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Heart rate variability predicts levels of inflammatory markers: Evidence for the vagal anti-inflammatory pathway

Timothy M. Cooper [a], Paula S. McKinley [b,c], Teresa E. Seeman [d], Tse-Hwei Choo [c], Seonjoo Lee [b,c], Richard P. Sloan [b,c,*]

[a] Columbia University College of Physicians and Surgeons, Columbia University Medical Center, New York, NY, United States
[b] Department of Psychiatry, Columbia University Medical Center, New York, NY, United States
[c] New York State Psychiatric Institute, New York, NY, United States
[d] Department of Medicine, David Geffen School of Medicine at UCLA, Los Angeles, CA, United States

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ABSTRACT

Evidence from numerous animal models shows that vagal activity regulates inflammatory responses by decreasing cytokine release. Heart rate variability (HRV) is a reliable index of cardiac vagal regulation and should be inversely related to levels of inflammatory markers. Inflammation is also regulated by sympathetic inputs, but only one previous paper controlled for this. In a larger and more representative sample, we sought to replicate those results and examine potential sex differences in the relationship between HRV and inflammatory markers. Using data from the MIDUS II study, we analyzed the relationship between 6 inflammatory markers and both HF-HRV and LF-HRV. After controlling for sympathetic effects measured by urinary norepinephrine as well as a host of other factors, LF-HRV was found to be inversely associated with fibrinogen, CRP and IL-6, while HF-HRV was inversely associated with fibrinogen and CRP. We did not observe consistent sex differences. These results support the existence of the vagal anti-inflammatory pathway and suggest that it has similar effects in men and women.

1. Introduction

The vagus nerve plays an important role in regulating inflammation and preventing tissue damage from excessive inflammatory responses. Vagal activity decreases production of pro-inflammatory cytokines such as TNF (Bernik et al., 2002) and inhibits the migration of leukocytes to sites of inflammation (Saeed et al., 2005), in part by its action on the reticuloendothelial system of the liver and spleen where cytokines are produced, and may function to dampen systemic inflammatory processes (Tracey et al., 2007). Data from numerous animal studies support this anti-inflammatory pathway. For example, administration of endotoxin in mice following vagotomy or in mice possessing knockout of the α7 subunit of the nicotinic acetylcholine receptor (α7nAChR) expressed in macrophages causes an unrestrained cytokine response (Borovikova et al., 2000; Wang et al., 2003). On the other hand, stimulation of the vagus nerve or administration of α7nAChR agonists has been found to decrease cytokine release (Wang et al., 2004).

Because heart rate variability (HRV) is a well-established and reliable index of cardiac vagal regulation, it should be inversely related to levels of inflammatory markers. Many studies show this predicted inverse relationship. For example, decreased low frequency HRV (LF-HRV) was found to be associated with increased levels of C-reactive protein (CRP) in a study of 1601 healthy young people (Haarala et al., 2011). A prospective cohort study of 188 middle-aged and older adults found an inverse relationship between high frequency HRV (HF-HRV) and CRP ($p < 0.01$) (Singh et al., 2009). A study of 264 middle-aged male twins found that ultra low frequency HRV and very low frequency HRV were inversely related to CRP and IL-6 after controlling for a host of factors ($p < 0.01$) (Lampert et al., 2008). IL-6 levels were shown to have an inverse relationship with HF-HRV and LF-HRV in a study of 682 patients after cardiac catheterization for acute myocardial infarction (MI) or unstable angina with elevated Troponin-T levels (Frasure-Smith et al., 2009). Inverse relationships between IL-6 and HRV have also been observed in patients with sepsis, type 1 diabetes and type 2 diabetes (Tateishi et al., 2007; Gonzalez-Clemente et al., 2007; Stuckey and Petrella, 2013).

* Corresponding author at: Presbyterian Hospital, Room 1540H, 622 West 168th St, New York, NY 10032, United States. Tel.: +1 (646) 774 8940; fax: +1 (212) 342 2006.

E-mail addresses: tmc2161@columbia.edu (T.M. Cooper), pm491@cumc.columbia.edu (P.S. McKinley), tseeman@mednet.ucla.edu (T.E. Seeman), tjc2143@columbia.edu (T.-H. Choo), sl3670@columbia.edu (S. Lee), rps7@columbia.edu (R.P. Sloan).

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Inflammatory processes are also influenced by the sympathetic nervous system (SNS), but its role is less well understood. The SNS possesses both pro- and anti-inflammatory properties and has been implicated in the production of cytokines (Koopman et al., 2011). Adrenergic signaling may activate or suppress macrophages depending on the subtype of adrenergic receptor they express (Bellinger et al., 2008). SNS activity can reduce Th1 response in favor of Th2 (Elenkov et al., 2000). Sympathetic activity has also been found to enhance leukocyte attraction (Viswanathan et al., 2005) and alter expression of cell adhesion markers (Redwine et al., 2003).

A thorough examination of the inflammatory role of the autonomic nervous system thus requires consideration of both vagally-mediated and sympathetically-mediated effects. With only a single exception, studies linking HRV and inflammation fail to control for levels of SNS activity. In that study, Thayer and Fischer found that even after controlling for SNS effects, measured by urinary epinephrine, the inverse relationships between HRV and CRP and between HRV and WBC count remained significant (Thayer and Fischer, 2009). In addition, they observed interesting sex differences in these relationships. For example, an increase of 1 SD in HRV measured as root mean square of successive interval differences was associated with a 48% decrease in CRP in men ($p = 0.05$), whereas in women, an increase of 1 SD in HRV was associated with a 104% decrease in CRP ($p = 0.008$). Larger differences in WBC count, another marker of inflammation, were also seen in women. This study suggests that there may be important sex differences in the relationship between parasympathetic activity and inflammatory markers. However, the study was limited by a small number of women ($n = 66$) relative to men ($n = 545$) and a relatively homogeneous sample of factory workers.

