Brain, Behavior, & Immunity - Health 15 (2021) 100273

Contents lists available at ScienceDirect

Brain, Behavior, & Immunity - Health

journal homepage: www.editorialmanager.com/bbih/default.aspx

Full Length Article

Heart rate variability and circulating inflammatory markers in midlife

Nicholas V. Alen [a], Anna M. Parenteau [a], Richard P. Sloan [b], Camelia E. Hostinar [a,*]

[a] Department of Psychology, University of California, Davis, United States
[b] Department of Psychiatry, Columbia University, United States

ARTICLE INFO

Keywords:
Inflammation
Heart rate variability
Immune system
Vagus nerve
Autonomic nervous system

ABSTRACT

Theoretical perspectives and empirical evidence suggest that the parasympathetic nervous system engages in active monitoring and moderating of inflammatory processes. A clearer understanding of the bidirectional communication between the parasympathetic nervous system and the immune system could lead to novel clinical interventions for inflammatory illnesses. The current study used a large (N = 836) nationally representative sample of adults in the United States to investigate the associations between resting parasympathetic modulation of the heart, indexed through both high frequency heart rate variability (HF-HRV) and low frequency heart rate variability (LF-HRV), and six circulating markers of inflammation. Statistical analyses revealed robust inverse associations of HF-HRV with interleukin-6 (IL6), C-reactive protein (CRP), and fibrinogen, with or without covariate adjustment. Similar inverse associations were observed between LF-HRV and IL6 and CRP. No significant associations were observed between HRV and either inflammatory adhesion molecules (E-selectin, intracellular adhesion molecule-1) or soluble IL6 receptor. Results are consistent with the cholinergic anti-inflammatory pathway and suggest that parasympathetic modulation of inflammation through the vagus nerve may act on specific inflammatory molecules more than others.

1. Introduction

Inflammatory processes are regulated through bidirectional communication between the nervous system and the immune system (Chiu et al., 2013; Hostinar et al., 2018). Evidence suggests that the parasympathetic branch of the autonomic nervous system (ANS) plays a particularly critical role in modulating inflammation (Borovikova et al., 2000). Specifically, through rapid afferent and efferent signaling of the vagus nerve, the parasympathetic nervous system (PNS) is involved in both monitoring current levels of inflammation and reducing production of inflammatory cytokines (Tracey, 2002). Parasympathetic modulation of inflammation is accomplished through targeted production of the neurotransmitter acetylcholine, which binds to nicotinic α7 receptors (α7 nAChRs) expressed by macrophages, leading to reduced production and recruitment of inflammatory molecules (Saeed et al., 2005). This inhibition of inflammatory processes by parasympathetic outflow has been labeled the *cholinergic anti-inflammatory pathway* (Tracey, 2002).

The existence of a cholinergic anti-inflammatory pathway has been supported by both animal experimental evidence (e.g., vagus nerve stimulation in rodents; Borovikova et al., 2000) and human correlational evidence. For instance, in humans greater resting PNS activity has been associated with lower levels of circulating inflammatory cytokines (Williams et al., 2019), reduced cytokine production in response to ex-vivo blood stimulation with a bacterial endotoxin (Marsland et al., 2007), and lower increases in serum cytokines during social threat (Alen et al., 2020). Most studies have used measures of resting heart rate variability (HRV), which can be portioned into different frequency domains (Laborde et al., 2017). High frequency HRV (HF-HRV) is a reliable marker of parasympathetic modulation of the heart through the vagus nerve (Cacioppo et al., 1994). Low frequency HRV (LF-HRV) also reflects ANS activity, though LF-HRV appears to be influenced by both the PNS and the sympathetic nervous system (SNS; Berntson et al., 1997) and may also reflect non-ANS related cardiovascular activity (e.g., baroreflex sensitivity; Goldstein et al., 2011).

In a recent meta-analysis, robust relations of both HF-HRV and LF-HRV with circulating inflammatory markers were observed (Williams et al., 2019). However, indices of HRV appear to be more strongly related to some inflammatory markers than others. Specifically, resting HF-HRV was negatively associated with circulating interleukin-6 (IL6), C-reactive protein (CRP), fibrinogen, and white blood cell count (WBC), but not with interleukin-1 (IL1) or tumor necrosis factor-alpha (TNFα). In addition, resting LF-HRV was negatively associated with IL6, CRP, and WBC,

* Corresponding author. Center for Mind and Brain, University of California, 202 Cousteau Place, Davis, CA, 95618, United States.
E-mail address: cehostinar@ucdavis.edu (C.E. Hostinar).

https://doi.org/10.1016/j.bbih.2021.100273
Received 30 March 2021; Received in revised form 9 May 2021; Accepted 10 May 2021
Available online 13 May 2021

but not with fibrinogen, IL1, or TNFα. Too few studies were available to test associations between HRV and other inflammatory markers (e.g., interleukin-10, interferon-gamma; Williams et al., 2019). These complex results suggest that parasympathetic modulation of inflammation may be stronger for specific inflammatory molecules. More research using large samples and multiple inflammatory markers could help clarify which inflammatory markers are linked to individual differences in parasympathetic activity.

There is also evidence that inflammation is modulated by SNS activity. Specifically, sympathetic release of norepinephrine can have both pro- and anti-inflammatory effects, depending on characteristics of the targeted inflammatory cell (e.g., number of adrenergic receptors; Koopman et al., 2011). Previous studies in humans have reported a positive association between overnight urinary norepinephrine output and peripheral inflammation (Hostinar et al., 2015). Given these associations, it is surprising most studies investigating the cholinergic anti-inflammatory pathway do not control for resting sympathetic activity. However, two relatively recent studies investigated HRV-inflammation links while controlling for SNS activity, indexed through urinary norepinephrine. Thayer and Fisher (2009) found inverse associations between HRV and CRP after controlling for urinary norepinephrine. In a more recent study, Cooper et al. (2015) examined the associations of both LF-HRV and HF-HRV with six inflammatory markers using a large nationally representative sample of adults from the Midlife in the United States (MIDUS) study. Results from this study revealed inverse relations of HF-HRV with both CRP and fibrinogen, and of LF-HRV with IL6, CRP, and fibrinogen, after controlling for a host of covariates including urinary norepinephrine. In addition, this study found comparable associations between indices of HRV and inflammation in males and females (Cooper et al., 2015), in contrast to previous evidence (Thayer and Fisher, 2009).

Elevated systemic inflammation has been implicated in both chronic somatic disease (e.g., atherosclerosis; Libby, 2002) and mental health disorders (e.g., depression; Dantzer and Kelley, 2007). A better understanding of the cholinergic anti-inflammatory pathway could aid ongoing efforts to use parasympathetic modulation of inflammation to improve clinical intervention. For example, monitoring parasympathetic activity in patients with inflammatory illnesses might help practitioners detect moments of relative risk (Huston and Tracey, 2011). In addition, increasing parasympathetic activity, through either patient training intervention (e.g., biofeedback training; Lehrer and Gevirtz, 2014) or clinical procedures (e.g., vagus nerve stimulation; Addorisio et al., 2019; Bonaz et al., 2020; Koopman et al., 2016), might improve prognosis in patients suffering from inflammation-mediated disease. Additional research into which specific inflammatory molecules are more strongly linked to parasympathetic activity could help identify which inflammatory conditions would be appropriate targets of these novel intervention methods.

