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Effects of Depressive & Anxious Symptoms on Norepinephrine and Platelet P-Selectin Responses to Acute Psychological Stress among Elderly Caregivers

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Abstract

Background—Caring for a spouse with Alzheimer's disease is associated with increased psychological distress, impaired immunity, and heightened cardiovascular risk. Hyperreactivity of sympathetic and platelet activation responses to acute psychological stress, or the failure to recover quickly from stressful events, may constitute an important pathway linking stress and negative affect with cardiovascular disease (CVD).

Objectives—1. To evaluate associations between negative affect (i.e., depressive and anxious symptoms) with increased norepinephrine and P-selectin responses to an acute psychological stress task, 2. To establish whether these associations are augmented among elderly spousal caregivers (CG) compared to non-caregivers (NC).

Methods—Depressive (DEP) and anxious (ANX) symptoms from the Brief Symptom Inventory were assessed among 39 CG & 31 NC. Plasma norepinephrine levels (NE) and percent platelet P-selectin (PSEL) expression were assayed at 3 time-points: rest, immediately following a laboratory speech test (reactivity), and after 14 minutes of recovery.

Results—Among CG, but not NC, increased symptoms of depression and anxiety were associated with delayed NE recovery (DEP: β=.460, p=.008; ANX: β=.361, p=.034), increased PSEL reactivity (DEP: β=.703, p<.001; ANX: β=.526, p=.002), and delayed PSEL recovery (DEP: β=.372, p=.039; ANX: β=.295, p=.092), while controlling for age, gender, aspirin use, antidepressant use, and preexisting CVD. Bivariate correlations showed delayed NE recovery was also associated with increased PSEL reactivity (r=.416) and delayed PSEL recovery (r=.372; all p's<.05) among CG but not NC.

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Discussion—Among chronically stressed caregivers, increased levels of depressive and anxious symptoms are associated with prolonged sympathetic activation and pronounced platelet activation. These changes may represent one pathway linking caregiving stress to cardiovascular risk.

Keywords

Coagulation; hemostasis; platelet hyperreactivity; prolonged platelet activation; chronic stress; cardiovascular disease; atherosclerosis; catecholamines; sympathetic nervous system; Alzheimer's disease; dementia; psychocardiology; negative affect

Introduction

Caregivers (CG) for a spouse with Alzheimer's disease face the chronic stress of anticipatory grief and coping with problematic changes in a spouse's personality and behavior (Acton and Kang, 2001; Chatterjee et al., 1992; Diwan et al., 2004; Vitaliano et al., 2003). Dementia CG also experience higher levels of psychological distress, negative affect (Schulz et al., 1995), sympathetic activity(Mills et al., 1997), proinflammatory activity(Kiecolt-Glaser et al., 1991), and cardiovascular disease risk(Shaw et al., 1999; Vitaliano et al., 2003) relative to their non-caregiving peers (NC). Caregivers who report greater subjective strain exhibit a 63% increased mortality risk(Schulz and Beach, 1999). Although the exact mechanisms underlying increased morbidity and mortality are unknown, elevated sympathetic tone and hypercoagulability may be contributing factors.

Some evidence suggests that chronic stress may sensitize acute stress responses as a means of allowing organisms to better adapt to the demands of their environment(Aschbacher et al., 2006; Chrousos, 1998; Ma and Morilak, 2005; Pike et al., 1997). Moreover, it has been proposed that changes in stress-induced release of NE or related alterations in alpha-adrenergic receptor sensitization may facilitate the adaptation to chronic stress(Ma and Morilak, 2005). Animal studies have found that chronic stress alters brain NE activity, thereby enhancing NE reactivity to novel stressors (Nisenbaum et al., 1991) and decreasing NE turnover (Roth et al., 1982). Previous research has raised the question of whether CG have elevated SNS arousal compared to NC(Mills et al., 2004; Mills et al., 1997). Although, to our knowledge, NE hyperreactivity among CG relative to NC has not specifically been shown, NE surge in response to acute stress is an outcome of particular interest, given its potential contribution to acute coronary events(Kop, 1999; von Känel and Dimsdale, 2000; von Känel et al., 2002).

