Cortisol, Testosterone, and Prospective Risk for War-zone Stress-Evoked Depression

Adam R. Cobb, MA*; Robert A. Josephs, PhD*; Cynthia L. Lancaster, PhD*; Han-Joo Lee, PhD†; Michael J. Telch, PhD*

ABSTRACT
Introduction: The major challenges of efforts to reveal biological risk factors and biomarkers of depression include the complexity of underlying systems, interactions with other systems, and contextual factors governing their expression. Altered endocrine function is believed to be a central contributor to depressive illness, but across studies, evidence for a link between endocrine markers and depression has been mixed, inconclusive, or conditional in nature. In the present study, we evaluated basal testosterone (T), cortisol (C), and CO2 inhalation-stress-reactivity measures of these hormones (TR, CR) as pre-deployment moderators of the later impact of war-zone stressors on depression symptoms in-theater. Materials and Methods: At pre-deployment, U.S. soldiers (N = 120) completed demographic, clinical and hormone measures, and during deployment, they completed monthly, web-based assessments of war-zone stressors and depression symptoms (N = 533 observations). Mixed effects models estimated the effects of the pre-deployment hormone profiles in moderating war-zone stressors’ impact on in-theater depression. Models also tested whether hormonally linked risk for later stress-evoked depression depends on pre-existing depression. Results: Controlling for pre-deployment depression, high T was protective; whereas TR had depressogenic effects that were amplified by pre-deployment depression. Further, high C was protective, but heightened CR was depressogenic, but only among those with elevated pre-deployment depression. Conclusions: Findings highlight the importance of examining basal and activity measures of endocrine function, and use of prospective, longitudinal models to test hypothesized causal pathways associated with depression vulnerability in the war-zone. Results also suggest that pre-existing depression and cortisol may work in tandem to increase vulnerability for later stress-evoked depression in the war-zone.

INTRODUCTION
Depression is the second most prevalent mental health problem in war veterans, after post-traumatic stress disorder. Further, depression most often follows PTSD onset, loads heavily on specific PTSD symptom factors (i.e., dysphoria and numbing), and contributes to negative mental and physical health outcomes beyond PTSD severity and prior combat exposure. This supports military research efforts to identify risk and resilience factors specific to depression, and person-level and contextual factors governing its emergence and course. The mechanisms of depression are not well understood, but it clearly involves biological vulnerability and stress. Depression is heritable, but two-thirds of the risk is environmental. Stressors are the most potent contributor, with 50–80% of individuals having experienced a major stressor 3–6 mo prior to depression onset. The impact of stress has also been observed to attenuate as a function of prior depressive episodes, suggesting recurrence may lead to greater autonomy (i.e., less stress-dependence) of endogenous depressogenic processes. This supports studying depression within a diathesis-stress framework, and determining whether risk for stress-linked depression depends on pre-existing depression. Among research efforts targeting biomarkers and biological risk factors, abnormal endocrine activity is regarded a key contributor to depression etiology. Impaired hypothalamic-pituitary-adrenal (HPA) axis negative feedback is the most cited endocrine abnormality implicated in depression. However, meta-analyses have revealed these effects are small, inconclusive or conditional in nature. Additionally, evidence supports the involvement of the hypothalamic-pituitary-gonadal (HPG) axis. However, testosterone-depression studies have similarly been inconsistent, with reliable effects observed only in certain sub-populations, especially older, hypogonadal men. Cortisol and testosterone are both stress-reactive, suggesting prior mixed findings may be due, in part, to a lack of considering environmental stress. Understanding the role of endocrine systems in depression may be informed by examining how hormones are influenced by stress, and in turn, how hormonal activity moderates the stress response. In support, evidence suggests a hormone-psychopathology association is informed by the presence of environmental stress, consistent with the view that endocrine activity is shaped by adaptation to environmental demands. Moreover, evidence for the dual involvement of cortisol and testosterone in risk for future stress-linked PTSD is consistent with the functional inhibitory cross-talk that exists between the HPA and HPG axes. Thus, a joint examination of cortisol and testosterone activity may be helpful in informing the association between environmental stress and depression.
The present study is among a series from the Texas Combat PTSD Risk Project, in which a primary aim was to identify biological risk factors for stress-related psychopathology in the war-zone. Soldiers (N = 120) awaiting deployment provided saliva for hormone assays before and 30 min. after a 35% CO2 / 65% O2 inhalation challenge – a well-established stressor in clinical and non-clinical samples. Basal and CO2 stress-reactivity hormone measures were examined as moderators of the later impact of war-zone stressors on depression symptoms in-theater.

