ORIGINAL ARTICLE

Emotional Reactivity to a Single Inhalation of 35% Carbon Dioxide and Its Association With Later Symptoms of Posttraumatic Stress Disorder and Anxiety in Soldiers Deployed to Iraq

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Context: The identification of modifiable predeployment vulnerability factors that increase the risk of combat stress reactions among soldiers once deployed to a war zone offers significant potential for the prevention of posttraumatic stress disorder (PTSD) and other combat-related stress disorders. Adults with anxiety disorders display heightened emotional reactivity to a single inhalation of 35% carbon dioxide (CO_2); however, data investigating prospective linkages between emotional reactivity to CO_2 and susceptibility to war-zone stress reactions are lacking.

Objective: To investigate the association of soldiers' predeployment emotional reactivity to 35% CO_2 challenge with several indices of subsequent war-zone stress symptoms assessed monthly while deployed in Iraq.

Design, Setting, and Participants: Prospective cohort study of 158 soldiers with no history of deployment to a war zone were recruited from the Texas Combat Stress Risk Study between April 2, 2007, and August 28, 2009.

Main Outcome Measures: Multilevel regression models were used to investigate the association between emotional reactivity to 35% CO_2 challenge (assessed before deployment) and soldiers' reported symptoms of general anxiety/stress, PTSD, and depression while deployed to Iraq.

Results: Growth curves of PTSD, depression, and general anxiety/stress symptoms showed a significant curvilinear relationship during the 16-month deployment period. War-zone stressors reported in theater were associated with symptoms of general anxiety/stress, PTSD, and depression. Consistent with the prediction, soldiers' emotional reactivity to a single inhalation of 35% CO_2-enriched air before deployment significantly potentiated the effects of war-zone stressors on the subsequent development of PTSD symptoms and general anxiety/stress symptoms but not on the development of depression, even after accounting for the effects of trait anxiety and the presence of past or current Axis I mental disorders.

Conclusion: Soldiers' emotional reactivity to a 35% CO_2 challenge may serve as a vulnerability factor for increasing soldiers' risk for PTSD and general anxiety/stress symptoms in response to war-zone stressors.

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THERE IS COMPELLING EVIDENCE that combat-related stress disorders, such as posttraumatic stress disorder (PTSD) and depression, are associated with several negative consequences, including somatic symptoms,[1,2] anger control,[3] substance use disorders,[4,5] low income and deficient job performance,[1,6] relationship problems,[7,8] and suicide.[9]

Exposure to trauma is a necessary criterion for the diagnosis of PTSD, and some soldiers are affected more than others by war-zone stress. This is not unique to military populations. Epidemiologic data indicate that most Americans (60.7%) have been exposed to a traumatic stressor sometime in their life, yet less than 10% develop PTSD.[10] Similarly, although life stress often precedes depression, few of those exposed to stressful life events become depressed.[11] These findings clearly point to the need to identify "modifiable" risk factors that increase soldiers' vulnerability to develop significant combat stress disorders in the face of war-zone stressors.

Inhalation of carbon dioxide (CO_2)–enriched air has been widely used as an investigative tool in the pathogenicity of anxiety disorders—particularly panic disorder.[12-18] Emotional reactivity to a 35% CO_2 challenge has been consistently observed in patients with panic disorder[19-22]

and their healthy first-degree relatives.[23,24] Moreover, monozygotic twins were significantly more likely than were dizygotic twins (56% vs 12%) to display CO_2-induced panic in response to a 35% CO_2 challenge,[25] thus suggesting the importance of genetic factors in determining sensitivity to CO_2 challenge–induced panic. In a prospective study[26] among nonclinical civilian samples, reactivity to CO_2 challenge has been shown to predict the subsequent development of anxiety disorders, even after controlling for anxiety sensitivity, which independently predicted increased risk for anxiety disorders.

Relatively few studies have examined emotional response to CO_2-enriched air among patients with anxiety disorders other than panic disorder. One study[27] has shown that patients with obsessive-compulsive disorder did not display heightened CO_2 sensitivity relative to healthy control individuals, whereas patients with panic disorder or panic disorder and obsessive-compulsive disorder showed heightened 35% CO_2 reactivity. In contrast, patients with social phobia displayed greater emotional reactivity to 35% CO_2 challenge relative to healthy controls while displaying levels of emotional reactivity similar to those of patients with panic disorder.[28,29] In generalized anxiety disorder samples, patients have shown increased anxiety symptoms in response to 7% CO_2 challenge[30] as well as reductions in CO_2-induced anxiety after a trial of lorazepam or paroxetine.[31] However, one study found that patients with generalized anxiety disorder did not differ significantly from healthy controls and that they were significantly less CO_2 reactive relative to those with panic disorder.[32]

Only 2 studies have reported on CO_2 reactivity among patients with PTSD. Talesnik and colleagues[33] administered a 35% CO_2 challenge to 20 drug-naive PTSD patients and compared them retrospectively with a sample of 39 healthy controls and 17 patients with panic disorder. The PTSD patients' response to CO_2 was indistinguishable from that of the healthy controls. Muhtz and colleagues[34] evaluated single administration of 35% CO_2 to 10 PTSD patients, 10 age- and sex-matched healthy controls, and 8 patients with panic disorder. In sharp contrast to the earlier study of Talesnik et al,[33] the PTSD group and the panic disorder group displayed significantly heightened emotional response to the CO_2 challenge relative to the healthy controls. Furthermore, some of the PTSD patients reported posttraumatic flashbacks in response to the CO_2 challenge.

To date, no studies have examined linkages between heightened emotional response to 35% CO_2 and war-zone stress reactions. Based on the aforementioned evidence linking heightened emotional response to CO_2 and anxiety disorders, as well as evidence suggesting that CO_2 reactivity may serve as a vulnerability marker for subsequent development of anxiety disorders,[26] we examined whether emotional reactivity to a 35% CO_2 stress challenge before deployment would predict soldiers' vulnerability for PTSD, depression, and general anxiety/stress symptoms in response to war-zone stressors. Based on a diathesis stress model of combat stress, we predicted that emotional reactivity to 35% CO_2 challenge (putative diathesis) would potentiate the effects of war-zone stressors on the subsequent development of war-zone stress reactions. The data reported herein are from the Texas Combat Stress Risk Project,[35-38] a prospective investigation of genetic, neuroimaging, psychosocial, and cognitive risk factors predicting soldiers' combat stress reactions while deployed in Iraq.

