Published in final edited form as:

Health Psychol. 2017 September ; 36(9): 852–862. doi:10.1037/hea0000517.

The Cortisol:C-reactive protein ratio and negative affect reactivity in depressed adults

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Abstract

Objective—We evaluated the effect of the cortisol (CORT) to high sensitivity C-reactive protein (hsCRP) ratio on stress-induced negative affect (NA) reactivity and whether the association was moderated by depressive symptom severity and gender. The CORT/CRP ratio was used to evaluate the integrity of the negative feedback loop between the hypothalamic-pituitary-adrenal (HPA) axis and inflammatory response system (IRS).

Methods—Basal CORT and hsCRP levels were measured in fasting blood samples from 198 medication-free and nonsmoking healthy men and women. Depressive symptom severity was assessed using the Hamilton Depression (HAMD) scale. NA ratings were collected at baseline and at the completion of the laboratory stressors, the Anger Recall Interview (ARI) and reading.

Results—Adjusting for potential confounders and baseline NA, analysis revealed a significant relationship between CORT/CRP ratio and NA reactivity to ARI as a function of depressive symptom severity. Simple effects revealed that for participants with high HAMD, decreasing CORT/CRP ratio, suggestive of an insufficient CORT release relative to higher hsCRP, predicted increasing stress-induced NA reactivity. For subjects with low HAMD, the CORT/CRP ratio failed to predict NA reactivity. Gender did not moderate the joint effect of depressive symptom severity and the CORT/CRP ratio on stress-induced NA reactivity.

Conclusions—This is the first study to document that a premorbid dysregulation of the neuro-immune relationship, characterized by an insufficient release of CORT in conjunction with higher CRP, plays a role in stress sensitivity, and specifically NA reactivity, in individuals with elevated levels of depression symptoms.

Keywords

Negative affect; Stress sensitivity; Depressive symptom severity; CORT/CRP ratio; Inflammation; Regulatory feedback mechanism; Cortisol; C-reactive protein

Meta-analytic studies have suggested that MDD, and to a lesser extent depressive symptom severity, are significantly associated with elevated basal levels of CRP, interleukin (IL)-6,

and IL-1 (Howren, Lamkin, & Suls, 2009; Valkanova, Ebmeier, & Allan, 2013). Depressive symptom severity and MDD have also been independently associated with HPA axis dysregulation with depressed individuals exhibiting heightened levels of CORT and adrenocorticotrophic hormone (ACTH) and flatter diurnal CORT (e.g., Knight, Avery, Janssen, & Powell, 2010; Knorr, Vinberg, Kessing, & Wetterslev, 2010; Stetler & Miller, 2011). Although a cause-and-effect has not been established, observational studies have reported a two-fold increase in the risk of MDD among a subgroup of patients treated with synthetic corticosteroids (Fardet, Petersen, & Nazareth, 2012). Similarly, patients with a history of MDD and elevated depressive symptom severity exhibit an increased risk for developing symptoms of depression during cytokine therapies (Udina et al., 2014). While the wealth of evidence for the individual contributions of each system to the etiology of MDD is notable, recent studies have suggested that MDD, and specifically depressive phenotypes, and sex-specific vulnerability to depression may best be characterized by a disorganization of inter-related systems, in this case the relationship between the HPA axis and the immune response system (IRS) (Gold & Chrousos, 2002; Lamers et al., 2013; Suarez, Sundry, & Erkanli, 2014).

Emerging evidence has suggested that depressive subtypes and vulnerabilities to depression are associated with distinct patterns of dysregulation between the HPA axis and immune response system (IRS) (Lamers, et al., 2013; Suarez, et al., 2014) and between the HPA axis and autonomic nervous system (ANS) (Laurent, Lucas, Pierce, Goetz, & Granger, 2016). Although different markers of system activities have been employed to evaluate individual system activities, the general conclusion derived by these study is that a disorganization between inter-related systems provides a more complete picture of underlying pathophysiology associated with MDD and vulnerability than dysregulation of any single system (Schumacher, Kirschbaum, Fydrich, & Strohle, 2013). Implementing a multisystem approach, one recent study explored the biological features of MDD in two clinical subtypes differentiated by the presence or absence of melancholic features. Melancholic features include anhedonia, physical agitation, insomnia or decreased appetite (American Psychiatric Association, 2013). Using two classifications, MDD with and with melancholic features, Kaestner et al. (2005) observed that patients admitted to a clinical unit with melancholia exhibited higher levels of CORT and ACTH but lower levels of proinflammatory cytokines relative to non-melancholic patients. Similarly, differences in patterns of neuro-immune activations have been reported in studies that compared atypical depression to melancholia (Gold, 2015; Gold & Chrousos, 2002, 2013; Lamers, et al., 2013). Atypical depression is a depressive subtype that is distinguished by mood reactivity with improvements in mood often preceded by positive events and mood deterioration associated with an intense reaction or increased sensitivity to rejection (American Psychiatric Association, 2013). Comparing these two depressive subtypes, Lamers et al (2013) observed that relative to atypical depression, melancholia was associated with higher CORT level at awakening and lower CRP whereas atypical depression was associated with a contrasting pattern of higher CRP and lower CORT levels (Lamers, et al., 2013). Although few in number, the combine results of those studies suggest that patterns of neuro-immune activities differ as a function of depressive subtypes and specifically atypical depression and melancholia.

