

# Individual Differences in Cardiac Sympathetic Control Predict Endocrine and Immune Responses to Acute Psychological Stress

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Potential mechanisms coordinating individual differences in cardiovascular reactivity and endocrine and immune responses to acute psychological stress were examined. Twenty-three young, healthy women performed a mental arithmetic challenge while measures of cardiovascular, endocrine, and immune function were assessed. Results revealed that the acute stressor was associated with changes in the cardiovascular, endocrine, and immune systems. More important analyses revealed that individual differences in cardiovascular reactivity predicted stress-induced cortisol changes. Furthermore, cardiac sympathetic control, as indexed by pre-ejection period, was specifically related to changes in natural killer cell activity. These results suggest that distinct physiological pathways are activated in response to acute psychological stress.

Exposure to acute stressors is a ubiquitous part of everyday life. Laboratory paradigms of acute psychological stress have demonstrated reliable changes in cardiovascular function that appear to provide information on an individual's reactions to daily hassles and stressors (Matthews, Owens, Allen, & Stoney, 1992; Pollak, 1991). Consistent with this position, there are stable individual differences in cardiovascular reactivity to acute psychological stress (Kamarck et al., 1992; Manuck, 1994; Manuck, Kasprowicz, Monroe, Larkin, & Kaplan, 1989). Recent research has begun to examine the relationship between individual differences in stress-induced cardiovascular reactivity and its association to endocrine and immune responses during brief psychological stress. More important, studies of immune

system alterations in response to acute psychological stress suggest that such changes are greater in individuals characterized by greater sympathetic-cardiovascular reactivity (see review by Kiecolt-Glaser, Cacioppo, Malarkey, & Glaser, 1992).

There appear to be at least two mechanisms coordinating individual differences in cardiovascular reactivity and alterations in certain aspects of the immune system during brief psychological stress. First, acute psychological stress activates the sympathetic adrenal medullary (SAM) system (Cacioppo et al., 1995; Manuck, Cohen, Rabin, Muldoon, & Bachen, 1991; Sgoutas-Emch et al., 1994), as evidenced by increased heart rate, blood pressure, and plasma catecholamines (i.e., epinephrine [EPI] and norepinephrine [NE]). More important, short-term activation of the SAM system may be associated with alterations in certain aspects of immune function. For instance, Manuck et al. (1991) classified individuals as high or low "sympathetic" reactors based on heart rate, blood pressure, and catecholamine reactivity to acute psychological stressors. As a measure of immune function, Manuck and colleagues examined changes in the proliferative response to the mitogen phytohemagglutinin (PHA). The proliferative response to mitogens provides an *in vitro* model of the functional ability of lymphocytes to respond to a challenge (see Kiecolt-Glaser & Glaser, 1995 for a review). Consistent with *in vivo* studies examining the effects of EPI infusions on aspects of immune function (Crary, Borysenko, et al., 1983), results revealed that high sympathetic reactors were characterized by a weaker proliferative response to PHA compared to low sympathetic reactors (also see Stone et al., 1993). Interestingly, Manuck et al. (1991) found that low and high sympathetic reactors did not differ in plasma cortisol changes, nor did the stressor result in alterations in cortisol levels. These data suggest that activation of the hypothalamic-pituitary axis (HPA) was not responsible for the results obtained by Manuck and colleagues.

The second potential mechanism coordinating individual

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differences in stress-induced cardiovascular reactivity and immune changes in response to acute psychological stress involves the HPA. The major hormones of the HPA include corticotropin releasing hormone (CRH), ACTH, and cortisol (see Baum & Grunberg, 1995 for a review). Lovallo, Pincomb, Brackett, and Wilson (1990) demonstrated that high, but not low, heart rate reactors showed significant changes in plasma cortisol levels during aversive incentives, but they did not assess aspects of immune function. Given the results of Lovallo et al. (1990) and the relatively large literature demonstrating the effects of HPA hormones on immune function (Cupps & Fauci, 1982), Sgoutas-Emch et al. (1994) identified individuals as low or high in heart rate reactivity to a speech stressor. Approximately 3 weeks later, Sgoutas-Emch and colleagues retested the low and high heart rate reactors and also examined endocrine (e.g., cortisol) and immune (e.g., natural killer cell activity [NKCA]) changes in response to a mental arithmetic challenge. Consistent with past research, results revealed that the heart rate reactivity classification was stable across testing sessions and only high heart rate reactors showed significant cortisol changes in response to the acute psychological stressor. As one measure of immune function, Sgoutas-Emch and colleagues examined natural killer (NK) cell, lysis which provides an *in vitro* model of the functional ability of NK cells to destroy tumor or virus-infected cells. Importantly, high heart rate reactors were also characterized by increased NKCA compared to low heart rate reactors. Analyses of catecholamine responses did not reveal the same pattern of results, suggesting that heart rate reactivity may be serving as a marker of HPA activation and, thus, as another potential mechanism coordinating certain aspects of immune response to acute psychological stress.