The notion that emotional stress elicits hypercoagulability via catecholamine surges was first proposed almost a century ago(Cannon and Mendenhall, 1914), but remains a topic of relevance in psychosomatic research today(von Känel and Dimsdale, 2000). Symptoms of negative affect have been associated elevated NE and cardiovascular reactivity to acute stress (Light et al., 1998; Mausbach et al., 2005; Suarez et al., 1998; von Känel et al., 2004a), as well as with heightened risk of cardiovascular morbidity and mortality (Brydon et al., 2006; Hamaad et al., 2003; Nemeroff and Musselman, 2000; Strike and Steptoe, 2003). Platelets may play an important role in understanding the mechanisms that underlie the relations among negative affect, stress, NE, and CVD.

Specifically, depressive symptoms, hostility and psychological stress are associated with platelet hyperreactivity (Lederbogen et al., 2004; Markovitz et al., 1996; Musselman et al., 1996; von Känel, 2004) and prolonged platelet activation (Strike et al., 2004). While little is known about the relationship between anxious symptoms and platelet hyperactivity, the association is of considerable interest given that anxious symptoms have been linked to elevated NE reactivity(Bremner et al., 1996; Cameron et al., 1996), down-regulation of alpha-adrenoreceptor density on platelets(Cameron et al., 1996; Freedman et al., 1990), hemostatic

hyperreactivity (von Känel et al., 2004a; von Känel et al., 2006b), and cardiovascular morbidity and mortality(Eaker et al., 1992; Frasure-Smith et al., 1995; Kawachi et al., 1994). Taken together, these findings imply that heightened sympathetic nervous system activity (SNS) could potentially mediate associations between negative affect (hencefore referring to depressive and anxious symptoms) and platelet reactivity (Light et al., 1998; Nemeroff and Musselman, 2000). Such research would help better understand the mechanisms underlying the development of CVD.

Previous researchers have suggested that the amount of NE secreted during stress in humans is sufficient to elicit platelet activation and aggregation, and that this process may be an important mechanism underlying CVD (Ardlie et al., 1985; Birk et al., 2003; Haft et al., 1972). The ex vivo application of catecholamines causes platelet activation, with some evidence suggesting a dose-dependent relationship(O'Brien, 1963; Olbrich et al., 1989), implying that greater in vivo NE responses to acute stress would likely be associated with an increased platelet response (i.e., increased hyperactivity). Although epinephrine has been more frequently studied as a platelet activator, NE may be of greater pathophysiological interest due to the physiological concentrations required to cause platelet activation (Larsson et al., 1994). Moreover, in vivo infusions of NE at physiologically plausible levels produces increased platelet aggregability(Larsson et al., 1994). In animals, prolonged NE infusion results in aggregated platelets and occlusive platelet thrombi in the small myocardial vessels(Haft et al., 1972). Such findings suggest that the duration of NE stimulation (i.e., NE recovery) may influence the degree of platelet response. Moreover, as concentrations of platelet-leukocyte aggregates can remain elevated for 30 minutes or longer in response to acute stress(Strike et al., 2004), the duration of both NE and platelet responses may be of potential interest.

The cell adhesion molecule P-selectin, expressed on the surface of activated platelets under conditions of stress, serves as a marker of platelet activation (Michelson, 2006). P-selectin plays numerous important roles, such as facilitating the formation of leukocyte-platelet aggregates, promoting atherogenic interactions between platelets and the vascular endothelium, and increasing the size and stability of platelet aggregates (Blann et al., 2003; Merten and Thiagarajan, 2000). Moreover, research suggests that P-selectin may have predictive value as a marker of vascular damage associated with hypertension, stroke, and acute coronary syndromes (Itoh et al., 1995; Thakore et al., 2007; Verhaar et al., 1998; Zeller et al., 2005) and cardiac mortality in older atrial fibrillation patients (Heeringa et al., 2006).

Platelet hyperactivity among CG has not been previously described. However, if we accept the notion that CG tend to have systems in which acute stress responses are “sensitized” by chronic stress, it is conceivable that the associations among affect, NE surge, and platelet hyperactivity may be enhanced or more readily detectable among CG. Thus, this study hypothesized that associations of negative affect with increased norepinephrine and P-selectin responses to an acute psychological stress task would be greater among caregivers than among non-caregivers. Moreover, it was hypothesized that an increased magnitude and prolonged elevation of the norepinephrine response among caregivers would be associated with increased magnitude (i.e., ‘platelet hyperreactivity’) and a slower recovery (i.e., ‘prolonged platelet activation’) of the P-selectin response.