Adopting a similar multisystem approach, we investigated whether vulnerability to depression, as indexed by trait and state factors, was also associated with distinct patterns of neuro-immune activities and whether gender influenced the associations (Suarez, et al., 2014). It is recognized that MDD affects women twice as often as men and such marked differences could potentially be associated with distinct patterns of neuro-immune activities. Using the CORT/CRP ratio, we observed vulnerability to depression was associated with gender specific and distinct patterns of neuro-immune dysregulation. For women, greater vulnerability was associated with higher hsCRP and lower CORT resulting in lower ratio values. In contrast, for men vulnerability was associated with higher CORT, lower CRP and higher ratio values. Interestingly, the pattern of neuro-immune activity (e.g., high CRP and low CORT) associated with vulnerability in women is consistent with that found for atypical depression. It has been reported that women are at greater risk not only for major depression but also atypical depression (Schuch, Roest, Nolen, Penninx, & de Jonge, 2014). While our findings require replication, the results suggests that distinct patterns of neuro-immune perturbations associated with sex-specific vulnerability are the same as those observed for depressive subtypes suggesting that the observed patterns of dysregulation may precede the onset of major depression.

In addition to difference in the etiologic and pathophysiologic mechanisms of MDD, emerging evidence also suggests that stress may further dysregulate neuro-immune functions. Stress sensitivity, and specifically stress-induced negative affect (NA) responses, has been associated with increased risk of MDD especially among individuals who exhibit genetic predispositions (Wichers et al., 2007). Although there is a lack of evidence for the relation of premorbid neuro-immune dysregulation to stress sensitivity, emerging evidence has suggested that stress evokes distinct patterns of CRP and CORT responses in patients with MDD versus relative to asymptomatic adults. Specifically, Miller et al. (2005) showed that exposure to acute laboratory stressors evoked synchronized *increases* in both CORT and hsCRP in non-depressed women whereas in clinically depressed women stress evoked *increases* in CRP with concomitant *decreases* in CORT. Differences in CORT and CRP responses to stress have also been reported in studies that compared cardiac patients to healthy controls. Relative to healthy controls, patients with coronary heart disease showed *increases* in CRP in tandem with *blunted* CORT in response to stress (Nijm, Kristenson, Olsson, & Jonasson, 2007). In another study of coronary patients, those patients who were depressed responded to stress with larger CRP increases and smaller CORT responses relative to patients who were not depressed (Taylor et al., 2006). Although preliminary, the combined evidence suggests that, in patient populations, stress evokes discordant CORT and CRP responses with coordinated neuroendocrine-immune reactivity observed in healthy adults and those without a diagnosis of depression. What is not understood is whether the interplay between the HPA axis and immune system predicts stress-induced negative affect responses implicated in the etiology of MDD (Gulley, Hankin, & Young, 2016).

In the current study we employed the ratio of CORT to hsCRP as an estimate of the status of the regulatory feedback between the HPA and IRS (Suarez, et al., 2014). The CORT/CRP ratio has been used in studies of patients with rheumatologic conditions characterized by heightened inflammation and hypoactivity of the HPA axis (Demir et al., 2006; Narvaez et al., 2006). In extending the use of the CORT/CRP ratio to biobehavioral research we have

shown that the full distribution of CORT/CRP values captures two distinct patterns of neuro-immune dysregulation (Suarez, et al., 2014). Higher ratio values are the result of higher CORT levels relative to lower CRP. Lower ratio values are suggestive of an inadequate release of CORT relative to higher levels of CRP. By interpreting high and low ratio values in this manner (see Figure 1) we proposed that mid-range ratio values are suggestive of coordinate activities or homeostasis (Suarez, et al., 2014). Although more complex strategies, such as tests of modeling statistical interactions, are useful when evaluating multi-system activities, the CORT/CRP ratio is unique in that it yields a single value with each end of the distribution capturing distinct and unique patterns of neuro-immune dysregulation and mid-range values indicative of homeostasis.