Findings of an inverse testosterone-depression association support the prediction that higher basal testosterone and greater testosterone stress reactivity (i.e., CO2 challenge-evoked change in testosterone), would be associated with a reduced impact of stressors on depression in-theater (see Models 2.1, 2.2, 2.5, and 3.1). Findings of hypercortisolism in depression, support the prediction that higher basal cortisol and greater CO2-evoked cortisol release would amplify the depressogenic effects of stressors (see Models 2.3, 2.4, 2.5, and 3.2). Additionally, evidence for inhibitory cross-talk between the HPA and HPG axes supports the prediction that higher basal testosterone and greater CO2-evoked testosterone release would mitigate the depressogenic effects of higher basal cortisol and greater CO2-evoked cortisol release (see Models 3.3–3.6).

Finally, we examined whether the hormones modulate risk conferred by pre-existing depression, by promoting later stress-evoked recurrence. Because depression is relapsing, and based on evidence that stress-evoked hormonal reactivity interacts with prior depressive episodes to predict future episodes, we hypothesized that hormonally linked risk might be further moderated by concurrent levels of depression (see Models 4.1–4.4). The prediction that risk would be conferred not only by in situ hormonal activity, but by concurrent linkage with depression is supported by evidence of hormonal involvement in emotional memory and affective processes, and consistent with the possibility that hormones, once linked with depression, may facilitate return to the depressed state.

METHODS
All study procedures were reviewed and approved by the Institutional Review Boards (IRB) at the University of Texas at Austin in Austin, TX, USA; and Brooke Army Medical Center in San Antonio, TX, USA.

Participants
Soldiers (N = 120; male: n = 104; female: n = 16) were recruited from 9 Army units scheduled to deploy from Ft. Hood to Iraq between August 2007 and August 2009. Eligibility required: (1) age of 18 or older, (2) no prior deployments, and (3) planned deployment within 3 mo. Among the 223 soldiers attending recruitment, 184 consented, 6 did not deploy, 1 withdrew consent, and 16 completed no in-theater measures. Of the remaining, 24 were excluded due to endocrine abnormalities or exclusionary medications (i.e., antidepressants, steroids), 8 had invalid assays, and 9 did not provide included measures, leaving 120 soldiers represented in the analyses (see Table I).

Participants reporting severe depression, or other severe mental or behavioral health problems, whether in interview or self-report assessments, were referred to the PI (M. Telch), and the Army PI and medical director for the project (W. Schirmer) for further evaluation. The medical director made all determinations of actions necessary to ensure participant welfare, including recommendations and referrals for treatment at Ft. Hood. Data on treatment utilization was unfortunately not available for the present analyses. However, all included soldiers were deemed fit for duty prior to deployment.

Pre-deployment Measures
Demographics and Lifetime Psychopathology
As part of a larger pre-deployment assessment, soldiers completed a demographics questionnaire, and the Structured Clinical Interview for Axis I DSM-IV Diagnoses (SCID-I-IV). The SCID-I-IV was administered by doctoral students, supervised by the PI, with > 1 yr of experience using this instrument.

Cortisol and Testosterone
Between 2:00 and 5:00 pm, two 3 mL saliva samples were collected for cortisol (C) and testosterone (T) assays – first following a 20-min rest period immediately preceding a 35% CO2 / 65% O2 inhalation challenge, and again 30 min after the challenge. The difference (post-CO2 minus pre-CO2 levels) was used to calculate cortisol (C_R) and testosterone reactivity

### TABLE I. Descriptive Statistics for Modeled Variables

<table>
<thead>
<tr>
<th>Variable</th>
<th>N</th>
<th>%</th>
<th>M</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>104</td>
<td>86.67</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Female</td>
<td>16</td>
<td>13.33</td>
<td>—</td>
<td>—</td>
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<td>53.33</td>
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<tr>
<td>Pre-Deployment Depression (CES-D-20)</td>
<td>120</td>
<td>10.99</td>
<td>8.36</td>
<td></td>
</tr>
<tr>
<td>Monthly average number of PTEs</td>
<td>120</td>
<td>1.83</td>
<td>1.85</td>
<td></td>
</tr>
<tr>
<td>CEL Entries</td>
<td>120</td>
<td>7</td>
<td>5.59</td>
<td></td>
</tr>
<tr>
<td>In-theater depression (CES-D-10)</td>
<td>120</td>
<td>7.65</td>
<td>5.11</td>
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<tr>
<td>C (pg/mL) Pre-CO2</td>
<td>120</td>
<td>12</td>
<td>.06</td>
<td></td>
</tr>
<tr>
<td>C (pg/mL) Post-CO2</td>
<td>120</td>
<td>.15</td>
<td>.13</td>
<td></td>
</tr>
<tr>
<td>C (pg/mL) Post-Pre CO2</td>
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<td>.03</td>
<td>.12</td>
<td></td>
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<tr>
<td>T (pg/mL) Pre-CO2</td>
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<td>26.74</td>
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<td>T (pg/mL) Post-CO2</td>
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<tr>
<td>T (pg/mL) Post-Pre CO2</td>
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<td>1.84</td>
<td>23.05</td>
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</tbody>
</table>

CES-D-10, Center for Epidemiological Studies Depression Scale – 10 items; CES-D-20, Center for Epidemiological Studies Depression Scale – 20 items.

a Dichotomously coded (yes or no) presence of lifetime, including current, DSM-IV-TR Axis I Disorders.

b Average monthly number of traumatic war-zone stressors across soldiers and deployment months.