1.1. Current study

The current study was designed to further investigate the association between the PNS, as indexed through resting HRV, and inflammation. The data used in the study by Cooper et al. (2015) came from MIDUS 2 Project 4, a subsample of MIDUS participants invited to provide biological data between 2004 and 2009. In 2012, data collection began on a separate sample of participants, called the MIDUS Refresher (MIDUS R) sample, meant to replenish the ongoing MIDUS study. Participants in the MIDUS R study engaged in the exact same study protocol and provided the exact same biological variables as the MIDUS 2 study, which presented an opportunity for direct replication of the Cooper et al. (2015) findings. The importance of replication studies for strengthening the reliability of biobehavioral science has recently been expressed by numerous researchers in the scientific community (e.g., NASEM, 2019; Zwaan et al., 2018). The current study was designed to promote transparency and replicability in biobehavioral science by both (1) replicating previous findings, and (2) providing software script in supplemental material detailing statistical procedures used to analyze these open access data.

Consistent with meta-analytic findings (Williams et al., 2019) and results from the Cooper et al. (2015) study, we hypothesized to find an inverse association between HRV and inflammation. Specifically, we expected to find negative associations of both indices of HRV (HF-HRV and LF-HRV) with IL6 and CRP, as well as negative associations between HF-HRV and fibrinogen. Whereas Cooper et al. (2015) observed associations between LF-HRV and fibrinogen, the meta-analysis by Williams et al. (2019) did not find this effect. We therefore did not have strong a priori expectations regarding this association. We expected to find weak or no evidence for an association of HRV with E-selectin, ICAM-1, and IL6r. In addition, based on a recent meta-analysis (Williams et al., 2019) we hypothesized that these associations would be similar for males and females.

2. Methods

2.1. Participants

Data for this study came from the Midlife in the United States (MIDUS) study, a longitudinal study of health and wellbeing among a nationally representative sample of adults (Brim et al., 2004). Complete details of the MIDUS study are available at www.midus.wisc.edu. Beginning in 2011, a separate sample of adults were recruited in order to replenish the MIDUS study, known as the MIDUS Refresher sample. The MIDUS Refresher sample included 3577 adults, who provided self-report written survey and phone interview data. Participants involved in the MIDUS Refresher study, included in the current analysis, were not involved in the original MIDUS study. This means there is zero overlap in participants between the current study and the Cooper et al. (2015) study. Starting in 2012, a subset of the Refresher sample was invited to participate in an overnight laboratory visit at one of three locations (University of California-Los Angeles, University of Washington, and Georgetown University) for the collection of biological data, called Project 4. The current analysis used data from the MIDUS Refresher Project 4 study, which included 863 adults between 26 and 78 years old (mean age = 52.7 years, *SD* = 13.4 years; 52% female). Included participants were 70% White, 18% Black or African American, 6% other race/ethnicity, 3% Native American or Alaskan Native, 1% Asian, and less than 1% Native Hawaiian or Pacific Islander, and had a mean yearly total income of $52,635 (*SD* = $50,446).

2.2. Measures

2.2.1. Heart rate variability

Heart rate variability (HRV) data were collected using a three-lead electrocardiogram (ECG) attached to the chest in Einthoven's triangle configuration. ECG recordings took place in the morning, after an overnight clinic stay, following breakfast, with no caffeine consumption permitted. ECG data were recorded during an 11-min seated, resting baseline period, as part of a larger ECG protocol involving cognitive tasks. The current study utilized data from the resting baseline measure only, during which participants were asked to breath naturally, to not talk, and to try to relax.

ECG data were digitized at 500 Hz by a 16-bit National Instruments analog-to-digital board and submitted to a custom-written program to generate an RR interval (RRI) time series. Ectopic beats or noisy signals were interpolated up to the equivalent of five consecutive RR intervals based on surrounding data. Longer segments were excluded from further analysis. Two 300-sec epochs from the resultant corrected RR interval time series were submitted to a Fast-Fourier transformation algorithm to estimate power in the low (0.04–0.15 Hz, LF) and high (0.15–0.40 Hz, HF) frequency bands based on method similar to that described by DeBoer et al. (1984). RRI time series shorter than 300 s due to noise or excess ectopic beats also were analyzed if they were at least 180 s in

length. Prior to computing Fourier transforms, the mean of the RRI series was subtracted from each value in the series. The series was filtered using a Hanning window (Harris, 1978) and the power (units = ms^2) over the LF and HF bands was summed. Estimates of spectral power were adjusted to account for attenuation produced by this filter (Harris, 1978). Values of LF and HF power from each of the two epochs were averaged.

Of the total sample, $n = 26$ declined to participate in the ECG data collection procedure, and for $n = 99$ participants, 180 s of clean data were unavailable; this resulted in available HRV data for $n = 738$ participants.

2.2.2. Inflammatory markers

Fasting blood draws were conducted in the morning following an overnight stay at the research site, prior to eating anything or engaging in any strenuous activity. From these blood draws a total of six inflammatory markers were measured: IL6, CRP, fibrinogen, soluble intracellular adhesive molecule 1 (ICAM-1), E-selectin, and soluble IL6 receptor (IL6r). IL6 was assayed from serum using a Quantikine® High-sensitivity ELISA kit #HS600B (R & D Systems, Minneapolis, MN). CRP was measured in plasma using a BNII nephelometer from Dade Behring utilizing a particle enhanced immunonepholometric assay. Fibrinogen was measured in plasma using the BNII nephelometer (N Antiserum to Human Fibrinogen; Siemens, Malvern, PA). ICAM-1 was assayed from serum using a sandwich ELISA Quantikine® kit #SCD540 (R&D Systems, Minneapolis, MN). E-selectin was assayed from serum with a sandwich ELISA Quantikine® kit #SSLE00 (R&D Systems, Minneapolis, MN). The concentration of IL6r was measured using a Quantikine® ELISA kit #DR600 (R & D Systems, Minneapolis, MN). The inter-assay CV was 15.66% for IL6, and 5.33% for IL6r. The inter-assay CVs had a range of 1.08–4.3% for CRP, 4.13–6.64% for fibrinogen, 7.49–8.16% for ICAM-1, and 7.1–11.15% for E-selectin. The intra-assay CV was 3.73% for IL6, 1.31% for IL6r, and 2.7% for fibrinogen. The intra-assay CVs ranged from 2.3 to 4.4% for CRP, 5.2–6.6% for E-selectin, and 3.7–5.2% for ICAM-1. Missingness for inflammatory markers was very low (less than 1%).

Table 1
Sample characteristics and primary study descriptives.

Continuous Variables	Mean	*SD*	Range
HF-HRV (units = ln [ms^2])	5.12	1.36	0.51–9.29
LF-HRV (units = ln [ms^2])	5.66	1.13	0.43–8.69
IL6 (units = ln [pg/mL])	0.72	0.80	−2.15–2.85
CRP (units = ln [ug/mL])	0.34	1.21	−3.0–4.37
Fibrinogen (units = mg/dL)	343.40	73.72	118–629
ICAM-1 (units = ln [ng/mL])	5.50	0.37	4.39–7.16
E-selectin (units = ln [ng/mL])	3.61	0.46	2.0–5.16
IL6r (units = pg/mL)	31771	8552	10983–61276
Norepinephrine*	5.33	0.95	1.75–9.54
Age in years	52.72	13.44	26–78
BMI	30.40	7.65	17.08–77.58
Categorical Variables	**Level**	***N***	**%**
Sex	Female	450	52.1
Heart disease	Yes	81	9.4
History of stroke	Yes	30	3.5
History of high blood pressure	Yes	334	38.7
Diabetes	Yes	93	10.8
Parkinson's disease	Yes	1	0.1
Other Neurological disorder	Yes	34	3.9
Statins	Yes	205	23.8
NSAIDs	Yes	395	45.8
Medications that increase HRV	Yes	78	9.0
Medications that lower HRV	Yes	219	25.4

Note. HF-HRV = high frequency heart rate variability. LF-HRV = low frequency heart rate variability. IL6 = interleukin-6. CRP = C-reactive protein. ILCAM-1 = intracellular adhesion molecule-1. IL6r = soluble interleukin-6 receptor. NSAIDs = non-steroidal anti-inflammatory drugs. *Norepinephrine values were adjusted for urinary creatinine using the following equation: adjusted values = (norepinephrine ug/dL)/(creatinine mg/d * 0.001) and natural log transformed.

