Psychophysiological Correlates of Systemic Inflammation in
Black and White Men

Kimberly G. Lockwood, MS ^{a*}

J. Richard Jennings, PhD ^{a,b}

Karen A. Matthews, PhD ^{a,b}

^aDepartment of Psychology, University of Pittsburgh, Pittsburgh, Pennsylvania 15260, USA

^bDepartment of Psychiatry, University of Pittsburgh, Pittsburgh, Pennsylvania 15260, USA

*Address correspondence to: Kimberly Lockwood, Department of Psychology, University of Pittsburgh, Room 506 Old Engineering Hall, 3943 O'Hara Street, Pittsburgh, PA, 15260. E-mail: KGL8@pitt.edu

Abstract

Inflammation plays a critical role in the pathophysiology of cardiovascular disease (CVD) and levels of circulating inflammatory markers are associated with future CVD risk. However, the physiological mechanisms that control systemic levels of circulating inflammatory markers are not well understood. Here, we explore possible autonomic nervous system mechanisms by testing whether resting and stressor-evoked cardiovascular responses are associated with two markers of systemic inflammation: interleukin(IL)-6 and C-reactive protein (CRP). Subjects were 159 Black and 129 White men ($M = 33.0$ years) who completed a laboratory protocol including an anger recall speech task. Electrocardiography and impedance cardiography data were collected during a resting baseline, the speech task, and a final recovery period. Hierarchical regressions tested whether resting or stressor-evoked levels of heart rate (HR), high-frequency heart rate variability (HF-HRV), pre-ejection period (PEP), and pulse transit time (PTT) were associated with CRP or IL-6. Higher resting HR was associated with higher CRP ($\beta = .19, p = .003$) and IL-6 ($\beta = .13, p < .05$). Similarly, shorter resting PTT was associated with higher CRP ($\beta = -.21, p < .001$) and IL-6 ($\beta = -.14, p = .02$). In addition, greater stressor-evoked decreases in HF-HRV were associated with higher CRP ($\beta = -.14, p = .01$). Associations were independent of age, race, body mass index (BMI), smoking behavior, and socioeconomic status. Resting HF-HRV and PEP were also associated with CRP and IL-6, but associations were not significant after controlling for BMI and smoking behavior. These findings indicate that resting HR and PTT, as well stressor-evoked HF-HRV reactivity, are associated with systemic inflammation. Our results suggest that both tonic and stressor-evoked sympathetic and parasympathetic nervous system activity may contribute to regulation of systemic inflammation.

KEYWORDS: Inflammation; Psychophysiology; Cardiovascular; Stress Reactivity

1.0 Introduction

Inflammatory processes play a key role in cardiovascular disease (CVD) pathophysiology (Libby et al., 2002; Ross, 1999). In addition, basal levels of inflammatory markers in the peripheral circulation predict future CVD risk. Specifically, higher levels of inflammatory markers, such as C-reactive protein (CRP) or interleukin (IL)-6, are associated with greater likelihood of developing CVD (Danesh et al., 2008, 2000). These basal levels of circulating inflammatory markers are commonly referred to as “systemic inflammation”, because they provide a non-specific indication of ongoing inflammatory processes in the body. Currently, the physiological mechanisms that drive and regulate systemic inflammation are not fully understood. The present study examined possible autonomic mechanisms underlying systemic inflammation by testing whether resting and stressor-evoked cardiovascular responses are associated with markers of systemic inflammation.

The autonomic nervous system (ANS) is one physiological system thought to regulate systemic inflammation. Two of the primary divisions of the ANS are the parasympathetic nervous system (PNS) and the sympathetic nervous system (SNS). Both divisions innervate multiple organs and regulate a wide variety of physiological functions outside of conscious control, including immune function (Kemeny, 2011). Animal and *in vitro* studies have provided evidence for associations between each division of the ANS and inflammation. Specifically, animal research indicates that tonic PNS input inhibits inflammation. This research is summarized by Tracey’s (2002; 2009) cholinergic anti-inflammatory pathway. According to this proposal, tonic stimulation of the vagus nerve promotes the release of acetylcholine (ACh), the primary neurotransmitter of the PNS. ACh acts on nicotinic, α -bungarotoxin-sensitive ACh

receptors on macrophages, inhibiting macrophage production of proinflammatory cytokines. Through this mechanism, tonic PNS activity prevents the overproduction of cytokines and protects the body from the harmful effects of excessive inflammation (Tracey, 2002).

The role of the SNS in driving systemic inflammation is less clear. The SNS innervates a number of key immune organs, but sympathetic activation can both stimulate and inhibit production of inflammatory cytokines (Nance and Sanders, 2007). Rodent and *in vitro* evidence indicates that SNS activation drives short-term increases in IL-6, which may contribute to higher basal levels of systemic inflammation. Specifically, increases in sympathetic catecholamines (e.g., norepinephrine) lead to increased activation of cellular nuclear factor κ B (NF- κ B), a transcription factor that triggers IL-6 production (Bierhaus et al., 2003; Johnson et al., 2005; Tracey, 2009). Direct human evidence for this mechanism is limited. Preliminary work aligns with rodent findings, indicating that healthy adults show a positive association between stress-induced increases in norepinephrine and IL-6 (Kop et al., 2008). These results suggest it is theoretically plausible that increased SNS activation may trigger increases in systemic inflammation.