METHODS

PARTICIPANTS AND RECRUITMENT PROCEDURES

The study sample (N=158) was drawn from Fort Hood soldiers recruited through announcements to unit leaders. The principal investigator (M.J.T.) and the project manager conducted briefing meetings for potential soldier volunteers from 8 combat and 2 combat support units at Fort Hood, Texas. To reduce the potential for soldiers to feel coerced to participate, unit leaders were not present at the briefing meetings, and an army ombudsman not connected to the study was present during all recruitment sessions. Of the 223 soldiers attending the group orientation sessions, 184 soldiers (82.5%) provided informed consent and completed an extensive 8-hour predeployment assessment at the Imaging Research Center at The University of Texas at Austin. Of the 184 soldiers completing the predeployment assessment, 6 were not deployed and 3 deployed soldiers withdrew from the study. Of the remaining 175 soldiers, 3 refused to participate in the CO_2 challenge and 14 soldiers failed to complete any assessments of war-zone stress while in theater.

Study inclusion/exclusion criteria included (1) current Army soldier scheduled to deploy to Iraq within 90 days, (2) no prior deployment to a war zone, and (3) age 18 years or older. The final sample was predominantly male (88.7%) with a mean (SD) age of 24.41 (6.12) years and the following race/ethnicity breakdown: white (72.2%); African American (10.1%); American Indian (12.0%); and Asian, Native Hawaiian, or Pacific Islander (5.7%). In addition, 29 of the 158 participants (18.4%) reported their ethnicity to be Hispanic. The educational level of the sample was as follows: some high school (53.1%), some college (37.9%), undergraduate degree (5.1%), and master's degree or higher (3.9%). Approximately one-third (31.3%) of the study participants were married, 2.8% were living with a partner, 7.9% were divorced or separated, and 57.6% had never been married.

INSTITUTIONAL REVIEW BOARD APPROVAL

This study was conducted under a human-use protocol approved by the Office of Research Support and Compliance at The University of Texas at Austin and the Brooks Army Medical Center Scientific and Human Use Review Committee. Informed consent was obtained from all study participants.

ASSESSMENT OF WAR-ZONE STRESSORS

Soldiers completed monthly assessments of their war-zone stress experiences using the Combat Experience Log (CEL), a web-based system for assessing war-zone stress in theater.[36] Soldiers identified stressors that they experienced from a list of 18 previously validated war-zone stressors (eg, received hostile incoming fire, had been wounded or injured in combat, and received bad news from home). These stressor items were derived from a modified version[39] of the Deployment Risk and Resilience Inventory.[40] Soldiers indicated which stressors they had experienced since their most recent in-theater CEL assessment (or since deployment to the combat zone in the event of

their first response to the CEL system). Moreover, soldiers were allowed to record up to 2 stressors not appearing on the standard list of 18. The number of combat stressors was summed to estimate the level of war-zone stress exposure and used to predict PTSD, depression, and general anxiety symptoms. Soldiers reported approximately 6 (mean [SD], 5.96 [5.34]) war-zone stress exposures per monthly assessment. Additional information on this assessment has been reported.[36]

ASSESSMENT OF PTSD, DEPRESSION, AND GENERAL ANXIETY/STRESS SYMPTOMS

Three major domains of deployment-related stress symptoms were assessed each month in theater using the CEL.[36] The PTSD symptoms were evaluated using the 4-item version of the PTSD Checklist (PCL-Short).[41] The PCL-Short addresses each of the 3 core PTSD symptom clusters: re-experiencing (2 items), avoidance (1 item), and increased arousal (1 item). Despite the brevity of the PCL-Short, a validation study[41] indicated that it has a diagnostic accuracy estimate equivalent to that of the 17-item PCL. For the current sample, the internal consistency coefficient of the PCL-Short was 0.72.

Depression during deployment was assessed in theater using the 10-item short version of the Center for Epidemiologic Studies Depression Scale (CES-D).[42] The CES-D was developed to screen general populations for the presence of depressive symptoms; thus, its items are designed to be understandable and relevant for all participants regardless of their clinical status. The CES-D has demonstrated excellent psychometric properties and has been widely administered in various measurement modalities, including web-based assessment.[43] The current 10-item short version is highly predictive of scores from the full 20-item version ($\kappa = 0.97$, $P < .001$).[42] Internal consistency computed from soldiers' first entry of the CESD-10 was 0.72.

The General Anxiety/Stress index included in the overall CEL was designed to provide a brief in-theater assessment of stress/anxiety symptoms during the past 30 days. Soldiers are presented with 18 stress/anxiety symptoms in 3 major domains: cognitive (eg, fear of losing control), emotional (eg, feeling scared), and somatic (eg, tension in muscles). Each symptom is rated on a 5-point scale (1, not at all, to 5, extremely). Internal consistency for this index was 0.92 for the current sample.[36]

PROCEDURE

Predeployment assessments were performed at the Imaging Research Center at The University of Texas at Austin. Soldiers typically arrived by 8 AM, usually in groups of 5 to 8, and were monitored by study personnel until dismissal approximately 7 hours later. Participants completed several study assessments, including many that are not the focus of this report. After providing informed consent, participants completed online questionnaires and were interviewed to assess for the presence of current and past *DSM-IV* diagnoses. The CO_2 inhalation challenge occurred between 2 PM and 4 PM and followed procedures similar to those described in other CO_2 challenge studies conducted in the Laboratory for the Study of Anxiety Disorders at The University of Texas at Austin.[17,44,45] Participants were seated individually in a soundproof room and fitted with an ambulatory heart rate monitor. After a 5-minute resting baseline heart rate was documented, participants watched a 3-minute video containing the rationale, procedural instructions, and a demonstration of the CO_2 inhalation procedure. They were then instructed to take a full vital capacity breath of the gas mixture containing 35% CO_2/65% oxygen through a plastic mask and to hold it in their lungs for 5 seconds. Participants were then instructed to breath normally until the effects of the gas subsided (approximately 30 seconds), at which point they completed the Acute Panic Inventory (API)[46]—a widely used self-report instrument for assessing emotional response to CO_2 challenge. Consistent with previous research[17,26,45] on emotional reactivity to CO_2 challenge in nonclinical samples, our primary index of emotional reactivity to CO_2 challenge was based on soldiers' rating of the highest level of fear experienced at any time during or after the inhalation. Responses were measured on a scale ranging from 0 (none) to 100 (extreme). In addition, using the work of Colasanti et al,[47] we constructed several other CO_2 reaction scales from the 29 API items (**Table 1**).