In the current study, we sought to replicate these findings on the relationship between HF-HRV and inflammatory markers using a larger, more diverse, and more representative sample. We tested the hypothesis that HF-HRV, as an index of cardiac vagal regulation, would be inversely related to inflammatory markers even after control for sympathetic effects. Because many studies also examine the relationship between LF-HRV and inflammatory markers, we also tested this relationship.

2. Methods

2.1. Participants

The data were collected from 1255 participants in Midlife Development in the U.S. (MIDUS), a study of the behavioral, psychological and social factors accounting for age-related variation in health and well-being in a national sample of middle-aged and older Americans (Brim et al., 2004). Data for the current study are from MIDUS II, a 9-year follow-up of the MIDUS I cohort, conducted between 2004 and 2006. MIDUS II consisted of five projects, including a self-administered survey of a wide array of behavioral, social and psychological factors and a Biomarker Project, with data collection conducted during a 1.5-day visit to a clinical research center (CRC) at the University of Wisconsin, UCLA, or Georgetown University. Biomarker data were collected from mid-2004 to mid-2009 (Ryff et al., 2012). IRB approval was obtained for data collection at the three sites, and written consent was obtained from all study participants.

2.2. Physical exam

Clinicians or trained staff evaluated vital signs, morphology, functional capacities, bone densitometry and medication usage and performed a physical exam. Medical history was obtained from participants.

2.3. Biomarker data

Subjects underwent fasting blood draws prior to breakfast. Samples were sent to the MIDUS Biocore Lab for analysis. Additionally, glycated hemoglobin and cholesterol panel assays were analyzed at Meriter Labs (Madison, WI) using a Cobas Integra® analyzer (Roche Diagnostics, Indianapolis, IN). IL-6 was measured using Quantikine® High-sensitivity ELISA kit #HS600B (R&D Systems, Minneapolis, MN). Soluble IL-6 receptor levels were measured using Quantikine® ELISA kit #DR600 (R&D Systems, Minneapolis, MN). Human soluble intercellular adhesion molecule-1 was measured by Parameter Human sICAM-1 Immunoassay (R&D Systems, Minneapolis MN). Soluble E-selectin was measured by Parameter Human sE-selectin Immunoassay (R&D Systems, Minneapolis, MN). Fibrinogen and CRP were measured by BNII nephelometer (Dade Behring Inc., Deerfield, IL). 12-h urine samples were collected overnight (7:00 PM–7:00 AM). Urinary catecholamine assays were performed using high-pressure liquid chromatography at the Mayo Medical Laboratory (Rochester, MN). Urinary norepinephrine levels were corrected for creatinine levels.

2.4. HRV assessment

After an overnight stay at the CRC, participants were provided with a light breakfast, but no caffeine consumption was permitted. Following breakfast, they began the HRV psychophysiology protocol.

ECG electrodes were placed on the left and right shoulders as well as in the left lower quadrant. Respiration bands were placed around the chest and abdomen, and the finger cuff of a Finometer beat-to-beat blood pressure monitor was placed around the middle finger of the non-dominant hand. Respiration was calibrated using an 800 cc spirobag. While participants were in the seated position, data were recorded during an 11-min baseline as part of a more extensive psychophysiology protocol with exposure to challenging stimuli and recovery periods. Here we report HRV data from this resting baseline.

Analog ECG signals were digitized at 500 Hz by a 16-bit A/D conversion board (National Instruments, Austin, TX) and passed to a microcomputer. The ECG waveform was submitted to an R-wave detection routine implemented by custom-written software, resulting in an RR interval series. Errors in marking R waves were corrected by visual inspection. Ectopic beats were corrected by interpolation.

HF-HRV (0.15–0.40 Hz) was computed based on 300-s epochs, using an interval method for computing Fourier transforms similar to that described by (DeBoer et al., (1984). The mean value of HF-HRV from the two baseline 300-s epochs was computed. The process was repeated for LF-HRV (0.04–0.15 Hz).

2.5. Respiration

Respiratory rate was measured using an Inductotrace respiration monitor (Ambulatory Monitoring Systems, Ardsley, NY). Signals from thoracic and abdominal stretch bands were collected by the A/D board at 20 Hz and submitted to a custom-written program that computed respiratory rate on a minute-by-minute basis. The mean respiratory rate for the baseline period was computed.

2.6. Statistical analysis

All analyses were carried out in SAS 9.3. The distributions of variables were examined and the right-skewed variables (HF- and LF-HRV, CRP, E-selectin, ICAM, IL-6, urinary norepinephrine) were natural log transformed prior to analysis. The associations of HF-HRV with each of the 6 inflammatory markers were separately tested within five hierarchical linear models, and the process was then repeated for LF-HRV. Individuals with data missing for a particular variable were removed from analyses involving that variable. Significance levels were corrected using the Bonferroni method to account for the 6 associations tested with each HRV variable, for each model.

In Model 1, each inflammatory marker was regressed on both HF- and LF-HRV without any covariates adjusted. Model 2 adjusted for urinary norepinephrine. Model 3 added sex, age, race, BMI, site of assessment, menstrual status, exercise and smoking status as covariates. Exercise was entered as a dichotomous variable indicating whether or not the subject engaged in at least 20 min of exercise at least 3 times a week. Smoking status was categorized into three components: current smoker, former smoker and never a smoker. In Model 4, data on use of statins, anti-inflammatory medications, and medications affecting parasympathetic activity were also adjusted for, as were heart problems and history of stroke, hypertension, diabetes, Parkinson's disease, and any other neurological conditions. Finally, Model 5 utilized the same covariates as Model 4, but both HRV variables were residualized for respiratory rate.

