Bidirectional Prospective Associations Between Cardiac Autonomic Activity and Inflammatory Markers

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ABSTRACT

Objective: Autonomic nervous system (ANS) imbalance has been cross-sectionally associated with inflammatory processes. Longitudinal studies are needed to shed light on the nature of this relationship. We examined cross-sectional and bidirectional prospective associations between cardiac autonomic measures and inflammatory markers.

Methods: Analyses were conducted with baseline ($n = 2823$), 2-year ($n = 2099$), and 6-year ($n = 1774$) data from the Netherlands Study of Depression and Anxiety. To compare the pattern of results, prospective analyses with ANS (during sleep, leisure time, and work) and inflammation were conducted in two data sets from the Netherlands Twin Register measured for 4.9 years ($n = 356$) and 5.4 years ($n = 472$). Autonomic nervous system measures were heart rate (HR) and respiratory sinus arrhythmia (RSA). Inflammatory markers were C-reactive protein (CRP) and interleukin (IL)-6.

Results: The Netherlands Study of Depression and Anxiety results showed that higher HR and lower RSA were cross-sectionally significantly associated with higher inflammatory levels. Higher HR predicted higher levels of CRP ($B = .065$, $p < .001$) and IL-6 ($B = .036$, $p = .014$) at follow-up. Higher CRP levels predicted lower RSA ($B = -.024$, $p = .048$) at follow-up. The Netherlands Twin Register results confirmed that higher HR was associated with higher CRP and IL-6 levels 4.9 years later. Higher IL-6 levels predicted higher HR and lower RSA at follow-up.

Conclusions: Autonomic imbalance is associated with higher levels of inflammation. Independent data from two studies converge in evidence that higher HR predicts subsequent higher levels of CRP and IL-6. Inflammatory markers may also predict future ANS activity, but evidence for this was less consistent.

Key words: autonomic nervous system, inflammation, longitudinal, vagal activity.

INTRODUCTION

Autonomic nervous system (ANS) imbalance, characterized by high sympathetic activity and low vagal activity, plays an important role in cardiovascular dysregulation and is a risk factor for coronary artery disease (CAD) (1,2). Inflammatory processes are thought to cause vascular endothelial dysfunction (3) and may provide a link between autonomic dysfunction and CAD.

Indicators of cardiac sympathetic and vagal activity, such as heart rate (HR) and respiratory sinus arrhythmia (RSA), can be noninvasively and unobtrusively measured by electrocardiography (ECG) and impedance cardiography (ICG) (1,2). Both high HR and low RSA have been associated with CAD (3,4). Two of the most important and most studied markers of inflammation are C-reactive protein (CRP) and interleukin (IL)-6, both of which have been implicated in atherogenesis (5). Given these findings, researchers have sought to associate measures of cardiac vagal activity with inflammatory markers and have indeed consistently found increased vagal activity to be cross-sectionally associated with decreased levels of CRP and IL-6 (6–12). This association corresponds with the cholinergic anti-inflammatory pathway, stating that high vagal activity has an anti-inflammatory effect by inhibiting the production of proinflammatory markers (13,14). In addition, studies have shown that high HR, indicative of relative sympathetic dominance (15), is associated with increased levels of proinflammatory markers (16–19).

ANS = autonomic nervous system, **BMI** = body mass index, **CAD** = coronary artery disease, **CRP** = C-reactive protein, **ECG** = electrocardiogram, **ELISA** = enzyme-linked immunosorbent assay, **GEE** = generalized estimated equations, **HF-HRV** = high-frequency HRV, **HR** = heart rate, **IBI** = interbeat interval, **ICG** = impedance cardiography, **IL-6** = interleukin 6, **IQR** = interquartile range, **METmin** = multiple of resting metabolic rate times minutes of physical activity per week, **NESDA** = Netherlands Study of Depression and Anxiety, **NTR** = Netherlands Twin Register, **RSA** = respiratory sinus arrhythmia, **SNRI** = selective serotonin and noradrenalin reuptake inhibitors, **SSRI** = selective serotonin reuptake inhibitors, **TCA** = tricyclic antidepressant, **VU-AMS** = Vrije Universiteit Ambulatory Monitoring System

SDC Supplemental Content

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Received for publication June 23, 2017; revision received November 27, 2017.