Soldiers were deployed to Iraq approximately 2 to 3 months after the predeployment assessment. In-theater assessments of war-zone stress experiences and war-zone stress reactions during deployment were obtained monthly using the CEL.

Table 1. Soldiers' Reactions on the API to a Single Inhalation of 35% CO_2 at the Predeployment Assessment

Soldiers' Reported Reactions to CO_2[a]	Mean (SD)	Soldiers Reporting Reaction, %
Feeling faint[1,3]	1.4 (0.8)	86
Afraid of dying[3,4]	0.1 (0.3)	4
Afraid in general[4]	0.4 (0.6)	32
Palpitations[1,3]	0.8 (0.9)	58
Hard to breathe[1,3]	1.2 (0.9)	75
Urge to urinate[3]	0.1 (0.3)	6
Urge to defecate[3]	0.0 (0.2)	4
Dizzy or lightheaded[1,3]	2.2 (0.7)	99
Confused[4]	0.6 (0.8)	47
Things and people unreal[2]	0.5 (0.8)	37
Detached from body[2]	0.6 (0.8)	39
Hard to concentrate	0.9 (0.8)	65
Sweating[1,3]	0.1 (0.4)	11
Difficult to speak	0.6 (0.8)	43
Difficult to do your job	1.6 (1.1)	80
Inner shakiness, twitching or trembling[3]	0.7 (0.9)	48
Nauseous or queasy[3]	0.4 (0.6)	27
Afraid of going crazy[4]	0.1 (0.3)	6
Afraid of losing control[4]	0.3 (0.6)	21
Tingling or numbness[3]	0.9 (0.9)	58
Chest pain or discomfort[1,3]	0.3 (0.6)	25
Difficulty in swallowing[3]	0.2 (0.5)	19
Choking or smothering sensations[1,3]	0.4 (0.6)	35
Hot or cold flashes[3]	0.4 (0.7)	30
Dry mouth[3]	0.4 (0.6)	28
Feelings of weakness[3]	0.9 (0.8)	66
Desire to flee[4]	0.2 (0.6)	18
Depressed	0.1 (0.3)	5
Embarrassed or humiliated	0.2 (0.6)	16
Anticipated panic (range, 0-100)	23.4 (23.1)	72
Peak fear during inhalation (range, 0-100)	26.1 (26.1)	68
Actual panic (no, 0; yes, 1)	0.1 (0.3)	11
Peak breathlessness during inhalation (range, 0-100)	39.7 (25.2)	94
Average respiratory reaction (cluster 1)	0.9 (0.4)	99
Average cognitive reaction (cluster 2)	0.7 (0.6)	77
Average neurovegetative/physical reaction (cluster 3)	0.7 (0.3)	100
Average emotional/fear reaction (cluster 4)	0.2 (0.3)	44

Abbreviations: API, Acute Panic Inventory; CO_2, carbon dioxide.
[a] Superscripts within the column indicate the cluster (1, 2, 3, or 4) within which the reaction belongs.

Table 2. Regression Coefficients for Each Class of War-Zone Stress Symptoms

Predictor	War-Zone Stress Reactions		
	PTSD Symptoms	General Anxiety Symptoms	Depressive Symptoms
Average stressors	0.53[a]	2.19[a]	0.30
Changes in stressors	0.23[a]	1.50[a]	0.36[b]
Predeployment CO_2 reactivity	0.23	1.29	0.34
Average stress × CO_2 reactivity	0.06	1.72[c]	0.21
Changes in stress × CO_2 reactivity	0.18[a]	−0.40[d]	0.21
Time[a]	0.02	−0.06	0.07
Time × time	−0.02[a]	−0.08[a]	−0.04[a]
Sex	0.82	−4.93[b]	2.33[b]

Abbreviations: CO_2, carbon dioxide; PTSD, posttraumatic stress disorder.
[a] $P \leq .001$.
[b] $P < .05$.
[c] $P \leq .01$.
[d] $P < .07$.

ANALYTIC PLAN

Multilevel, mixed-effects random coefficient regression models (MRMs) were used to analyze the data. Our dependent variables were PTSD symptoms, general anxiety/stress symptoms, and depressive symptoms (together referred to as *war-zone stress reactions*), measured monthly during deployment. The predictors of war-zone stress reactions in the MRMs included time (months since deployment), time × time, war-zone stressors (assessed monthly during deployment), CO_2 reactivity (assessed before deployment), and the interaction between CO_2 reactivity and level of war-zone stressor exposure. All predictors (except time) were *z*-transformed to facilitate the interpretation of results. We used an unstructured covariance matrix to model the relationships between the random effects because all tested restrictions on the covariance matrix significantly increased the deviance statistics for the models.

Following Hedeker and Gibbons' recommendation,[48] we decomposed the monthly measure of war-zone stressors into a between-soldier effect (the mean level of stressors reported during the deployment period) and a within-soldier effect (the deviation from the mean level of stressors for each soldier at each point in time, referred to as *change in war-zone stressors*). Failing to decompose these effects would confound the between- and within-soldier effects, resulting in potentially misleading results.[48] Results of the model are reported in **Table 2**.

RESULTS

PARTICIPANTS

A total of 158 soldiers were included in the present analysis. Mean (SD) duration of deployment was 386 (71.75) days. Soldiers provided a total of 1021 monthly assessments with a mean (SD) of 6.5 (5.5) assessments per soldier (range, 1-16). Initial MRM analyses found no effects for race or ethnicity, so that variable was not included in the final analyses. Because sex was associated with the level of depressive symptoms, it was retained in the final models.