Methods

Participants

Thirty-nine caregivers providing in-home care for spouses with Alzheimer's disease (AD) and 31 gender-matched non-caregivers participated in the study. On average, caregivers' spouses had received the AD diagnosis 9 years previously. The mean age of all participants was 70 years, 47 (67%) of the participants were women, and 65 (93%) were Caucasian. Participants

taking anticoagulants (e.g., coumadin), antiplatelet drugs (e.g., clopidogrel), and beta-blockers were originally excluded from the study, given the effects of such drugs on sympathetic and coagulant activity; however, two non-caregivers and one caregiver began taking beta-blockers following enrollment. two caregivers were also taking alpha-blockers. No participants were taking anti-platelet or anti-coagulant drugs. Data on P-selectin expression following the acute speech stress were not available for two caregivers. All volunteers provided written consent to participate in the study, which was approved by the University of California, San Diego Institutional Review Board.

Stressor & Blood Draw Procedure

To provoke cardiovascular reactivity, participants were requested to prepare a 3-minute impromptu speech on one of two possible topics involving conflicts of an interpersonal nature (e.g., “the stolen belt paradigm”(Saab et al., 1992) that have previously been shown to exhibit similar stress reactivity (Adler et al., 2002; Mills et al., 1995; von Känel et al., 2004b). To maximize ecological validity, the stress tests were conducted in the participants' homes by trained research nurses. Blood was drawn using an indwelling 22-gauge venous forearm catheter at 3 points: baseline (following 20-minutes of quiet rest), speech (immediately following the stress test), and recovery (14-minutes following the speech task). This recovery period was selected based on the balance of two factors: 1. minimizing participant burden associated with asking elderly participants to sit for extended periods, and 2. recent research demonstrating that the hemostatic factors D-dimer and fibrinogen returned to baseline in less than 20 minutes in the absence of hypertension (Wirtz et al., 2007). The first 2 ml of blood was discarded to control for the potential effects of artificial platelet activation evoked by drawing blood.

Norepinephrine Levels

Blood samples were spun and plasma was frozen at -80° until assay. Norepinephrine levels were determined using a catechol-o-methyltransferase (COMT)-based radioenzymatic assay with a preconcentration step that extracted norepinephrine from 1 ml plasma and concentrated it in 0.1 ml of dilute acid. The assay is 10 times as sensitive as prior methods. The assay procedure concentrates catecholamines from plasma with 81% efficiency and also removes components of plasma such as Ca^{++} that inhibit the COMT assay. The inter-assay of coefficient of variation is 11% and the intra-assay coefficient of variation is 6.5% (Kennedy and Ziegler, 1990a). This technique is considered highly sensitive relative to standard catecholamine assays (Kennedy and Ziegler, 1990b).

Platelet Measures & Assay Procedure

Flow cytometry (Harrison, 2000; Michelson and Furman, 1999) was utilized to assess the percentage of platelets expressing the cell adhesion molecule P-selectin, a well-recognized surface marker of platelet activation (Blann et al., 2003). Two fluorochrome-conjugated antibodies that bind membrane glycoproteins on the activated platelet surface were utilized: 1. CD61-PerCP (an activation-independent marker of platelets), and 2. CD62P-PE (a marker of platelet P-selectin). Blood was stained with antibodies and fixed immediately after each blood draw in order to avoid in vitro platelet activation, and samples were brought back to the lab for flow cytometry analyses. Five μl of whole blood was incubated with 30 μl of fluorochrome-conjugated antibodies (Becton Dickenson Immunocytometry Systems, San Jose, CA) at their pre-determined saturating concentrations for 15 minutes in the dark at room temperature. Samples were fixed with 1 ml 1% formaldehyde in PBS containing 0.1% NaN_3. Samples were analyzed within 24 hours of staining using a Beckman Coulter EPICS Elite flow cytometer and Expo32 Software. Platelets were identified by forward and side scatter and distinguished from red blood cells by the presence of the CD61 antigen. Ten thousand events were collected

per tube. Single platelets were distinguished from aggregated platelets by size discrimination using forward scatter and gated separately (Linden et al., 2004; Shattil et al., 1987). Initially, PSEL expression was assessed separately on singulates and aggregates. However, given the correlation between P-selectin expressed on singulates and aggregates ranged from .85 (for circulating levels) to .97 (following the speech test), they were averaged to create a single measure of P-selectin expression.