DOI: 10.1097/PSY.0000000000000589

Studies investigating the relationship between ANS activity and inflammation have mostly been cross-sectional, whereas few studies have investigated this relationship longitudinally. A study by Jarczok and colleagues (20) investigated this longitudinal association unidirectionally and found that higher high-frequency HR variability (HF-HRV), indicative of higher vagal activity, at baseline predicted lower levels of CRP 4 years later. Singh and colleagues (21) investigated the bidirectional longitudinal relationship between HF-HRV and CRP for 3 years and, surprisingly, found that higher CRP predicted subsequent increases in vagal activity. Thus, it remains unclear whether there is a unidirectional causal effect of ANS activity on inflammation, a reverse effect of inflammation on ANS activity, a bidirectional effect, or an underlying mechanism causing parallel changes of autonomic functioning and inflammation levels over time. Additional longitudinal analyses are needed to investigate whether longer-term exposure to deviant ANS functioning may lead to accumulative effects on inflammation or vice versa.

The current study determined whether HR and RSA were cross-sectionally associated with IL-6 and CRP levels. In addition, we investigated bidirectional prospective associations between cardiac autonomic activity and inflammatory markers. These analyses were conducted with baseline, 2-, and 6-year data from the Netherlands Study of Depression and Anxiety (NESDA). We additionally conducted prospective analyses with ANS and inflammation data of two studies from the Netherlands Twin Register (NTR) measured for 5 years to compare the pattern of results.

METHODS

Participants

NESDA

Participants were recruited from community, primary care, and mental health care in the Netherlands as part of the NESDA study, an ongoing longitudinal cohort study to examine the long-term course of depression and anxiety. The NESDA sample consists of 2981 participants aged 18 to 65 years with a current diagnosis of depression and/or anxiety disorder, a history of these disorders, and healthy controls. Exclusion criteria were a primary clinical diagnosis of other severe psychiatric disorders and nonfluency in Dutch. The baseline assessment, conducted between 2004 and 2007, lasted approximately 4 hours, and follow-up assessments took place after 2, 4, and 6 years. A detailed description of the rationale, objectives, and methods of the NESDA study can be found elsewhere (22). The study protocol was performed conform the declaration of Helsinki and approved by the ethical review board of each participating center. All participants provided written informed consent.

For the present study, data were drawn from baseline (n = 2981), 2-year (n = 2596), and 6-year (n = 2256) follow-up assessments. Of the participants, 118, 334, and 375 people were excluded at baseline, 2-, and 6-year follow-up, respectively, because of missing physiological data (due to telephone or at-home interviews without ANS recording, equipment failure during assessment, or poor electrocardiogram quality). Another 40, 161, and 107 participants were excluded at baseline, 2-, and 6-year follow-up, respectively, because of missing data on inflammatory markers. This resulted in a total of 2823 participants at baseline, 2099 participants at 2-year follow-up, and 1774 participants at 6-year follow-up.

NTR

Study 1 (1998–2003) consisted of 816 participants of a large cardiac ambulatory monitoring study conducted in families registered with the NTR. Exclusion criteria for this study were heart disease, diabetic neuropathy, and pregnancy. The number of participants with valid data on ANS activity was 741. Of these participants, we had valid data on inflammatory markers of 356 persons, because they had also participated in a large NTR biobank study (2004–2008) (23), conducted on M (SD) of 4.9 (1.7) years later.

Study 2 composed of 592 participants who participated in the NTR biobank study and, in the second part of the cardiac ambulatory monitoring study (2010–2012), conducted on M (SD) of 5.4 (1.1) years later. The same exclusion criteria were applied as for study 1. Of the total sample, 472 participants had valid data on inflammatory markers and ANS activity.

Physiological Measurements

For both NESDA and NTR, physiological data were recorded with the "Vrije Universiteit Ambulatory Monitoring System" (VU-AMS), an unobtrusive lightweight portable device containing a six-electrode configuration. These electrodes measure electrocardiograms (ECG) and changes in thorax impedance (ICG) (15). HR was directly derived from the ICG interbeat interval (IBI) time series (15). RSA combined ECG with ICG and was obtained by subtracting the shortest IBI at inhalation from the longest IBI at exhalation for all breaths (5).