2.2.3. Urinary norepinephrine and creatinine

Urine samples were collected over a 12-h period (7:00pm–7:00am) during the overnight laboratory stay. Each urine void was separated into two containers: one with 25 mL of 50% acetic acid added to it, used for assaying norepinephrine; and a second container without additives, used in assaying creatinine. Thirteen mL aliquots from each container were then stored at −60 °C to −80 °C before being shipped to the MIDUS Biocore lab for assaying. High-Pressure Liquid Chromatography (HPLC) was used for free norepinephrine fractionation, which was quantified using electrochemical detection. Creatinine was assayed by a colorimetric assay known as the Jaffe reaction: the urine was mixed with an alkaline picrate solution, which reacts with creatinine to produce an orange-yellow end product. Urinary norepinephrine values were adjusted for urinary creatinine using the following equation: adjusted values = (norepinephrine ug/dL)/(creatinine mg/d * 0.001). Missingness for urine data was very low (less than 1%).

2.2.4. Respiration rate

Participant respiration rate was recorded during the baseline ECG data collection period using two stretch bands, one around the chest and one around the abdomen. Analog signals from the two bands were sampled at 20 Hz and digitized, then averaged into a single waveform for analysis. Using event detection software, respiratory excursions were identified automatically then corrected based on visual inspection to produce a respiration time series, which was used to calculate respiration rate during 60 s epochs. Individual epochs were then averaged together to produce a baseline respiration rate. Of the total sample, respiration data was available for $n = 763$ ($n = 26$ declined to participate; $n = 69$ had less than 60 s of clean continuous respiration data).

2.2.5. Covariates

The following covariates were included in analysis: participant age, sex, race, BMI, menopausal status, smoking history, exercise habits, heart disease, history of stroke, high blood pressure, diabetes, Parkinson's disease, other neurological disorder, medication regimen, and site of data collection. This specific list of covariates was chosen in order to replicate the methodology used in the Cooper et al. (2015) paper, which used the exact same covariates. BMI was calculated from participant height and weight measured by project nurses during the laboratory visit. All other covariates were self-reported. Given the distribution of race, we created two dummy coded variables representing Black/African American compared to white, and other race compared to white. Menopausal status was coded as: males and pre-menopausal females = 0; menopausal and post-menopausal females = 1. Smoking history was coded as: never-smoker = 0; previous-smoker = 1; current-smoker = 2. Exercise habits were scored such that participants that engaged in at least 20 min of exercise at least 3 times a week were scored as 1, all others were scored as 0. Six dichotomous variables were created reflecting whether a participant had ever been diagnosed with: heart disease, stroke, high blood pressure, diabetes, Parkinson's disease, or other neurological disorder. Medication regimen was coded into four dichotomous variables reflecting whether the participant was currently taking: (1) statins, (2) anti-inflammatory drugs, (3) medication that can increase heart rate variability (parasympathomimetic agents, beta-blockers), and (4) medication that can decrease heart rate variability (anti-cholinergic agent, antidepressants, sedatives, anti-psychotics). Missingness for covariates was very low (less than 1%).

2.3. Statistical analysis

Statistical analyses were performed using RStudio version 1.3.959, running R version 4.0.0. Complete R script and details on acquiring the data used in the current study are available in supplemental material (see supplemental Appendix S1). Variables that exhibited skewed distribution were log transformed (HF-HRV, LF-HRV, IL6, CRP, ICAM-1, E-selectin, norepinephrine adjusted for creatinine). After log transformation, outliers were observed in ICAM-1 ($n = 7$) and E-selectin ($n = 1$), defined as

values more than 4 *SD* away from the mean; these values were Winsorized to the closest value within 4 *SD* of the mean. In order to test the association between HRV and inflammation, we conducted a series of multiple linear regressions, regressing each of the six inflammatory markers onto HF-HRV and LF-HRV individually, for a total of 12 regression models. Each regression model was performed in five steps. In step 1, inflammation was regressed onto HRV unadjusted for covariates. In step 2, the model was adjusted for urinary norepinephrine. In step 3, age, sex, race, BMI, menopausal status, smoking history, and exercise habits were added to the model as covariates. In step 4, health condition and medication regimen data were added to the model as covariates. In step 5, HRV residualized for respiration rate was used as the primary independent variable. In order to account for multiple comparisons (i.e., 12 models), test significance was determined using Benjamini-Hochberg False Discovery Rate (FDR; Benjamini and Hochberg, 1995; see supplemental Table S1 for FDR calculations).

Lastly, we tested moderation by participant sex by creating interaction terms between each HRV index and sex and including this variable in the final model. Each HRV measure was mean-centered prior to calculating the interaction term.

Exploratory analyses were conducted to (1) examine the association between HRV and two additional inflammatory markers not included in the original study by Cooper et al. (2015): interleukin-10 (IL10) and TNFα, and (2) test the robustness of our findings to the inclusion of mental health variables (anxious and depressive symptoms) as covariates, and to the exclusion of individuals diagnosed with an arrhythmia. These additional analyses are described in Supplemental Appendix S2. R script for running these additional analyses is included in Supplemental Appendix S1.

2.3.1. Missing data handling

A Little's MCAR test rejected the null hypothesis that data were missing completely at random (Chi-square (69) = 152.91, $p < .001$). Probing of missing data patterns revealed that, compared to participants with available HRV data, participants with missing HRV data tended to be older (t (861) = 6.08, $p < .001$), to have higher levels of urinary norepinephrine (t (860) = 3.80, $p < .001$), and to have higher levels of circulating IL6 (t (850) = 3.26, $p = .001$). Because of this, we employed a two-stage approach to handling missing data: first (1) calculating all models using available data only, and then (2) imputing missing data using multiple imputations (10 imputations) with the R package *mice,* which uses multivariate imputation by chained-equations (van Buuren and Groothuis-Oudshoorn, 2011). However, all results were comparable and inferences identical using either method, we therefore present the results from the simpler method (list-wise deletion).

3. Results

3.1. Preliminary analysis

Sample descriptive statistics are presented in Table 1. Bivariate correlations between primary variables of interest are presented in Table 2. As expected, HF-HRV and LF-HRV were highly correlated ($r = 0.68$, $p < .001$). In addition, all inflammatory markers were correlated with one another (r's ranged from 0.11 to 0.58), with the following exceptions: IL6r was not correlated with CRP or fibrinogen. Urinary norepinephrine was negatively correlated with both measures of HRV (with HF-HRV, $r = -0.23$, $p < .001$; with LF-HRV, $r = -0.24$, $p < .001$). Urinary norepinephrine was also positively correlated with IL6 ($r = 0.23$, $p < .001$), CRP ($r = 0.08$, $p = .02$), and fibrinogen ($r = 0.16$, $p < .001$), and negatively correlated with ICAM-1 ($r = -0.16$, $p < .001$).