An important issue raised by Sgoutas-Emch et al. (1994) concerns the underlying autonomic substrates of heart rate reactivity that contributed to the differential endocrine and immune changes found in individuals dispositionally low and high in heart rate reactivity. Previous research has typically treated heart rate reactivity to acute psychological stress as a unidimensional construct, ranging from low to high, primarily reflecting sympathetic beta-adrenergic activation (see reviews by Berntson, Cacioppo, & Quigley, 1991; Berntson, Cacioppo, & Quigley, 1993a). Heart rate reactivity, however, is a joint function of sympathetic and parasympathetic influences. Importantly, psychological stressors have been shown to alter parasympathetic activity, and the neural determinants of heart rate reactivity can be uncoupled (e.g., increased sympathetic with no change in parasympathetic), or nonreciprocally coupled (e.g., coactivation of both the sympathetic and parasympathetic nervous systems). Ignoring the autonomic substrates of heart rate reactivity, therefore, may obscure relationships with psychological, physiological, and health-related outcomes (Cacioppo, 1994).

Two of the most promising noninvasive measures of sympathetic and vagal activation of the heart are preejection period (PEP) and respiratory sinus arrhythmia (RSA), respectively. PEP is the time interval from the onset of ventricular depolarization to the onset of left ventricular ejection. Therefore, it represents the time from ventricular depolarization that is necessary to generate enough force to eject blood from the left ventricle. PEP is thought to reflect sympathetic activation of the heart because the ventricular myocardium is innervated pri-

marily by the sympathetic nervous system (Randall, Randall, & Ardell, 1991) and PEP is strongly correlated with invasive measures of contractility (Ahmed, Levinson, Schwartz, & Etinger, 1972; Martin, Shaver, Thompson, Reddy, & Leonard, 1971). Cacioppo et al. (1994) used relatively selective sympathetic and parasympathetic pharmacological blockades (i.e., metoprolol and atropine sulfate, respectively) to evaluate several proposed noninvasive measures of cardiac chronotropy (i.e., timing of cardiac activity). Of the noninvasive indexes that were assessed, only PEP reflected sympathetic but not parasympathetic activation of the basal cardiac chronotropy.

RSA is the rhythmical fluctuations in heart period within the respiratory frequency band (i.e., .12 to .40 Hz; see review by Berntson, Cacioppo, & Quigley, 1993b). The phase-dependent relationship between heart period and respiration is thought to reflect modulation by a central respiratory generator that inhibits vagal motor neurons during inspiration (shortens heart period) but slightly activates it during expiration (lengthens heart period). Consistent with the notion that RSA reflects vagal activity to the heart, RSA is greatly attenuated by parasympathetic blockade but relatively unaffected by sympathetic blockade (Cacioppo et al., 1994).

To examine the autonomic substrates of heart rate reactivity responsible for the effects obtained by Sgoutas-Emch et al. (1994), Cacioppo et al. (1995) utilized PEP and RSA as noninvasive indexes of sympathetic and parasympathetic activation of cardiac chronotropy and examined its relationships to neuroendocrine and immune responses to brief psychological stress in an elderly sample. Prior research has demonstrated the cross-task or temporal stability in individuals' stress-induced PEP and RSA changes to psychological stressors (Berntson et al., 1994; Cacioppo, Uchino, & Berntson, 1994; Kamarck et al., 1992). Results revealed that the psychological stressors significantly affected aspects of cardiovascular, endocrine, and immune function. More important, analyses revealed that the sympathetic substrate of heart rate reactivity (i.e., PEP reactivity) and not RSA reactivity was more strongly related to stress-induced activation of the HPA (e.g., cortisol changes). In addition, plasma catecholamine changes were only moderately related to PEP changes and did not evidence the same pattern of associations as PEP. These data suggest that individual differences in stress-induced cardiac sympathetic control may be serving as a marker of HPA activation and not more general sympathetic activation. Furthermore, consistent with the notion that individual differences in cardiac sympathetic control reflect an individual's reactions to daily stressors, Kiecolt-Glaser and colleagues (see Cacioppo, 1994) found that interindividual variations in stress-induced PEP and cortisol changes were associated not with short-term but with longer term immune effects (i.e., response to an influenza vaccine).