The combined evidence suggests that differential patterns of neuro-immune dysregulation are associated not only with depressive subtypes (Lamers, et al., 2013) but also with gender-specific vulnerabilities to depression (Suarez, et al., 2014). Moreover, among patient populations, and specifically individuals with clinical depression, cardiovascular disease or both, stress appears to further amplify the dysregulation of the feedback loop as suggested by desynchronized CORT and CRP responses to psychological challenges. What remains unknown is whether neuro-immune dysregulation, as indexed by the CORT/CRP ratio, also predicts emotional responses to stress and specifically negative affect (NA) reactivity. Given evidence suggesting that inflammation influences mood states most notably among those with a history of depression or elevated depressive symptom severity (Loftis et al., 2013), we hypothesized that low CORT/CRP ratio values, suggestive of a pro-inflammatory state accompanied by insufficient CORT release, would be predictive of greater NA reactivity but only among individuals with higher levels of depressive symptom severity. Given additional evidence suggesting sex differences in the relation of inflammation and CORT to depression (Hiles et al., 2015; Moieni et al., 2015; Suarez, et al., 2014) and risk of depression (Owens et al., 2014), we also examined whether gender, in conjunction with depressive symptom severity, moderated the hypothesized association between the CORT/CRP ratio and stress-induced NA reactivity.

Method

Participants

Study sample included 198 (aged 18–65 yr., 45% women) adults recruited from the surrounding communities. Subjects were recruited to participate in a study of the relation of stress to various health measures. Individual were enrolled if they were non-smokers and reported being in ‘good health’, defined as negative history of medical and psychiatric illnesses, no acute medical conditions or injuries within the past 2-weeks, and no current or recent (< 1 month) use of medications, whether over-the-counter or prescribed. Individuals were excluded if they reported use of low-dose aspirin on a regular basis and, for women, use of exogenous hormones (e.g., oral contraceptives, hormone replacement therapy) during the previous 6 months. Women suspected of being perimenopausal (i.e., irregular menstrual cycle during the previous 6 months) were also excluded. Men were excluded if treated with androgen therapy during the 6 months prior to study participation. For premenopausal women, laboratory studies were conducted during the follicular phase (days 5–10) of the

menstrual cycle, a period when estrogen levels are at their lowest. Conducting laboratory studies during the follicular phase of the menstrual cycle minimized the influences of ovarian hormones, and specifically estrogen, which have been shown to stimulate corticotropin-releasing hormone (Vamvakopoulos & Chrousos, 1993). The Institutional Review Board (IRB) of Duke Medicine approved this study and appropriate informed consent was obtained from participants prior to data collection. Subjects were compensated for their participation.

Laboratory Stressors

Two laboratory stressors were used in the study. The Anger Recall Interview (ARI) is an acute psychological stressor with high ecological validity due to its reliance on the subject's own stressful experiences. Recalling stressful life events, even after some period of time has passed, has been shown to evoke comparable psychophysiological responses to those that occurred during actual events (Glynn, Christenfeld, & Gerin, 2007). As important, and relevant to the current study, evidence suggests that for persons with elevated levels of depressive symptoms, recalling negative life events is enhanced by rumination, a cognitive process associated with depression and neuroticism (Nolen-Hoeksema, Wisco, & Lyubomirsky, 2008). Evidence has also suggested that individuals who are depressed have difficulties in removing negative thoughts and memories from working memory even after those memories are no longer relevant (Foland-Ross et al., 2013). Although other stressors have been used in studies of stress sensitivity and NA, we have shown that the ARI is an effective acute stressor that evokes significant arousal of NA (Suarez, Saab, Llabre, Kuhn, & Zimmerman, 2004). Its relevance to depression is further supported by studies suggesting that, relative to adults with low depressive symptom severity, adults with elevated symptoms of depression are characterized by more hostile and confrontative coping and greater vulnerability to daily life stressful encounters (Folkman & Lazarus, 1986).

Instructions to the ARI required subjects to recall a stressful life event that recently had occurred (4 months) and specifically one that evoked anger when it occurred and "when thinking about it today" continued to arouse anger (Ironson et al., 1992). After a 1-min period for preparation and visualization, participants verbally described the event for approximately a 4-min period. The experimenters used prompts such as "How did that make you feel when that happened?" to engage participants during the recall period.

Subjects also participated in a neutral reading task. We have shown that reading alone increases blood pressure and heart rate even when individuals report minimal emotional arousal (Suarez, et al., 2004). Participants were instructed to read aloud a passage about the ocean using "a normal tone of voice and normal rate of speech." Participants were told that they would not be evaluated for either reading style or comprehension. Using a 10-point Likert scale [1=easy, 10=difficult, participants rated the text as easy to read [Mean = 1.8, SD= 0.8].

The timeline for the laboratory visit is as follows: two 5-min baseline periods during which subjects rested quietly, a 5-min ARI and a 5-min neutral reading task counterbalanced, followed by a 15-min recovery.

Negative Affect Ratings

Participants rated intensity of NA at the end of the two 5-min baseline periods, 5-min reading, and 5-min ARI. Using a 10-point Likert scale with 1 representing “not at all” and 10 representing “extreme or very strong feelings” (Orr, Pitman, Lasko, & Herz, 1993; Pitman et al., 1990; Suarez, Harlan, Peoples, & Williams, 1993; Suarez & Williams, 1990), subjects were asked to rate their level of anger, anxiety, depression, frustration, irritation, nervousness, sadness, and upset. Individual ratings were summed to yield a total NA score (range: 8 – 80). The total NA score was used in all analysis. Using this assessment tool, we have observed significant associations between arousal of NA during the ARI and stress-induced increases in blood pressure, catecholamines, and inflammatory biomarkers (Suarez, Boyle, Lewis, Hall, & Young, 2006; Suarez, Kuhn, Schanberg, Williams, & Zimmerman, 1998).