To address missing covariates in the data, a multiple imputation analysis was also performed, for which missing data were imputed using all variables included in Model 5 regressions using PROC MI in SAS 9.3 (Little and Rubin, 2002; Rubin, 1976). This uses a Markov Chain Monte Carlo method (Schafer, 1997) assuming arbitrary missing data patterns.

3. Results

3.1. Demographic data

Analysis was carried out on participants with data for HF-HRV and LF-HRV as well as at least one inflammatory marker ($n = 1153$). 91.3% of study participants were white. Demographic data are shown in Tables 1a and 1b. Men and women did not significantly differ in age, BMI, smoking status, history of hypertension, history of diabetes, LDL levels, ratio of total cholesterol to HDL, 12-h urinary norepinephrine, or urinary norepinephrine adjusted for creatinine. Men had significantly higher levels of heart disease, blood pressure, triglycerides and urinary norepinephrine, and significantly lower levels of glycated hemoglobin, total cholesterol, HDL and CRP.

3.2. Relationships between HRV and inflammatory markers

As Table 2 indicates, univariate analyses showed significant inverse relationships between HF-HRV and fibrinogen, soluble IL-6 receptor, ICAM and IL-6. After controlling for urinary norepinephrine, sex, age, race, BMI, study site, menstrual status, exercise, smoking, medications affecting parasympathetic activity, cardiac disease, stroke, hypertension, diabetes, Parkinson's disease and other neurological conditions, and respiratory rate, HF-HRV was significantly and inversely related to levels of fibrinogen and CRP.

In univariate analyses, LF-HRV was significantly inversely related to all inflammatory markers except E-selectin. After controlling for all covariates, LF-HRV was significantly inversely related to fibrinogen, CRP and IL-6 but lost significance for ICAM and soluble IL-6 receptor.

As Table 2 indicates, the number of subjects available for each analysis varied, due to differences in information about the covariates available for each model. Missing data were imputed using all

Table 1a
Demographic information on subjects included in the analyses.

Label	Males (497)		Females (656)		*P*-value
	N	%	*N*	%	
Current smoker	79	16	94	14	0.4522
Ever heart disease	66	13	46	7	0.0004
Ever hypertension	166	34	236	36	0.3623
Ever diabetes	64	13	79	12	0.6703

Table 1b
Demographic information on subjects included in the analyses.

Variable	Males (497)		Females (656)		Difference		*P*-value
	Mean	SD	Mean	SD	Mean	SD	
Age (years)	57.23	11.55	56.38	11.09	0.85	11.29	0.2060
Glycated hemoglobin (%)	6.39	7.36	7.59	12.64	−1.21	10.69	0.0421
SBP 1 (mm Hg)	134.30	15.12	131.30	19.17	3.00	17.54	0.0030
SBP 2 (mm Hg)	132.85	15.98	129.43	19.81	3.42	18.26	0.0012
SBP 3 (mm Hg)	131.74	15.36	128.04	19.25	3.71	17.68	0.0003
SBP mean of All 3 (mm Hg)	132.47	14.99	129.13	18.95	3.34	17.35	0.0009
SBP mean of 2 and 3 (mm Hg)	132.52	15.14	128.99	19.07	3.53	17.49	0.0005
DBP 1 (mm Hg)	79.30	10.09	74.08	10.90	5.22	10.55	<0.0001
DBP 2 (mm Hg)	78.45	9.94	73.22	10.51	5.23	10.27	<0.0001
DBP 3 (mm Hg)	77.95	9.69	72.89	9.98	5.06	9.86	<0.0001
DBP mean of all 3 (mm Hg)	78.44	9.53	73.25	10.16	5.19	9.89	<0.0001
DBP mean of 2 and 3 (mm Hg)	78.43	9.52	73.29	9.89	5.14	9.73	<0.0001
BMI (kg/m^2)	29.73	5.34	29.81	7.42	−0.08	6.61	0.8302
Total cholesterol (mm/dl)	183.07	41.03	190.31	38.85	−7.24	39.81	0.0023
HDL (mm/dl)	47.73	15.32	60.85	17.46	−13.12	16.57	<0.0001
LDL (mm/dl)	105.80	34.95	106.33	35.24	−0.53	35.11	0.8005
Ratio total cholesterol/HDL	4.75	7.58	4.25	9.28	0.49	8.59	0.3208
Triglycerides (mm/dl)	156.86	189.05	116.22	68.13	40.64	134.53	<0.0001
CRP (μg/ml)	2.17	3.22	3.36	4.88	−1.19	4.24	<0.0001
Urinary NE (μg/dl)	2.34	1.71	1.86	1.55	0.48	1.62	<0.0001
12-h Urinary NE (μg/12 h)	402.01	1915.52	518.32	2183.90	−116.30	2072.51	0.3369
Urinary NE adjusted for creatinine (μg/g)	124.19	996.64	89.95	776.93	34.24	878.37	0.5263

Table 2
Univariate and adjusted relationships between HRV and inflammatory markers in sequential Models 1 through 5.