Movement registration through vertical accelerometry was used to remove nonstationary periods. Suspicious IBIs and breathing cycles were investigated with automated and visual data cleaning and corrected or discarded when necessary.

NESDA

The participants wore the VU-AMS device during the assessments at baseline and follow-up. The assessments were divided into different conditions by an event marker. Autonomic nervous system data of the following four conditions were present at all waves: a supine rest condition with blood pressure measurement (±11 min) and three sitting conditions: a psychiatric interview (±41 min), a general interview (±33 min), and a computer task (±13 min). Cardiac autonomic variables during the separate conditions were highly correlated and previous research has shown that an average score of these conditions had higher temporal stability than the individual scores (24). Therefore, data during the four conditions were collapsed to create one single HR and RSA value per subject per wave.

NTR

The participants wore the device an entire day and night, starting in the morning before their normal daily activities until awakening the next morning. In addition, they kept a diary in which they wrote down a chronological account of activity, posture, location, and social situation for the past period. This was done every 30 minutes for study 1 and every 60 minutes for study 2. The activity diary entries were used in combination with visual inspection of the movement registration to divide the 24-hour recording into fixed periods. An average score of HR and RSA was calculated for the following three conditions: (1) sleep, (2) leisure: defined as the period with the lowest HR in the evening from 6:00 PM until bedtime during which the participant was sitting, and (3) work: all periods in which the participant was engaged in sitting activities at work between 9:00 AM and 6:00 PM during a reported working day.

Inflammatory Markers

NESDA

At each wave, fasting blood samples were obtained in the morning between 8:00 and 9:00 AM and kept frozen at −80°C. CRP and IL-6 were assayed at the Clinical Chemistry Department of the VU University Medical Center.

At baseline, plasma levels of CRP were measured in duplicate by an in-house high-sensitivity enzyme-linked immunosorbent assay (ELISA) based on purified protein and polyclonal anti-CRP antibodies (Dako, Glostrup, Denmark). The lower detection limit of CRP was 0.1 mg/L, and the sensitivity was 0.05 mg/L. Intra- and interassay coefficients of variation

were 5% and 10%, respectively. Plasma levels of CRP at 2- and 6-year follow-up were measured in duplicate by a high-sensitivity particle-enhanced immunoturbidimetric assay (CRPHS, Roche Diagnostics, Indianapolis, IN). In this kit, the lower detection limit of CRP was 0.15 mg/L and the sensitivity was 0.3 mg/L. Intra-assay coefficients of variation were 5% for 2-year follow-up and 7% for 6-year follow-up. Interassay coefficients of variation were 4% for 2-year follow-up and 9% for 6-year follow-up. Individuals with CRP values of greater than 10 mg/L were considered outliers and excluded from all analyses at that same wave.

Plasma IL-6 levels at baseline were measured in duplicate by a high-sensitivity ELISA (PeliKine CompactTM ELISA, Sanquin, Amsterdam, the Netherlands). The lower detection limit of IL-6 was 0.35 pg/ml and the sensitivity was 0.10 pg/ml. Intra- and interassay coefficients of variation were 8% and 12%, respectively. At the 2- and 6-year follow-up, IL-6 was measured in duplicate by a high-sensitivity solid phase ELISA (Human IL-6 Quantikine HS kit, R&D systems, Minneapolis, MN). In this kit, the lower detection limit of IL-6 was 0.08 pg/ml and the sensitivity range was 0.016 to 0.110 pg/ml. Intra- and interassay coefficients of variation were 8% and 7%, respectively.

NTR

After an overnight fast, blood samples were collected in EDTA, heparin and CTAD blood tubes stored at −30°C (23). CRP was measured in heparin plasma, using Immulite 1000 CRP assay (Diagnostic Product Corporation). Interassay coefficients of variation were less than 5%. Again, individuals with CRP values of greater than 10 mg/L were excluded for all inflammatory markers. IL-6 was measured in EDTA plasma, using an UltraSensitive ELISA (Quantikine HS HSTA00C; R&D Systems, Minneapolis, MN) (25). Interassay coefficients of variation were less than 12%.