Similar to animal and *in vitro* models, human studies employing non-invasive psychophysiological methods to indirectly assess ANS activity also support regulation of systemic inflammation by the ANS. Such studies report positive correlations between resting HR and systemic inflammation (Piwońska et al., 2008; Rogowski et al., 2007; Whelton et al., 2014). Although the authors of these studies suggest that higher HR indicates greater sympathetic control over the cardiovascular system, HR is influenced by both the PNS and SNS (Cacioppo et al., 2007). Thus, it is unclear from this literature whether associations between HR and systemic inflammation in humans are driven primarily by the SNS.

Other psychophysiological measures of the distinct branches of the ANS may also be associated with systemic inflammation. Specifically, high frequency heart rate variability (HF-HRV), a marker of PNS input to the heart (Berntson et al., 1997), is negatively associated with circulating CRP and IL-6 (Cooper et al., 2015; Sajadieh et al., 2004; Singh et al., 2009; Sloan et al., 2007). Lower resting HF-HRV is also associated with greater stimulated production of cytokines, including IL-6, in response to an *in vitro* inflammatory challenge (Marsland et al., 2007). These findings are consistent with the existence of the cholinergic anti-inflammatory pathway (Tracey, 2009, 2002), indicating that lower levels of tonic PNS activity are associated with heightened systemic inflammation. SNS activity can also be assessed using cardiovascular measures, such as pre-ejection period (PEP). However, there is little human evidence linking SNS responses with systemic inflammation. Only one study has assessed this relationship, finding that PEP was not significantly associated with CRP (Singh et al., 2009). It is unknown whether PEP associates with IL-6.

It is important to note that current psychophysiological evidence linking ANS activity with inflammatory markers has largely focused on resting levels of these cardiovascular ANS indices. However, previous studies have shown that greater chronic and acute psychological stress are also associated with heightened systemic inflammation (Rohleder et al., 2014) and affect the ANS (Carter and Goldstein, 2015; Nater et al., 2013). Cardiovascular reactions to laboratory stressors have been found to be consistent within an individual over time (Debski et al., 1993; Kamarck et al., 1992), raising the possibility that these responses may influence chronic inflammation. Moreover, rodent and *in vitro* models indicate that the ANS regulates transient, stressor-evoked inflammatory responses (Johnson et al., 2005). These findings suggest

that individual differences in ANS stress reactivity may also be associated with levels of systemic inflammation. This possibility remains largely unexplored in the existing literature.

Taken together, this evidence suggests that the ANS plays a role in stimulating and inhibiting systemic inflammation. There is, however, a paucity of studies examining 1) the association of systemic inflammation with concurrent psychophysiological measures of both SNS and PNS activity, and 2) the role of individual differences in ANS stress reactivity in relation to systemic inflammation. Thus, the first aim of the present study was to assess associations between systemic inflammation and resting psychophysiological measures of PNS and SNS activity. Specifically, we hypothesized that higher levels of systemic inflammation would be associated with 1) lower resting levels of PNS activity (HF-HRV), 2) higher SNS activity (PEP), and 3) higher HR, which reflects both PNS and SNS activity. Additionally, because previous studies have largely focused on resting levels of ANS activity, the second aim of the current study was to examine whether systemic inflammation is associated with individual differences in SNS and PNS reactivity to a stressful laboratory task. In particular, we hypothesized that higher levels of systemic inflammation would be associated with 1) greater stress-related decreases in PNS activity (HF-HRV), 2) larger increases in SNS activity (PEP), and 3) greater increases in combined SNS and PNS activity (HR), independent of resting SNS and PNS levels.

2.0 Methods

2.1 Sample

Participants were drawn from the Pittsburgh Youth Study (PYS). Initiated in 1987, PYS is an ongoing study of black and white men originally designed to examine the development of delinquency and antisocial behavior. Details of the original study and follow-up recruitment are

published elsewhere (Loeber et al., 1998; Lynam et al., 2008). Briefly, a population-based sample of boys was drawn from Pittsburgh public school students enrolled in either first or seventh grade, with approximately 500 in each cohort. Recruitment involved an initial screening process to identify males at high risk for antisocial and delinquent behavior later in life. Risk status was classified as high if students in the younger cohort exhibited one of seven antisocial behaviors or those in the older cohort exhibited three antisocial behaviors. Approximately half of the original sample was classified as high risk. Participants have been followed since study initiation.

A subsample of adult participants from the original PYS study was recruited to participate in the laboratory-based Pathways to Healthy Hearts Study. Participants were excluded if they were incarcerated, lived more than 75 miles away from Pittsburgh and were not planning to return, were severely mentally disabled, had withdrawn from the original PYS study, or were deceased. Of the 322 eligible men in the original younger cohort, 267 participated in the laboratory study. Because the target sample size was 300 men, we then recruited 40 additional participants from eligible men in the older cohort. In total, 307 men participated in the laboratory protocol. This study was approved by the Institutional Review Board at the University of Pittsburgh and all participants provided informed consent.

Of the 307 men who participated in the laboratory protocol, 19 were excluded from the present analyses due to CRP values greater than 10 mg/dL. This resulted in an analytic sample of 288 men. Although most participants had complete data for all variables of interest, a small number had incomplete data for the psychophysiological variables due to data collection issues. For this reason, the N for each regression analysis varied slightly; the N for each set of regressions is noted in regression tables.