In the present study, aspects of cardiovascular, endocrine, and immune function were assessed in young, healthy women at rest, and in response to an acute psychological stressor (i.e., mental arithmetic challenge). Consistent with past research, we predicted that the acute psychological stressor would significantly affect aspects of the cardiovascular (e.g., increased heart rate), endocrine (e.g., increased plasma catecholamines), and immune (e.g., increased NKCA) systems (Kiecolt-Glaser et al., 1992). We further predicted that (a) individual differences in

stress-induced PEP reactivity would predict cortisol changes to acute psychological stress (Cacioppo, 1994; Cacioppo et al., 1995) and (b) PEP reactivity would predict short-term changes in NKCA.

## Method

### Participants

Twenty-four undergraduate women ( $M$  age = 18.96) were paid \$40.00 for approximately 2.5 hr of participation in the study. One participant was excluded from the analyses because of an inability to perform the experimental task, resulting in a final sample of 23 women. The inclusion criteria were as follows: (a) good health and normotensive, (b) no history of psychological disorder or chronic illness, (c) no prescription medication, nonprescription drugs, or tobacco products, (d) exercised less than 10 hr per week, (e) consumed less than 10 alcoholic beverages a week, (f) within 20% of their ideal body weight, and (g) not math, speech, or needle phobic. Participants were asked to drink nothing except water after midnight of the test day, and to refrain from ingesting antiinflammatory agents, antihistamines, or alcohol during the 24 hr preceding the test day.

### Procedure

All participants were instructed before their appointment to (a) reschedule if they became ill or experienced a major negative life event (e.g., death in the family), (b) not consume any alcohol or take any nonprescription medication the day before the study, (c) refrain from any exercise the day before the study, and (d) refrain from eating or drinking anything but water after midnight of the test day.

The study was run in the mornings and consisted of five stages: (a) informed consent and explanation of the task, (b) placement of an occluding cuff of appropriate size and four spot electrodes for blood pressure and impedance cardiograph recordings, (c) insertion of an 18-gauge indwelling catheter into the antecubital vein of the participant's arm, (d) 30-min supine rest period followed by a 4-min resting cardiovascular assessment and blood draw, and (e) 12 min of mental arithmetic with continuous cardiovascular assessments and a posttask blood draw.

The mental arithmetic task consisted of 12 min of serial subtractions that participants performed without stopping. Participants were told that any errors would be corrected by the experimenter and to continue from the corrected number. In addition, participants were prompted to speed up their responses at the start of minutes 3, 7, and 11. The serial subtraction problems were as follows: minutes 1 and 2: 2907 by 3s, minutes 3 and 4: 6828 by 7s, minutes 5 and 6: 9561 by 13s, minutes 7 and 8: 5113 by 8s, minutes 9 and 10: 8318 by 14s, and minutes 11 and 12: 9994 by 17s. During minutes 7 through 12, participants were also presented with a series of random 100-dB noise blasts administered by means of a headphone set. The experimenter explained to the participant prior to the task that the noise blasts were intended to make the task more challenging.

### Measures

**State anxiety scale.** A short form of the Spielberger state-trait anxiety scale was given to participants prior to and following the acute stressor (Marteau & Bekker, 1992). Responses to the six items range from 1 (*not at all*) to 4 (*very much*). Marteau and Bekker (1992) reported that the short form correlates .95 with the original state-anxiety scale.

**Cardiovascular assessments.** A Minnesota Impedance Cardiograph (Model 304B) was used to measure electrocardiogram (ECG), basal thoracic impedance ( $Z_0$ ), and the first derivative of the impedance

signal ( $dZ/dt$ ). Disposable spot electrodes were placed in the tetrapolar configuration as proposed by Qu, Zhang, Webster, and Tompkins (1986).<sup>1</sup> The two outer (current) electrodes were placed over the fourth cervical vertebra and the ninth thoracic vertebra, whereas the two inner (voltage) electrodes were placed 4 cm above the clavicle, and over the sternum at the fourth rib. A 4-mA AC current at 100 kHz was passed through the two outer electrodes, and  $Z_0$  and  $dZ/dt$  were recorded from the two inner electrodes. The ECG,  $Z_0$ , and  $dZ/dt$  were digitized at 500 Hz, and the interbeat intervals (IBI) were derived from a custom software package.<sup>2</sup> The impedance data were ensemble averaged within 1-min epochs, and each waveform was verified or edited prior to analyses. PEP was quantified as the time interval in milliseconds from the ECG Q-point (corresponding to the onset of ventricular depolarization) to the B-point of the  $dZ/dt$  waveform (corresponding to the onset of left ventricular ejection). Mean PEP was calculated by averaging across minutes within the resting and stressor periods to increase reliability.