High sensitivity C-reactive protein and Cortisol

Blood samples for CORT and hsCRP were taken sequentially over the two 5-min baseline periods. Samples for all subjects were collected between 0830 and 0930. C-reactive protein is a sensitive systemic marker of inflammation that is produced by the liver following stimulation by interleukin (IL)-6 (Ockene et al., 2001). In contrast to other biomarkers of inflammation, CRP level is relatively stable over time, has no diurnal fluctuations, and is easily measured using commercially available high-sensitivity assays. In the current study, high sensitivity CRP was assessed in duplicate using an ultrasensitive, enzyme-linked, immunometric latex-enhanced assay (Diagnostic Products Corporation, Los Angeles, CA) using purified protein and polyclonal anti-CRP antibodies from Diagnostic Products Corporation (Los Angeles, CA). This system has a lower detection threshold of < 0.10 mg/L with coefficients of variation ranging from 6.6% to 9.3%. For healthy individuals, a single measure of hsCRP has been shown to have high reliability (Navarro et al., 2012). For the study sample, the median hsCRP was 0.72 mg/dl (interquartile (IQ) range: 0.28 – 1.65).

Assessment of CORT was accomplished using a commercially available radioimmunoassay kit (Radioassay System Laboratories, Carson City, CA). To determine the relation of time to CORT levels we conducted correlational analysis. The correlation was not significant (Suarez, et al., 2014). The resulting CORT values from the two samples were averaged and mean CORT level was used to calculate the ratio. For the study sample, mean baseline CORT was 14.5 µg/dl (SD = 5.8) values consistent with reference ranges for morning serum CORT. The intra- and inter-assay coefficients of the assay were 5.3% and 8.4%, respectively.

Depressive Symptom Severity

The Hamilton Depression (HAMD) rating scale was used to assess severity of depressive symptoms (Williams, 1988). The HAMD is composed of 21-items of which the first 17-items are used to calculate a total score (Brown, Shulberg, & Madonia, 1995; Rehm & O'Hara, 1985). The total HAMD score reflects depressive symptom severity (Williams, 2001) and it is significantly correlated with the total score on the Beck Depression Inventory (Beck, Steer, & Garbin, 1988) as well as other self-report scales used to assess depressive symptom severity (Aben, Verhey, Lousberg, Lodder, & Honig, 2002). The HAMD was administered by members of the research staff trained in administering the Structure

Interview HAMD. For the sample, the median HAMD score was 4 (IQ range: 1 – 9) with a range of 0 to 17. Although the HAMD total score was not used to determine categories of symptom severity, the following has been used to guide severity classification: no depression (0–7), mild depression (8–16), moderate depression (17–23), and severe depression (> 24) (Furukawa et al., 2002; Zimmerman, Martinez, Young, Chelminski, & Dalrymple, 2013). Using these *a priori* criteria, approximately 20% of the sample exhibited mild to moderate depression. No subject met HAMD criteria for severe depression.

Inter-rater reliability for the HAMD total scores was calculated using a randomly selected subset of interviews (n = 20). The inter-rater reliability coefficient was 0.93 for the total score which is consistent with previously reported reliability coefficients between raters (Morris, Leese, Chatwin, & Baldwin, 2008).

Demographic and Anthropomorphic Variables

Age, level of education and race/ethnicity were ascertained via self-report. Body mass index was calculated from height (cm) and weight (kg) measured on the day of the laboratory visit.

Analysis

Statistical analyses were performed with SAS 9.3 (Research Triangle Park, NC, 27710). Due to skewness of the distributions, the HAMD score was log-transformed. Following the recommendations of Sollberger and Ehlert (2016), CORT/CRP ratio values were log-transformed. To examine the relationship between CORT and CRP, regression analysis was performed to determine if the intercept of the regression line went through the origin (Sollberger & Ehlert, 2016). Results revealed that the relationship between log(hsCRP) and log(CORT) was a straight line with the intercept going through the origin (intercept: $\beta = -0.24$, $SE = 0.94$, $t = -0.26$, ns). In this study the ratio was not used to control for the effect of the denominator, in this case CRP, but as a metric to characterize the relative proportion between CORT and CRP levels allowing inferences to be made regarding the integrity of the neuro-immune feedback loop between the HPA axis and IRS. Additional details regarding the ratio and corresponding levels of CORT and CRP associated with high and low values can be found in Suarez et al. (Suarez, et al., 2014).