Average total number of in-theater Combat Experience Log (CEL) entries, across soldiers. Basal salivary cortisol (C) and testosterone (T) were measured before and 30 min after a single inhalation of 35% CO2 / 65% O2 gas at pre-deployment, and reactivity measures (C_R, T_R) were derived by subtracting pre-CO2 from post-CO2 hormone levels.
Hormone concentrations were assayed by previous definitions.14 Hormone concentrations were assayed in duplicate by Salimetrics (State College, PA, USA). Intra-assay and inter-assay variances were acceptable (4.6% and 9.9%, respectively, for testosterone, 3.5% and 5.1% for cortisol).

35% CO₂ Inhalation Stress Challenge
The CO₂ stressor followed standard procedures.31–34 A 3-min video presented the rationale, instructions, and demonstration of procedures. Participants took one full capacity breath of the gas through a plastic mask, and held this breath for 5 s, followed by normal breathing until the effects of the gas subsided (~30 s).

Pre-deployment Depression Symptoms
Depression was assessed with the 20-item Center for Epidemiological Studies Depression Scale (CES-D-20).40 This validated instrument assesses depressive symptomology, with items rated according to frequency (0 = “Rarely / None of the time”; 3 = “Most / All of the time”). Internal consistency for the CES-D-20 was good in the present sample (α = 0.90).

In Theater Measures
During deployment, monthly emails were sent with reminders and web links to complete the Combat Experiences Log (CEL),41 which assesses the occurrence of stressors and severity of symptoms.

Potentially Traumatic War-zone Stressors
CEL items adapted from the Deployment Risk and Resilience Inventory42 assessed stressors experienced in the past 30 d (present vs. absent). Three of the authors (AC, CL, and MT) selected items that would meet PTSD DSM-5 criteria A1 for a trauma26 and excluded items not meeting these criteria (e.g., “conflict(s) with other soldiers”). Two free-response items allowing reporting of stressors that were not included in the checklist were coded by hand. The coding of these items produced perfect agreement among raters (see supplementary Table S1 for items).

In-Theater Depression Symptoms
In-theater depression was assessed with the 10-item Center for Epidemiological Studies Depression Scale (CES-D-10).43 Items assessing core depression symptoms were rated according to frequency (0 = “Rarely / None of the time”; 3 = “Most / All of the time”). The CES-D-10 is well validated highly correlated with the 20-item version.44,45 Internal consistency in the present sample was good (α = 0.81).

Statistical Analyses
Mixed effects models estimated the main and interactive moderation effects of basal cortisol (C), basal testosterone (T), and cortisol (Cr) and testosterone reactivity (Tr) to the 35% CO₂ challenge on the later impact of war-zone stressors (PTEs) on monthly in-theater depression (CES-D-10) (Full maximum likelihood estimation was used to determine functional forms of in-theater symptoms, and to compare deviance statistics of nested models, whereas restricted maximum likelihood produced the final estimates.46 Unstructured variance-covariance matrices were specified across models. Degrees of freedom were derived using Satterthwaite approximations.47 Local effect sizes were derived from t-statistics with the following formula: \( r = \sqrt{(t^2 / (df + t^2))} \). The number of days deployed was centered at 8 mo, and entered in its raw metric. Gender (male = 0, female = 1) and past or current psychopathology (absent = 0, present = 1) were entered dichotomously. All other continuous predictors, including pre-deployment depression (DEP) (CES-D-20), hormone indices, and war-zone stressor indices, were z-transformed for ease of interpretation.

Basal and reactivity hormone variables (C, T, Cr, and Tr) were z-transformed separately for each sex in order to remove sex differences in absolute levels and variability. We further assessed the validity of including both sexes by examining effects and residuals for all tested models, which indicated comparable patterns of effects and prediction accuracy (or equivalently, error) for males and females. For all models including hormone reactivity, we controlled for basal levels corresponding to each reactivity index. Stressor variables were parsed into between- and within-subject components to avoid imposing the assumption that between- and within-subject effects are equal.48 Between-subject stressor effects reflect average monthly exposure to potentially traumatic stressors (PTEWP), whereas within-subject stressor effects reflect monthly deviation around the individual’s own average (PTEWP). Moderation effects were probed by centering each moderator 1 SD above and below the respective means to estimate the conditional effects of stressors given a particular pre-deployment profile of the moderators (i.e., high vs. low basal hormones, CO₂-evoked hormone reactivity, and depression symptom severity).49