3.2. Heart rate variability and inflammation

Multiple linear regression results, presented in Table 3, revealed unadjusted negative associations of HF-HRV with IL6 ($B = -0.14$, 95% CI [-0.18, −0.10]), CRP ($B = -0.14$, 95% CI [-0.20, −0.07]), fibrinogen ($B = -8.9$, 95% CI [-12.77, −5.03]), and IL6r ($B = -761$, 95% CI [−1216, −305]). After adjusting for urinary norepinephrine, age, sex, race, BMI, menopause, smoking, exercise, site of data collection, health disorders, medication regimen, and respiration rate, HF-HRV was significantly and inversely associated with IL6 ($B = -0.07$, 95% CI [-0.11, −0.03]), CRP ($B = -0.15$, 95% CI [-0.21, −0.09]), and fibrinogen ($B = -5.06$, 95% CI [-8.98, −1.14]). HF-HRV was not significantly associated with either ICAM-1 or E-selectin.

Linear regression results also revealed unadjusted negative associations between LF-HRV and IL6 ($B = -0.22$, 95% CI [-0.27, −0.17]), CRP ($B = -0.24$, 95% CI [-0.32, −0.16]), and fibrinogen ($B = -15.75$, 95% CI [-20.33, −11.17]). After adjusting for all covariates and respiration rate, LF-HRV was negatively associated with IL6 ($B = -0.08$, 95% CI [-0.13, −0.03]), and CRP ($B = -0.15$, 95% CI [-0.23, −0.07]), but was no longer significantly associated with fibrinogen ($B = -4.74$, 95% CI [-9.6, 0.12]). The associations between LF-HRV and ICAM-1, E-selectin, or IL6r were not significant. Please see Fig. 1 for scatterplots depicting HRV's associations with IL6, CRP, and fibrinogen, respectively.

Moderation analysis revealed no significant interaction between HF-HRV and sex in predicting inflammation (p's > 0.09). There were also no significant interactions between LF-HRV and sex in predicting inflammation (p's > 0.14). These results suggest the association between HRV and inflammation is similar in male and female adults.

Table 2
Bivariate correlations among primary variables.

	2	3	4	5	6	7	8	9
1. HF-HRV	.68**	-.23**	-.24**	-.16**	-.17**	-.02	-.09*	-.12*
2. LF-HRV	–	-.24**	-.32**	-.22**	-.24**	.03	-.05	-.01
3. Norepinephrine		–	.23**	.08*	.16**	-.16**	-.02	.03
4. IL6			–	.58**	.46**	.08*	.28**	.12**
5. CRP				–	.57**	.14**	.24**	.01
6. Fibrinogen					–	.11*	.20**	.05
7. ICAM-1						–	.37**	.18**
8. E-selectin							–	.12*
9. IL6r								–

Note. HF-HRV = high frequency heart rate variability. LF-HRV = low frequency heart rate variability. IL6 = interleukin-6. CRP = C-reactive protein. ILCAM-1 = intracellular adhesion molecule-1. IL6r = soluble interleukin-6 receptor. Norepinephrine is adjusted for creatinine.
*$p < .05$. **$p < .001$.

Table 3
Step wise linear relation between heart rate variability and inflammation.

		Step 1			Step 2			Step 3			Step 4			Step 5		
Predictor	Outcome	*B*	*SE*	*p*	*B*	*SE*	*p*	*B*	*SE*	*p*	*B*	*SE*	*p*	*B*	*SE*	*p*
HF-HRV	log IL6	**-.14**	.02	<.001	**-.12**	.02	<.001	**-.08**	.02	<.001	**-.07**	.02	<.001	**-.07**	.02	<.001
	log CRP	**-.14**	.03	<.001	**-.13**	.03	<.001	**-.15**	.03	<.001	**-.15**	.03	<.001	**-.15**	.03	<.001
	Fibrinogen	**−8.9**	1.97	<.001	**−7.45**	2.01	<.001	**−6.76**	1.92	<.001	**−6.15**	1.96	.002	**−5.06**	2.00	.01
	log ICAM 1	-.01	.01	.55	-.02	.01	.11	-.02	.01	.03	-.02	.01	.03	-.02	.01	.06
	log E-selectin	-.03	.01	.02	-.03	.01	.01	-.03	.01	.03	-.02	.01	.05	-.02	.01	.10
	IL6 Receptor	**−757**	232	.001	**−734**	239	.002	−429	251	.08	−448	257	.08	−511	265	.05
LF-HRV	log IL6	**-.22**	.02	<.001	**-.20**	.02	<.001	**-.10**	.02	<.001	**-.09**	.02	<.001	**-.08**	.03	.001
	log CRP	**-.24**	.04	<.001	**-.23**	.04	<.001	**-.16**	.04	<.001	**-.16**	.04	<.001	**-.15**	.04	<.001
	Fibrinogen	**−15.75**	2.33	<.001	**−14.25**	2.39	<.001	**−7.1**	2.33	.002	**−6.08**	2.46	.01	−4.74	2.47	.06
	log ICAM 1	.01	.01	.40	.00	.01	.87	-.01	.01	.59	.00	.01	.76	.00	.01	.94
	log E-selectin	-.02	.01	.18	-.02	.01	.10	-.01	.02	.41	-.01	.02	.61	-.01	.02	.73
	IL6 Receptor	−79.40	282	.78	−2.34	290	.99	141	305	.64	119	322	.71	224	328	.50

Note. HF-HRV = high frequency heart rate variability. LF-HRV = low frequency heart rate variability. IL6 = interleukin-6. CRP = C-reactive protein. ICAM 1 = soluble intracellular adhesion molecule 1.
Step 1: unadjusted model.
Step 2: adjusted for urinary norepinephrine.
Step 3: adjusted for urinary norepinephrine, age, sex, race, BMI, menopause, smoking, exercise, and data collection site.
Step 4: adjusted for urinary norepinephrine, age, sex, race, BMI, menopause, smoking, exercise, data collection site, heart disease, stroke, high blood pressure, diabetes, Parkinson's disease, other neurological disorders, and medication regimen.
Step 5: adjusted for all covariates listed in Step 4, using HRV index residualized for respiration rate.
Bolded coefficients are significant after controlling for multiple comparisons using Benjamini-Hochberg False Discovery Rate.