2.2 Procedures

Prior to the laboratory session, participants were instructed to abstain from food and beverages for 8 hours and caffeine, tobacco, and exercise for at least three hours. After arriving at the laboratory, participants completed a blood draw and anthropometric measures. Participants were then prepared for psychophysiological recording and seated upright in a comfortable chair in a separate room. The time between the blood draw and the beginning of the reactivity protocol was approximately 45 minutes, with the exception of a few subjects ($N=13$) who were ill or failed to fast prior to the lab visit; these subjects returned within three weeks for the fasting blood draw. To begin the reactivity protocol, participants completed a 10-minute resting baseline period while watching a nature video. The rest period was followed by three tasks: mental arithmetic (3 minutes), mirror tracing (3 minutes), and an anger recall speech task (3 minutes preparation, 4 minutes speech). The anger recall task asked participants to think about something that made them very angry in the last 6 months and to describe in their speech what happened, why they were angry, and the outcome. During the tasks, an experimenter remained in the room to provide instructions for the stress tasks and add a social evaluation element. After the first two tasks, participants completed ratings of task stressfulness and demand on a 5-point Likert-type scale, followed by a 5-minute recovery period. After the speech task ratings, participants completed another set of ratings and a 10-minute final recovery period. During each recovery period, participants watched a nature video. For the purposes of this study, we elected to focus on the preparation period for the anger recall speech task because it did not involve speaking, which interferes with multiple cardiovascular psychophysiological measures.

During the laboratory session, HR and HF-HRV were collected continuously using electrocardiogram (ECG) with a modified lead II electrode placement following published

guidelines (Jennings et al., 1981). Respiration rate was collected using a respiratory belt. Pre-ejection period (PEP), calf pulse transit time (PTT), and pulse-wave velocity (PWV) were assessed using impedance cardiography (ICG). Impedance signals were collected using bands on the thorax and the left calf following standard impedance guidelines (Sherwood et al., 1990). Chest signals were collected using Biopac System (Goleta, CA) modules (100C series), whereas calf signals were collected using a Minneapolis 504B cardiograph.

2.3 Psychophysiological Data Processing

ECG signals were converted to digital format (1000 Hz sampling, 16 bit analog to digital converter) and prepared for analysis using Mindware Technology (Gahanna, OH) software. Mean HR and HRV were derived with the MindWare HRV module (Mindware Technologies, Gahanna, OH). Prior to calculating psychophysiological measures, trained study staff inspected ECG signals for irregularities and corrected artifactual R-waves. Such corrections were required for less than 5% of any data file. Average measures were calculated for the resting baseline, speech preparation, and final recovery periods. We randomly selected 10% of the data and calculated inter-rater reliability among staff (4-5 individuals depending on the measure) (Bartko, 1966). Mixed effects models with random effects for the subject and rater were used to calculate intraclass correlations (ICC; median ICC = .90, range .72-.998).

Psychophysiological measures were processed using established guidelines (Berntson et al., 1997; Jennings et al., 1981; Sherwood et al., 1990); specifically, we assessed HR, HF-HRV, PEP, PTT, and PWV. We employed spectral analysis to estimate the different frequency components of HRV. HF-HRV (0.12-0.40 Hz) was used as a marker of PNS input to the heart; higher HF-HRV reflects more PNS input (Berntson et al., 1997). PEP is an indicator of cardiac contractility that is largely driven by β -adrenergic (SNS) mechanisms; shorter PEP reflects

greater SNS input (Sherwood et al., 1990). PEP was estimated using the time between the q wave of the ECG and the b point in the thoracic impedance signal.

We also included two vascular measures, PTT and PWV, which reflect a combination of autonomic influence and arterial stiffness. PTT refers to the time it takes a pulse wave to travel to an arterial point; it is thought to be driven primarily by β -sympathetic influence on the heart and vasculature, as well as the characteristics of the arterial walls (Contrada et al., 1995; Obrist et al., 1979; Weiss et al., 1980). Here, calf PTT was assessed from the r-wave of the electrocardiogram to the b point of the calf impedance wave. PWV refers to the speed with which pulse wave travels up from the aorta and out to the arterial system (Nürnberg et al., 2003). Clinically, PWV is widely accepted as a measure of arterial stiffness (Berntson et al., 2007), but it is also influenced by SNS control of the vasculature (Nürnberg et al., 2003). In the present study, PWV was calculated as $PWV (m/s) = \frac{body\ length\ (m)}{calf\ PTT - PEP\ (s)}$. To test our assessment procedure, the PWV measure was evaluated in a prior study of 27 men of comparable age/race to the present sample (Jennings et al., unpublished results). Measures using the impedance technique were compared with measures taken (on a separate occasion) using clinical instruments employing Doppler ultrasound and tonometry measures (model 810-a, 10 MHz, Parks Medical Electronics, Aloha, OR). Used in epidemiological studies, the latter PWV is derived using proprietary formulae executing a number of corrections related to age, gender, and weight. The impedance measures of PWV correlated highly ($r > .65$) with the clinical measures and estimates of arterial PWV were closer to those in the literature than the ‘corrected’ measures from the clinical assessment.