Predictor	Response	Model 1				Model 2				Model 3				Model 4				Model 5			
		N	Estimate	SE	*P*-value	*N*	Estimate	SE	*P*-value	*N*	Estimate	SE	*P*-value	*N*	Estimate	SE	P-Value	*N*	Estimate	SE	P-Value
log HF-HRV	Fibrinogen	1138	−6.68	1.943	0.0006^	1129	−5.21	1.944	0.0074^	946	−6.91	2.042	0.0007^	931	−6.89	2.085	0.0010^	928	−8.78	2.706	0.0012^
	Soluble IL-6R	1145	−1005	233.4	<0.0001^	1136	−985	236.2	<0.0001^	950	−585	270.9	0.0311	935	−505	278.1	0.0696	932	−670	360.9	0.0637
	log CRP	1138	−.053	0.026	0.0450	1129	−.035	0.026	0.1863	946	−.076	0.027	0.0045^	931	−.086	0.027	0.0016^	928	−.110	0.035	0.0019^
	log E-Selectin	1153	−.016	0.014	0.2338	1144	−.019	0.014	0.1633	956	−.019	0.015	0.2143	941	−.013	0.016	0.3886	938	−.016	0.020	0.4186
	log ICAM	1153	−.034	0.011	0.0029^	1144	−.029	0.012	0.0109	956	−.004	0.011	0.7393	941	0.003	0.011	0.7518	938	0.003	0.014	0.8115
	log IL-6	1145	−.050	0.017	0.0026^	1136	−.039	0.017	0.0200	950	−.037	0.017	0.0295	935	−.041	0.017	0.0176	932	−.051	0.022	0.0228
log LF-HRV	Fibrinogen	1138	−15.6	2.115	<0.0001^	1129	−13.8	2.142	<0.0001^	946	−10.3	2.299	<0.0001^	931	−10.6	2.350	<0.0001^	928	−12.1	2.761	<0.0001^
	Soluble IL-6R	1145	−801	259.7	0.0021^	1136	−761	265.2	0.0042^	950	−743	306.5	0.0155	935	−701	315.1	0.0263	932	−835	370.0	0.0242
	log CRP	1138	−.182	0.029	<0.0001^	1129	−.164	0.029	<0.0001^	946	−.157	0.030	<0.0001^	931	−.162	0.031	<0.0001^	928	−.188	0.036	<0.0001^
	log E-Selectin	1153	−.035	0.015	0.0216	1144	−.041	0.015	0.0090^	956	−.025	0.017	0.1480	941	−.022	0.018	0.2192	938	−.025	0.021	0.2380
	log ICAM	1153	−.046	0.013	0.0003^	1144	−.040	0.013	0.0021^	956	−.012	0.012	0.3344	941	−.006	0.013	0.6260	938	−.008	0.015	0.6020
	log IL-6	1145	−.154	0.018	<0.0001^	1136	−.141	0.018	<0.0001^	950	−.093	0.019	<0.0001^	935	−.085	0.019	<0.0001^	932	−.098	0.023	<0.0001^

Model 1: Unadjusted for any covariates.
Model 2: Adjusted for log urinary norepinephrine (adjusted for creatinine).
Model 3: Adjusted for log urinary norepinephrine, sex, age, race, BMI, site, menstrual status, exercise, and smoking.
Model 4: Adjusted for log urinary norepinephrine, sex, age, race, BMI, site, menstrual status, exercise, smoking, statins, anti-inflammatories, medications affecting parasympathetic activity positively or negatively, history of heart problems, history of stroke, hypertension, diabetes, Parkinson's disease, and any other neurological condition.
Model 5: Adjusted for log urinary norepinephrine, sex, age, race, BMI, site, menstrual status, exercise, smoking, statins, anti-inflammatories, medications affecting parasympathetic activity positively or negatively, history of heart problems, history of stroke, hypertension, diabetes, Parkinson's disease, and any other neurological condition, corrected for respiratory rate.
^ Significant after Bonferroni correction for 6 comparisons ($p < 0.01$).

variables included in Model 5 regressions. At least one value was imputed for approximately 18% of the subjects. A total of 10 imputed datasets were created and analyzed, which is considered sufficient to yield relatively high efficiency (Graham et al., 2007). The significant findings were the same for the imputed and non-imputed analyses.

We found little evidence for sex differences in the HRV-inflammatory marker relationships for both HF-HRV and LF-HRV (Tables 3a and 3b).

4. Discussion

This study provides further support for the anti-inflammatory role of the vagus nerve, even after controlling for SNS activity. Using a large, diverse and nationally representative sample, we found that HF-HRV and LF-HRV were significantly and inversely related to several inflammatory markers after controlling for relevant covariates. These results confirm and extend those of Thayer and Fischer (2009), which demonstrated an inverse relationship between an index of HF-HRV and both CRP and WBC count after controlling for sympathetic activity in a smaller and more homogeneous sample composed mostly of men. The inverse relationship between HRV and inflammatory markers supports the role of vagus nerve activity in limiting and preventing excessive inflammatory reactions.

Previous studies have tended to examine the relationship between HRV and relatively few inflammatory markers (Haarala et al., 2011; Singh et al., 2009; Lampert et al., 2008). Unlike these studies, we examined a larger panel of inflammatory markers while adjusting for multiple comparisons. Even after adjustment, CRP and fibrinogen showed the predicted significant inverse relationships with both HF- and LF-HRV. Additionally, IL-6 showed a significant inverse relationship with LF-HRV. As von Känel et al. point out, control for multiple comparisons may be excessively conservative (von Känel et al., 2008). Without this adjustment, soluble IL-6R and IL-6 also would have attained a significant or marginally significant inverse relationship to measures of HRV. Overall, the consistency of these findings across several inflammatory markers and with previous studies further strengthens the role of the vagal anti-inflammatory pathway as a regulator of systemic inflammation.