Our analyses can be summarized in four steps. In step 1, we examined main effects by entering the hormone indices separately (C, T, Cr, or Tr), followed by all hormone indices to estimate incremental main effects (C, T, Cr, and Tr; Model 1; see supplementary Table S2). In step 2, we examined singular stress-moderation effects by entering two-way interactions between each hormone variable and PTEs (e.g., T × PTEWP, Cr × PTEWP, etc.) in separate models (referred to as “singlular hormone diathesis-stress models”) (Hypotheses 1.1–1.4; Models 2.1–2.4), followed by modeling them together to estimate incremental stress-moderation effects (Model 2.5) (referred to as “incremental hormone diathesis-stress models”). In step 3, we tested whether basal and reactivity hormone measures interact within (e.g., C × Cr × PTEs) and across the HPA and HPG axes (e.g., C × Tr × PTEs) as stress moderators (Models 3.1–3.6) (referred to as “interacting diathesis-stress models”). In step 4, we tested whether any of the hormone diathesis-stress effects, modeled separately, were further moderated.

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by DEP (Models 4.1–4.4). See supplementary Table S2 for a list of these models (Models 1–4.4) with detailed specifications.

RESULTS

Preliminary Models of DEP

Table I provides demographics and descriptive statistics for all modeled variables. Preliminary models of in-theater depression revealed sex (b = 4.48, SE = 2.20, t = 2.04, p = 0.044), and lifetime psychopathology (b = 3.24, SE = 1.56, t = 2.08, p = 0.039) were significantly associated with DEP (see supplementary Table S3). In contrast, there were no associations between DEP and any of the hormone measures (all p’ s > 0.10).

Preliminary Models of In-Theater Depression

Unconditional growth models of in-theater depression revealed an intra-class correlation of 50.80%. The best fitting change trajectories were similar to those previously reported. There were 533 observations of 120 soldiers, who each completed an average of 4.44 (SD = 3.37; range: 1–14) in-theater assessments. Among the 120 soldiers, 29 (24.17%) fell above, and 91 (75.83%) fell below the cutoff for clinically significant depression at pre-deployment (CES-D-20 ≥ 16). Among those depressed at pre-deployment, 19 (65.15%) scored above the clinical cutoff for depression at some point during deployment (CES-D-10 ≥ 10). Among those not depressed at pre-deployment, 35 (38.46%) scored above the cut-off in-theater.

Main Effect Predictors of In-Theater Depression

Supplementary Table S4 presents main effects including all covariates (see Statistical Analyses; Step 1, Model 1). Race and ethnicity were non-significant predictors in preliminary models, and so were excluded. Females reported higher levels of in-theater depression than males (b = 2.10, SE = 1.01, t = 2.09, p = 0.039, r = 0.20) (note the smaller number of women in the sample (n = 16)). Lifetime psychopathology was a non-significant predictor of in-theater depression (p = 0.810), whereas DEP significantly predicted higher in-theater depression (b = 1.42, SE = 0.38, t = 3.70, p < 0.000, r = 0.31). Monthly within-soldier increases in stressors (PTEWP), significantly predicted higher in-theater depression (b = 0.41, SE = 0.17, t = 2.43, p = 0.016, r = 0.11), whereas the effect of average stressor exposure (PTEBp), was non-significant (p = 0.357). Neither T (p = 0.910), Tp (p = 0.084), C (p = 0.319), nor CR (p = 0.073) predicted in-theater depression directly. (Additionally, in models with each hormone index entered separately, but controlling for all other covariates, none of the hormone measures were found to directly predict in-theater depression (all p’s > 0.10). Furthermore, these null findings held irrespective of whether models controlled for the main effects of PTE exposure.)

Singular and Incremental Hormone Diathesis-Stress Models of In-Theater Depression

The following results are limited to tests of our primary hypotheses, evaluating the interactive effects of DEP, hormone indices, and war-zone stress on in-theater depression.

Singular Hormone Diathesis-Stress Models

Supplementary Table S5 presents findings for the singular hormone stress-moderation models (see Statistical Analyses, Step 2, Models 2.1–2.4), which tested whether any of the hormone indices, modeled separately, moderate the effects of PTEs.

Testosterone and testosterone reactivity × war-zone stressors. Neither C (Model 2.3) nor CR (Model 2.4) moderated the effects of either stressor index (PTEWP or PTEBp) on in-theater depression (C × PTEWP: p = 0.914; C × PTEWP: p = 0.137; CR × PTEBP: p = 0.471; CR × PTEBP: p = 0.998).

Incremental Hormone Diathesis-Stress Models

Table II presents findings for the incremental hormone stress-moderation model, in which all hormone indices were entered to estimate independent stress-moderation effects (see Statistical Analyses, Step 2; Model 2.5). Only Tp emerged as a significant moderator of PTEWP on depression (Tp × PTEWP: b = 0.49, SE = 0.25, t = 1.99, p = 0.048, r = 0.09; see supplementary Figure S3), indicating a significant impact of PTEWP on depression for those who exhibited increases in Tp (b = 0.94, SE = 0.30, t = 3.17, p = 0.002, r = 0.15), but not decreases in Tp (b = 0.821; Model 2.2; see supplementary Figure S2). Neither T (p = 0.654) nor Tp (p = 0.611) moderated PTEBP effects on depression.