2.4 Inflammatory Markers

Systemic inflammation was measured with two circulating inflammatory markers: CRP and IL-6. Participants with CRP values > 10 mg/dL were not included in the analytic sample. Assays were carried out by the Heinz Laboratory at the University of Pittsburgh using standard techniques. High sensitivity CRP was measured turbidimetrically, with intra- and inter-assay coefficients of variation (CVs) of 5.5% and 3.0%, respectively. IL-6 was measured in duplicate using a high sensitivity ELISA kit (R&D Systems, HS600) with intra-assay CV of 9.1% and an inter-assay CV of 10.2%.

2.5 Covariates

Age, race, body mass index (BMI), cigarette use, marijuana use, and current SES were included as covariates. BMI was determined using participants' height and weight measurements and calculated as weight (kg)/height (m^2). Participants completed questionnaire measures of cigarette and marijuana use. For cigarette use, participants were placed into four categories: never smoked, not smoked in past year, <10 cigarettes/day in past year, or >10 cigarettes/day in past year. Marijuana use was dichotomized as having used marijuana in the past year or not. Current SES was self-reported and measured using the two-factor Hollingshead Index (Hollingshead, 1975), which incorporates occupational status and educational attainment. Participants also reported years of education completed and whether they were employed at least part-time. Participant ratings of task demand and stressfulness were included as covariates in any regression models with psychophysiological reactivity as a predictor variable. Participants also reported medication use, including high blood pressure medication, beta-blockers, depression medication, or anti-inflammatory drugs.

2.6 Statistical Analysis

Statistical analyses were carried out with SPSS. CRP, IL-6, HF-HRV, and BMI were not normally distributed and were natural log transformed prior to analyses; these transformations ameliorated outlier issues with IL-6 and BMI detected in preliminary descriptive analyses. To control for the effects of respiration on HF-HRV, we regressed HF-HRV on respiration rate for each corresponding period (i.e., rest, speech preparation, recovery). Saved residuals were used for the rest of the analyses.

Effects of the speech preparation task on HR, HF-HRV, PEP, and PTT were assessed using repeated measures ANOVAs. Huynh-Feldt corrections were applied if the assumption of sphericity was violated. Planned contrasts assessed whether mean values during the speech preparation task and recovery periods differed significantly from resting levels.

Residualized reactivity scores were created for HR, HF-HRV, PEP, and PTT by regressing speech preparation task levels on resting levels. These residuals were used in correlation and regression analyses. Preliminary correlation analyses were used to characterize relationships between study variables.

Any significant correlation between resting levels or reactivity and either CRP or IL-6 was further probed with hierarchical linear regressions. These regressions used resting levels or reactivity scores to predict CRP and IL-6. Each association was tested with three regressions, adding in additional covariates with each model to determine independence of the association between psychophysiological and inflammatory variables. Covariates were added in the following order:

Model 1: Age, Race, Risk Group

Model 2: Model 1 + BMI, Smoker Category, Marijuana User

Model 3: Model 2 + Hollingshead

Regression analyses for PTT also controlled for distance between electrodes on the chest and calf, as measured by centimeters between the lower chest impedance band and the upper calf impedance band; this variable was included in Model 1 of PTT regressions.

Ancillary analyses also tested whether recovery levels of each psychophysiological variable were correlated with CRP and IL-6. For these analyses, we created residualized recovery scores for HR, HF-HRV, PEP, and PTT by regressing recovery levels onto resting and speech preparation task levels.

Finally, we tested race as a moderator in each of the associations between psychophysiological predictors and systemic inflammation. To do this, we initially probed possible interactions with scatterplots broken down by race. We further tested any possible interactions by creating a race x psychophysiological predictor interaction term and used it to predict CRP and IL-6 in regression models.

3.0 Results

3.1 Descriptive Statistics

Participants were between the ages of 30-41 and 55% of the participants were Black and 45% were White (Table 1). Approximately half of the sample was categorized as “high risk” during the original PYS study. Twenty-eight percent of the sample had not completed high school, 34% had completed high school, and 38% had at least one year of college education. The majority of the sample reported at least part-time employment (78.1%). On average, the sample was overweight and had moderate levels of inflammatory markers. Approximately half of the sample reported smoking cigarettes and half reported using marijuana in the past year. Medication use was not common amongst the analytic sample, with 10% reporting use of any of the four categories of medications.

3.2 Speech Preparation Task Effects

Across participants, the majority of the psychophysiological variables followed the anticipated pattern of changes across the testing session (Table 2). HR increased from resting to the speech preparation task and remained slightly elevated during recovery HR ($\eta_p^2 = .60$). HF-HRV decreased from resting to task and returned to resting levels during recovery ($\eta_p^2 = .05$). Respiration rate increased during the task and decreased during recovery ($\eta_p^2 = .05$). The main effect of the task on HF-HRV remained significant when controlling for task-evoked respiratory changes ($F(2, 556) = 9.32, p < .001, \eta_p^2 = .03$). There was a significant main effect of task period on PEP ($\eta_p^2 = .02$). However, contrasts indicated that this effect was largely driven by higher PEP levels during recovery compared to resting; PEP during the task was not significantly different compared to resting. Finally, PTT decreased during the task and returned to resting levels during recovery ($\eta_p^2 = .06$).