The proposed hypothesis, that the CORT/CRP ratio would predict arousal of NA as a function of depressive symptom severity, was tested using the interaction between log-transformed CORT/CRP and HAMD in a multiple regression model that included age, race, educational status, and baseline NA score as covariates. Age, race, and educational status were used as covariates to control for possible confounding of these variables on the relationship between NA reactivity and the components of the ratio (Friedman & Herd, 2010; Khera et al., 2005; Woloshin & Schwartz 2005). We also performed parallel analysis testing the 3-way interaction between CORT $\times$ CRP $\times$ HAMD. Results of these analyses and accompanying figure are provided in the Supplemental Material. Effect size was calculated using partial eta squared, η^2_p (Richardson, 2011). The following values were used to characterize small, medium, and large effect sizes: 0.0099, 0.0588, and 0.1379, respectively (Richardson, 2011).

As recommended by Aiken and West (1991), we probed the significant two-way interaction using simple slope with centered covariate. A simple effect (e.g., coefficient of the slope) is the effect of one factor conditioned on the level of a second factor. In this study, we examined the effect of CORT/CRP on NA reactivity at ± 1 SD of HAMD.

Results

Of 198 subjects, two subjects were excluded from the analysis due to hemolysis of blood samples. Therefore, results are based on 196 subjects. Inspection of raw HAMD total score using non-parametric statistics failed to show gender differences (see Table 1). Preliminary analysis of NA scores revealed a significant main effect for experimental task ($F(2, 390) = 610.18, p < .0001, \eta^2_p = .88$) with ARI evoking significant increases in the arousal of NA (see Table 1)

Adjusting for baseline NA and age, comparison between men and women on NA reactivity during the ARI and reading revealed a marginally significant gender effect ($F(1, 193) = 3.34, p < 0.07, \eta^2_p = 0.02$) with women responding to the ARI with slightly greater arousal of NA ($M = 40.9, SE = 1.4$) relative to men ($M = 37.3, SE = 1.3$). No gender differences ($F(1, 197) = 0.00, ns$) were observed for NA response to reading.

Main Effect of Depressive Symptom Severity on Negative Affect at Baseline and in Response to Stress

We first conducted analysis to examine the main effect of HAMD on NA at baseline and in response to reading and the ARI. Baseline NA was not associated with HAMD ($r = 0.08, ns$). Similarly, NA responsivity to the reading task was not associated with HAMD ($r = 0.03, ns$). As anticipated, NA reactivity to ARI was associated with HAMD ($r = 0.24, p < .001$) with higher HAMD scores associated with greater NA reactivity. In multivariate regression analysis adjusting for sex, age, race, educational status, and baseline NA, HAMD ($\beta = 3.24, [95\% \text{ CI}: 0.33 \text{ to } 20.67], F = 6.62, p = .01, \eta^2_p = 0.04$) significantly predicted NA reactivity to the ARI.

CORT/CRP and Negative Affect Reactivity: Test of moderation by Depressive Symptom Severity

We conducted a multivariate analysis with HAMD score, the CORT/CRP ratio and the CORT/CRP by HAMD interaction as predictors and age, sex, race, educational level and baseline NA as covariates. The CORT/CRP ratio by HAMD interaction was significant ($\beta = -2.16, [95\% \text{ CI}: -0.10 \text{ to } -4.15], F = 4.31, p = .038, \eta^2_p = 0.02$) in predicting NA reactivity to the ARI (see Online Supplement for results of complementary analysis of test of 3-way interaction between $\text{CORT} \times \text{CRP} \times \text{HAMD}$).

To probe the interaction we performed test of simple effects. As illustrated in Figure 2, at 1 SD above the group mean for HAMD, the coefficient of the regression line was significant ($\beta = -2.65, t = -1.99, p < 0.05$) suggesting that as the value of the CORT/CRP ratio decreased the intensity of NA responses increased. In contrast, the coefficient of the regression line at HAMD -1 SD below the group mean was not significant ($\beta = 0.75, t =$

0.65, ns) suggesting that the CORT/CRP ratio did not predict NA reactivity to ARI for subjects with low HAMD scores.

Tests of potential gender differences in the relation of the CORT/CRP Ratio to Negative Affect Response as a function of Depressive Symptom Severity

We have shown that the relationship between vulnerability to depression and CORT/CRP ratio differed as a function of gender (Suarez, et al., 2014). Others have suggested that inflammation has a greater influence on behavioral and mood responses in females than in males (Tonelli, Holmes, & Postolache, 2008). Given these two independent lines of evidence, we examined whether gender further moderated the prediction of NA reactivity by log(CORT/CRP) by evaluating the 3-way interaction between HAMD, CORT/CRP ratio, and gender. Using the same set of covariates as in the primary analysis, the 3-way interaction was not significant ($\beta = 0.19$, [95% CI: -3.97 to 4.36], $F = 0.01$, ns) suggesting that the CORT/CRP ratio predicted stress-induced NA reactivity to the ARI in a similar manner in men and women.

Exploratory Analysis: Is the CORT/CRP ratio a proxy for adiposity?