Cholinergic vagal input is transmitted to the celiac ganglia and thence to the splenic nerve and beta-receptors on memory T-lymphocytes via noradrenergic signaling. T-lymphocytes go on to stimulate macrophages via the α7nAChR. The activated macrophages then produce acetylcholine, which acts to decrease levels of inflammation (Rosas-Ballina et al., 2011). Vagotomy or knockout of α7nAChR in animal models causes an unrestrained cytokine response (Borovikova et al., 2000; Wang et al., 2003), while vagal stimulation or administration of α7nAChR agonists decreases cytokine release (Wang et al., 2004). In a study of humans who had experienced traumatic injury, 56 patients who underwent vagotomy were compared with 115 controls with similar injury severity. The vagotomy group showed increased levels of ulcer disease (71.43% vs. 2.61%; $p < 0.001$), septicemia (26.79% vs. 3.48%; $p < 0.001$), and mortality (27.27% vs. 9.57%; $p = 0.003$) (Peterson et al., 2012). Taken together, these studies suggest a critical role for the vagus nerve in regulating inflammatory processes.

Thayer and Fischer reported significant sex differences in the relationship between HRV and inflammatory markers. However, this conclusion was limited by a sample in which only 10% of participants were women. In our sample composed of 656 women and 497 men, we did not observe significant sex differences in the relationship between HRV and inflammatory markers after controlling for covariates. These results suggest that the vagal

Table 3a
Comparison of the relationship and interaction between HF-HRV and inflammatory markers in men and women.

Model	Response	Men			Women			Interaction		
		Estimate	SE	*P*-value	Estimate	SE	*P*-value	Estimate	SE	*P*-value
Model 1	Fibrinogen	−9.66	2.799	0.0006^	−5.79	2.611	0.0268	3.861	3.898	0.3221
	Sol. IL-6R	−587	333.1	0.0787	−1329	322.6	<0.0001^	−742	475.4	0.1187
	log CRP	−.069	0.039	0.0737	−.056	0.035	0.1090	0.014	0.053	0.7957
	log E-Sel	0.002	0.019	0.9260	−.027	0.019	0.1701	−.028	0.028	0.3120
	log ICAM	−.029	0.015	0.0600	−.039	0.016	0.0175	−.010	0.023	0.6794
	log IL-6	−.065	0.025	0.0088^	−.045	0.022	0.0415	0.019	0.034	0.5636
Model 2	Fibrinogen	−8.21	2.791	0.0034^	−4.77	2.650	0.0724	4.298	3.880	0.2682
	Sol. IL-6R	−591	338.4	0.0814	−1307	327.7	<0.0001^	−730	476.4	0.1257
	log CRP	−.052	0.039	0.1820	−.044	0.035	0.2119	0.018	0.053	0.7330
	log E-Sel	0.005	0.019	0.8097	−.034	0.020	0.0791	−.029	0.028	0.2945
	log ICAM	−.019	0.015	0.2033	−.038	0.017	0.0218	−.008	0.023	0.7195
	log IL-6	−.054	0.025	0.0280	−.033	0.022	0.1363	0.021	0.033	0.5256
Model 3	Fibrinogen	−7.25	2.804	0.0100	−6.06	2.506	0.0159	1.716	3.654	0.6387
	Sol. IL-6R	−293	344.4	0.3958	−1024	336.0	0.0024^	−600	471.9	0.2035
	log CRP	−.047	0.037	0.2033	−.095	0.032	0.0031^	−.043	0.047	0.3577
	log E-Sel	−.006	0.019	0.7496	−.061	0.020	0.0021^	−.052	0.027	0.0551
	log ICAM	−.007	0.015	0.6629	−.034	0.017	0.0483	−.014	0.023	0.5337
	log IL-6	−.039	0.023	0.0907	−.057	0.020	0.0048^	−.021	0.030	0.4752
Model 4	Fibrinogen	−6.86	3.111	0.0281	−6.35	2.907	0.0293	0.178	4.048	0.9650
	Sol. IL-6R	−29.4	386.8	0.9394	−1011	409.5	0.0139	−822	540.3	0.1284
	log CRP	−.047	0.041	0.2575	−.123	0.037	0.0010^	−.067	0.053	0.2020
	log E-Sel	0.004	0.020	0.8480	−.027	0.023	0.2482	−.033	0.030	0.2697
	log ICAM	0.008	0.013	0.5233	0.002	0.018	0.9320	−.003	0.022	0.8893
	log IL-6	−.025	0.025	0.3293	−.063	0.024	0.0092^	−.035	0.033	0.2960
Model 5	Fibrinogen	−8.45	4.035	0.0368	−8.32	3.774	0.0280	−.059	5.278	0.9911
	Sol. IL-6R	−58.3	502.6	0.9077	−1334	531.3	0.0124	−1093	704.5	0.1211
	log CRP	−.056	0.054	0.2946	−.161	0.048	0.0009^	−.093	0.069	0.1774
	log E-Sel	0.005	0.026	0.8538	−.033	0.030	0.2714	−.041	0.039	0.2934
	log ICAM	0.011	0.017	0.5146	−.001	0.023	0.9507	−.008	0.028	0.7834
	log IL-6	−.031	0.033	0.3383	−.080	0.031	0.0111	−.042	0.044	0.3353

^ $p < 0.01$.

Table 3b
Comparison of the relationship and interaction between LF-HRV and inflammatory markers in men and women.