3.3 Correlations

There were a number of significant correlations between primary study variables (Table 3). CRP was positively correlated with IL-6, BMI, and resting HR ($ps < .001$) and negatively correlated with resting HF-HRV ($p = .001$), HF-HRV reactivity ($p = .001$), and resting PTT ($p < .001$). IL-6 was positively correlated with smoking status ($p = .02$), BMI ($p < .001$), and resting HR ($p = .001$) and negatively correlated with education ($p = .006$), Hollingshead score ($p = .003$), and resting PTT ($p = .03$). Regression analyses were conducted for psychophysiological variables that were significantly correlated with either CRP or IL-6.¹

3.4 Psychophysiological Variables and CRP

¹ We also conducted regression analyses for the association between resting PWV and CRP, given the marginal correlation ($r = .11, p = .07$). The association did not reach significance after adjusting for covariates.

3.4.1 Resting

Regression model results for the associations between resting psychophysiological variables and CRP are shown in Table 4. As predicted, resting HR was significantly associated with CRP across each of the three models. Specifically, higher HR was associated with higher CRP (Fig. 1a). In the fully adjusted model, resting HR explained 3% of the variance in CRP. As hypothesized, resting HF-HRV was negatively associated with CRP in the minimally adjusted model (Table 4, Model 1); however, this association was no longer significant after adjusting for BMI and smoking variables in Model 2. Similarly, resting PEP was negatively associated with CRP in the minimally adjusted model (Table 4, Model 1), but was not independent of BMI and smoking variables in Model 2. Resting PTT was negatively associated with CRP across all three models. Specifically, shorter PTT was associated with higher CRP (Fig. 1b). In the fully adjusted model, resting PTT accounted for 4% of the overall variance in CRP.

3.4.2 Speech Preparation Task Reactivity

As hypothesized, HF-HRV reactivity was negatively associated with CRP across each of the three regression models (Table 4). Specifically, greater stressor-evoked decreases in HF-HRV were associated with higher CRP (Fig. 2). In the fully adjusted model, HF-HRV reactivity explained 2% of the overall variance in CRP. To ensure that this association was not explained by confounding variables, follow-up analyses were conducted. First, participants' ratings of task demand, subjective stress, and self-reported ratings of feelings about their speech were included as covariates in the regression model; these variables did not account for significant variance in CRP and did not diminish the relationship between HF-HRV reactivity and CRP. In another analysis, residualized speech preparation task scores for respiration rate were included as a

covariate in the regression model; inclusion of respiration task scores did not decrease the relationship between HF-HRV reactivity and CRP.

3.4.3 Recovery

There were no significant correlations between any psychophysiological variable during recovery and CRP.

3.5 Psychophysiological Variables and IL-6

3.5.1 Resting

Regression models for associations between resting psychophysiological variables and IL-6 are shown in Table 5. The same sequence of regression models was used as with the CRP analyses. As predicted, resting HR was significantly associated with IL-6 across each of the three regression models. Specifically, higher resting HR was associated with higher IL-6 (Fig. 3a). In the fully adjusted model, resting HR explained 1% of the variance in IL-6. Resting HF-HRV and resting PEP were significantly associated with IL-6 in the minimal model (Table 5, Model 1), but these associations were attenuated after adjustment for BMI and smoking variables in Model 2. Finally, resting PTT was significantly associated with IL-6 across each of the three models. Specifically, shorter PTT was associated with higher IL-6 (Fig 3b). In the fully adjusted model, PTT accounted for 2% of the variance in IL-6.

3.5.2 Speech Preparation Task Reactivity

There were no significant correlations between any speech preparation task-evoked psychophysiological response and IL-6.

3.5.3 Recovery

There were no significant correlations between recovery levels of any psychophysiological variable and IL-6.

3.6 Medication Status

All significant regression analyses were re-run including a dichotomous medication covariate, indicating whether the participant was taking high blood pressure medication, beta-blockers, depression medication, or anti-inflammatory drugs. Inclusion of this variable did not affect any significant regression results.

3.7 Moderation by Race

Race did not moderate the association between any psychophysiological predictor variable and CRP or IL-6.

4.0 Discussion

In this study, we tested whether resting and stressor-evoked psychophysiological responses were associated with two markers of systemic inflammation, CRP and IL-6. After controlling for a number of covariates, resting HR and PTT were the strongest resting predictors of both CRP and IL-6. In contrast, HF-HRV and PEP did not show robust associations with CRP and IL-6. In addition, HF-HRV task reactivity was significantly associated with CRP in fully adjusted models. These significant results were independent of age, race, BMI, cigarette and marijuana use, and SES. Our findings suggest that the ANS may be one physiological system that participates in the regulation of systemic inflammation. In the following section, we discuss how these results provide insight into the autonomic mechanisms that participate in the regulation of systemic inflammation

4.1 Resting Psychophysiological Measures and Systemic Inflammation

Our results indicated that higher CRP and IL-6 were associated with higher resting HR and shorter PTT. Both HR and PTT reflect combined input from multiple sources: HR reflects SNS and PNS input, whereas PTT reflects autonomic control of the heart and vasculature

(Cacioppo et al., 1994a, 1994b; Contrada et al., 1995; Obrist et al., 1979). Although the type of autonomic influence differs between HR and PTT, our results suggest that more complex ANS measures may be more robust predictors of systemic inflammation in this sample. Interestingly, the SNS- and PNS-specific markers did not show robust associations with systemic inflammation. Although resting HF-HRV and PEP were significantly associated with both CRP and IL-6 in minimally adjusted models, these associations were attenuated after controlling for BMI and smoking variables. Our results suggest that the SNS and PNS may indeed play distinct roles in regulating systemic inflammation, but these effects are not independent of body composition or smoking behavior in the present sample.