The IBIs were checked and edited for artifacts using the detection algorithm of Berntson, Quigley, Jang, and Boysen (1990). The IBI data were then converted to a timeseries of successive 500-ms samples. RSA amplitude was extracted as a noninvasive measure of cardiac vagal activity using a PC-based software package (MXedit 2.01, Delta-Biometrics, Bethesda, MD) with a bandpass of .12 to .40 Hz. The natural logarithm of the variance in heart period within the respiratory frequency (i.e., .12 to .40 Hz) served as the estimate of cardiac vagal activity. Mean RSA was calculated during the rest and stressor periods by averaging across minutes.

A Cortronic Model 7000 continuous blood pressure monitor was used to assess systolic and diastolic blood pressure. An appropriate size occluding cuff was placed over the brachial artery and provided continuous blood pressure measurements at a constant cuff pressure of 20 mmHg.<sup>3</sup>

**Endocrine and immune assays.** The blood draws prior to and following the stressor provided the materials for the endocrine and immune assays. Plasma catecholamine concentrations of epinephrine and norepinephrine were determined by high performance liquid chroma-

<sup>1</sup> The focus of our study was on the assessment of the systolic time intervals (i.e., PEP). Spot electrodes measure the systolic time intervals at comparable reliabilities to band electrodes (Sherwood, Royal, Hutcheson, & Turner, 1992) but are more convenient and comfortable for participants.

<sup>2</sup> We thank Robert Kelsey and William Guethien for providing us with copies of their data acquisition and reduction software for impedance cardiography and for their helpful advice.

<sup>3</sup> Recently, data have been reported on the accuracy of the Cortronic monitor (e.g., Numaguchi et al., 1994). Numaguchi et al. (1994) found that blood pressure readings from the Cortronic monitor were not significantly correlated with blood pressure readings from either the Finapres blood pressure monitor or intermittent measurements from a mercury sphygmomanometer taken from the opposite arm. However, the Cortronic monitor used by Numaguchi et al. (1994) had not been serviced for approximately 8 years prior to data collection (J.C. Kircher, personal communication, January 27, 1995). Therefore, it is possible that the Cortronic monitor may have been out of calibration. Due to the relatively new technology of the Cortronic blood pressure monitor, we have been careful to monitor the reliability of the Cortronic by periodically checking it against a standard ocillometric monitor and readings from a mercury sphygmomanometer. In the rare case that the Cortronic needed recalibration, we have sent it back to the manufacturer for maintenance. Our ability to replicate research within and across laboratories on stress-induced changes in blood pressure (e.g., Cacioppo et al., 1995; Knapp et al., 1992) and age-related differences in blood pressure (e.g., Uchino, Kiecolt-Glaser, & Cacioppo, 1992) gives us confidence in the reliability of the Cortronic model 7000 monitor.

tography (HPLC), using a Waters system with an electrochemical detector. The HPLC system has a sensitivity level of 10pg/ml for EPI and 20 pg/ml for NE. This assay has an intra- and interassay variation coefficient of 15% for EPI and 9% for NE. Plasma ACTH levels were assayed using immunoradiometric assays supplied by Nichols Institute (Capistrano, CA). This assay has an intra- and interassay coefficient of variation less than 10% with a sensitivity of 1 pg/ml. Plasma cortisol levels were assayed using a florescent polarization technique (TDX-Abbott Lab, Chicago, IL). This assay has an intra- and interassay coefficient of variation of less than 10%.

Immune assessments were performed using peripheral blood leukocytes (PBLs) isolated by density gradient centrifugation on Ficoll-Metrazaote gradients from 40 cc of heparinized venous blood, washed with complete RPMI 1640 medium, counted, and then prepared for the following assays.

**Proliferative response to concanavalin A (Con A) and PHA.** The procedures used in this study are described in Kiecolt-Glaser, Dura, Speicher, Trask, & Glaser (1991). Cells were prepared ( $5 \times 10^6$ ) in RPMI 1640 medium supplemented with 10% fetal bovine serum, treated with Con A and PHA and incubated for 48 hr. The concentrations for Con A and PHA were 2.5, 5.0, 10.0, and 20.0 ug. All samples were run in triplicate and counts per minute were determined by averaging the triplicate samples and reported as the log 10 transformed values of the counts per minute.