Cortisol and cortisol reactivity × war-zone stressors. Neither C (Model 2.3) nor CR (Model 2.4) moderated the effects of either stress loop index (PTEWP or PTEBp) on in-theater depression (C × PTEWP: p = 0.914; C × PTEWP: p = 0.137; CR × PTEBP: p = 0.471; CR × PTEBP: p = 0.998).

Interacting Hormone Diathesis-Stress Models of In-Theater Depression

Next, we examined whether basal and reactivity measures interact both within (e.g., C × CR × PTEs) and across the HPA and HPG axes (e.g., C × Tp × PTEs) as moderators of stressors (see Statistical Analyses, Step 3; Models 3.1–3.6). Results are presented in supplementary Table S6. T and Tp did not significantly

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interact in moderating the effects of PTEWP on depression (T × T × PTEWP; $p = 0.900$). However, the T × T × PTEWP interaction was significant (PTEWP: $b = -0.49$, SE = 0.23, $t = -2.14$, $p = 0.033$, $r = 0.10$), indicating a pronounced impact of PTEWP on depression given low T and high Tg (PTEWP: $b = 1.66$, SE = 0.41, $t = 4.10$, $p < 0.000$, $r = 0.20$). All other high-low combinations of T and Tg were non-significant (all $p’$s > 0.10; see Fig. 1; Model 3.1). In contrast, interactive effects including C and Cg were non-significant (all $p’$s > 0.10; Model 3.2).

Finally, all three-way interactions consisting of each possible cross-axis combination of hormone indices (i.e., T × C, T × Cg, T × C, T × Cg) and one of the two stressor variables (i.e., PTEBP, PTEWP) were non-significant (all $p’$s > 0.10; Models 3.3–3.6).

The effects of PTEWP on in-theater depression. In contrast, given low DEP, Tg was non-significantly related to the impact of PTEWP ($p > 0.10$).

DEP significantly moderated the C × PTEWP interaction on in-theater depression (DEP × C × PTEWP $b = -1.02$, SE = 0.35, $t = -2.91$, $p = 0.004$, $r = 0.13$; see Fig. 3; Model 4.3). High depression and low C at pre-deployment significantly potentiated the impact of PTEWP ($b = 2.04$, SE = 0.46, $t = 4.40$, $p < 0.000$, $r = 0.20$), whereas high depression and high C predicted a non-significant relation between PTEWP and in-theater depression ($p = 0.077$). In contrast, given low DEP, the impact of PTEWP was not significant, irrespective of C ($p’$s > 0.10).

Finally, DEP significantly moderated the Cg × PTEWP interaction on in-theater depression ($b = 0.78$, SE = 0.27, $t = 2.87$, $p = 0.004$, $r = 0.13$; Model 4.4; see Fig. 4). Given high DEP, Cg significantly amplified the impact of PTEWP (Cg+: $b = 2.00$, SE = 0.45, $t = 3.64$, $p < 0.000$, $r = 0.17$; Cg−: $b = 0.11$, SE = 0.46, $t = 0.24$, $p = 0.811$, $r = 0.01$). In contrast, given low DEP, there is no significant relation between Cg and the impact of PTEWP on in-theater depression (PTEWP | Cg+: $p = 0.058$; PTEWP | CR−: $p = 0.262$).

**DISCUSSION**

These findings revealed profiles of DEP, basal hormones and hormone stress-reactivity that predict depressogenic reactions to

<table>
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<tr>
<th>Parameter</th>
<th>b</th>
<th>SE</th>
<th>df</th>
<th>t</th>
<th>p</th>
<th>Effect Size</th>
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<td>(Intercept)</td>
<td>7.72</td>
<td>0.59</td>
<td>119.70</td>
<td>13.13</td>
<td>0.000</td>
<td>**** 0.77</td>
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<td>0.01</td>
<td>475.60</td>
<td>-4.73</td>
<td>0.000</td>
<td>**** 0.21</td>
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<td>113.90</td>
<td>1.66</td>
<td>0.100</td>
<td>* 0.15</td>
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<td>Pre-Deployment CES-D-20</td>
<td>1.45</td>
<td>0.41</td>
<td>122.10</td>
<td>3.56</td>
<td>0.001</td>
<td>*** 0.31</td>
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<td>1.65</td>
<td>0.099</td>
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<td>0.717</td>
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<td>0.22</td>
<td>479.40</td>
<td>-1.15</td>
<td>0.250</td>
<td>0.05</td>
</tr>
<tr>
<td>Tg × PTEBP</td>
<td>-0.08</td>
<td>0.38</td>
<td>131.70</td>
<td>-0.21</td>
<td>0.834</td>
<td>0.02</td>
</tr>
<tr>
<td>Tg × PTEWP</td>
<td>0.49</td>
<td>0.25</td>
<td>460.40</td>
<td>1.99</td>
<td>0.048</td>
<td>** 0.09</td>
</tr>
<tr>
<td>PTEWP</td>
<td>Tg</td>
<td>-0.18</td>
<td>0.28</td>
<td>449.30</td>
<td>-0.63</td>
<td>0.530</td>
</tr>
<tr>
<td>PTEWP</td>
<td>Tg</td>
<td>0.81</td>
<td>0.34</td>
<td>473.40</td>
<td>2.37</td>
<td>0.018</td>
</tr>
</tbody>
</table>