Depressive & Anxious Symptoms

The depressive and anxious symptom subscales from the Brief Symptom Inventory (BSI) were utilized to characterize negative affect (Derogatis, 1993). The depression and anxiety subscales consist of 6 items each, rated on a 5-point Likert scale (responses range from “not at all” to “extremely”), with good internal consistency (.85 and .81 respectively) and test-retest reliability (.84 and .79). All BSI subscales have been normed in the elderly (Hale et al., 1984). Studies among similar samples of community-dwelling elderly caregivers and non-caregivers, speak to the validity of the BSI depressive subscale in gerontological research (Stukenberg et al., 1990). Although the anxiety subscale is less well-researched among older adults, some evidence is available supporting its construct and divergent validity from the BSI depression subscale among older adults(Chester, 2001), and suggesting good convergent validity with other anxiety measures (Osman et al., 1997).

Role Overload

In order to provide an assessment of global burden associated with life stressors, all participants completed the Role Overload scale developed by Pearlin (Pearlin et al., 1990). This scale includes 4 items rated on a 4-point scale(1 = “not at all” to 4 = “completely”), where scores are summed, and higher scores indicate greater stress.

Participant Health

The self-reported medical histories of all participants were assessed during in-home interviews on a separate day from the blood draw. Reports of preexisting cardiovascular conditions - i.e., physician's diagnosis of cerebrovascular disease (previous stroke or transient ischemic attack), coronary artery disease (CAD; previous myocardial infarction (MI), angina, coronary artery bypass surgery, other unspecified CAD), diabetes, hypertension, or hypercholesterolemia were taken. An aggregate variable “CVD” was formed to represent the presence of any of the aforementioned conditions (Table 1). Additionally, the use of aspirin (both prescription and non-prescription) and any antidepressant medication (i.e., selective serotonin reuptake inhibitors, atypicals, tricyclics) were noted.

Data Analysis

The primary hypotheses were tested using multiple regression analyses in SPSS 13.0 for Windows. In order to obtain normal distributions, all norepinephrine (NE) values were square-root transformed, and all P-selectin (PSEL) values were log-transformed (raw values presented in Table 1). The distributions for depressive and anxious symptoms did not significantly differ from normality according to the Kolmogorov-Smirnov test (K-S)(Maxwell and Delaney, 2004). Reactivity and recovery variables for NE and PSEL were formed by forming standardized residual scores in SPSS, and K-S tests confirmed that none of these variables exhibited significant non-normality. “Reactivity” was conceptualized as the magnitude of the increase from baseline to speech, whereas “recovery” was conceptualized as a participant's ability to quickly recover, or return to a resting level of functioning, following a stressful event. Reactivity scores represent the value drawn immediately following the speech test regressed on the basal value, such that participants with positive reactivity scores had higher than average elevations from baseline to speech, and participants with negative reactivity scores had lower

than average elevations from baseline to speech. Similarly, the residualized recovery scores were defined as the recovery value (14-minutes following the speech) regressed on the baseline. Thus, participants with positive recovery scores remained elevated in recovery relative to the sample average. In order to evaluate whether the presence of 5 participants taking either alpha- or beta-blocking medications would impact the results, analyses were conducted with and without these 5 participants. Given that the overall pattern of significance in the results did not differ, we report the results of the full sample.

Results

Caregiver versus Non-Caregiver Comparisons

Caregivers (CG) had significantly higher levels of depressive and anxious symptoms than non-caregivers (NC) (all p's $\leq .01$), as well as significantly greater scores on role overload and raw NE levels following acute stress (Table 1). CG and NC did not significantly differ on age, gender, the prevalence of physician-diagnosed cardiovascular disease, aspirin use, or the use of antidepressant drugs (includes selective serotonin reuptake inhibitors (SSRIs), atypicals, and tricyclics) (Table 1).

Negative Affect & Norepinephrine Response to Acute Stress

Table 2, which depicts bivariate correlations among measures of negative affect (depressive and anxious symptoms), NE (reactivity & recovery), and PSEL (reactivity & recovery), demonstrates that the overall pattern of associations differs between CG and NC. In the first series of regression analyses, regressions among caregivers only were conducted to determine whether the associations between negative affect and NE responses were maintained when controlling for age, gender, aspirin use, antidepressant use, and the presence of CVD. All covariates were entered in block 1, mood in block 2, and NE reactivity or recovery was entered as the dependent variable.