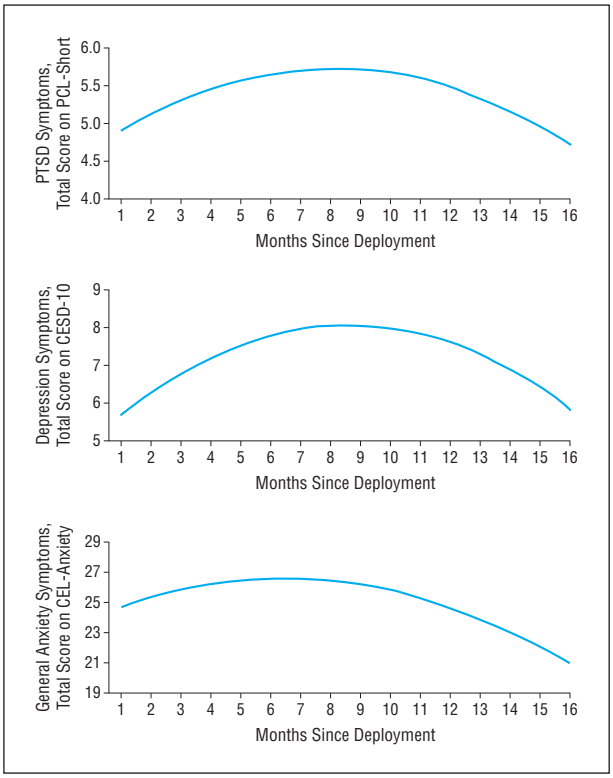

Figure 1. Growth curves of war-zone stress symptoms over time. CEL-Anxiety indicates the General Anxiety/Stress index included in the Combat Experiences Log[36]; CESD-10, the 10-item Center for Epidemiologic Studies Depression Scale[43]; and PCL-Short, the 4-item version of the PTSD (Posttraumatic Stress Disorder) Checklist.[41]

Responses to the CO_2 challenge varied widely (Table 1 reports the means and SDs on all the API items). Our primary measure of fear reactivity to the CO_2 challenge was the soldiers' highest level of fear in response to the challenge (possible range, 0-100). The level of fear reported ranged from 0 to 90, with a mean (SD) of 26.1 (26.2); 50 of the 158 soldiers (31.6%) reported no fear, and 18 soldiers (11.4%) reported actual panic in response to the challenge.

GROWTH CURVE ANALYSES OF WAR-ZONE STRESS REACTIONS OVER TIME

The quadratic growth curves for each measure during the 16-month deployment period are displayed in **Figure 1**. These curves are similar to those reported[36] in an analysis of a subset of these soldiers. The quadratic trend over time was significant for all 3 war-zone stress reactions: PTSD symptoms (b=−0.02, $P<.001$), general anxiety symptoms (b=−0.06, $P<.001$), and depression symptoms (b=−0.04, $P<.001$). The linear trend over time was significant only for general anxiety symptoms (b=−0.19, $P=.02$), indicating that anxiety symptoms near the end of deployment were lower than those at the beginning of deployment. In general, symptoms steadily increased immediately after deployment, but the increase peaked approximately 8 months into deployment and gradually returned to initial levels (or below, for general anxiety symptoms) by approximately 16 months after deployment.

MAIN EFFECTS OF STRESS AND CO_2 REACTIVITY ON WAR-ZONE STRESS REACTIONS IN THEATER

The MRM analyses indicated that soldiers who reported higher average war-zone stressors had higher levels of PTSD symptoms (b=0.53, P<.001) and higher general anxiety/stress symptoms (b=2.19, P=.001). Similarly, changes in a soldier's war-zone stressors were related to concomitant changes in PTSD symptoms, general anxiety/stress symptoms, and depression symptoms (b=0.23, P<.001; b=1.50, P<.001; and b=0.36, P=.02, respectively). Time from deployment was controlled in the MRM models; thus, relationships between changes in stress over time and changes in symptoms were not merely a result of both measures changing in concert over time. The CO_2 reactivity was not related to any of our war-zone stress reactions (P>.10 for all comparisons).

POTENTIATING EFFECTS OF PREDEPLOYMENT CO_2 REACTIVITY

We also investigated whether predeployment CO_2 reactivity would moderate the soldiers' response to stressful events as they occurred during deployment. Consistent with our hypotheses, predeployment CO_2 reactivity interacted with the soldiers' average stress level during deployment to affect anxiety symptoms (b = 0.17, P < .001). Predeployment CO_2 reactivity also interacted with monthly changes in war-zone stressors to affect PTSD symptoms and anxiety symptoms, although the relationship with anxiety symptoms did not reach conventional levels of significance (b = 0.18, P = .001 for PTSD symptoms and b = 0.40, P < .07 for anxiety symptoms). These interactions all indicated that soldiers with higher predeployment CO_2 reactivity responded to war-zone stressors with more symptoms than did soldiers with lower predeployment CO_2 reactivity. To examine the nature of these significant interactions, we used the approach described by Aiken and West[49] to compare soldiers with low predeployment CO_2 reactivity (1 SD below the mean) with those high in CO_2 reactivity (1 SD above the mean). In our sample, CO_2 reactivity ranged from 0 to 90 (scale, 0-100), with a mean (SD) of 26.1 (26.2). We examined the MRM-predicted relationship between war-zone stressors and war-zone stress symptoms for soldiers displaying low CO_2 reactivity compared with the model-predicted relationship between war-zone stressors and war-zone symptoms for soldiers displaying high CO_2 reactivity. This analytic approach has the advantage of probing the CO_2 reactivity × war-zone stress interaction using model-based predictions from the full sample as opposed to restricting the analysis to the subsample of soldiers who had either high or low CO_2 reactivity,

Consistent with prediction, for soldiers with low predeployment CO_2 reactivity, the model predicted no relationship between level of war-zone stressors and either PTSD symptoms (b = 0.05, P = .53) or anxiety symptoms (b = 0.47, P = .58) (**Figure 2** and **Figure 3**). However, for those with high predeployment CO_2 reactivity, the MRM predicted a strong relationship between the average level of war-zone stressors and PTSD symptoms (b = 0.41, P < .001) as well as anxiety symptoms (b = 3.91, P < .001) (Figures 2 and 3).