It has been suggested that obesity-related inflammation promotes emotional distress and behavioral symptoms (Capuron et al., 2011; Daly, 2013). To examine if our primary findings were due, in part, to the effects of adiposity, we performed univariate analysis and multiple linear regression. Consistent with current understandings of obesity as an inflammatory condition (Das, 2001), BMI was significantly correlated with the CORT/CRP ratio ($r = -0.46$, $p < .0001$) with higher BMI associated with lower CORT/CRP. We then examined if the interaction between CORT/CRP ratio and HAMD remained significant in a model that included the BMI by HAMD interaction. The same set of covariates as in the primary analysis was used. In these analysis, the CORT/CRP by HAMD interaction remained significant ($\beta = -2.41$, [95% CI: -4.65 to -0.17], $F = 4.57$, $p = .003$, $\eta^2_p = 0.02$) whereas the interaction between BMI and HAMD was not significant ($\beta = -0.25$ [95% CI: -0.84 to 0.35], $F = 0.66$, ns) suggesting that the effect of the CORT/CRP ratio on stress-reactivity in subjects with elevated symptoms of depression was independent of BMI.

Discussion

Differences in vulnerability to depression are thought to involve complex interactions among predisposing factors that include experience and chronicity of stress, early life stress, personality, genetic vulnerability, and biological perturbations across multiple systems. Although neurobiologic precursors of MDD have not been well elucidated, dysregulation of the HPA axis and IRS have been independently implicated (Gold, 2015). We recently proposed that vulnerability to depression may involve dysregulation of feedback mechanisms critical to maintaining homeostasis between the HPA axis and IRS (Suarez, et al., 2014). In further testing this general hypothesis, the current study examined the association between the CORT/CRP ratio and stress-induced NA reactivity, an intermediate phenotype of MDD, and whether this association was moderated by depressive symptom severity and gender. As hypothesized, the CORT/CRP ratio significantly predicted stress-induced NA responses to the ARI as function of depressive symptom severity. In participants with higher levels of depressive symptoms, decreasing CORT/CRP ratio value predicted

increasing stress-induced NA reactivity to the ARI. For individuals with minimal to no symptoms of depression, the CORT/CRP ratio failed to predict NA responses to the ARI. The results were independent of age, race, and educational status as well as baseline NA and gender. Moreover, BMI did not significantly alter the relationship between CORT/CRP ratio and NA reactivity. Thus, only in the subset of participants with higher HAMD scores was lower CORT/CRP values, suggestive of a heightened inflammatory state coupled with an insufficient glucocorticoid release, predictive of NA hyperreactivity to the ARI. To our knowledge, these data are the first to suggest that preexisting levels of depressive symptomatology and perturbation of the negative feedback loop between the HPA axis and IRS combine to predict stress-induced NA hyperreactivity thought to play a role in the etiology of MDD (Parrish, Cohen, & Laijrenceaij, 2011; Wichers et al., 2009).

The present study adds to an emerging body of evidence suggesting that distinct patterns of physiological disruptions among inter-related stress systems are associated not only with MDD subtypes (Lamers, et al., 2013) but also with vulnerability to depression in a sex-dependent manner (Suarez, et al., 2014). A wealth of evidence suggests that inflammation and dysregulation of HPA axis are independently associated with MDD (Pariante & Lightman, 2008) including onset of MDD among at-risk individuals (Mannie, Harmer, & Cowen, 2007; Owens, et al., 2014). Only recently have studies begun to employ a multisystem approach to explore the complex biological underpinnings of MDD and vulnerability with preliminary results underscoring differences in patterns of neuro-immune perturbations associated with depressive subtypes (e.g., Lamers, et al., 2013; Laurent, et al., 2016) and gender-specific vulnerability. To date, however, no study had employed a multisystem approach when examining NA reactivity to stress as a function of depressive symptom severity. By using the CORT/CRP ratio, the current study employed a multisystem approach by to evaluate the integrity of the feedback loop between the HPA axis and IRS and it role in stress-induced NA reactivity. While the primary analysis used the CORT/CRP ratio, parallel analysis using linear regression and test of the interaction between CORT, hsCRP, and HAMD yielded concordant results (see Supplemental Material).

Cytokines stimulate not only the synthesis of CRP by the liver but also the HPA axis to respond with a release of glucocorticoid that protects the body from the potentially damaging effects of prolonged immune activation (Kapcala, Chautard, & Eskay, 1995; Pace, Raison, & Miller, 2009; Sapolsky, Romero, & Munck, 2000). In animal studies that have abolished or attenuated the release of CORT or studies that have used inbred rat strains characterized by a blunted HPA axis response (e.g., Lewis, LEW/N), exposure to lipopolysaccharides, a potent inflammatory stimuli, evoked a sustained activation of the immune system that promoted clinical pathology and increased mortality risk (Perritti & Ahluwalia, 2000; Sternberg, 1995). In humans, an attenuated release of CORT has been observed in patients with polymyalgia rheumatica (Straub & Cutolo, 2006) and in coronary heart disease patients with comorbid depression who, relative to coronary patients without depression, exhibit heightened levels of inflammation (Nikkheslat et al., 2015). Similarly, lower CORT levels have been observed in patients with post-traumatic stress disorder (PTSD) (Meewisse, Reittma, De Vries, Gersons, & Olff, 2007) and atypical depression (Gold & Chrousos, 2013), conditions also characterized by heightened inflammation. Lastly, lower CORT levels and elevated CRP have also been observed in hypertensive women