**NKCA.** The procedures used for NKCA in this study have been described in detail elsewhere (Glaser, Rice, Speicher, Stout, & Kiecolt-Glaser, 1986). Briefly, PBLs were prepared in complete RPMI 1640 medium.  $^{51}\text{Cr}$  labeled K562 target cells were added to make 100:1, 50:1, 25:1, 12.5:1, 6.25:1, and 3.12:1 effector to target cell ratios and were seeded in triplicates, in 96-well microtiter plates (Costar Corp.). Additional wells containing only  $^{51}\text{Cr}$  labeled target cells in RPMI 1640 medium and wells containing medium plus 1% sodium dodecyl sulfate were used to determine spontaneous and maximum release of  $^{51}\text{Cr}$ , respectively. NKCA values were standardized at the 25:1 effector to target ratio using a logistic regression that used NKCA values across concentrations (Kazimer, Whisler, Stephens, Pearl, & Yates, 1989).

It is also important to monitor the nutritional status of the participant to rule out changes in the immune response that may be related to malnutrition. Therefore, we measured serum albumin at rest, as described in Kiecolt-Glaser et al. (1991). All participants' data fell within the normal range.

## Results

We first examined the efficacy of the acute stressor in altering levels of psychological stress. A one-way repeated measures analysis of variance (ANOVA; Assessment period: baseline,

Table 1  
Mean (SEM) Cardiovascular Baseline and Reactivity Assessments

Measure	Baseline	Reactivity
Heart rate (BPM)	70.77 (1.89)	12.83 (1.13)**
SBP (mmHg)	110.35 (2.05)	7.94 (2.77)**
DBP (mmHg)	69.34 (1.27)	6.0 (1.68)**
PEP (ms)	101.30 (2.30)	-7.94 (1.73)**
RSA	6.87 (.20)	-0.39 (.17)*

Note. BPM = beats per minute; SBP = systolic blood pressure; DBP = diastolic blood pressure; PEP = pre-ejection period; RSA = respiratory sinus arrhythmia.  
\* $p < .05$ . \*\* $p < .01$ .

Table 2  
Mean (SEM) Endocrine Baseline and Reactivity Assessments

Measure	Baseline	Reactivity
NE (pg/ml)	279.83 (21.97)	123.91 (14.90)**
EPI (pg/ml)	24.45 (1.80)	5.04 (2.14)*
ACTH	14.33 (1.49)	1.26 (3.37)
Cortisol	14.70 (1.35)	0.33 (0.88)

Note. NE = norepinephrine; EPI = epinephrine.  
\* $p < .05$ . \*\* $p < .01$ .

stressor) was conducted on the state anxiety scale. Results confirmed that the acute stressor significantly increased state anxiety levels,  $F(1, 22) = 10.86, p < .01$  ( $M_{\text{baseline}} = 1.72, M_{\text{reactivity}} = .40$ ).<sup>4</sup>

### Effects of Acute Psychological Stress on Cardiovascular, Endocrine, and Immune Function

One aim of this study was to examine the effects of an acute psychological stressor on multiple aspects of physiological functioning. One-way repeated measures ANOVAS (Assessment period: baseline, stressor) were performed on the cardiovascular, endocrine, and immune measures. Results confirmed that the acute psychological stressor affected multiple aspects of physiological function. Table 1 contains the cell means for the cardiovascular measures. The acute psychological stressor was associated with increased heart rate,  $F(1, 22) = 128.51, p < .001$ , systolic blood pressure,  $F(1, 22) = 8.24, p < .01$ , and diastolic blood pressure,  $F(1, 22) = 16.39, p < .001$ . Replicating Cacioppo et al. (1995), PEP was shortened,  $F(1, 22) = 21.02, p < .001$ , and RSA decreased,  $F(1, 22) = 5.37, p < .05$ , in response to the stressor, suggesting reciprocal sympathetic activation and parasympathetic withdrawal of cardiac chronotropy at the group level of analysis.

We next examined endocrine changes in response to the acute psychological stressor (see Table 2). Consistent with prior evidence indicating that acute stressors activate the SAM system, both plasma NE,  $F(1, 22) = 69.17, p < .001$ , and EPI,  $F(1, 22) = 5.57, p < .05$  were elevated in response to the psychological stressor. Also consistent with prior research, the main effects for period were nonsignificant for plasma ACTH and cortisol levels,  $F_s < 1$ .

Analyses conducted on the immune assessments revealed that NKCA increased in response to the acute stressor,  $F(1, 22) = 70.64, p < .001$  ( $M_{\text{baseline}} = 28.86, M_{\text{reactivity}} = 13.47$ ). A 2 (Assessment period: baseline, stress)  $\times$  4 (Mitogen concentration: 2.5, 5.0, 10.0, 20.0) repeated measures ANOVA was conducted on the proliferative response to Con A and PHA. No changes in Con A or PHA were found in response to the acute psychological stressor,  $F_s < 1$ .