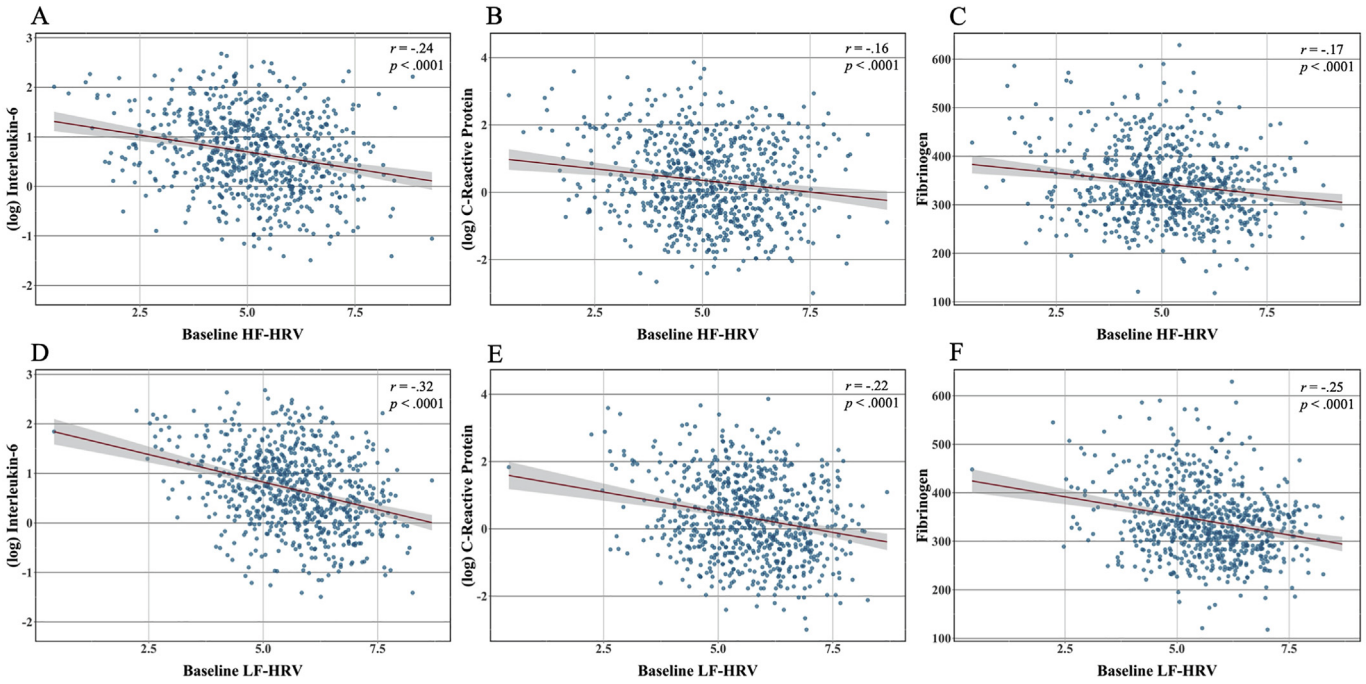

Fig. 1. Associations between high frequency heart rate variability (HF-HRV) and (A) interleukin-6, (B) C-reactive protein, and (C) fibrinogen, and between low frequency heart rate variability (LF-HRV) and (D) interleukin-6, (E) C-reactive protein, and (F) fibrinogen. Shaded region represents 95% confidence interval.

4. Discussion

The present study tested the associations between markers of inflammation and parasympathetic activity (indexed by HRV) in humans. We observed inverse associations between HF-HRV and measures of inflammation (IL6, CRP, fibrinogen) before and after adjusting for a host of covariates. LF-HRV was also inversely related to some of the measures of inflammation (IL6 and CRP), again before and after adjusting for covariates. These findings parallel previous studies which indicate an inverse relationship between HRV and inflammation, as well as work that supports the existence of a vagus nerve-driven *cholinergic anti-inflammatory pathway* (Pavlov and Tracey, 2005; Williams et al., 2019). Importantly, this study replicates previous findings from the MIDUS 2 study (Cooper et al., 2015), in a separate sample of participants (MIDUS R) who completed the same protocol as the original study.

Our findings are consistent with those of previous studies that have shown an inverse relation between HRV and inflammation in both healthy individuals and patient populations with cardiovascular risk or disease (Haensel et al., 2008; Lampert et al., 2008; Frasure-Smith et al., 2009; Singh et al., 2009; von Känel et al., 2011; Williams et al., 2019). Many of these studies primarily focused on CRP and IL6, which are included in the present analyses, but the MIDUS data collection efforts allowed us to include additional indices of inflammation (fibrinogen, ICAM-1, E-Selectin, IL6r). Although testing a panel of several

inflammatory markers is somewhat novel in the extant literature regarding HRV and inflammation, the findings from Cooper et al. (2015) in the MIDUS 2 study and a recent meta-analysis (Williams et al., 2019) led us to hypothesize inverse associations of HF-HRV with IL6 and CRP and inverse associations of LF-HRV with IL6 and CRP. Based on previous findings, the potential association of HRV with fibrinogen was unclear. Analyses revealed that HF-HRV was inversely related to IL6, CRP, fibrinogen, and IL6r; however, after adjusting for covariates (urinary norepinephrine, age, sex, race, BMI, menopause, smoking, exercise, study site, health disorders, medication regimen, and respiration rate), and controlling for multiple comparisons, IL6r was no longer significantly related to HF-HRV. In addition, LF-HRV was inversely related to IL6, CRP, and fibrinogen. After adjusting for our panel of covariates and controlling for multiple comparisons, fibrinogen was no longer significantly related to LF-HRV. Overall, the other markers tested (ICAM-1 and E-selectin, IL6r) were not significantly related to either measure of heart rate variability.

SNS activity has also been implicated in the regulation of inflammatory processes (Koopman et al., 2011). Indeed, we found significant positive correlations of urinary norepinephrine with IL6, CRP, and fibrinogen, and a negative correlation between urinary norepinephrine and ICAM-1. Importantly, after controlling for urinary norepinephrine, the associations with HRV and inflammation remained significant, as previous studies have shown (Cooper et al., 2015; Thayer and Fischer, 2009).

Notably, this study replicates findings from Cooper et al. (2015) collected in an earlier sample of the MIDUS study, with a large sample ($N = 1153$) and thorough data collection efforts. The present study also has a large sample size ($N = 738$) and a more nationally representative sample compared to the original study, which was 91.3% White. In contrast, the participants recruited for the present analyses (MIDUS R) were 70% White, with the largest minority group being African American/Black at 18% and 12% belonging to other minority groups. The present study adds to previous evidence by analyzing multiple inflammatory markers in a large and representative sample, helping to clarify which inflammatory molecules are more likely influenced by parasympathetic-driven modulation of inflammation.

Further replicating Cooper's findings, there was not a significant difference in the strength of the association between HRV and markers of inflammation between male and female participants (Cooper et al., 2015). This is in contrast to some previous studies (Thayer and Fischer, 2009; von Känel et al., 2009), which did not have a large proportion of female participants. In the present sample, 52.1% of participants were female ($n = 450$), and moderation analyses did not reveal any significant difference between males and females in the association between HRV and inflammation. Recent meta-analytic results revealed similar findings, such that sex did not moderate the link between HF-HRV or LF-HRV and inflammation (Williams et al., 2019). Future studies should continue to examine sex as a moderator in the association between HRV and inflammation.

Despite the similarities in findings, it is worth noting that we found a stronger association between HF-HRV and IL6, compared to the study by Cooper et al. (2015), which found an association in the same negative direction. In their study, this weaker negative association did not survive correction for multiple comparisons in covariate-adjusted models. Although the covariates were the same in the two studies, the earlier study utilized Bonferroni correction for multiple comparisons, which may be more conservative than necessary (Cooper et al., 2015; von Känel et al., 2009).

The present study found significant associations of HRV with IL6, CRP, and fibrinogen, but not the other inflammatory markers assessed (ICAM-1, IL6r, E-Selectin). This suggests that there may be specific biomarkers that are more closely implicated in the cholinergic anti-inflammatory pathway than others. Indeed, the activation/regulation of different inflammatory markers by the SNS and PNS are triggered at different points along the immune response (Bellinger and Lorton, 2014; Madden, 2017; Pavlov and Tracey, 2017). Previous work has implicated TNFα, IL1β, and IL6, in studies demonstrating nicotinic receptor α7 as a mediator of cholinergic anti-inflammatory pathway output (Pavlov and Tracey, 2005; Wang et al., 2003). However, in a recent meta-analysis (Williams et al., 2019), both TNFα and IL1 were not found to be associated with any measures of cardiac/autonomic physiology, citing a lack of studies with large enough sample sizes, and a scarcity of studies altogether. However, the meta-analysis revealed significant inverse relations of both IL6 and CRP with HF-HRV and LF-HRV. In our exploratory analyses (Supplemental Material Appendix 1), TNFα showed a weak negative association with both HF-HRV and LF-HRV, but neither survived correction for multiple comparisons and adjustment for covariates.