(Tolmay, Malan, & Van Rooyen, 2012). Interestingly, a recent study observed that short telomere length was associated not only with depression but also with hypocortisolism and high CRP in healthy community volunteers (Wikgren et al., 2012). It is notable that heightened stress sensitivity and NA reactivity have been associated with all of the aforementioned conditions (Fries, Hesse, Hellhammer, & Hellhammer, 2005) including short telomere length (Mathur et al., 2016).

If disruptions of the neuro-immune negative feedback loop underlie MDD, it is reasonable to speculate that re-establishing regulatory influences is associated with amelioration of clinical symptoms. In one study of patients undergoing treatment for MDD, those achieving remission showed normalization of the relationship between the HPA axis and TNF- α system (Himmerich et al., 2006). In another study of patients with treatment resistant MDD, the anti-TNF agent infliximab reduced symptoms of depression but only among those patients with elevated hsCRP at entry (Raison et al., 2013). In individuals with low hsCRP, depressive symptoms failed to improve following infliximab administration. While preliminary, it may be that restoring the regulatory influences between the HPA axis and immune system is important not only for reducing the risk of MDD but also for improving clinical symptoms in patients undergoing treatment (Himmerich, et al., 2006). In light of the findings of those studies, especially among depressed patients with elevated CRP, we speculate that the CORT/CRP ratio may be a promising biomarker for evaluating treatment efficacy in patients receiving antidepressant therapies and in persons who receive stress management as part of comprehensive interventions program (Wheat et al., 2015).

As previously noted, evidence suggests that psychological stress and depression influence inflammatory responses suggesting that inflammation is one potential link between stress and MDD (Fagundes, Glaser, Hwang, Malarkey, & Kiecolt-Glaser, 2013; Sin, Graham-Engeland, & Almeida, 2015; Slavich & Irwin, 2014). Others have shown that stress increases the vulnerability of the prefrontal cortex to heightened levels of inflammation (de Pablos et al., 2006). It has been suggested that emotional states are indicators of underlying active disease processes and vulnerability to disease processes (Leventhal, 2000). Thus, increased stress-induced NA reactivity in individuals with elevated levels of depressive symptoms may not only be a factor contributing to the onset and progression of MDD, but also an indicator of existing biological vulnerability attributed to disruptions of neuro-immune regulatory feedback loop.

It is important to note that stress-induced negative emotions by themselves do not confer increased risk for MDD or other stress-related mood disorders. Nevertheless, stress sensitivity and specifically NA reactivity represents an important mediator between vulnerability and clinical manifestations (Hammen, 2005; Slavich & Irwin, 2014; Wichers, et al., 2007). Similarly, stress alone is not considered a causal factor but its depressogenic potential appears to be more pronounced in individuals at increased risk for clinical depression such as those with subsyndromal depressive symptoms (SSD) (Sadek & Bona, 2000). In one large epidemiological study, the experiences of daily stress predicted increases in NA arousal but only among individuals predisposed to depression due to high scores on neuroticism (Mroczek & Almeida, 2004). In a study of formerly depressed patients, individuals continued to exhibit greater arousal of NA in response to daily life stressors

suggesting that stress-induced NA reactivity may be a defining and stable characteristic even among remitted MDD patients (O'Hara, Armeli, Boynton, & Tennen, 2014). Taken together, the interaction between vulnerability and NA reactivity to daily life stressors may be an important psycho-dynamic feature that precedes the onset of MDD (Aldinger et al., 2014; Hammen, 2005; Kendler, Kuhn, & Prescott, 2004; McLaughlin et al., 2010; Mennin, Holaway, Fresco, Moore, & Heimberg, 2007; Wichers, et al., 2007). In line with this speculation, evidence suggests that genetic liability to depression is expressed, in part, as the tendency to display NA in response to minor stressors in daily life (Wichers, et al., 2007).

The current study has limitations. First, the findings are based on a measure of depressive symptom severity and not clinical diagnosis. Although not all individuals who exhibit elevated depressive symptoms go on to develop a depressive episode, heightened levels of depressive symptoms are associated with an increased risk of future MDD (Lewinsohn, Solomon, Seeley, & Zeiss, 2000). At this time, however, the extent to which the current findings generalize to individuals with MDD is not known. A second limitation is the frequency to which CORT and hsCRP were measured. In this study, we assessed level of hsCRP only once. There is evidence to suggest that in healthy individuals a single measure of hsCRP captures short term (4–6 weeks) variability (Navarro, et al., 2012) with reliability being slightly lower for long-term (~1.8 years) comparisons. In the current study, morning CORT was determined in two sequentially collected blood samples with results being highly correlated. (Suarez, et al., 2014). In one previous longitudinal study, morning CORT levels were relatively stable over a 2.5 year period with individuals who exhibited higher levels of CORT at entry exhibiting higher levels at follow-up (Huizenga et al., 1998). Although a single measure of each component of the ratio appears reliable, the test-retest reliability of the CORT/CRP ratio remains to be established.