**TABLE II.** Incremental Hormone-Stress Models

This table presents results from multi-level growth models including each of the hormone indices in interaction with both the between-soldier (PTEWP) and within-soldier (PTEWP) variance component of stressors in order to evaluate the incremental contribution of the hormone variables to the impact of in-theater stressors on depression symptoms. PTEBP reflects the total between-soldier effect of having a higher monthly average exposure to PTEs, across deployment months. PTEWP indicates the effect of having 1 additional PTE relative to the individual soldier’s monthly average number of stressors, in any single deployment month. Effect sizes (r) were derived from the t-statistics, using the following formula: $r = \sqrt{(t^2 / (df + t^2))}$, where $r$ equals the value of the t-statistic for each parameter, and df equals the degrees of freedom associated with the t-statistics. *p < 0.10, **p < 0.05, ***p < 0.01, ****p < 0.000.
FIGURE 1. Basal testosterone and testosterone reactivity to a 35% CO2 challenge jointly moderate the impact of within-soldier deviations from average monthly exposure to traumatic war-zone stressors on in-theater depression symptoms. The main plot (A) represents the effects of within-soldier deviations from their own average monthly exposure to potentially traumatic war-zone stressors (PTEWP) for soldiers exhibiting low basal testosterone (dotted lines) or high basal testosterone (solid lines) and decreases (empty triangles) or increases (filled triangles) in salivary testosterone levels from pre-to-post CO2 inhalation at pre-deployment. Models controlled for gender, past and current Axis I DSM-IV-TR psychopathology, and DEP severity. Shaded regions (subplots B and C) indicate asymmetric bootstrap-derived 95% confidence limits. Undulations reflect variation in data density across the length of each regression line.

TABLE III. Single Basal and Reactivity Hormone-Stress Moderation Models Further Moderated by DEP Symptoms