### Coordination of Cardiovascular, Endocrine, and Immune Responses to Acute Psychological Stress

The primary aim of this study was to examine potential mechanisms coordinating individual differences in stress-in-

<sup>4</sup> The means for reactivity were calculated as stressor minus baseline levels.

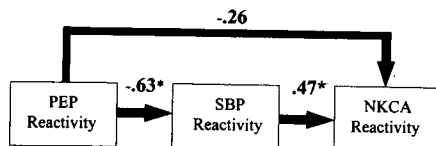


Figure 1. Path model examining the mediational effect of systolic blood pressure (SBP) reactivity on the stress-induced preejection period (PEP) and natural killer cell activity (NKCA) relationship ( $*p < .05$ ).

duced cardiovascular reactivity and endocrine and immune responses to brief psychological stress. Residualized change scores were calculated by regressing task values on baseline values (see Llabre, Spitzer, Saab, Ironson, & Schneiderman, 1991), and correlations were then computed between the cardiovascular, endocrine, and immune measures.<sup>5</sup>

Replicating past research (Lovallo et al., 1990; Sgoutas-Emch et al., 1994), results revealed that heart rate reactivity was significantly correlated with plasma ACTH ( $r = .50, p < .02$ ) and cortisol changes ( $r = .62, p < .01$ ). Consistent with Cacioppo et al. (1995), subsequent analysis revealed that PEP reactivity was significantly correlated with cortisol reactivity ( $r = -.45, p < .05$ ), whereas RSA reactivity was only weakly correlated with cortisol changes ( $r = -.18, ns$ ). Both SBP reactivity and DBP reactivity also predicted changes in cortisol levels ( $r_s = .42$  and  $.41$ , respectively,  $ps < .05$ ). None of the cardiac measures were significantly associated with catecholamine reactivity to the brief psychological stressor ( $-.35 < r < .20, ps > .10$ ).

Consistent with Sgoutas-Emch et al. (1994), heart rate reactivity tended to predict greater NKCA ( $r = .29, p < .09$ , one-tailed). Analyses aimed at examining the autonomic substrates of this association revealed that stress-induced PEP reactivity was a strong predictor of changes in NKCA ( $r = -.56, p < .01$ ). In contrast, RSA reactivity was uncorrelated with changes in NKCA ( $r = -.12, ns$ ). SBP reactivity was also a predictor of NKCA changes in response to the acute stressor ( $r = .63, p < .01$ ). Finally, the only significant association between the endocrine and immune indexes was a positive correlation between cortisol and NKCA changes ( $r = .51, p < .02$ ).<sup>6</sup>

Based on the simple correlational analyses, we used path analyses to directly examine potential mediational processes (see Baron & Kenny, 1986). A conceptual model was tested using RAMONA PC that utilized maximum likelihood estimates to derive the parameter estimates of the model (Browne & Mels, 1990). In the path model, we tested the possibility that cardiac sympathetic reactivity, as indexed by PEP changes, was having an effect on NKCA via stress-related changes in SBP. As a noninvasive measure of sympathetic cardiac chronotropy, a shortening of PEP is associated with greater beta-adrenergic activation of the heart and an increase in both the rate and force of contractility. SBP reactivity, in turn, may impact on NKCA partly through mechanical and/or soluble immune factors (Ottaway & Husband, 1992). For instance, the increased vascular pressure may lead to a migration of NK cells from lymphoid tissues into the peripheral circulation, which would produce an increase in the *in vitro* NKCA (Benschop, Godaert, et al., 1994; Benschop, Nieuwenhuis, et al., 1994; Ottaway & Husband, 1992).

The results of the path analysis are depicted in Figure 1, and

provide strong evidence for mediation. In this model, all three criteria for mediation were established (see Baron & Kenny, 1986). First, PEP reactivity was a significant predictor of SBP reactivity. Second, SBP reactivity was significantly associated with NKCA while statistically controlling for the effects of PEP. Finally, the direct path between PEP and NKCA was rendered nonsignificant when controlling for the effects of SBP reactivity (i.e., the mediator).<sup>7</sup>

## Discussion

Prior research on physiological changes to acute psychological stress has reliably demonstrated changes in cardiovascular, endocrine, and immune function. Consistent with past research, we found that brief psychological stress was associated with increased heart rate and blood pressure, shortened PEP, lowered RSA, increased plasma catecholamine levels, and increased NKCA. However, few studies have examined such changes within the same study. With these multiple assessments, we further demonstrated that the cardiovascular measures were consistently related to plasma cortisol changes and that individual differences in cardiovascular sympathetic control in particular, was related to NKCA. In addition, path analyses revealed that the effect of PEP on short-term changes in NKCA was mediated, in part, by SBP reactivity.