Regression analyses revealed that among CG, increased symptoms of depression (β=.460, $t(32)$=2.830, p=.008) and anxiety (β=.361, $t(32)$=2.221, p=.034) were significantly associated with delayed NE recovery (i.e., slower return to baseline) following the acute stress test, while controlling for age, gender, aspirin use, antidepressant use, and preexisting CVD. Greater depressive, but not anxious, symptoms were associated with a trend toward increased NE reactivity among CG (β=.313, $t(32)$=1.904, p=.066). No significant associations between any negative affect and any NE measure were found among NC.

Negative Affect & P-Selectin Response to Acute Stress

Among CG, the magnitude of PSEL reactivity to stress remained strongly associated with higher levels of depressive symptoms (β=.703, $t(302)$=5.135, p<.001) and anxiety symptoms (β=.526, $t(30)$=3.397, p=.002), while controlling for all covariates. Similarly, PSEL expression in recovery remained elevated among CG with greater depressive symptoms (β=.372, $t(32)$=2.149, p=.039) and exhibited a similar trend among those with elevated anxiety symptoms (β=.295, $t(32)$=1.738, p=.092). Among non-caregivers, although bivariate correlations (Table 2) had suggested that there might be a significant negative association between anxious symptoms and PSEL recovery, this association was no longer significant (p>.10) when controlling for all covariates. Moreover, for NC, the use of antidepressants was associated with significantly decreased PSEL reactivity (e.g., in analysis of DEP; β= -.430, $t(30)$=-2.069, p=.049). All of the regression analyses were repeated after removing the 5 participants who were taking either beta or alpha blockers, and the overall pattern of significance in the results did not change.

Role Overload in relation to Norepinephrine & P-Selectin Responses

Regression analyses of the relations between role overload and all NE and PSEL response variables, while controlling for all covariates, yielded the same pattern of significance as depicted in Table 2. Among CG, when controlling for all covariates, overload remained significantly associated with NE recovery (β=.387, t(32)= 2.355, *p*=.025) and PSEL reactivity (β=.477, t(32)= 2.919, *p*=.007). In the interests of brevity, the remaining non-significant associations are not reported. Notably, overload was not significantly associated with any physiological outcome among NC.

Post-Hoc Mediation Analyses

Post-hoc analyses were conducted to better understand the differential pattern of results among CG versus NC. Noting that overload was associated with PSEL reactivity among CG but not NC, we hypothesized that chronic caregiving stress might lead to an increase in depressive symptoms, which in turn would be associated with elevated PSEL reactivity. Thus, we investigated whether DEP would mediate the association between overload and PSEL reactivity among CG. According to standard statistical procedures (Cohen et al., 2003), both DEP and overload were centered by subtracting the respective mean from each individual score in order to minimize collinearity between these predictors. First, we verified that overload remained a significant predictor of DEP among CG when adjusting for all covariates (β=.522, t(32)= 3.663, *p*=.001). Next, all covariates were entered in the first block of the regression model, with centered overload and DEP in the second. Consistent with a mediation model (Baron and Kenny, 1986), the significance of overload as a predictor of PSEL reactivity disappeared upon the inclusion of DEP (β=.144, t(29)= .891, *p*=.38), while DEP remained a significant predictor of PSEL reactivity (β=.624, t(29)= 3.821, *p*=.001). The Sobel test of mediation (Sobel, 1982) revealed that DEP significantly mediated the relationship between overload and PSEL reactivity (z=2.644, p=.008). According to Holmbeck's algorithm (Holmbeck, 1997, 2002), the magnitude of the indirect effect was 69.60, indicating that depressive symptoms accounted for 70% of the relationship between overload and PSEL reactivity among caregivers.