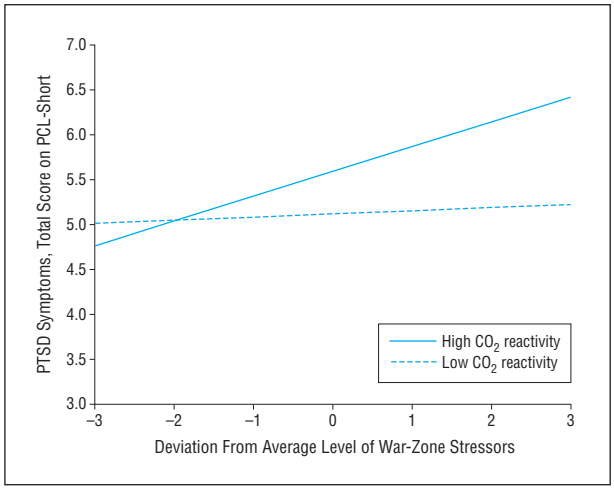

Figure 2. The effect of war-zone stressors on posttraumatic stress disorder (PTSD) symptoms for high and low carbon dioxide (CO_2) reactivity at predeployment. PCL-Short indicates the 4-item version of the PTSD Checklist.[41]

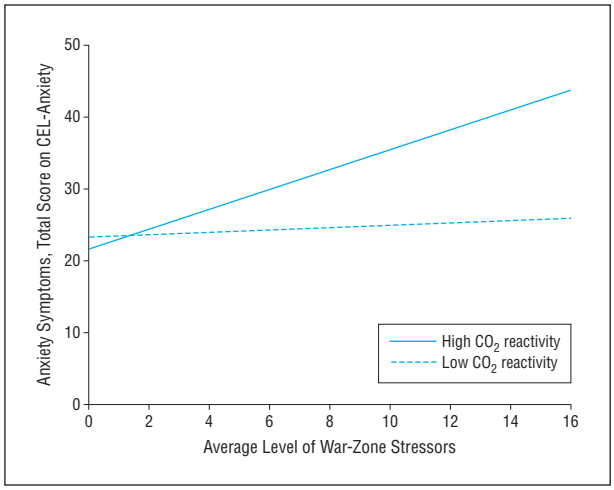

Figure 3. The effect of war-zone stressors on anxiety symptoms for high and low carbon dioxide (CO_2) reactivity at predeployment. CEL-Anxiety indicates the General Anxiety/Stress index included in the Combat Experiences Log.[36]

To assess the clinical significance of the potentiation effect of CO_2 peak fear on the relationship between war-zone stressors and PTSD and anxiety symptoms, we examined the differences in the expected outcomes for soldiers with high and low CO_2 reactivity (1 SD above the mean vs 1 SD below the mean). When war-zone stressors increased a moderate amount (by 2 stressors) in a particular month, the expected difference in PTSD symptoms between soldiers high and low in CO_2 reactivity was predicted to be 0.95, which is equivalent to a moderate to large effect size (Cohen d = 0.72). Similarly, for soldiers who experienced a moderately high level of stressors (6, equivalent to 1 SD above the mean number of stressors), the difference in anxiety symptoms predicted by the MRM for soldiers with high vs those with low CO_2 reactivity was 5.68, which is equivalent to a large effect size (Cohen d = 0.91).

Furthermore, we investigated whether the potentiating effects of CO_2 reactivity on the effect of war-zone stressors on PTSD symptoms, general anxiety, and depression was specifically related to CO_2 reactivity or merely a result of the relationship between CO_2 reactivity and other predeployment psychological variables (which could affect war-zone stress reactions). Thus, we repeated the MRM analyses, adding different relevant predeployment control variables (and their interactions with the average level of war-zone stressors and monthly changes in war-zone stressors) as additional predictors of the war-zone stress reactions. The control variables included in the model were trait anxiety (measured by the State-Trait Anxiety Inventory[50]) lifetime diagnosis of any Axis I disorder (yes/no), and current diagnosis of any Axis I disorder (yes/no). All the significant effects of war-zone stressors and CO_2 reactivity remained significant, even when each of these control variables (and their interactions with war-zone stressors) were added (separately) to the MRMs.

EXPLORATORY ANALYSES

Finally, we performed exploratory analyses to determine whether other API-derived CO_2 reaction indices explained the significant variance in symptoms more than what was explained by our primary index of CO_2-induced fear. Colasanti et al[47] used factor analysis to derive 3 separate CO_2-induced symptom clusters (respiratory, cognitive, and neurovegetative) based on responses of healthy volunteers to a double inhalation of 4 CO_2 mixtures. They found that the respiratory cluster was the best predictor of CO_2 fear/discomfort. Although the 29 CO_2 reaction items from the API do not directly map onto the 13 *DSM-IV* panic symptoms, many of the items on the API can be grouped into 1 of these 3 clusters either directly (the API item is virtually identical to the item used by Colasanti et al[47]) or conceptually (the item clearly fits into 1 these 3 clusters [Table 1]). Because the API included more items than the 13 *DSM-IV* symptoms, we created 1 additional response cluster (emotional/fear) to separate the cognitive cluster into 2 categories: cognitive and emotional/fear. This was done because some items reflected changes in perception that were not necessarily fearful (Do things and people seem unreal?), whereas others were primarily emotional/fear related (Were you afraid of dying?). We formed scale scores for the 4 clusters of CO_2 reactions by adding the items from the API that matched each category. Table 1 reports the means and SDs for these 4 CO_2 reaction scales. The internal consistencies of the 4 clusters of responses to CO_2 challenge were adequate: respiratory, $\alpha = 0.64$; cognitive, $\alpha = 0.78$; neurovegetative/physical, $\alpha = 0.79$; and emotional/fear, $\alpha = 0.73$.

To determine whether any of these reaction cluster scores explained significant variance in symptoms beyond what was explained by our primary index of CO_2-induced fear, we added each scale, in turn, to our MRMs for each of the 3 war-zone symptoms. Each scale was added as an additional predictor of outcome and as an additional moderator of the relationship between war-zone stressors and war-zone symptoms. Results showed that none of these scales explained significant variance beyond our original CO_2 peak fear index for either PTSD or general anxiety symptoms. However, higher cognitive, neurovegetative/physical, and emotional/fear responses to the CO_2 challenge were related to higher depression symptoms in theater ($b = 1.12$, $P = .003$; $b = 0.83$, $P = .04$; and $b = 0.96$, $P = .04$, respectively). In addition, higher scores on the emotional/fear composite interacted with changes in war-zone stressors over time ($b = 0.49$, $P = .005$), such that the relationship between war-zone stressors and depression symptoms was greater for soldiers with higher emotional/fear reactions to the CO_2 challenge.