A discussion of the potential mechanisms underlying the mediational effect of SBP reactivity on the PEP and NKCA relationship is warranted. As mentioned earlier, increased cardiac sympathetic control, as indexed by PEP, may be partly responsible for changes in SBP. This study, and others, have found stress-induced changes in SBP to be associated with an increase in the number of circulating NK cells and increased NKCA (Benschop, Godaert, et al., 1994; Knapp et al., 1992). The effect

<sup>5</sup> To assess the potential influence of variations in female reproductive hormone levels on interactions between the cardiovascular, endocrine, and immune measures, we also assessed levels of estrogen and progesterone during baseline. Results of simple correlational analyses revealed that levels of progesterone were significantly associated with heart rate reactivity ( $r = .42, p < .05$ ), SBP reactivity ( $r = .42, p < .05$ ), and ACTH reactivity ( $r = .47, p < .05$ ). However, an examination of the scatterplots revealed that the association between progesterone and ACTH reactivity was primarily due to one outlier. In comparison, levels of estrogen were associated with greater changes in NE ( $r = .46, p < .05$ ) and EPI ( $r = .42, p = .05$ ). To examine the contribution of these associations, we duplicated the analyses reported below while statistically controlling for levels of estrogen and progesterone. Importantly, the magnitude of the significant results were unchanged when statistically controlling for these baseline reproductive hormone levels.

<sup>6</sup> There was a tendency for DBP changes to be associated with a weaker stress-induced proliferative response to PHA at concentration levels 2.5 ug ( $r = -.51, p < .05$ ), 5.0 ug ( $r = -.36, p < .10$ ), 10.0 ug ( $r = -.38, p < .08$ ), and 20.0 ug ( $r = -.40, p < .06$ ).

<sup>7</sup> Based on the simple correlational analyses, one could argue that cortisol changes may also be mediating the association between PEP and NKCA. However, we know of no simple mechanism by which short-term increases in cortisol should be associated with increases in NKCA (Cupps & Fauci, 1982). Therefore, the theoretical justification for this model is weak. However, we examined this ancillary model with cortisol reactivity as the mediator of the PEP and NKCA relationship and found no evidence of mediation.

of SBP reactivity on NK cell parameters may be achieved through mechanical and/or soluble immune factors (Ottaway & Husband, 1992). The increased vascular pressure may lead to a migration of NK cells from lymphoid tissues into the peripheral circulation with a subsequent increase in NK cell numbers and NKCA (Benschop, Godaert, et al., 1994; Benschop, Neuwnehuis, et al., 1994; Ottaway & Husband, 1992). Consistent with this possibility, Benschop, Neuwnehuis, et al. (1994) found that the increased NKCA in response to acute psychological stress was primarily due to a concomitant increase in the number of NK cells. However, there is also evidence for increased NKCA independent of NK cell numbers during more intense acute stressors. For instance, Schedlowski et al. (1993) found that the increased NKCA during the stress of first-time parachute jumping was apparent when examining the lytic activity per NK cell.

NK cells express beta-adrenergic receptors of relatively greater affinity and density than other lymphocyte subsets (see Mills & Dimsdale, 1993). In addition, *in vivo* infusions of catecholamines and beta-agonists have been associated with increased NK cell numbers and NKCA (Crary, Hauser, et al., 1983; Tonnesen, Christensen, & Brinklov, 1987; Van Tits et al., 1990). As a result, it has been hypothesized that some of the short-term immune changes in response to acute psychological stress are mediated by changes in catecholamines (Benschop, Neuwnehuis, et al., 1994; Manuck et al., 1991). For instance, Benschop, Neuwnehuis, et al. (1994) found that administration of propranolol, a sympathetic blocker, abolished PEP and NKCA changes in response to acute psychological stress. Benschop and colleagues interpreted their results as reflecting activation of the sympathetic nervous system, however, their general sympathetic blockade did not allow them to examine specific mechanisms. Our data suggest specifically that stress-induced cardiac sympathetic control was associated with increased NKCA through changes in SBP as (a) PEP was only moderately related to changes in catecholamine levels (also see Cacioppo et al., 1995) and (b) statistical controls for both NE and EPI did not change the association between NKCA and PEP ( $r = -.51, p < .03$ ).