The possibility that NE reactivity might act as a mediator of the relationship between depressive symptoms and PSEL recovery among caregivers was also investigated. All covariates were entered in the first block of the regression model, with NE reactivity and centered depression in the second. If a mediation effect were present, it would be expected that the significance of depression as a predictor of PSEL recovery would decline substantially in the presence of NE reactivity (Baron and Kenny, 1986). Indeed, both centered depression (β= .290, t(31)=1.614, *p*=.117) and NE reactivity (β= .261, t(31)= 1.429, *p*=.163) became non-significant predictors of PSEL recovery among caregivers, when entered simultaneously. The Sobel test of mediation (Sobel, 1982) was non-significant (z=1.143, p=.25); however, research suggests that this test may have insufficient power in small samples (MacKinnon et al., 2002). As the potency of NE reactivity as a potential mediator may be relevant to power concerns in future studies, we calculated the percentage of the total effect attributable to potential mediation effects (i.e., the indirect effect), per Holmbeck's algorithm(Holmbeck, 1997, 2002). The indirect effect suggested that NE reactivity accounted for 22% of the total relationship between depressive symptoms and P-selectin recovery.

Discussion

This study found that elderly caregivers of spouses with Alzheimer's disease who endorsed increased levels of depressive and anxious symptoms exhibited hyperreactivity of platelet P-selectin expression immediately following exposure to an acute psychological stressor, as well as elevated norepinephrine and P-selectin recovery values 14 minutes following the stressor.

The fact that a similarly-sized gender and age-matched non-caregiving group did not exhibit this pattern of associations may indicate that the chronic stress of caregiving renders elderly caregivers more susceptible to mood-related increases in sympathetic and procoagulant activity. In support of this interpretation, post-hoc analyses revealed that among caregivers, depressive symptoms accounted for 70% of the association between overload, a measure of global burden, and P-selectin reactivity, a significant mediation effect. However, this finding should not be over-interpreted until replicated with longitudinal data. Relative to their non-caregiving peers, caregivers were found to have significantly elevated norepinephrine reactivity, independent of mood symptoms. Theoretically, the failure of norepinephrine levels to return to basal levels among caregivers with greater psychological distress could reflect overall prolonged sympathetic stimulation and resulting increases in cardiovascular risk. This study also found evidence that elevated norepinephrine was associated with increased P-selectin expression on activated platelets among caregivers. Elevated P-selectin may contribute to the initial development and evolution of atherosclerotic plaques (Blann et al., 2003; Prescott et al., 1996) and increase the likelihood of atherothrombotic events (Libby et al., 2002; Merten and Thiagarajan, 2000). In sum, these findings demonstrate the links between chronic caregiving stress, mood, sympathetic activation and platelet activation in response to acute psychological stress.

The current study extends the previous research demonstrating an association between depressive symptoms and norepinephrine reactivity among elderly dementia caregivers (Mausbach et al., 2005) in several important ways. This study demonstrates that negative affect not only affects norepinephrine reactivity, but may result in prolonged elevations in norepinephrine, or a failure to quickly return to baseline levels. Moreover, whereas anxious symptoms were not significantly associated with norepinephrine reactivity, they were associated with impaired recovery. Theoretically, prolonged norepinephrine stimulation could provoke a stronger procoagulant response as well as potentially amplifying the response to other platelet agonists, including 5-hydroxytryptamine (serotonin)(Ardlie et al., 1985), that may be altered in major affective disorders. One previous review of cardiovascular reactivity suggested that prolonged activation may be of equal or greater importance than hyperreactivity, given that cardiovascular responses to stress are likely most deleterious when prolonged (Schwartz et al., 2003). Moreover, the current study demonstrates that negative affect, norepinephrine responses, and platelet P-selectin activation in response to acute stress are all interrelated among caregivers. Although this study does not conclusively demonstrate mediational effects by the sympathetic nervous system, the fact that both depressive symptoms and norepinephrine reactivity became non-significant when simultaneously entered as predictors of P-selectin recovery remains consistent with a model in which the relationship between negative affect and platelet P-selectin response to acute stress may be partially mediated by norepinephrine reactivity or recovery. However, such models remain speculative, bearing further examination with larger samples and longitudinal data.

An important contribution of the current study is the finding that the interrelations between negative affect, norepinephrine, and P-selectin are significant among caregivers, but not among non-caregivers. This supports previous research indicating that chronic stress may be associated not only with increased psychological distress, but also with elevated sympathetic nervous system, immune, and procoagulant activity (Kiecolt-Glaser et al., 1996; McEwen, 1998; Vitaliano et al., 2003; von Känel et al., 2006a). It remains possible that the lack of significant associations between negative affect and either norepinephrine or P-selectin responses in the non-caregiving sample may be due in part to the restricted range of depressive and anxious symptoms in this group. However, the fact that the effect size was quite large among caregivers (equivalent to an r of .7), but not even close to statistical significance among non-caregivers, speaks against this interpretation. Moreover, overload was associated with P-selectin responses among caregivers but not among non-caregivers, and depressive symptoms

significantly mediated this relationship among caregivers in post-hoc analyses. These findings further support the interpretation that the relationship between depressive symptoms and P-selectin among caregivers reflects a state of physiological dysregulation due to chronic stress.