COMMENT

We sought to test whether emotional reactivity to enriched CO_2 inhalation before deployment would predict soldiers' psychological adjustment while deployed in Iraq. A unique design feature of this study was the use of a web-based in-theater assessment of war-zone stress exposure and war-zone stress symptoms. To our knowledge, this is the first investigation to link a potentially modifiable predeployment risk factor to soldiers' level of exposure to war-zone stressors and war-zone stress reactions assessed repeatedly during soldiers' deployment.

The growth pattern of war-zone stress reactions over time was more complex than expected given previous reports[51] showing a positive association between war-zone stress reactions and length of deployment. Consistent with an earlier report[36] on a subset of soldiers from the present study, all 3 war-zone stress reactions—PTSD symptoms, depression symptoms, and general anxiety symptoms—showed a significant inverted U-pattern in their respective growth curves over time. Stress reactions increased during the first 8 months of deployment but decreased to their earlier levels (or below) during the final 8 months. Perhaps this pattern reflects the effects of habituation or an increased sense of mastery in response to the repeated confrontation of similar war-zone stressors.

Soldiers' reactions to CO_2 inhalation at the predeployment assessment were similar to those reported for nonclinical civilian samples.[52] The 3 most frequently reported physical reactions to CO_2 challenge were lightheadedness, feelings of faintness, and breathlessness—all expected reactions to acute hypercapnia. Soldiers' fear reactions to the CO_2 challenge varied markedly across soldiers, ranging from no fear (32%) to panic (11%), with the average soldier reporting mild fear. These data for CO_2-induced fear are in sharp contrast to those observed for patients with panic disorder, who report extreme fear, with more than 60% experiencing panic in response to the CO_2 challenge.[53]

In line with previous reports, soldiers reporting greater exposure to war-zone stressors reported higher levels of anxiety, depression, and PTSD symptoms. However, consistent with a stress-diathesis formulation, the impact of war-zone stressors on soldiers' psychological symptoms was potentiated by their emotional reactivity to CO_2 assessed before deployment. Specifically, soldiers displaying heightened reactivity to CO_2 before deployment re-

ported greater PTSD and anxiety symptoms in response to increased war-zone stressors relative to soldiers displaying low levels of predeployment CO_2 reactivity.

It is noteworthy that fear responding to CO_2 showed a significant potentiation effect for PTSD and anxiety symptoms but not for symptoms of depression. The observed specificity is consistent with studies[54] with civilian samples showing increased fear responding to CO_2 challenge among patients with anxiety but not those with clinical depression.

What might explain the observed relationship between heightened emotional response to CO_2 and the increased vulnerability to develop anxiety symptoms in response to war-zone stressors? One possibility is that CO_2 reactivity is simply serving as a proxy for soldiers' predeployment trait anxiety or the presence of past or current mental illness. We tested this possibility by adding soldiers' level of trait anxiety, presence of any past or current mental illness, and their interaction with stress exposure to our MRM models and found that CO_2 reactivity retained its predictive status even after controlling for these variables. These data suggest that the observed potentiation effect of CO_2 reactivity is not simply a consequence of its association with other psychological trait variables.

A second possibility is that heightened reactivity to CO_2 challenge represents a behavioral marker for a neurobiological hypersensitivity of one's suffocation alarm that would be triggered under times of heightened stress exposure.[55] Although the design of our study does not provide a stringent test of this formulation, our exploratory analyses showing that the respiratory reaction cluster did not account for additional variance in war-zone stress reactions beyond that found for CO_2-induced fear seems at odds with this formulation.

A third possibility is that CO_2 reactivity may function as a more specific vulnerability to respond fearfully to respiratory distress. Hyperventilation is a common reaction to stress in civilian[56] and military[57] samples. Because respiratory distress often occurs in response to stress-induced hyperventilation,[58] it would be expected that soldiers who respond fearfully to the respiratory distress elicited during 35% CO_2 challenge might be particularly likely to show increased anxiety symptoms in response to war-zone stressors. Although our data are consistent with this formulation, we cannot rule out the possibility that other genetic or neurobiological factors are responsible for the CO_2 challenge findings. For example, there is exciting emergent evidence in rodents linking neurobiological substrates and genetic markers, such as acid-sensing ion channels and the genes that code for them, to fear of suffocation associated with inhalation of CO_2.[59]

Several limitations of the study deserve comment. First, the small sample size may have limited our ability to detect significant relationships between CO_2 reactivity and depressive symptoms as well as our power to examine the stability of our findings across subgroups, such as women or racial/ethnic minority participants. Replication with a larger sample is warranted. Although participants were recruited from 10 different army units, we cannot rule out the possibility that our findings may not generalize to soldiers from outside Fort Hood.

The potential significance of these findings for the prevention of combat stress disorders deserves comment. Studies using civilian samples have shown that CO_2 reactivity can be lowered significantly with brief cognitive-behavioral interventions.[17] Given the safety and ease of administration of CO_2 challenge, it would be feasible to integrate these brief cognitive-behavioral interventions into the military with the aim of reducing fear responding to CO_2 challenge. Whether such training would reduce the development of PTSD and anxiety symptoms among soldiers deployed to a war zone awaits research.