<table>
<thead>
<tr>
<th>Parameter</th>
<th>b</th>
<th>SE</th>
<th>df</th>
<th>t</th>
<th>p</th>
<th>Effect Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dep × T × PTEBP</td>
<td>0.09</td>
<td>0.44</td>
<td>120</td>
<td>0.40</td>
<td>0.21</td>
<td>0.02</td>
</tr>
<tr>
<td>Dep × T × PTEWP</td>
<td>−0.20</td>
<td>0.24</td>
<td>450</td>
<td>−0.81</td>
<td>0.417</td>
<td>0.04</td>
</tr>
<tr>
<td>Dep × TR × PTEBP</td>
<td>0.11</td>
<td>0.45</td>
<td>142</td>
<td>0.20</td>
<td>0.835</td>
<td>0.02</td>
</tr>
<tr>
<td>Dep × TR × PTEWP</td>
<td>0.86</td>
<td>0.28</td>
<td>449</td>
<td>3.10</td>
<td>0.002</td>
<td>0.14</td>
</tr>
<tr>
<td>PTEWP</td>
<td>Low Dep, Tr−</td>
<td>0.36</td>
<td>0.35</td>
<td>433.60</td>
<td>1.04</td>
<td>0.300</td>
</tr>
<tr>
<td>PTEWP</td>
<td>High Dep, Tr−</td>
<td>−0.55</td>
<td>0.46</td>
<td>445.50</td>
<td>−1.18</td>
<td>0.238</td>
</tr>
<tr>
<td>PTEWP</td>
<td>Low Dep, Tr+</td>
<td>−0.41</td>
<td>0.47</td>
<td>460.40</td>
<td>−0.87</td>
<td>0.384</td>
</tr>
<tr>
<td>PTEWP</td>
<td>High Dep, Tr+</td>
<td>2.10</td>
<td>0.43</td>
<td>437.70</td>
<td>4.86</td>
<td>0.000</td>
</tr>
<tr>
<td>Dep × C × PTEBP</td>
<td>0.64</td>
<td>0.48</td>
<td>169</td>
<td>1.34</td>
<td>0.253</td>
<td>0.10</td>
</tr>
<tr>
<td>Dep × C × PTEWP</td>
<td>−1.02</td>
<td>0.35</td>
<td>469</td>
<td>−2.91</td>
<td>0.004</td>
<td>0.13</td>
</tr>
<tr>
<td>PTEWP</td>
<td>Low Dep, Low C</td>
<td>−0.37</td>
<td>0.41</td>
<td>455.50</td>
<td>−0.92</td>
<td>0.357</td>
</tr>
<tr>
<td>PTEWP</td>
<td>High Dep, Low C</td>
<td>0.20</td>
<td>0.46</td>
<td>461.10</td>
<td>4.40</td>
<td>0.000</td>
</tr>
<tr>
<td>PTEWP</td>
<td>Low Dep, High C</td>
<td>0.27</td>
<td>0.33</td>
<td>451.00</td>
<td>0.83</td>
<td>0.407</td>
</tr>
<tr>
<td>PTEWP</td>
<td>High Dep, High C</td>
<td>−1.39</td>
<td>0.78</td>
<td>480.10</td>
<td>−1.77</td>
<td>0.077</td>
</tr>
<tr>
<td>Dep × C × PTEBP</td>
<td>−0.17</td>
<td>0.38</td>
<td>182</td>
<td>−0.44</td>
<td>0.663</td>
<td>0.03</td>
</tr>
<tr>
<td>Dep × C × PTEWP</td>
<td>0.78</td>
<td>0.27</td>
<td>484</td>
<td>2.87</td>
<td>0.004</td>
<td>0.13</td>
</tr>
<tr>
<td>PTEWP</td>
<td>Low Dep, Cr−</td>
<td>0.38</td>
<td>0.34</td>
<td>449.20</td>
<td>1.12</td>
<td>0.262</td>
</tr>
<tr>
<td>PTEWP</td>
<td>High Dep, Cr−</td>
<td>0.11</td>
<td>0.46</td>
<td>476.50</td>
<td>0.24</td>
<td>0.811</td>
</tr>
<tr>
<td>PTEWP</td>
<td>Low Dep, Cr+</td>
<td>−0.85</td>
<td>0.45</td>
<td>442.90</td>
<td>−1.90</td>
<td>0.058</td>
</tr>
<tr>
<td>PTEWP</td>
<td>High Dep, Cr+</td>
<td>2.00</td>
<td>0.55</td>
<td>458.20</td>
<td>3.64</td>
<td>0.000</td>
</tr>
</tbody>
</table>

This table presents results from multi-level growth models including three-way interactions of testosterone indices and stressors in interaction with DEP symptoms, based on scores on the CES-D-20 (Dep), to examine whether baseline depression moderates the hormone stress-moderation effects (e.g., Dep × T × PTEBP). Omnibus effects (e.g., Dep × TR × PTEBP, etc.) are followed by conditional effects (PTEWP | Low Dep, Tr−, etc.), which indicate the effects of PTEs given each combination of high (+1 SD from the mean) or low (−1 SD from the mean) basal hormone levels (i.e., C, T) measured prior to the pre-deployment CO2 challenge, or increases (+1 SD from 0) or reductions (−1 SD from 0) in salivary hormone levels (i.e., CR−/+, TR−/+, etc.) from pre-to-post CO2 challenge. PTEWP reflects the total between-soldier effect of having a higher monthly average exposure to PTEs, across deployment months. PTEBP indicates the effect of having 1 additional PTE relative to the individual soldier’s monthly average number of stressors, in any single deployment month. Effect sizes (r) were derived from the t-statistics, using the following formula: $r = \sqrt{t^2 / (df + t^2)}$, where t equals the value of the t-statistic for each parameter, and df equals the degrees of freedom associated with the t-statistics. *p < 0.10, **p < 0.05, ***p < 0.01, ****p < 0.000.
monthly increases in war-zone stressors. Specifically, low testosterone and elevated stress-evoked testosterone release strengthened the association between monthly increases in stressors and depression, and the effects of testosterone stress-reactivity were more pronounced in soldiers who were depressed at pre-deployment. In contrast, whereas testosterone’s effects were also independent of DEP, the stress-moderation effects of cortisol critically depended on concurrent depression – among
depressed soldiers, high basal cortisol protected against war-zone stress-evoked depression, whereas elevated stress-evoked cortisol release amplified stressors’ impact. Finally, contrary to predictions based on HPA-HPG reciprocal suppression, no cross-axis effects were found, suggesting the hormone effects operated independently. Overall, the findings support simultaneous examination of basal and endocrine stress-reactivity markers, and baseline depression severity within a diathesis-stress framework when estimating risk for depression.

The finding that low testosterone elevated risk for stress-evoked depression is consistent with sex differences in depression prevalence, and evidence that testosterone ameliorates depression. However, contrary to predictions, testosterone reactivity amplified the depressogenic impact of monthly stressors. Supporting this possibility is evidence that short-term elevations in testosterone followed by declines that can last up to several days – effects possibly mediated by auto-regulation.