The finding that norepinephrine and P-selectin were significantly associated among caregivers but not non-caregivers suggests the potential utility of assessing platelet-serotonin interactions or other synergistic agonists in future research. Catecholamines are typically described as "weak" platelet agonists that act primarily in a synergistic fashion, by potentiating the activity of other agonists(Birk et al., 2003; Jin and Kunapuli, 1998; Olbrich et al., 1989). Thus, catecholamines might evoke weak or undetectable activation in some individuals and large responses in others, depending on other unidentified synergistic agonists or amplification via secretion of intracellular platelet granules, whose combined effects *in vivo* are not well-characterized(Birk et al., 2003; De Clerck et al., 1988; Hergovich et al., 2000; Jin and Kunapuli, 1998; Li et al., 1997). Previous studies have linked a functional polymorphism in the promoter region of the serotonin transporter gene (5HTTLPR) to depression, stress, 24-hour urinary norepinephrine excretion, cardiovascular reactivity and elevated platelet activation (Otte et al., 2007; Whyte et al., 2001; Williams et al., 2001). Human platelets express serotonin transporter sites that are genetically identical to those expressed in the brain(Lesch et al., 1993). Moreover, evidence suggests that stress interacts with this polymorphism in predicting depression(Caspi et al., 2003). While purely speculative and outside the scope of the current study, this suggests the possibility that caregiving stress predisposes genetically susceptible individuals to higher levels of negative affect, norepinephine levels and platelet activation, in part via serotonergic mechanisms, offering a promising topic for future study. Alternatively, it could also be that because non-caregivers mounted a significantly lower norepinephrine peak response during the speech task than caregivers (p=.03), the amount of norepinephrine spillover in non-caregivers might have to too low to elicit a statistically significant PSEL response. Some evidence suggests that weak stimulation may not be sufficient to cause platelets to secrete their dense granules, which contain components important for potentiating responses and recruiting platelets to sites of injury(Murugappan et al., 2004); however, further research is needed to confirm these speculations.

While the current study broadly controls for the influence of antidepressant drugs, due to sample size limitations it was not possible to examine the specific influences of various pharmacological factors such as drug classification (e.g., atypical versus SSRI), dosage, duration of use, medication adherence, or primary mechanism of action (e.g., NE versus 5HT-mediated), which existing evidence suggests may be relevant(Hergovich et al., 2000; Piletz et al., 2000). Thus, it is possible that the lack of significant antidepressant effects on PSEL among CGs could reflect the small sample size (10 CG were taking antidepressants) and heterogeneity of pharmacological factors in that group. Three NC taking antidepressants did exhibit significantly decreased P-selectin reactivity relative to NC not taking antidepressants. Further study in randomized clinical trials of antidepressants would be needed to fully investigate these issues. Future studies may also benefit from including a longer recovery period, as these findings support one previous study by Strike et al. (2004) suggesting that platelet activation may remain elevated for 30 to 75 minutes following acute stress.

This may be one of the first studies to show significant associations between anxious symptoms and P-selectin reactivity, although anxiety has been previously related to platelet alpha-adrenoreceptor density(Cameron et al., 1996; Freedman et al., 1990), D-dimer reactivity(von Känel et al., 2004a), and fatal coronary heart disease(Kawachi et al., 1994). These results suggest that while depressive symptoms exhibited the strongest relationships with physiological reactivity, clinicians should additionally monitor anxious symptoms among older caregivers with high CVD risk.

In summary, the current study demonstrates that the combination of chronic caregiving stress and depressive or anxious symptoms is associated with elevated sympathetic and platelet P-selectin responses to acute psychological stress. This suggests a potential physiological pathway by which negative affect and chronic stress may increase the risk of cardiovascular disease. Moreover, it raises the possibility that psychological interventions to reduce negative affect or caregiving burden could potentially mitigate these harmful effects.