Notwithstanding the above described limitations, the current findings are the first to describe the effect of putative loss of regulatory influences between the HPA axis and IRS on stress sensitivity in individuals with elevated depressive symptoms. Until now it was not known if loss of regulatory feedback between the IRS and HPA axis was associated or predictive of stress-induced NA reactivity. For individuals with mild-to-moderate symptoms of depression, a disturbance of the neuro-immune feedback loop characterized by a pro-inflammatory state accompanied by insufficient release of CORT may represent biological vulnerability associated with context-dependent NA reactivity, an intermediate phenotype associated with increased risk of MDD. For those with minimal depressive symptoms, the CORT/CRP was not associated with stress induced NA responses, suggesting the importance of depressive symptom severity as a key early moderator of the relation of premorbid biological determinants of stress sensitivity. It remains to be determined if the CORT/CRP ratio is useful in predicting NA responses to naturalistic stressors in at risk individuals and whether treatment-related changes in the ratio value correlate with reduction in stress sensitivity and symptom remission. At this time it is reasonable to conclude that the CORT/CRP ratio is a promising biomarker that reflects multi-system interactions that distinguish MDD subtypes, gender-specific risks of depression, and predicts stress-induced NA reactivity in at-risk individuals.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Interpretation of the distribution of CORT/CRP ratio values. Values at the two extreme of the distribution capture distinct patterns of dysregulation with higher ratio values the result of an excessive CORT release relative to low levels of hsCRP. Lower values are the result of an insufficient release of CORT relative to high levels of hsCRP. Within this schema moderate values are suggestive of balanced and coordinated responses between the HPA axis and IRS indicative of homeostasis.

Figure 2.

Severity of depressive symptoms, assessed using the Hamilton Depression Inventory (HAMD), moderated the relationship between stress-induced negative affect (NA) reactivity to the anger recall interview (ARI) and baseline values of CORT/CRP ratio. Comparison of slopes for the regression lines at ± 1 SD on the HAMD revealed significant differences. For subjects with higher HAMD scores, the slope of the regression line was significant ($\beta = -2.65$, $p < .05$) indicating that increasing values of the CORT/CRP ratio were associated with increasing stress-induced NA reactivity. For non-depressed subjects, the slope of the regression line was not significant ($\beta = -1.05$, ns) suggesting that the CORT/CRP ratio did not predict stress-induced NA responses.

Table 1

Subject characteristics for total sample and by gender.

	Men	Women	Total
N	109	89	198
Age (y)	28 (9.7)	29.6 (9.5)	28.4 (9.6)
BMI (kg/m ²)	25.4 (4.7)	25.0 (4.9)	25.5 (4.8)
Race (%)			
White	57.4	51.1	54.6
African American	22.2	35.2	28.1
Asian	16.7	12.5	14.8
Hispanic	0.9	0	0.5
Multi-racial	2.8	1.2	2.0
Education (%)			
Less than high school	0.5	0.0	0.5
High School	5.6	4.6	5.1
Some college	33.3	28.8	31.3
College degree	22.2	31.0	26.1
Post-college	38.0	35.6	37.0
Hamilton Depression *	4.4 (1–9)	4.7 (1–9)	4.5 (1–9)
Cortisol (µg/dl)	14.4 (5.0)	14.6 (6.6)	14.5 (5.8)
hsCRP (mg/L) *	0.72 (0.29–1.64)	0.74 (0.28–1.65)	0.72 (0.28–1.65)
CORT/CRP *	17.9 (9.3 – 41.8)	21.1 (7.9 – 50.7)	19.3 (8.1 – 45.1)
Negative Affect			
Baseline	15.1 (6.3)	12.3 (6.5)	13.8 (6.5)
Reading	13.9 (6.4)	12.2 (6.2)	13.1 (6.4)
Anger Recall	37.9 (14.2)	40.1 (14.4)	38.9 (14.3)

Data are presented as percent, means (SD) and median (interquartile range) if not normally distributed.

* Median (Interquartile range)

Table 2

Results of linear regression analysis of the Hamilton Depression (HAMD) score, CORT/CRP ratio, and their interaction in predicting Negative Affect Reactivity to the Anger Recall Interview (ARI).

Predictor	Negative Affect Reactivity		
	β	SE	P-value
Hamilton Depression (HAMD)	9.61	3.30	0.004
Log(CORT/CRP)	2.42	1.67	0.149
HAMD * CORT/CRP	-2.16	1.03	0.031
Gender	-2.00	2.08	0.339
Race	3.83	6.95	0.582
Education	7.69	16.46	0.641
Age	0.31	0.12	0.013
Baseline Negative Affect	0.56	0.16	0.001