Given evidence for hippocampal atrophy in depression, and that hippocampal neurogenesis mediates treatment effects in depression, the literature examining testosterone as a hippocampal neurotrophic factor offers a related account. For instance, testosterone may protect against glucocorticoid-mediated hippocampal neural degeneration by suppressing HPA function, and directly enhancing neuron survival. However, consistent with the depressogenic effects of testosterone reactivity, evidence suggests these effects depend on the duration of testosterone exposure. In animals, testosterone administration for at least 30 d has produced neurotrophic effects, whereas shorter treatments (15 d; 21 d) have produced no effects, and still shorter durations have actually diminished new neuron survival in the hippocampus (5 d).

In contrast to the testosterone findings, the stress-moderating effects of basal cortisol and cortisol reactivity were observed only among those with elevated depression at pre-deployment. Contrary to predictions based on evidence for hyper-corticolism in depression, among soldiers with high DEP, elevated cortisol predicted an inverse relation, whereas low cortisol predicted a strong positive relation between monthly stressors and in-theater depression. These findings are consistent with evidence that low cortisol predicts earlier relapse in recurrent major depression, and with the interpretation that adaptive benefits may explain observations of hyper-corticolism in depression.

The present findings suggest these benefits may arise only in the context of elevated stress, consistent with observations of a reduced impact of stressors given a greater number of previous depressive episodes. However, the present findings suggest that cortisol elevations in depression may be an adaptive response that buffers the impact of stressors, thereby reducing risk for recurrence, but only in the context of elevated stress.

FIGURE 4. Baseline depression and cortisol reactivity to a 35% CO2 challenge jointly moderate the impact of war-zone stressors on in-theater depression symptoms. The main plot (A) represents the effects of within-soldier deviations from their own average monthly exposure to potentially traumatic war-zone stressors (PTEWP) for soldiers exhibiting cortisol decreases (empty squares) or cortisol increases (filled squares) in response to the 35% CO2 inhalation challenge, and low (dotted lines) or high (solid lines) depression based on scores on the CES-D-20 at pre-deployment. Models controlled for gender, and past and current Axis I DSM-IV-TR psychopathology. Shaded regions (subplots C and B) indicate asymmetric bootstrap-derived 95% confidence limits. Undulations reflect variation in data density across the length of each regression line.
The protection conferred by high basal cortisol did not extend to reactivity, although the findings for cortisol reactivity were consistent with predictions and existing literature. Burke et al’s meta-analysis found a pattern of blunted acute stress reactivity in depressed individuals, but higher cortisol levels at least 25-min post-stressor onset, and this pattern was more pronounced in studies of more severely depressed patients. Other evidence has shown that a history of major depression combined with greater cortisol release during anticipatory stress enhances risk for future depressive episodes. Extending these findings, we found that soldiers depressed at pre-deployment who also exhibited pronounced stress-evoked increases in cortisol (pre-to 30 min. post-CO2 challenge) were most susceptible to the later depressogenic impact of monthly elevations in war-zone stressors.

**Limitations**

A few study limitations should be noted. First, most soldiers provided in-theater data, but missing observations were not uncommon. However, there were no patterns in missing data across deployment months, and our statistical approach is robust to missing observations. Second, because depression is multifactorial and multiply determined, and given the inherent constraints of model complexity, the present approach is necessarily limited in terms of attending to the constellation of factors that contribute to depression. For instance, we were unable to validly assess the effects of several potentially important variables, including sex, age, and physical health. Third, hormone sampling under conditions of fasting would be less than ideal for indexing hormonal reactivity to a laboratory stress challenge, but it may be worthwhile for future studies to also obtain fasting levels, as this may help guide use of routine tests of endocrine function for informing risk assessments. Fourth, we relied on self-report measures of in-theater stressors and depression, and did not have repeated hormone measures in the war-zone, which would be useful for determining how hormone profiles may be functionally impacted by stress exposure, and influence stress reactions over time. Still, the prospective design enhances confidence in evaluating relations between hormone profiles and the depressogenic impact of stressors, and the use of a diathesis-stress framework is consistent with most etiological models, in which stress is given a determinant role in the pathophysiology of depression.

**Conclusions**

The major challenges of discovering biomarkers and biological risk factors for depression and other stress-linked psychopathologies include the complexity of biological systems, their reciprocal interactions with other systems, and the variety of contextual factors governing their expression. The present study addressed these issues in evaluating a more complete and functional etiological model that views endocrine systems as nested within individuals, interacting with other endogenous factors, and subject to the fluctuating contextual influence of environmental stress. Future work should similarly implement dynamic measures of stress-reactivity, and aim for identifying susceptible individuals by examining clusters of individual difference and contextual factors. In addition to informing the etiology of stress-related psychopathology, such an approach may contribute to enhancing identification of those most vulnerable to depression and other stress-related mental disorders.

**SUPPLEMENTARY MATERIAL**

Supplementary material is available at Military Medicine online.

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