Model	Response	Men			Women			Interaction		
		Estimate	SE	*P*-value	Estimate	SE	*P*-value	Estimate	SE	*P*-value
Model 1	Fibrinogen	−17.9	2.946	<0.0001^	−11.7	2.951	<0.0001^	6.181	4.241	0.1452
	Sol. IL-6R	−547	359.7	0.1287	−950	370.0	0.0105	−402	527.3	0.4455
	log CRP	−.207	0.041	<0.0001^	−.136	0.039	0.0006^	0.071	0.057	0.2151
	log E-Sel	−.025	0.020	0.2214	−.048	0.022	0.0284	−.023	0.031	0.4513
	log ICAM	−.054	0.017	0.0012^	−.039	0.018	0.0355	0.015	0.026	0.5626
	log IL-6	−.163	0.026	<0.0001^	−.139	0.025	<0.0001^	0.024	0.036	0.5012
Model 2	Fibrinogen	−16.1	2.985	<0.0001^	−10.8	2.987	0.0003^	6.125	4.234	0.1483
	Sol. IL-6R	−557	369.8	0.1323	−896	374.5	0.0170	−383	529.2	0.4690
	log CRP	−.187	0.042	<0.0001^	−.129	0.040	0.0013^	0.068	0.057	0.2353
	log E-Sel	−.021	0.021	0.3142	−.056	0.022	0.0113	−.023	0.031	0.4536
	log ICAM	−.039	0.017	0.0202	−.038	0.019	0.0406	0.012	0.026	0.6283
	log IL-6	−.153	0.026	<0.0001^	−.130	0.025	<0.0001^	0.022	0.036	0.5457
Model 3	Fibrinogen	−13.5	3.172	<0.0001^	−8.19	2.856	0.0043^	5.826	4.025	0.1480
	Sol. IL-6R	−364	394.5	0.3562	−927	384.2	0.0161	−339	523.6	0.5180
	log CRP	−.154	0.042	0.0002^	−.136	0.037	0.0002^	0.035	0.052	0.5021
	log E-Sel	−.023	0.022	0.2995	−.055	0.022	0.0140	−.032	0.030	0.2896
	log ICAM	−.020	0.017	0.2584	−.031	0.019	0.1144	0.006	0.025	0.8238
	log IL-6	−.095	0.026	0.0003^	−.106	0.023	<0.0001^	−.004	0.033	0.9082
Model 4	Fibrinogen	−11.1	3.576	0.0020^	−10.9	3.204	0.0007^	0.631	4.395	0.8858
	Sol. IL-6R	−261	447.5	0.5602	−1002	455.3	0.0282	−470	590.8	0.4263
	log CRP	−.145	0.047	0.0023^	−.191	0.041	<0.0001^	−.024	0.057	0.6796
	log E-Sel	−.031	0.023	0.1874	−.022	0.026	0.3978	−.007	0.033	0.8184
	log ICAM	−.008	0.015	0.5783	0.005	0.020	0.8022	0.016	0.024	0.5111
	log IL-6	−.066	0.029	0.0237	−.116	0.027	<0.0001^	−.024	0.036	0.5102
Model 5	Fibrinogen	−12.2	4.209	0.0039^	−12.7	3.759	0.0008^	0.238	5.187	0.9635
	Sol. IL-6R	−332	527.2	0.5297	−1194	534.0	0.0258	−564	697.2	0.4184
	log CRP	−.164	0.056	0.0035^	−.226	0.048	<0.0001^	−.035	0.067	0.6019
	log E-Sel	−.036	0.028	0.1919	−.024	0.030	0.4158	−.008	0.038	0.8340
	log ICAM	−.009	0.018	0.5932	0.003	0.023	0.9076	0.015	0.028	0.5946
	log IL-6	−.075	0.034	0.0286	−.134	0.031	<0.0001^	−.026	0.043	0.5494

^ $p < 0.01$.

anti-inflammatory pathway has similar effects on inflammation in both men and women.

While studies consistently have shown significant inverse relationships between HRV and a series of inflammatory markers (Haarala et al., 2011; Lampert et al., 2008; Frasure-Smith et al., 2009), the magnitude of these effects generally has been small, as they were in this study. These small magnitude effects raise questions about the vagal pathways that regulate the heart and the inflammatory reflex. The vagus nerve contains A, B, and C fiber subtypes, only the latter two of which are involved in heart rate regulation (Jones et al., 1995). This neuroanatomy suggests the possibility of a dissociation of efferent vagal regulation of the heart and inflammation. Consistent with this point, Huston et al. have shown that electrical stimulation of the distal end of the transected vagus nerve (1 V, 5 Hz, 2 ms), while sufficient to elicit anti-inflammatory effects, had no effect on heart rate (Huston et al., 2007). More intense stimulation had the expected cardioinhibitory effect. This finding implicates vagal A fibers in anti-inflammatory signaling and suggests a lower activation threshold for this effect. Because inflammatory and cardiac effects appear to be activated by different levels of vagal stimulation via different fibers, the limited relationship between HRV and vagally-mediated anti-inflammatory effects may not be surprising.

Our study had the advantage of a large, representative sample that controlled for sympathetic effects in examining the relationship between HRV and inflammation. However, there were several limitations to our study. Participation required traveling to one of three testing centers and participating in research over an extended period of time, which may limit the generalizability of these results. In this respect, our study is similar to that of Thayer and Fischer, whose research participants all were currently employed. Thus, in both studies, participants were likely to be healthier than if they were drawn from community samples. However, studies from community samples also show inverse relationships between HRV and inflammatory markers (Sloan et al., 2007). Additionally, given the cross-sectional nature of our study, we were unable to assess possible bidirectional relationships between HRV and inflammation. Overall, our study adds to the growing literature reporting significant inverse relationships between HRV and several inflammatory markers after controlling for numerous relevant covariates including urinary norepinephrine. Our results provide further support for the existence of the vagal anti-inflammatory pathway and suggest that it has similar effects in men and women. These findings on the role of the vagus nerve may be of clinical significance in the development of new therapies for inflammatory processes.