It is difficult to know whether IL6 and CRP serve as the main biomarkers implicated in the cholinergic anti-inflammatory pathway, or if simply most previous studies reported/measured associations between IL6 and CRP instead of other markers of inflammation and indices of HRV. Together, these findings demonstrate a need for studies with larger sample sizes that assess a broader panel of multiple inflammatory markers to further explore mechanisms of the anti-inflammatory pathway in humans. Although the majority of the studies described, the present study included, are correlational, previous studies show a potential causal role of the vagus nerve as a regulator of inflammation in the cholinergic anti-inflammatory pathway. Vagotomy in non-human animal models results in an increased immune response (Borovikova et al., 2000; Karimi et al., 2010) and vagal stimulation or use of α7 nAChR agonists limits the release of inflammatory cytokines (Wang et al., 2004). However, more recent studies of vagotomy in animals are proving this relationship to be more complex (Kobrzycka et al., 2019), as communication between the immune system and brain was found to be largely preserved following vagotomy, via alternative compensatory mechanisms in the periphery (PGE2). In humans, a group of patients who underwent vagotomy showed significantly increased levels of ulcer disease, septicemia, and overall mortality in comparison to healthy controls (Peterson et al., 2012). In a study of patients with various of forms of brain damage, the patient group with likely the most increased vagus nerve activity (those with intracranial hemorrhage, ICH) demonstrated the lowest cytokine production in response to ex-vivo stimulation of whole blood (Kox et al., 2012). Further, bioelectronic stimulation of the vagus nerve has proven to be an effective treatment of chronic inflammatory disorders in humans (Bonaz et al., 2016; Koopman et al., 2016; Pavlov et al., 2020).

4.1. Clinical implications

While many previous studies on PNS-inflammation links have been conducted in patients with brain injury or cardiovascular diseases, the present study demonstrates a potential role of the PNS in anti-inflammatory processes in a large, representative sample. Exploring evidence supporting the cholinergic anti-inflammatory pathway has potential implications for both therapies in clinical populations and overall public health promotion.

Noninvasive measures of HRV may serve as potential diagnostic biomarkers to identify periods of inflammatory risk in individuals with autoimmune or other chronic diseases. Huston and Tracey (2011) propose monitoring HRV function with ambulatory devices which would therein serve to monitor inflammation risk in individuals with cytokine-mediated diseases (e.g., hypertension). In addition, acetylcholinesterase inhibitors, such as galantamine, have been approved to treat Alzheimer's disease but have begun to be examined in other disorders such as metabolic syndrome. A randomized, double-blind, placebo-controlled trial demonstrated galantamine to have anti-inflammatory effects (suppressing TNFα), suppress insulin levels, and modulate heart rate variability in patients with metabolic syndrome (Consolim-Colombo et al., 2017; Pavlov et al., 2018). Thus, metabolic syndrome, characterized by chronic inflammation and insulin resistance, may be effectively treated by drugs such as galantamine, which promote acetylcholine in the central nervous system.

Recently, as the SARS-CoV-2/COVID-19 global pandemic persists, there has been an interest in vagal stimulation as a potential treatment. Bonaz and colleagues suggest that stimulation of the vagus nerve could be of interest in treating patients with SARS-CoV-2 infection, as this stimulation could decrease the "cytokine storm" that is seen in critical COVID-19 patients (Bonaz et al., 2020; Ye et al., 2020), if future clinical trials support its efficacy in this patient population.

4.2. Conclusions

Our findings are consistent with the existence of a cholinergic anti-inflammatory pathway, demonstrating inverse relationships of both HF- and LF-HRV with multiple inflammatory markers in a large, representative sample. Importantly, our findings replicate a previous study examining the same associations in another publicly available dataset (Cooper et al., 2015). Recent calls encourage scientists to both engage in replication studies and to freely share research materials (datasets, code) in order to reproduce results (Kubilius, 2014). The shift toward reproducible and transparent science is supported by data and analysis-generating code being available (Stevens, 2017).

Despite the several strengths of our study, there are some limitations that should be described in order to inform future research efforts. First, the observed effect sizes were small (r's < 0.50) according to the definition by Cohen (1988). However, small differences in circulating inflammation levels observed at a single timepoint could accumulate to meaningful differences in risk over the lifespan, particularly in individuals at high risk (Abelson, 1985). Nevertheless, these results suggest other factors should be considered to fully explain variance in inflammatory processes. Second, although participants were asked to relax and breath naturally during the resting HRV measurement, it is possible that some individuals may have engaged in modulation of breathing rate, which could influence resting HRV measures. Adjusting for respiratory rate helps alleviate concerns over this potential limitation. Future studies could benefit from explicitly requesting participants to not engage in modulation of respiration. Third, the list of medical conditions and medication regimens included as covariates was extensive but not exhaustive. Considering the old age of some of our participants, and therefore the large range of possible health issues and medication taken, it is possible that our findings could be influenced by some illness or medication not accounted for in the current study. Future research may also benefit from testing moderation effects of illness and medication (i.e., does the relation between HRV and inflammation depend on medication regimen?). Lastly, the list of inflammatory markers in the current study was not exhaustive. Our exploratory analyses expanded upon the original study of Cooper et al. (2015; see Supplemental Appendix S2) to include IL10 and TNFα, resulting in eight inflammatory markers in total. Nevertheless, future research could benefit from including inflammatory markers that appear to be less common in studies of the cholinergic anti-inflammatory pathway (e.g., interleukin-2; Williams et al., 2019).

In addition to replicating the findings by Cooper and colleagues in a different sample, both the previous and present study did not detect moderation by sex in the association of HRV and inflammation. As emphasized in a recent meta-analysis (Williams et al., 2019), future studies should continue to examine sex as a moderator in the cholinergic anti-inflammatory pathway. Additionally, there is a need for studies with greater detail, testing multiple inflammatory markers in large, nationally representative samples and to examine the relationship between ANS and inflammation in controlled experimental settings (Williams et al., 2019). Notably, this study features a sample (MIDUS R) with greater representation of African American participants than the previously conducted study (MIDUS 2); however, future work is needed to examine any racial differences in the relation between PNS activity and inflammatory markers. The findings presented, which emphasize the potential role of the vagus nerve in the anti-inflammatory pathway, are of clinical significance, especially during the COVID-19 pandemic.

Declaration of competing interest

The authors report no conflicts of interest.

Acknowledgements

Data used for this research were provided by the longitudinal study titled "Midlife in the United States" (MIDUS), managed by the Institute on Aging, University of Wisconsin, and supported by Grants P01-AG020166 and U19-AG051426 from the National Institute on Aging; and Grants UL1TR001409, UL1TR001881, and 1UL1RR025011 from the NIH National Center for Advancing Translational Sciences (NCATS) Clinical and Translational Science Award (CTSA) program. The authors assume full responsibility for this work. The ideas expressed herein do not represent the views of the original collector of the data, the Inter-university Consortium for Political and Social Research (ICPSR), or that of the funding agencies.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbih.2021.100273.