Covariates

NESDA

Adjustments were made for sociodemographics: age, sex, and years of education. We further adjusted for other covariates measured at all time points: physical activity measured by the International Physical Activity Questionnaire (26), number of smoked cigarettes per day, alcohol use (units per week), body mass index (BMI), number of treated chronic diseases (cardiovascular disease, epilepsy, diabetes, osteoarthritis, stroke, cancer, chronic lung disease, thyroid disease, liver disease, intestinal disorders and ulcer), current (6-month recency) depression and/or anxiety disorder (according to the DSM-IV based Composite International Diagnostic Interview), and use of antidepressants (tricyclic antidepressants, ATC code N06AA; selective serotonin reuptake inhibitors, ATC code N06AB; and selective serotonin and noradrenalin reuptake inhibitors, ATC code N06AX), heart medication (ATC codes C01, C02, C03, C04, C05, C07, C08), anti-inflammatory agents (ATC codes M01A, M01B, A07EB, A07EC), and statins (ATC codes C10AA, C10B). Because it has been linked to RSA (27), respiration rate was included as a covariate when analyzing RSA.

NTR

NTR covariates were measured before the start of ANS activity recording and before blood collection. Comparable with NESDA, adjustments were made for age, sex, level of education, smoking (yes/no), and BMI. The number of participants using medication (antidepressants, heart medication, and anti-inflammatory agents) was negligible and therefore not included as covariate. The number of chronic diseases or mental health status was not registered at the moment of ANS activity recording or blood collection. Respiration rate was included as a covariate when analyzing RSA.

Statistical Analyses

Data were analyzed using SPSS, Version 22.0. All autonomic and inflammation variables were z-transformed to make comparisons between estimated effects. Because RSA, CRP, and IL-6 values were skewed, these were first ln-transformed and then z-transformed when analyzed as outcome (dependent) variables.

NESDA

We used generalized estimated equations (GEE) analyses with an exchangeable correlation structure to examine the consistency of the cross-sectional association between autonomic variables and inflammatory markers across waves. To investigate whether these associations differed between waves, the previous analyses were repeated with the inclusion of an interaction term between ANS activity and categorical time. In addition, effects of sex were investigated by including a sex-interaction term in the analyses. Covariates age, sex, and years of education were held at baseline. Other covariates could vary over time.

Autoregression GEE models with an independent correlation structure were used to investigate whether autonomic activity at one time point (t) predicted levels of inflammatory markers at the next time point ($t + 1$), while accounting for covariates and inflammatory markers at t. This analysis was repeated with inflammatory markers as predictors and autonomic activity as outcome. To investigate whether these prospective relationships differed over waves, we added an interaction term between the predictor and categorical time.

Because BMI is suggested to be in the pathway between ANS activity and inflammatory markers, adjusting for it might be considered overcorrection (28). Therefore, we first ran the previous analyses adjusting for all covariates excluding BMI. In a second model, we included BMI.

To investigate whether results were independent from disease status, sensitivity analyses were performed that excluded people with diabetes or cardiovascular disease.

NTR

Because blood samples for NTR were processed in different batches (NESDA used one batch), we used a residual score of the inflammatory markers correcting for batch and season effects (25). For study 1, GEE analyses with an exchangeable correlation structure were used to investigate the association between ANS activity at one time point and inflammatory markers 4.9 years later. These analyses were repeated for study 2, with inflammatory markers at one time point and ANS activity 5.4 years later. Adjustments were made for covariates at baseline. Again, we first adjusted for all covariates excluding BMI and included BMI in a second model. Family was included as variable random factor to account for the familial clustering of the data.

Because RSA is prone to a ceiling effect during sleep due to low HR (29), we performed additional sensitivity analyses by excluding participants (study 1: $n = 47$; study 2: $n = 20$) who showed a quadratic relationship between RSA and HR. In addition, effects of sex were investigated by including a sex-interaction term in the analyses.