Although it may be premature to rule out the influence of the catecholamines on the PEP and NKCA relationship due to measurement issues (e.g., the error inherent in these assays), a consistent picture is beginning to emerge regarding the coordination of individual differences in cardiovascular reactivity and endocrine and immune responses to brief psychological stress. Relatively general stress-induced activation of the SAM system appears to be associated with increased plasma catecholamines, increased numbers of suppressor/cytotoxic T-cells, and weaker lymphocyte proliferation to mitogens (Bachen et al., 1992; Manuck et al., 1991). In comparison, specific changes in stress-induced cardiac sympathetic control, as indexed by PEP, appear to be more closely related to short-term changes in NKCA by means of changes in SBP reactivity. These data suggest that distinct pathways may be activated in response to brief psychological stress and highlight the value of examining specific patterns of physiological function to elucidate potential mechanisms responsible for the covariations (or lack thereof) reported in studies of psychosocial factors and physiological processes (Cacioppo et al., 1992).

We previously found changes in PEP and cortisol to covary in a study of the effects of acute psychological stressors in a sam-

ple of older women (Cacioppo et al., 1995). This association was replicated in the present research: Brief psychological stressors activated the HPA in individuals who were characterized by high cardiac sympathetic activation to routine psychological stressors. Given the potential long-term immunosuppressive effects of repeated HPA activation, cardiac sympathetic activation, as indexed by stress-induced abbreviations of PEP, may be related to short-term NK cell function by means of its effects on vascular processes but to long-term immune function by means of the actions of HPA hormones. Consistent with this reasoning, Kiecolt-Glaser and colleagues examined the utility of individual differences in PEP reactivity to brief psychological stress in predicting older participants' reaction to an influenza vaccination given 7 to 8 months earlier (see Cacioppo, 1994). The response to the influenza vaccine was quantified *in vitro* by measuring virus specific T-cell production of interleukin-2 (IL-2), a protein hormone that serves as an important growth factor for T-cells, B-cells, and NK cells. Although these results are preliminary, influenza virus-induced IL-2 levels *in vitro* during the third month was predicted only by stress-induced individual differences in PEP ( $r = .68$ ) and not RSA ( $r = -.12$ ) or heart rate reactivity ( $r = -.17$ ). Furthermore, these longer term associations appear to be due to activation of the HPA, as cortisol changes in response to acute psychological stress was a strong predictor of influenza virus-induced IL-2 levels ( $r = -.56$ ), whereas stress-induced epinephrine levels were unrelated to the vaccine response ( $r = .13$ ).

There are several additional issues that warrant discussion. The present study used the same experimental paradigm as Sgoutas-Emch et al. (1994) but did not find as strong a relationship between heart rate reactivity and changes in NKCA. An important difference between the two studies, however, is that Sgoutas-Emch et al. (1994) used a prospective design and pre-selected individuals as low and high in heart rate reactivity (also see Lovallo et al., 1990). Therefore, assessments of relatively extreme and stable individual differences in heart rate reactivity may be contributing to the stronger associations obtained in Sgoutas-Emch et al. (1994). In addition, Sgoutas-Emch et al. (1994) and others (e.g., Bachen et al., 1992) found that acute psychological stress significantly decreased the blastogenic response to mitogens (e.g., Con A). It is possible that differences in the gender relevance of the task may have rendered the math stressor less evocative for the present sample of women (Matthews, Davis, Stoney, Owens, & Caggiula, 1991; Stoney, Matthews, McDonald, & Johnson, 1988).

Although the gender relevance of the stressor may have resulted in a weaker effect on the proliferative response to mitogens, it is important to note that the present study replicated and extended much of the past research using men (e.g., Lovallo et al., 1990; Sgoutas-Emch et al., 1994). For instance, both Lovallo et al. (1990) and Sgoutas-Emch et al. (1994) examined only men and found that heart rate reactivity predicted cortisol responses during acute psychological stress. We documented the same mechanism and further demonstrated that individual differences in cardiovascular sympathetic control was related to (a) cortisol reactivity and (b) short-term changes in NKCA by means of mediation by SBP reactivity.

Although the present study suggests that several endocrine hormones were not responsible for the associations between

cardiovascular sympathetic control and short-term changes in NKCA, it is important to note that additional hormones such as hypothalamic CRH may have contributed to the association reported in this study. Consistent with this possibility, CRH has been shown to have joint effects on both the autonomic and immune systems (Irwin, Hauger, Brown, & Britton, 1988). Research is now being conducted to examine such potential pathways and their associated time course of action.

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