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REFERENCES

1. Hoge CW, McGurk D, Thomas JL, Cox AL, Engel CC, Castro CA. Mild traumatic brain injury in U.S. soldiers returning from Iraq. *N Engl J Med*. 2008;358(5):453-463.
2. Hoge CW, Terhakopian A, Castro CA, Messer SC, Engel CC. Association of posttraumatic stress disorder with somatic symptoms, health care visits, and absenteeism among Iraq war veterans. *Am J Psychiatry*. 2007;164(1):150-153.
3. Chemtob CM, Hamada RS, Roitblat HL, Muraoka MY. Anger, impulsivity, and anger control in combat-related posttraumatic stress disorder. *J Consult Clin Psychol*. 1994;62(4):827-832.
4. Bremner JD, Southwick SM, Darnell A, Charney DS. Chronic PTSD in Vietnam combat veterans: course of illness and substance abuse. *Am J Psychiatry*. 1996;153(3):369-375.
5. Tanielian T, Jaycox LH, eds. *Invisible Wounds of War: Psychological and Cognitive Injuries, Their Consequences, and Services to Assist Recovery*. Santa Monica, CA: Rand Corp; 2008.
6. Savoca E, Rosenheck R. The civilian labor market experiences of Vietnam-era veterans: the influence of psychiatric disorders. *J Ment Health Policy Econ*. 2000;3(4):199-207.
7. Riggs DS, Byrne CA, Weathers FW, Litz BT. The quality of the intimate relationships of male Vietnam veterans: problems associated with posttraumatic stress disorder. *J Trauma Stress*. 1998;11(1):87-101.
8. MacDonald C, Chamberlain K, Long N, Flett R. Posttraumatic stress disorder and interpersonal functioning in Vietnam War veterans: a mediational model. *J Trauma Stress*. 1999;12(4):701-707.
9. Hendin H, Haas AP. Suicide and guilt as manifestations of PTSD in Vietnam combat veterans. *Am J Psychiatry*. 1991;148(5):586-591.
10. Kessler RC, Sonnega A, Bromet E, Hughes M, Nelson CB. Posttraumatic stress disorder in the National Comorbidity Survey. *Arch Gen Psychiatry*. 1995;52(12):1048-1060.
11. Kessler RC. The effects of stressful life events on depression. *Annu Rev Psychol*. 1997;48:191-214.