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Abbreviations Used

AD
Alzheimer's disease

CG
Caregivers

NC
non-caregivers

CVD
cardiovascular disease

CAD
coronary artery disease

MI
myocardial infarction

DEP
depressive symptoms

ANX
anxious symptoms

BSI
Brief Symptom Inventory

NE
norepinephrine

SNS
sympathetic nervous system activity

PSEL
% P-selectin expression

K-S
Kolmogorov-Smirnov test

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Table 1

Comparison of Caregivers and Non-Caregivers on Negative Affect, Role Overload, Norepinephrine, Platelet P-Selectin, and Medical Factors

	Caregivers (n=39)	**Non-Caregivers (n=31)**	**Statistical Test**	***P* Value**
Demographics				
Age, M (se), years	68.74 (1.28)	71.87 (1.27)	t(68) = -1.72	.09
Male Gender, n (%)	14 (36%)	9 (29%)	$\chi^2(1) = .37$	.54
Role Overload, M (se)	9.13 (.49)	6.23 (.36)	t(66.05) = -4.79*	<.01
Brief Symptom Inventory				
Depression, M (se)	.74 (.11)	.26 (.07)	t(60.23) = -3.69*	<.01
Anxiety, M (se)	.57 (.08)	.26 (.05)	t(59.49) = -3.29*	<.01
Raw Norepinephrine Values				
Baseline, M (se), pg/ml	442.68 (34.36)	382.18 (18.94)	t(68) = -1.30	.20
Speech, M (se), pg/ml	569.74 (34.57)	464.18 (30.58)	t(68) = -2.23	.03
Recovery, M (se), pg/ml	524.41 (43.24)	456.84 (31.70)	t(68) = -1.20	.23
Raw % P-Selectin Expression				
Baseline, M (se), %	3.11 (.63)	2.16 (.57)	t(68) = -1.10	.28
Speech, M (se), %	19.03 (3.81)	15.62 (3.55)	t(66) = -.65†	.52
Recovery, M (se), %	21.00 (3.88)	15.29 (3.45)	t(68) = -1.07	.29
Medical Conditions				
Cerebrovascular disease, n (%)	3 (8%)	0 (0%)	FET‡	.25
Coronary artery disease, n (%)	11 (28%)	6 (19%)	$\chi^2(1) = .74$	.42
Diabetes, n (%)	3 (8%)	3 (10%)	FET	1.00
Hypercholesterolemia, n (%)	17 (44%)	14 (45%)	$\chi^2(1) = .02$	.90
Hypertension, n (%)	13 (33%)	7 (23%)	$\chi^2(1) = .978$	.32
Any CVD condition, n (%)	25 (64%)	21 (68%)	$\chi^2(1) = .102$	.75
Medications				
Aspirin, n (%)	11 (28%)	6 (19%)	$\chi^2(1) = .74$	.39
Anti-depressants, n (%)	10 (26%)	3 (10%)	FET	.12

*Unequal variances assumed.

†Speech P-Selectin values were not available for two participants.

‡FET = Fisher's Exact Test, 2-sided.

Note: M (se) = Mean (standard error of the mean).

Table 2

Bivariate Correlations among Negative Affect, Overload, Norepinephrine (NE), and P-Selectin (PSEL) Responses to Acute Stress for Caregivers versus Non-caregivers

NC = top-right/ CG = lower left	Depression	Anxiety	Overload	NE Reactivity	NE Recovery	PSEL Reactivity	PSEL Recovery
Depression	-	.692*	.249	-.182	-.238	-.167	-.255
Anxiety	.721**	-	.367*	-.078	-.203	-.180	-.369*‡
Overload	.489**	.340*	-	-.187	-.029	-.063	-.274
NE Reactivity	.231	.068	.232	-	.517**	-.109	.114
NE Recovery	.485**	.407**	.364*	.195	-	.145	.123
PSEL Reactivity	.704**	.524**	.416**	.185	.416**	-	.534**
PSEL Recovery	.409**	.311†	.173	.297†	.372*	.681**	-

Note: Top-right correlations refer to non-caregivers (NC), and lower-left refer to caregivers (CG). Reactivity/recovery scores are standardized residuals (see data analysis).

** $p \leq .01$.

* $p \leq .05$.

† $p \leq .07$.

‡ This association became non-significant when controlling for age, gender, aspirin use, antidepressant use, and preexisting CVD.