References

Addorisio, M.E., Imperato, G.H., de Vos, A.F., Forti, S., Goldstein, R.S., Pavlov, V.A., van der Poll, T., Yang, H., Diamond, B., Tracey, K.J., Chavan, S.S., 2019. Investigational treatment of rheumatoid arthritis with a vibrotactile device applied to the external ear. Bioelectronic Medicine 5 (1), 1–11. https://doi.org/10.1186/s42234-019-0020-4.

Alen, N.V., Deer, L.B.K., Hostinar, C.E., 2020. Autonomic nervous system activity predicts increasing serum cytokines in children. Psychoneuroendocrinology 119, 104745. https://doi.org/10.1016/j.psyneuen.2020.104745.

Abelson, R.P., 1985. A variance explanation paradox: when a little is a lot. Psychol. Bull. 97 (1), 129–133. https://doi.org/10.1037/0033-2909.97.1.129.

Bellinger, D.L., Lorton, D., 2014. Autonomic regulation of cellular immune function. Auton. Neurosci. : basic & clinical 182, 15–41. https://doi.org/10.1016/j.autneu.2014.01.006.

Benjamini, Y., Hochberg, Y., 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J. R. Stat. Soc. Ser. B 57, 289–300.

Berntson, G.G., Bigger, J.T., Eckberg, D.L., Grossman, P., Kaufmann, P.G., Malik, M., van, d.M., 1997. Heart rate variability: origins, methods, and interpretive caveats. Psychophysiology 34 (6), 623–648.

Bonaz, B., Sinniger, V., Hoffmann, D., Clarençon, D., Mathieu, N., Dantzer, C., Vercueil, L., Picq, C., Trocmé, C., Faure, P., Cracowski, J.-L., Pellissier, S., 2016. Chronic vagus nerve stimulation in Crohn's disease: a 6-month follow-up pilot study. Neuro Gastroenterol. Motil. 28 (6), 948–953. https://doi.org/10.1111/nmo.12792.

Bonaz, B., Sinniger, V., Pellissier, S., 2020. Targeting the cholinergic anti-inflammatory pathway with vagus nerve stimulation in patients with Covid-19? Bioelectronic Medicine 6 (1), 15. https://doi.org/10.1186/s42234-020-00051-7.

Borovikova, L.V., Ivanova, S., Zhang, M., Yang, H., Botchkina, G.I., Watkins, L.R., Wang, H., Abumrad, N., Eaton, J.W., Tracey, K.J., 2000. Vagus nerve stimulation attenuates the systemic inflammatory response to endotoxin. Nature 405 (6785), 458–462. https://doi.org/10.1038/35013070.

The John D. and Catherine T. MacArthur foundation series on mental health and development. In: Brim, O.G., Ryff, C.D., Kessler, R.C. (Eds.), 2004. Studies on Successful Midlife Development. How Healthy Are We?: A National Study of Well-Being at Midlife. The University of Chicago Press.

Cacioppo, J.T., Berntson, G.G., Binkley, P.F., Quigley, K.S., Uchino, B.N., Fieldstone, A., 1994. Autonomic cardiac control. II. Noninvasive indices and basal response as revealed by autonomic blockades. Psychophysiology 31, 586–598. https://doi.org/10.1111/j.1469-8986.1994.tb02351.x.

Chiu, I.M., Heesters, B.A., Ghasemlou, N., Von Hehn, C.A., Zhao, F., Tran, J., Wainger, B., Strominger, A., Muralidharan, S., Horswill, A.R., Bubeck Wardenburg, J., Hwang, S.W., Carroll, M.C., Woolf, C.J., 2013. Bacteria activate sensory neurons that modulate pain and inflammation. Nature 501 (7465), 52–57. https://doi.org/10.1038/nature12479.

Cohen, J., 1988. Statistical Power Analysis for the Behavioral Sciences, second ed. Lawrence Erlbaum Associates, Publishers, Hillsdale, NJ.

Consolim-Colombo, F.M., Sangaleti, C.T., Costa, F.O., Morais, T.L., Lopes, H.F., Motta, J.M., Irigoyen, M.C., Bortoloto, L.A., Rochitte, C.E., Harris, Y.T., Satapathy, S.K., Olofsson, P.S., Akerman, M., Chavan, S.S., MacKay, M., Barnaby, D.P., Lesser, M.L., Roth, J., Tracey, K.J., Pavlov, V.A., 2017. Galantamine alleviates inflammation and insulin resistance in patients with metabolic syndrome in a randomized trial. JCI Insight 2 (14), e93340. https://doi.org/10.1172/jci.insight.93340.

Cooper, T.M., McKinley, P.S., Seeman, T.E., Choo, T.H., Lee, S., Sloan, R.P., 2015. Heart rate variability predicts levels of inflammatory markers: evidence for the vagal anti-