12. Gorman JM, Fyer MR, Goetz R, Askanazi J, Liebowitz MR, Fyer AJ, Kinney J, Klein DF. Ventilatory physiology of patients with panic disorder. *Arch Gen Psychiatry*. 1988;45(1):31-39.
13. Schmidt NB, Telch MJ, Jaimez TL. Biological challenge manipulation of PCO_2 levels: a test of Klein's (1993) suffocation alarm theory of panic. *J Abnorm Psychol*. 1996;105(3):446-454.
14. Freire RC, Lopes FL, Valença AM, Nascimento I, Veras AB, Mezzasalma MA, de-Melo-Neto VL, Zin WA, Nardi AE. Panic disorder respiratory subtype: a comparison between responses to hyperventilation and CO_2 challenge tests. *Psychiatry Res*. 2008;157(1-3):307-310.
15. Sanderson WC, Rapee RM, Barlow DH. The influence of an illusion of control on panic attacks induced via inhalation of 5.5% carbon dioxide–enriched air. *Arch Gen Psychiatry*. 1989;46(2):157-162.
16. Zucker D, Taylor CB, Brouillard M, Ehlers A, Margraf J, Telch M, Roth WT, Agras WS. Cognitive aspects of panic attacks: content, course and relationship to laboratory stressors. *Br J Psychiatry*. 1989;155(1):86-91.
17. Telch MJ, Smits JA, Brown M, Dement M, Powers MB, Lee H, Pai A. Effects of threat context and cardiac sensitivity on fear responding to a 35% CO_2 challenge: a test of the context-sensitivity panic vulnerability model. *J Behav Ther Exp Psychiatry*. 2010;41(4):365-372.
18. Griez E. Experimental induction of anxiety: the case of carbon dioxide [in French]. *Encephale*. 1987;13(6):335-339.
19. Griez EJ, Lousberg H, van den Hout MA, van der Molen GM. CO_2 vulnerability in panic disorder. *Psychiatry Res*. 1987;20(2):87-95.
20. Woods SW, Charney DS, Goodman WK, Heninger GR. Carbon dioxide–induced anxiety: behavioral, physiologic, and biochemical effects of carbon dioxide in patients with panic disorders and healthy subjects. *Arch Gen Psychiatry*. 1988; 45(1):43-52.
21. Perna G, Gabriele A, Caldirola D, Bellodi L. Hypersensitivity to inhalation of carbon dioxide and panic attacks. *Psychiatry Res*. 1995;57(3):267-273.
22. Battaglia M, Perna G. The 35% CO_2 challenge in panic disorder: optimization by receiver operating characteristic (ROC) analysis. *J Psychiatr Res*. 1995;29(2): 111-119.
23. Coryell W, Pine D, Fyer A, Klein D. Anxiety responses to CO_2 inhalation in subjects at high-risk for panic disorder. *J Affect Disord*. 2006;92(1):63-70.
24. van Beek N, Griez E. Reactivity to a 35% CO_2 challenge in healthy first-degree relatives of patients with panic disorder. *Biol Psychiatry*. 2000;47(9):830-835.
25. Bellodi L, Perna G, Caldirola D, Arancio C, Bertani A, Di Bella D. CO_2-induced panic attacks: a twin study. *Am J Psychiatry*. 1998;155(9):1184-1188.
26. Schmidt NB, Maner JK, Zvolensky MJ. Reactivity to challenge with carbon dioxide as a prospective predictor of panic attacks. *Psychiatry Res*. 2007;151 (1-2):173-176.
27. Perna G, Bertani A, Arancio C, Ronchi P, Bellodi L. Laboratory response of patients with panic and obsessive-compulsive disorders to 35% CO_2 challenges. *Am J Psychiatry*. 1995;152(1):85-89.
28. Caldirola D, Perna G, Arancio C, Bertani A, Bellodi L. The 35% CO_2 challenge test in patients with social phobia. *Psychiatry Res*. 1997;71(1):41-48.
29. Gorman JM, Papp LA, Martinez J, Goetz RR, Hollander E, Liebowitz MR, Jordan F. High-dose carbon dioxide challenge test in anxiety disorder patients. *Biol Psychiatry*. 1990;28(9):743-757.
30. Seddon K, Morris K, Bailey J, Potokar J, Rich A, Wilson S, Bettica P, Nutt DJ. Effects of 7.5% CO_2 challenge in generalized anxiety disorder. *J Psychopharmacol*. 2011;25(1):43-51.
31. Bailey JE, Kendrick A, Diaper A, Potokar JP, Nutt DJ. A validation of the 7.5% CO_2 model of GAD using paroxetine and lorazepam in healthy volunteers. *J Psychopharmacol*. 2007;21(1):42-49.
32. Perna G, Bussi R, Allevi L, Bellodi L. Sensitivity to 35% carbon dioxide in patients with generalized anxiety disorder. *J Clin Psychiatry*. 1999;60(6):379-384.
33. Talesnik B, Berzak E, Ben-Zion I, Kaplan Z, Benjamin J. Sensitivity to carbon dioxide in drug-naïve subjects with post-traumatic stress disorder. *J Psychiatr Res*. 2007;41(5):451-454.
34. Muhtz C, Yassouridis A, Daneshi J, Braun M, Kellner M. Acute panicogenic, anxiogenic and dissociative effects of carbon dioxide inhalation in patients with posttraumatic stress disorder (PTSD). *J Psychiatr Res*. 2011;45(7):989-993.
35. Beevers CG, Marti CN, Lee HJ, Stote DL, Ferrell RE, Hariri AR, Telch MJ. Associations between serotonin transporter gene promoter region (5-HTTLPR) polymorphism and gaze bias for emotional information. *J Abnorm Psychol*. 2011; 120(1):187-197.
36. Lee H-J, Goudarzi K, Baldwin B, Rosenfield D, Telch MJ. The Combat Experience Log: a web-based system for the in theater assessment of war zone stress. *J Anxiety Disord*. 2011;25(6):794-800.
37. Josephs RA, Telch MJ, Hixon JG, Evans JJ, Lee H, Knopik VS, McGeary JE, Hariri AR, Beevers CG. Genetic and hormonal sensitivity to threat: testing a serotonin transporter genotype x testosterone interaction. *Psychoneuroendocrinology*. 2011; 37(6):752-761.
38. Beevers CG, Lee HJ, Wells TT, Ellis AJ, Telch MJ. Association of predeployment gaze bias for emotion stimuli with later symptoms of PTSD and depression in soldiers deployed in Iraq. *Am J Psychiatry*. 2011;168(7):735-741.
39. Vasterling JJ, Proctor SP, Amoroso P, Kane R, Gackstetter G, Ryan MA, Friedman MJ. The Neurocognition Deployment Health Study: a prospective cohort study of Army soldiers. *Mil Med*. 2006;171(3):253-260.
40. King DW, King LA, Vogt DS. *Manual for the Deployment Risk and Resilience Inventory (DRRI): A Collection of Measures for Studying Deployment Related Experiences of Military Veterans*. Boston, MA: National Center for PTSD; 2003.
41. Bliese PD, Wright KM, Adler AB, Cabrera O, Castro CA, Hoge CW. Validating the primary care Posttraumatic Stress Disorder Screen and the Posttraumatic Stress Disorder Checklist with soldiers returning from combat. *J Consult Clin Psychol*. 2008;76(2):272-281.
42. Andresen EM, Malmgren JA, Carter WB, Patrick DL. Screening for depression in well older adults: evaluation of a short form of the CES-D (Center for Epidemiologic Studies Depression Scale). *Am J Prev Med*. 1994;10(2):77-84.
43. Eaton W, Smith C, Ybarra M, Muntaner C, Tien A. Center for Epidemiologic Studies Depression Scale: review and revision (CESD and CESD-R). In: Maruish M, ed. *The Use of Psychological Testing for Treatment Planning and Outcomes Assessment*. Vol 3. 3rd ed. Mahwah, NJ: Lawrence Erlbaum Associates Inc; 2004:363-377.
44. Harrington PJ, Schmidt NB, Telch MJ. Prospective evaluation of panic potentiation following 35% CO_2 challenge in nonclinical subjects. *Am J Psychiatry*. 1996; 153(6):823-825.
45. Telch MJ, Harrington PJ, Smits JA, Powers MB. Unexpected arousal, anxiety sensitivity, and their interaction on CO_2-induced panic: further evidence for the context-sensitivity vulnerability model. *J Anxiety Disord*. 2011;25(5):645-653.
46. Liebowitz MR, Gorman JM, Fyer AJ, Dillon DJ, Klein DF. Effects of naloxone on patients with panic attacks. *Am J Psychiatry*. 1984;141(8):995-997.
47. Colasanti A, Salamon E, Schruers K, van Diest R, van Duinen M, Griez EJ. Carbon dioxide–induced emotion and respiratory symptoms in healthy volunteers. *Neuropsychopharmacology*. 2008;33(13):3103-3110.
48. Hedeker D, Gibbon RD. *Longitudinal Data Analysis*. Hoboken, NJ: Wiley-Interscience; 2006.
49. Aiken LS, West SG. *Multiple Regression: Testing and Interpreting Interactions*. Thousand Oaks, CA: Sage Publications Inc; 1991.
50. Spielberger CD, Gorssuch RL, Lushene PR, Vagg PR, Jacobs GA. *Manual for the State-Trait Anxiety Inventory*. Palo Alto, CA: Consulting Psychologists Press Inc; 1983.
51. Shen YC, Arkes J, Pilgrim J. The effects of deployment intensity on posttraumatic stress disorder: 2002-2006. *Mil Med*. 2009;174(3):217-223.
52. Griez EJ, Colasanti A, van Diest R, Salamon E, Schruers K. Carbon dioxide inhalation induces dose-dependent and age-related negative affectivity. *PLoS One*. 2007;2(10):e987. doi:10.1371/journal.pone.0000987.
53. Perna G, Romano P, Caldirola D, Cucchi M, Bellodi L. Anxiety sensitivity and 35% CO_2 reactivity in patients with panic disorder. *J Psychosom Res*. 2003;54(6): 573-577.
54. Perna G, Barbini B, Cocchi S, Bertani A, Gasperini M. 35% CO_2 challenge in panic and mood disorders. *J Affect Disord*. 1995;33(3):189-194.
55. Klein DF. Testing the suffocation false alarm theory of panic disorder. *Anxiety*. 1994;1(1):1-7.
56. Suess WM, Alexander AB, Smith DD, Sweeney HW, Marion RJ. The effects of psychological stress on respiration: a preliminary study of anxiety and hyperventilation. *Psychophysiology*. 1980;17(6):535-540.
57. Solomon Z. Somatic complaints, stress reaction, and posttraumatic stress disorder: a three-year follow-up study. *Behav Med*. 1988;14(4):179-185.
58. Griez E, Perna G. Respiration and anxiety. In: Nutt D, Ballinger J, eds. *Anxiety Disorders*. Oxford, England: Blackwell Science Ltd; 2007.
59. Maren S. An acid-sensing channel sows fear and panic. *Cell*. 2009;139(5):867-869.