, Wang Y, Liu L, Du H, Wang W, Ren X, Lutfy K, Friedman TC. Reduction of hepatic glucocorticoid receptor and hexose-6-phosphate dehydrogenase expression ameliorates diet-induced obesity and insulin resistance in mice. J Mol Endocrinol. 2008 Aug;41(2):53-64. doi: 10.1677/JME-08-0004. Epub 2008 Jun 4.

Longui CA, Giusti MM, Calliari LE, Katiki T, Kochi C, Monte O. Partial glucocorticoid resistance in obese children detected by very low dose dexamethasone suppression test. J Pediatr Endocrinol Metab. 2003 Dec;16(9):1277-82.

Mattsson C, Reynolds RM, Simonyte K, Olsson T, Walker BR. Combined receptor antagonist stimulation of the hypothalamic-pituitary-adrenal axis test identifies impaired negative feedback sensitivity to cortisol in obese men. J Clin Endocrinol Metab. 2009 Apr;94(4):1347-52. doi: 10.1210/jc.2008-2054. Epub 2009 Jan 13.

McGowan PO, Sasaki A, D'Alessio AC, Dymov S, Labonté B, Szyf M, Turecki G, Meaney MJ. Epigenetic regulation of the glucocorticoid receptor in human brain associates with childhood abuse. Nat Neurosci. 2009 Mar;12(3):342-8. doi: 10.1038/nn.2270.

Mokhtari M, Arfken C, Boutros N. The DEX/CRH test for major depression: a potentially useful diagnostic test. Psychiatry Res. 2013 Jul 30;208(2):131-9. doi: 10.1016/j.psychres.2012.09.032. Epub 2013 Jan 3. Review.

Neels JG, Olefsky JM. Inflamed fat: what starts the fire? J Clin Invest. 2006 Jan;116(1):33-5.

Pace TW, Miller AH. Cytokines and glucocorticoid receptor signaling. Relevance to major depression. Ann N Y Acad Sci. 2009 Oct;1179:86-105. doi: 10.1111/j.1749-6632.2009.04984.x. Review.

Paslakis G, Krumm B, Gilles M, Schweiger U, Heuser I, Richter I, Deuschle M. Discrimination between patients with melancholic depression and healthy controls: comparison between 24-h cortisol profiles, the DST and the Dex/CRH test. Psychoneuroendocrinology. 2011 Jun;36(5):691-8. doi: 10.1016/j.psyneuen.2010.10.002. Epub 2010 Oct 28.

Rickard AJ, Young MJ. Corticosteroid receptors, macrophages and cardiovascular disease. J Mol Endocrinol. 2009 Jun;42(6):449-59. doi: 10.1677/JME-08-0144. Epub 2009 Jan 21. Review.

Scheiermann C, Kunisaki Y, Frenette PS. Circadian control of the immune system. Nat Rev Immunol. 2013 Mar;13(3):190-8. doi: 10.1038/nri3386. Epub 2013 Feb 8. Review.

Sickmann HM, Li Y, Mørk A, Sanchez C, Gulinello M. Does Stress Elicit Depression? Evidence From Clinical and Preclinical Studies. Curr Top Behav Neurosci. 2014 Mar 15. [Epub ahead of print]

Silverman MN, Sternberg EM. Glucocorticoid regulation of inflammation and its functional correlates: from HPA axis to glucocorticoid receptor dysfunction. Ann N Y Acad Sci. 2012 Jul;1261:55-63. doi: 10.1111/j.1749-6632.2012.06633.x. Review.

Simen BB, Duman CH, Simen AA, Duman RS. TNFalpha signaling in depression and anxiety: behavioral consequences of individual receptor targeting. Biol Psychiatry. 2006 May 1;59(9):775-85. Epub 2006 Feb 3.

Smarr KL, Keefer AL. Measures of depression and depressive symptoms: Beck Depression Inventory-II (BDI-II), Center for Epidemiologic Studies Depression Scale (CES-D), Geriatric Depression Scale (GDS), Hospital Anxiety and Depression Scale (HADS), and Patient Health Questionnaire-9 (PHQ-9). Arthritis Care Res (Hoboken). 2011 Nov;63 Suppl 11:S454-66. doi: 10.1002/acr.20556. Review.

Tchernof A, Després JP. Pathophysiology of human visceral obesity: an update. Physiol Rev. 2013 Jan;93(1):359-404. doi: 10.1152/physrev.00033.2011. Review.

Thaler JP, Yi CX, Schur EA, Guyenet SJ, Hwang BH, Dietrich MO, Zhao X, Sarruf DA, Izgur V, Maravilla KR, Nguyen HT, Fischer JD, Matsen ME, Wisse BE, Morton GJ, Horvath TL, Baskin DG, Tschöp MH, Schwartz MW. Obesity is associated with hypothalamic injury in rodents and humans. J Clin Invest. 2012 Jan 3;122(1):153-62. doi: 10.1172/JCI59660. Epub 2011 Dec 27. Erratum in: J Clin Invest. 2012 Feb 1;122(2):778.

van Rossum EF, Binder EB, Majer M, Koper JW, Ising M, Modell S, Salyakina D, Lamberts SW, Holsboer F. Polymorphisms of the glucocorticoid receptor gene and major depression. Biol Psychiatry. 2006 Apr 15;59(8):681-8.

van Zuiden M, Heijnen CJ, Maas M, Amarouchi K, Vermetten E, Geuze E, Kavelaars A. Glucocorticoid sensitivity of leukocytes predicts PTSD, depressive and fatigue symptoms after military deployment: A prospective study. Psychoneuroendocrinology. 2012 Nov;37(11):1822-36. doi: 10.1016/j.psyneuen.2012.03.018. Epub 2012 Apr 12.

Weaver IC, Cervoni N, Champagne FA, D'Alessio AC, Sharma S, Seckl JR, Dymov S, Szyf M, Meaney MJ. Epigenetic programming by maternal behavior. Nat Neurosci. 2004 Aug;7(8):847-54. Epub 2004 Jun 27.

Wirtz PH, von Känel R, Rohleder N, Fischer JE. Monocyte proinflammatory cytokine release is higher and glucocorticoid sensitivity is lower in middle aged men than in women independent of cardiovascular risk factors. Heart. 2004 Aug;90(8):853-8.

Zunszain PA, Anacker C, Cattaneo A, Carvalho LA, Pariante CM. Glucocorticoids, cytokines and brain abnormalities in depression. Prog Neuropsychopharmacol Biol Psychiatry. 2011 Apr 29;35(3):722-9. doi: 10.1016/j.pnpbp.2010.04.011. Epub 2010 Apr 18. Review.