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References

Bellinger, D.L., Millar, B.A., Perez, S., Carter, J., Wood, C., ThyagaRajan, S., et al., 2008. Sympathetic modulation of immunity: relevance to disease. Cell. Immunol. 252 (1–2), 27–56. http://dx.doi.org/10.1016/j.cellimm.2007.09.005, Epub 2008/03/01; PubMed PMID:18308299; PubMed Central PMCID: PMC3551630.

Bernik, T.R., Friedman, S.G., Ochani, M., DiRaimo, R., Susarla, S., Czura, C.J., et al., 2002. Cholinergic antiinflammatory pathway inhibition of tumor necrosis factor during ischemia reperfusion. J. Vasc. Surg. 36 (6), 1231–1236. http://dx.doi.org/10.1067/mva.2002.129643, Epub 2002/12/07.

Borovikova, L.V., Ivanova, S., Zhang, M., Yang, H., Botchkina, G.I., Watkins, L.R., et al., 2000. Vagus nerve stimulation attenuates the systemic inflammatory response to endotoxin. Nature 405 (6785), 458–462. http://dx.doi.org/10.1038/35013070, Epub 2000/06/06.

Brim, O.G., Ryff, C.D., Kessler, R.C., 2004. The MIDUS national survey: an overview. In: Brim, O.G., Ryff, C.D., Kessler, R.C. (Eds.), How Healthy Are We? A National Study of Well-Being at Midlife. University of Chicago Press, Chicago, pp. 1–36.

DeBoer, R.W., Karemaker, J.M., Strackee, J., 1984. Comparing spectra of a series of point events particularly for heart rate variability data. IEEE Trans. Biomed. Eng. 31 (4), 384–387. http://dx.doi.org/10.1109/tbme.1984.325351, Epub 1984/04/01.

Elenkov, I.J., Wilder, R.L., Chrousos, G.P., Vizi, E.S., 2000. The sympathetic nerve – an integrative interface between two supersystems: the brain and the immune system. Pharmacol. Rev. 52 (4), 595–638, Epub 2000/12/21.

Frasure-Smith, N., Lesperance, F., Irwin, M.R., Talajic, M., Pollock, B.G., 2009. The relationships among heart rate variability, inflammatory markers and depression in coronary heart disease patients. Brain Behav. Immun. 23 (8), 1140–1147. http://dx.doi.org/10.1016/j.bbi.2009.07.005, Epub 2009/07/29.

Gonzalez-Clemente, J.M., Vilardell, C., Broch, M., Megia, A., Caixas, A., Gimenez-Palop, O., et al., 2007. Lower heart rate variability is associated with higher plasma concentrations of IL-6 in type 1 diabetes. Eur. J. Endocrinol./Eur. Fed. Endocr. Societies 157 (1), 31–38. http://dx.doi.org/10.1530/eje-07-0090, Epub 2007/07/05.

Graham, J.W., Olchowski, A.E., Gilreath, T.D., 2007. How many imputations are really needed? Some practical clarifications of multiple imputation theory. Prev. Sci. 8 (3), 206–213. http://dx.doi.org/10.1007/s11121-007-0070-9, Epub 2007/06/06.

Haarala, A., Kahonen, M., Eklund, C., Jylhava, J., Koskinen, T., Taittonen, L., et al., 2011. Heart rate variability is independently associated with C-reactive protein but not with Serum amyloid A. The cardiovascular risk in Young Finns study. Eur. J. Clin. Invest. 41 (9), 951–957. http://dx.doi.org/10.1111/j.1365-2362.2011.02485.x, Epub 2011/02/18.

Huston, J.M., Gallowitsch-Puerta, M., Ochani, M., Ochani, K., Yuan, R., Rosas-Ballina, M., et al., 2007. Transcutaneous vagus nerve stimulation reduces serum high mobility group box 1 levels and improves survival in murine sepsis. Crit. Care Med. 35 (12), 2762–2768. http://dx.doi.org/10.1097/01.ccm.0000288102.15975.ba, Epub 2007/09/29.

Jones, J.F., Wang, Y., Jordan, D., 1995. Heart rate responses to selective stimulation of cardiac vagal C fibres in anaesthetized cats, rats and rabbits. J. Physiol. 489 (Pt 1), 203–214, Epub 1995/11/15. PubMed PMID: 8583404; PubMed Central PMCID: PMC1156804.

Koopman, F.A., Stoof, S.P., Straub, R.H., Van Maanen, M.A., Vervoordeldonk, M.J., Tak, P.P., 2011. Restoring the balance of the autonomic nervous system as an innovative approach to the treatment of rheumatoid arthritis. Mol. Med. (Cambridge, Mass) 17 (9–10), 937–948. http://dx.doi.org/10.2119/molmed.2011.00065, Epub 2011/05/25; PubMed PMID: 21607292; PubMed Central PMCID: PMC3188868.

Lampert, R, Bremner, JD, Su, S, Miller, A, Lee, F, Cheema, F, 2008. Decreased heart rate variability is associated with higher levels of inflammation in middle-aged men. Am. Heart J. 156 (4), 759e1-7. http://dx.doi.org/10.1016/j.ahj.2008.07.009, Epub 2008/10/18; PubMed PMID: 18926158; PubMed Central PMCID: PMC2587932.

Little, R.J.A., Rubin, D.B., 2002. Statistical Analysis with Missing Data, . 2nd ed., xv. Wiley, Hoboken, N.J., 381 pp.