The relationship between resting HF-HRV and systemic inflammation was only present in the basic analyses before adjustment for BMI and smoking status. As such, the relationship is not as robust as expected, given previous evidence for the cholinergic anti-inflammatory pathway (Tracey, 2002; 2009) and associations between resting HF-HRV and systemic inflammation in prior work with other samples (Cooper et al., 2015; Jarczok et al., 2014; Sajadieh et al., 2004; Sloan et al., 2007). However, there are other human studies that also fail to find strong support for the cholinergic anti-inflammatory hypothesis using psychophysiological measures (Lampert et al., 2008; von Känel et al., 2011). Furthermore, a review of the literature concluded that associations between HRV and systemic inflammation are often driven by non-vagal HRV measures whose autonomic origins are less well understood (e.g., low frequency HRV) (Haensel et al., 2008). The reason for these inconsistencies is not entirely clear, but could be attributed to sample differences, measurement issues, or simply a weak relationship between resting HF-HRV and systemic inflammation. Our discrepant findings could be due to the fact that our sample differs from previous reports on gender and racial/ethnic breakdown, as well as

differences in smoking behavior and BMI. Body composition and smoking behavior may have a substantial impact on the relationship between resting HF-HRV and systemic inflammation as obtained in the present study. Although some prior studies continue to find robust associations after controlling for smoking and BMI in regression analyses, there are complex effects of body composition and smoking on inflammatory processes that may not be accounted for by statistical control. Thus, our results highlight the importance of considering health behaviors and body composition when characterizing the association between PNS activity and systemic inflammation.

The lack of robust association between resting PEP and systemic inflammation was less surprising for two reasons. First, our results replicate those of Singh et al. (2009), who also found univariate associations between PEP and CRP that were not significant in multivariate models. Here, our results expand on previous work by showing that PEP is not robustly associated with IL-6. Second, the role of the SNS in regulating inflammation is complex. The SNS innervates a number of immune organs (Nance & Sanders, 2007) and sympathetic catecholamines (e.g., norepinephrine) can both inhibit and facilitate production of IL-6 and other inflammatory cytokines (Meltzer et al., 2004; Nance and Sanders, 2007). Thus, resting assessment of one indicator of SNS input to the heart may not provide a complete picture of sympathetic regulation of systemic inflammation.

Our results suggest that HR, a cardiovascular indicator of combined SNS and PNS activity is a more robust predictor of systemic inflammation than markers of SNS or PNS specific activity. This has important implications for work on the physiological mechanisms that regulate systemic inflammation. Given our interest in elucidating the mechanisms that drive systemic inflammation in humans, it is natural to try to parse the distinct roles of the SNS and

PNS. However, the SNS and PNS do not just have opposing effects on psychophysiological responses, but can also work synergistically (Cacioppo et al., 2007). Moreover, the ANS has specific effects on immune function via effector organs outside of the cardiovascular system (Jänig, 2014). By attempting to deconstruct the disparate influences of the SNS and PNS from cardiovascular indices, we are likely oversimplifying ANS regulation of systemic inflammation. Thus, the field would benefit from mechanistic work focused on how the SNS and PNS work synergistically to regulate inflammation through different effector organs in humans.

4.2 Psychophysiological Reactivity and Systemic Inflammation

To our knowledge, this is the first study linking greater HF-HRV reactivity (greater decrease in response to stress) with heightened systemic inflammation. The cholinergic anti-inflammatory pathway provides a mechanistic explanation for this association: vagal output stimulates production of ACh, which inhibits production of proinflammatory cytokines like IL-6 and eventually CRP (Tracey, 2002, 2009). Based on this model, reduced vagal input will result in less cholinergic inhibition of inflammation and, ultimately, to higher levels of systemic inflammation. Notably, the cholinergic anti-inflammatory pathway is largely based on tonic levels of vagal input. However, it is plausible to extend this model to stressor-evoked changes in PNS activity, provided that these changes reflect an individual's typical response to mild stressors. In other words, frequent exaggerated stressor-evoked decreases in PNS activity would result in overall reduced cholinergic inhibition of inflammation and higher levels of inflammatory markers like IL-6 and CRP. To date, however, it is unknown whether individual differences in laboratory stressor-evoked HF-HRV represent a trait-like characteristic that parallel responses to stress outside of the laboratory. However, one study did find that reactivity of another HRV metric (LF/HF ratio) to a brief, mildly stressful laboratory task was significantly

correlated with reactivity to an acute stressor in the “real world” ($r=0.69$) (Dikecligil and Mujica-Parodi, 2010). Thus, future work should explore whether transient reductions in HF-HRV during stress are similarly representative of an individual’s typical responses to acute stress outside of the laboratory. Such work could also help determine whether greater HF-HRV reactivity is clinically relevant for CVD risk.