inflammatory pathway. Brain Behav. Immun. 49, 94–100. https://doi.org/10.1016/j.bbi.2014.12.017.
Dantzer, R., Kelley, K.W., 2007. Twenty years of research on cytokine-induced sickness behavior. Brain Behav. Immun. 21 (2), 153–160. https://doi.org/10.1016/j.bbi.2006.09.006.
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. https://doi.org/10.1109/TBME.1984.325351.
Frasure-Smith, N., Lespérance, 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. https://doi.org/10.1016/j.bbi.2009.07.005.
Goldstein, D.S., Bentho, O., Park, M.Y., Sharabi, Y., 2011. Low-frequency power of heart rate variability is not a measure of cardiac sympathetic tone but may be a measure of modulation of cardiac autonomic outflows by baroreflexes. Exp. Physiol. 96 (12), 1255–1261. https://doi.org/10.1113/expphysiol.2010.056259.
Haensel, A., Mills, P.J., Nelesen, R.A., Ziegler, M.G., Dimsdale, J.E., 2008. The relationship between heart rate variability and inflammatory markers in cardiovascular diseases. Psychoneuroendocrinology 33 (10), 1305–1312. https://doi.org/10.1016/j.psyneuen.2008.08.007.
Harris, F.J., 1978. On the use of windows for harmonic analysis with the discrete Fourier transform. Proceesings of the IEEE 66 (1), 51–83.
Hostinar, C.E., Lachman, M.E., Mroczek, D., Seeman, T.E., Miller, G.E., 2015. Additive roles of childhood adversity and recent stressors in explaining inflammation at midlife: findings from the MIDUS study. Dev. Psychol. 51 (11), 1630–1644.
Hostinar, C.E., Nusslock, R., Miller, G.E., 2018. Future directions in the study of early-life stress and physical and emotional health: implications of the neuroimmune network hypothesis. J. Clin. Child Adolesc. Psychol. 47 (1), 142–156. https://doi.org/10.1080/15374416.2016.1266647.
Huston, J.M., Tracey, K.J., 2011. The pulse of inflammation: heart rate variability, the cholinergic anti-inflammatory pathway and implications for therapy. J. Intern. Med. 269 (1), 45–53. https://doi.org/10.1111/j.1365-2796.2010.02321.x.
Karimi, K., Bienenstock, J., Wang, L., Forsythe, P., 2010. The vagus nerve modulates CD4 + T cell activity. Brain Behav. Immun. 24 (2), 316–323. https://doi.org/10.1016/j.bbi.2009.10.016.
Kobrzycka, A., Napora, P., Pearson, B.L., Pierzchała-Koziec, K., Szewczyk, R., Wieczorek, M., 2019. Peripheral and central compensatory mechanisms for impaired vagus nerve function during peripheral immune activation. J. Neuroinflammation 16 (1), 150. https://doi.org/10.1186/s12974-019-1544-y.
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. (Camb.) 17 (9–10), 937–948. https://doi.org/10.2119/molmed.2011.00065.
Koopman, F.A., Chavan, S.S., Miljko, S., Grazio, S., Sokolovic, S., Schuurman, P.R., Mehta, A.D., Levine, Y.A., Faltys, M., Zitnik, R., Tracey, K.J., Tak, P.P., 2016. Vagus nerve stimulation inhibits cytokine production and attenuates disease severity in rheumatoid arthritis. Proc. Natl. Acad. Sci. Unit. States Am. 113 (29), 8284–8289. https://doi.org/10.1073/pnas.1605635113.
Kox, M., Vrouwenvelder, M.Q., Pompe, J.C., van der Hoeven, J.G., Pickkers, P., Hoedemaekers, C.W., 2012. The effects of brain injury on heart rate variability and the innate immune response in critically ill patients. J. Neurotrauma 29 (5), 747–755. https://doi.org/10.1089/neu.2011.2035.
Kubilius, J., 2014. I-review: sharing code. I-Perception 5 (1), 75–78. https://doi.org/10.1068/i004ir.
Laborde, S., Mosley, E., Thayer, J.F., 2017. Heart rate variability and cardiac vagal tone in psychophysiological research - recommendations for experiment planning, data analysis, and data reporting. Front. Psychol. 8 (FEB), 1–18. https://doi.org/10.3389/fpsyg.2017.00213.
Lampert, R., Bremner, J.D., Su, S., Miller, A., Lee, F., Cheema, F., Goldberg, J., Vaccarino, V., 2008. Decreased heart rate variability is associated with higher levels of inflammation in middle-aged men. Am. Heart J. 156 (4) https://doi.org/10.1016/j.ahj.2008.07.009, 759.e1-759.e7.
Lehrer, P.M., Gevirtz, R., 2014. Heart rate variability biofeedback: how and why does it work? Front. Psychol. 5 (JUL), 1–9. https://doi.org/10.3389/fpsyg.2014.00756.
Libby, P., 2002. Inflammation in atherosclerosis. Nature 420, 868–874. https://doi.org/10.1038/nature01323.
Madden, K.S., 2017. Sympathetic neural-immune interactions regulate hematopoiesis, thermoregulation and inflammation in mammals. Dev. Comp. Immunol. 66, 92–97. https://doi.org/10.1016/j.dci.2016.04.015.
Marsland, A.L., Gianaros, P.J., Prather, A.A., Jennings, J.F., Neumann, S.A., Manuck, S.B., 2007. Stimulated production of proinflammatory cytokines covaries inversely with heart rate variability. Psychosom. Med. 69 (8), 709–716. https://doi.org/10.1097/PSY.0b0136181576118.
National Academies of Sciences, Engineering, and Medicine, 2019. Reproducibility and Replicability in Science. The National Academies Press, Washington, DC. https://doi.org/10.17226/25303.
Pavlov, V.A., Chavan, S.S., Tracey, K.J., 2018. Molecular and functional neuroscience in immunity. Annu. Rev. Immunol. 36 (1), 783–812. https://doi.org/10.1146/annurev-immunol-042617-053158.
Pavlov, V.A., Chavan, S.S., Tracey, K.J., 2020. Bioelectronic medicine: from preclinical studies on the inflammatory reflex to new approaches in disease diagnosis and treatment. Cold Spring Harbor Perspectives in Medicine 10 (3), a034140. https://doi.org/10.1101/cshperspect.a034140.
Pavlov, V.A., Tracey, K.J., 2005. The cholinergic anti-inflammatory pathway. Brain Behav. Immun. 19 (6), 493–499. https://doi.org/10.1016/j.bbi.2005.03.015.
Pavlov, V.A., Tracey, K.J., 2017. Neural regulation of immunity: molecular mechanisms and clinical translation. Nat. Neurosci. 20 (2), 156–166. https://doi.org/10.1038/nn.4477.
Peterson, C.Y., Krzyzaniak, M., Coimbra, R., Chang, D.C., 2012. Vagus nerve and postinjury inflammatory response. Arch. Surg. 147 (1), 76. https://doi.org/10.1001/archsurg.2011.237.
Saeed, R.W., Varma, S., Peng-Nemeroff, T., Sherry, B., Balakhaneh, D., Huston, J., Tracey, K.J., Al-Abed, Y., Metz, C.N., 2005. Cholinergic stimulation blocks endothelial cell activation and leukocyte recruitment during inflammation. J. Exp. Med. 201 (7), 1113–1123. https://doi.org/10.1084/jem.20040463.
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), 367–374. https://doi.org/10.1007/s10286-009-0019-0.
Stevens, J.R., 2017. Replicability and reproducibility in comparative psychology. Front. Psychol. 8, 862. https://doi.org/10.3389/fpsyg.2017.00862.
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. https://doi.org/10.1111/j.1365-2796.2008.02023.x.
Tracey, K., 2002. The inflammatory reflex. Nature 420, 853–859. https://doi.org/10.1038/nature01321.
van Buuren, S., Groothuis-Oudshoorn, K., 2011. Mice: multivariate imputation by chained equations in R. J. Stat. Software 45 (3), 1–67. https://doi.org/10.18637/jss.v045.i03.
von Känel, R., Carney, R.M., Zhao, S., Whooley, M.A., 2011. Heart rate variability and biomarkers of systemic inflammation in patients with stable coronary heart disease: findings from the Heart and Soul Study. Clin. Res. Cardiol. 100 (3), 241–247. https://doi.org/10.1007/s00392-010-0236-5.
von Känel, R., Thayer, J.F., Fischer, J.E., 2009. Nighttime vagal cardiac control and plasma fibrinogen levels in a population of working men and women. Ann. Noninvasive Electrocardiol. 14 (2), 176–184. https://doi.org/10.1111/j.1542-474X.2009.00293.x.
Wang, H., Liao, H., Ochani, M., Justiniani, M., Lin, X., Yang, L., Al-Abed, Y., Wang, H., Metz, C., Miller, E.J., Tracey, K.J., Ulloa, L., 2004. Cholinergic agonists inhibit HMGB1 release and improve survival in experimental sepsis. Nat. Med. 10 (11), 1216–1221. https://doi.org/10.1038/nm1124.
Wang, H., Yu, M., Ochani, M., Amella, C.A., Tanovic, M., Susarla, S., Li, J.H., Wang, H., Yang, H., Ulloa, L., Al-Abed, Y., Czura, C.J., Tracey, K.J., 2003. Nicotinic acetylcholine receptor alpha7 subunit is an essential regulator of inflammation. Nature 421 (6921), 384–388. https://doi.org/10.1038/nature01339.
Williams, D.W.P., Koenig, J., Carnevali, L., Sgoifo, A., Jarczok, M.N., Sternberg, E.M., Thayer, J.F., 2019. Heart rate variability and inflammation: a meta-analysis of human studies. Brain Behav. Immun. 80, 219–226. https://doi.org/10.1016/j.bbi.2019.03.009.
Ye, Q., Wang, B., Mao, J., 2020. The pathogenesis and treatment of the `Cytokine Storm' in COVID-19. J. Infect. 80 (6), 607–613. https://doi.org/10.1016/j.jinf.2020.03.037.
Zwaan, R.A., Etz, A., Lucas, R.E., Donnellan, M.B., 2017. Making replication mainstream. Behav. Brain Sci. 1–50 https://doi.org/10.1017/S0140525X17001972.