Peterson, C.Y., Krzyzaniak, M., Coimbra, R., Chang, D.C., 2012. Vagus nerve and postinjury inflammatory response. Arch. Surg. (Chicago, Ill: 1960) 147 (1), 76–80, Epub 2012/02/23.

Redwine, L., Snow, S., Mills, P., Irwin, M., 2003. Acute psychological stress: effects on chemotaxis and cellular adhesion molecule expression. Psychosom. Med. 65 (4), 598–603, Epub 2003/07/29.

Rosas-Ballina, M., Olofsson, P.S., Ochani, M., Valdes-Ferrer, S.I., Levine, Y.A., Reardon, C., et al., 2011. Acetylcholine-synthesizing T cells relay neural signals in a vagus nerve circuit. Science (New York, NY) 334 (6052), 98–101. http://dx.doi.org/10.1126/science.1209985, Epub 2011/09/17.

Rubin, D.B., 1976. Inference and missing data. Biometrika 63 (3), 581–590. http://dx.doi.org/10.1093/Biomet/63.3.581, PubMed PMID: ISI:A1976CP66700021.

Ryff, C., Almeida, D.M., Ayanian, J.S., Carr, D.S., Cleary, P.D., Coe, C., et al., 2012. National Survey of Midlife Development in the United States (MIDUS II), 2004–2006. ICPSR04652-v6 ed: Inter-university Consortium for Political and Social Research (ICPSR).

Saeed, R.W., Varma, S., Peng-Nemeroff, T., Sherry, B., Balakhaneh, D., Huston, J., et al., 2005. Cholinergic stimulation blocks endothelial cell activation and leukocyte recruitment during inflammation. J. Exp. Med. 201 (7), 1113–1123. http://dx.doi.org/10.1084/jem.20040463, Epub 2005/04/06; PubMed PMID: 15809354; PubMed Central PMCID: PMC2213139.

Schafer, J.L., 1997. Analysis of Incomplete Multivariate Data. Chapman & Hall, New York.

Singh, P., Hawkley, L.C., McDade, T.W., Cacioppo, J.T., Masi, C.M., 2009. Autonomic tone and C-reactive protein: a prospective population-based study. Clin. Auton. Res. 19 (6), 367–741950. http://dx.doi.org/10.1007/s10286-009-0019-0, Epub 2009/06/09; PubMed PMID:4232; PubMed Central PMCID: PMC2783459.

Sloan, R.P., McCreath, H., Tracey, K.J., Sidney, S., Liu, K., Seeman, T., 2007. RR interval variability is inversely related to inflammatory markers: the CARDIA study. Mol. Med. (Cambridge, Mass) 13 (3–4), 178–184. http://dx.doi.org/10.2119/2006-00112.Sloan, Epub 2007/06/27.PubMed PMID: 17592552; PubMed Central PMCID: PMC1892756.

Stuckey, M.I., Petrella, R.J., 2013. Heart rate variability in type 2 diabetes mellitus. Crit. Rev. Biomed. Eng. 41 (2), 137–147, Epub 2013/01/01.

Tateishi, Y., Oda, S., Nakamura, M., Watanabe, K., Kuwaki, T., Moriguchi, T., et al., 2007. Depressed heart rate variability is associated with high IL-6 blood level and decline in the blood pressure in septic patients. Shock (Augusta, Ga) 28 (5), 549–553. http://dx.doi.org/10.1097/shk.0b013e3180638d1, Epub 2007/12/14.

Thayer, J.F., Fischer, J.E., 2009. Heart rate variability, overnight urinary norepinephrine and C-reactive protein: evidence for the cholinergic anti-inflammatory pathway in healthy human adults. J. Intern. Med. 265 (4), 439–447. http://dx.doi.org/10.1111/j.1365-2796.2008.02023.x, Epub 2008/11/21.

Tracey, K.J., 2007. Physiology and immunology of the cholinergic antiinflammatory pathway. J. Clin. Investig. 117 (2), 289–296. http://dx.doi.org/10.1172/jci30555, Epub 2007/02/03; PubMed PMID: 17273548; PubMed Central PMCID: PMC1783813.

Viswanathan, K., Dhabhar, F.S., 2005. Stress-induced enhancement of leukocyte trafficking into sites of surgery or immune activation. Proc. Natl. Acad. Sci. U.S.A. 102 (16), 5808–5813. http://dx.doi.org/10.1073/pnas.0501650102, Epub 2005/04/09; PubMed PMID: 15817686; PubMed Central PMCID: PMC556309.

von Känel, R., Nelesen, R.A., Mills, P.J., Ziegler, M.G., Dimsdale, J.E., 2008. Relationship between heart rate variability, interleukin-6, and soluble tissue factor in healthy subjects. Brain Behav. Immun. 22 (4), 461–468. http://dx.doi.org/10.1016/j.bbi.2007.09.009, Epub 2007/11/06; PubMed PMID: 17977694; PubMed Central PMCID: PMC2373608.

Wang, H., Liao, H., Ochani, M., Justiniani, M., Lin, X., Yang, L., et al., 2004. Cholinergic agonists inhibit HMGB1 release and improve survival in experimental sepsis. Nat. Med. 10 (11), 1216–1221. http://dx.doi.org/10.1038/nm1124, Epub 2004/10/27.

Wang, H., Yu, M., Ochani, M., Amella, C.A., Tanovic, M., Susarla, S., et al., 2003. Nicotinic acetylcholine receptor alpha7 subunit is an essential regulator of inflammation. Nature 421 (6921), 384–388. http://dx.doi.org/10.1038/nature01339, Epub 2003/01/01.