The association between HF-HRV reactivity and CRP could be driven by a common mechanism, such as adiposity. Because adiposity has complex and profound effects on both cardiovascular function and systemic inflammation, it is difficult to parse out its role in the associations reported here. In the present data, HF-HRV reactivity and CRP were significantly correlated with BMI, but regression models showed that the association between HF-HRV reactivity and CRP was independent of BMI. In addition, follow-up analyses also indicated that obesity ($BMI > 30$) did not moderate the association between HF-HRV reactivity and CRP. Elucidating whether adiposity is a common mechanism linking HF-HRV reactivity with systemic inflammation requires more sophisticated measures of adiposity.

In contrast to CRP, IL-6 was not significantly associated with HF-HRV reactivity. The reason for this is not entirely clear but could be related to differences in stability between these two inflammatory markers. In general, CRP is a more stable marker of systemic inflammation than IL-6, with levels remaining consistent across relatively long periods (1-5 years) (Ridker, 2007). Although IL-6 is fairly stable compared to other proinflammatory cytokines, it is prone to diurnal rhythms and fluctuations due to acute stress, exercise, and other behavioral factors (O’Connor et al., 2009; Steptoe et al., 2007). Thus, HF-HRV reactivity may be more likely to correlate with acute IL-6 stress reactivity, rather than basal levels of IL-6.

4.3 Strengths and Limitations

There are a number of limitations to the present study. First, our results are limited to cardiovascular ANS relationships with inflammatory measures. Cardiovascular indices of ANS function have long been shown to covary with individual differences in immune responses (Cacioppo et al., 1995; Uchino et al., 1995) and are a useful noninvasive tool for assessing SNS and PNS activity in humans. Importantly, such indices are indirect and do not necessarily reflect direct regulation of the immune system. There is ample animal and *in vitro* evidence that the ANS regulates different immune organs (e.g., spleen, lymphoid organs, bone marrow) with a high degree of specificity (Jänig, 2014). To our knowledge, however, assessing the direct effects of the ANS on effector organs in humans is substantially more invasive than the cardiovascular psychophysiological measures used in the present study. Thus, our results should be interpreted with the understanding that cardiovascular psychophysiological measures are an indirect proxy for ANS regulation of inflammation. Future work would benefit from the development of tools for noninvasively measuring regulation of systemic inflammation via ANS input to other immune organs in humans.

In terms of our methods, these data are cross-sectional, so any directional inferences are purely speculative. Future longitudinal work with multiple assessments of systemic inflammation could provide further insight on this issue. In addition, our sample was composed only of men, so we are not able to comment on gender differences in associations between psychophysiological variables and systemic inflammation. These associations should be tested in a female sample to determine whether there are similar patterns. Also, this study did not use strict health-related exclusion criteria, because participants were drawn from the previously recruited PYS sample. As such, some of the participants smoked tobacco or marijuana, used medications, or had a high BMI. We statistically controlled for these variables and excluded

participants with clinically elevated levels of CRP, but there may be unmeasured health or health behavior factors that account for our results.

Despite these limitations, this study has several notable strengths. First, we had sufficient power to control for a wide range of covariates; this allowed us to rule out a number of alternative explanations. Also, we measured a variety of psychophysiological indicators, allowing us to test whether systemic inflammation showed divergent associations with PNS, SNS, or combined ANS indicators. Additionally, we were able to explore associations between systemic inflammation and both resting and stressor-evoked psychophysiological responses. These unique aspects of our data allowed us to explore possible physiological mechanisms that might drive heightened systemic inflammation. Taken together, our results indicate that both basal and stressor-evoked psychophysiological responses are associated with systemic inflammation, supporting growing evidence that the ANS contributes to the regulation of systemic inflammation.

Acknowledgments

This work was supported by National Institutes of Health grant HL111802 (KAM), HL07560, and the National Science Foundation Graduate Research Fellowship Program (DGE-1247842).

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Table 1
Descriptive Statistics

	N	Mean or n	SD or %
Age (years)	288	33.0	2.6
Race (Black)	288	159	55.2%
Risk Group (high)	288	142	49.3
Education (grade)	288	12.7	2.5
Employed (at least part-time)	288	225	78.1%
Hollingshead Score	286	31.59	14.5
BMI (kg/m ²)	288	29.4	7.0
CRP (mg/L)	288	2.1	2.1
IL-6 (pg/mL)	288	2.0	1.7
Smoker Category	288		
Never Smoked		85	29.3%
Not in Past Year		44	15.3%
< 10/Day in Past Year		103	35.8%
> 10/Day in Past Year		56	19.4%
Marijuana User (past year)	288	141	49.0%
Medication Use			
High Blood Pressure	288	13	4.5%
Beta Blockers	288	5	1.7%
Anti-Inflammatory	288	9	3.1%
Anti-Depressants	288	11	3.8%

Note: BMI = body mass index; CRP = C-reactive protein; IL-6 = interleukin-6;

Table 2
Mean HR, HF-HRV, respiration rate, and PEP across the testing session

	Baseline		Task		Recovery		<i>F</i>	<i>p</i>
	Mean	<i>SD</i>	Mean	<i>SD</i>	Mean	<i>SD</i>		
HR (bpm)	68.12	11.38	74.34*	12.21	69.31*	11.3	353.28	<.001
HF-HRV (ln)	6.22	1.31	5.92*	1.57	6.16	1.34	14.15	<.001
Resp (breaths/min)	16.30	3.53	17.50*	5.14	16.54	3.63	12.72	<.001
PEP (ms)	98.46	19.37	98.36	21.02	100.55*	18.62	5.05	.007
PTT (ms)	207.72	18.38	203.36*	19.48	208.41	18.74	18.37	<.001
PWV (m/s)	9.70	1.82	-	-	-	-	-	-

Note: HF-HRV is log transformed. HR = heart rate; HF-HRV = high-frequency heart rate variability; Resp = respiration rate; PEP = pre-ejection period; PTT = pulse transit time; PWV = pulse wave velocity

* Contrasts indicate a significant difference from baseline ($p < .001$)

Table 3

Bivariate Correlations

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1. Age	-															
2. Race	-.03	-														
3. Smoker	-.06	-.12	-													
4. Education	-.04	.20**	-.42***	-												
5. Hollingshead	.01	.24***	-.37***	.64***	-											
6. BMI	.04	.02	-.32***	.07	.01	-										
7. HR	-.09	.16**	-.19**	-.12	-.09	.43***	-									
8. HR Δ	-.03	.15**	-.17	.22***	.10	.05	.02	-								
9. HF-HRV	-.06	-.24***	.23***	-.05	-.03	-.41***	-.62***	.04	-							
10. HF-HRV Δ	.20***	.03	.01	.01	.08	-.14*	-.18**	-.31***	-.02	-						
11. PEP	-.17**	-.01	.04	.02	-.02	-.13*	-.10	-.08	.01	.05	-					
12. PEP Δ	.01	-.13*	.18**	-.14*	.09	-.07	-.10**	-.31***	.10	.03	-.01	-				
13. PTT	-.25***	.08	.09	.12*	.06	-.13*	-.26***	-.09	.10	.10	.56***	.16**	-			
14. PTT Δ	.03	-.08	.08	-.09	.01	-.04	-.11	-.19**	.06	.10	.12	.29***	-.01	-		
15. PWV	.06	-.10	-.03	-.07	-.06	.04	.19**	-.40	-.09	.05	.54***	.14*	-.34***	.13*	-	
16. CRP	-.01	.10	-.02	-.06	-.10	.41***	.31***	.07	-.19**	-.19**	-.12*	.03	-.23***	.04	.11	-
17. IL-6	-.06	.00	.13*	-.16**	-.19**	.25***	.20**	-.03	-.11*	-.08	-.11	-.01	-.14*	-.01	.03	.44***

Note: Δ denotes reactivity; BMI = body mass index; CRP = C-reactive protein; IL-6 = interleukin-6; HR = heart rate; HF-HRV = high-frequency heart rate variability; PEP = pre-ejection period; PTT = pulse transit time; PWV = pulse wave velocity

* $p < .05$

** $p < .01$

*** $p < .001$

Table 4

Associations between Resting and Task Physiological Indices and CRP

	N	Model 1		Model 2		Model 3	
		β	<i>p</i>	β	<i>p</i>	β	<i>p</i>
Resting HR	283	.31	<.001	.19	.002	.19	.003
Resting HF-HRV	283	-.18	.003	-.04	.51	-.03	.60
Resting PEP	276	-.12	0.04	-.07	.22	-.06	.26
Resting PTT	274	-.26	<.001	-.21	<.001	-.21	<.001
HF-HRV Reactivity	281	-.20	.001	-.14	.01	-.14	.01

Notes: CRP = C-reactive protein; HR = heart rate; HF-HRV = high-frequency heart rate variability; PEP = pre-ejection period; PTT = pulse transit time

Model 1: Age, Race, Risk group

Model 2: Age, Race, Risk group, BMI, Smoker Category, Marijuana User

Model 3: Age, Race, Risk group, BMI, Smoker Category, Marijuana User, Hollingshead

Table 5

Associations between Resting Physiological Indices and IL-6

	N	Model 1		Model 2		Model 3	
		β	<i>p</i>	β	<i>p</i>	β	<i>p</i>
Resting HR	283	.20	.001	.14	.03	.13	<.05
Resting HF-HRV	283	-.12	.04	-.06	.41	-.05	.48
Resting PEP	276	-.13	.04	-.10	.09	-.09	.11
Resting PTT	274	-.17	.009	-.14	.02	-.14	.02

Notes: IL-6 = interleukin-6; HR = heart rate; HF-HRV = high-frequency heart rate variability; PEP = pre-ejection period; PTT = pulse transit time

Model 1: Age, Race, Risk group

Model 2: Age, Race, Risk group, BMI, Smoker Category, Marijuana User

Model 3: Age, Race, Risk group, BMI, Smoker Category, Marijuana User, and Hollingshead

2 column

Figure 1. Associations between resting psychophysiological variables and CRP. (a) Higher heart rate is associated with higher CRP. (b) Shorter PTT is associated with higher CRP. *Notes:* CRP = C-reactive protein; HR = heart rate; PTT = pulse transit time

1.5 column

Figure 2. Association between stressor-evoked HF-HRV reactivity to the speech preparation task and CRP. (a) Greater stressor-evoked decreases in HF-HRV are associated with higher CRP. *Notes:* CRP = C-reactive protein; HF-HRV = high frequency heart rate variability.

2 column

Figure 3. Associations between resting psychophysiological variables and IL-6. (a) Higher heart rate is associated with higher IL-6. (b) Shorter PTT is associated with higher IL-6. *Notes:* IL-6 = interleukin-6; HR = heart rate; PTT = pulse transit time