RESULTS

NESDA

At baseline, the NESDA sample ($n = 2823$) had a M (SD) age of 41.8(13.1) years and 66.5% were female (Table 1). Table 2 shows the cross-sectional associations between cardiac autonomic activity with inflammatory markers across waves. In the models without adjustments for BMI, a higher HR was significantly associated with higher levels of CRP ($B = .179$; $p < .001$) and IL-6 ($B = .141$; $p < .001$). A higher RSA was associated with lower levels of CRP ($B = -.041$; $p = .013$) and IL-6 ($B = -.073$;

TABLE 1. Sample Characteristics NESDA

Characteristics	Baseline (n = 2823)	2-y Follow-Up (n = 2099)	6-y Follow-Up (n = 1774)
Social demographics			
Age, M (SD), y	41.8 (13.1)	44.3 (13.2)	48.3 (13.1)
Female sex, %	66.5	65.4	65.2
Education, M (SD), y	12.2 (3.3)	12.6 (3.3)	12.9 (3.3)
Life-style			
Physical activity, median (IQR), 1000 MET min/wk	2.8 (1.4–5.0)	3.1 (1.5–5.4)	2.9 (1.5–5.4)
Alcohol use, median (IQR), drinks/week	3.7 (0.2–8.7)	3.7 (0.2–8.7)	3.7 (0.2–8.2)
Smokers, %	35.7	30.6	25.8
No. cigarettes/day, median (IQR)	14.0 (7.0–20.0)	12.0 (7.0–20.0)	12.0 (5.7–20.0)
Health factors			
BMI, M (SD), kg/m^2	25.6(5.0)	25.7(4.8)	26.1(5.0)
No. chronic diseases, %			
0 chronic diseases	58.3	61.3	57.3
1 chronic disease	27.9	27.1	29.3
>2 chronic diseases	13.8	11.6	13.4
Use of cardiac medication	12.2	14.9	17.0
Use of anti-inflammatory agents	18.6	17.6	18.2
Use of statins	6.8	7.1	10.0
Current psychopathology	57.0	37.3	26.9
Use of TCA	2.7	3.1	3.2
Use of SNRI	4.1	4.0	3.9
Use of SSRI	17.0	14.1	12.0
Cardiac autonomic measures			
HR, M(SD), beats/min	72.0 (9.6)	72.7 (9.7)	71.7 (9.5)
RSA, median (IQR), ms	38.8 (27.2–55.5)	37.2 (25.1–52.9)	39.4 (26.9–55.5)
Respiration rate, M(SD), breaths/min	17.1 (1.2)	17.3 (1.3)	16.3 (1.4)
Inflammatory markers			
CRP, median (IQR), mg/l	1.1 (0.5–2.6)	1.0 (0.4–2.3)	1.1 (0.5–2.4)
IL-6, median (IQR), pg/ml	0.8 (0.5–1.3)	1.0 (0.7–1.7)	0.9 (0.6–1.6)

M (SD) = mean (standard deviation); IQR = interquartile range; METmin = multiple of resting metabolic rate times minutes of physical activity per week; TCA = tricyclic antidepressant; SNRI = selective serotonin and noradrenalin reuptake inhibitors; SSRI = selective serotonin reuptake inhibitors; HR = heart rate; RSA = respiratory sinus arrhythmia; CRP = C-reactive protein; IL-6 = interleukin 6.

$p < .001$). When additionally adjusting for BMI, the strength of associations slightly decreased but remained significant between HR with CRP ($B = .141$; $p < .001$) and IL-6 ($B = .141$; $p < .001$). RSA and Il-6 also remained significantly associated ($B = -.063$; $p < .001$), but the association between a higher RSA and a lower CRP level rendered nonsignificant. The fully adjusted cross-sectional

TABLE 2. Adjusted Association Between ANS Values With Inflammatory Markers Across Waves in NESDA

	CRP				IL-6			
	No. Observations	B	SE	p	No. Observations	B	SE	p
HR[a]	6398	**.179**	**.014**	**<.001**	6688	**.162**	**.013**	**<.001**
HR[b]		**.141**	**.013**	**<.001**		**.141**	**.013**	**<.001**
RSA[a]	6398	**−.041**	**.016**	**.013**	6689	**−.073**	**.016**	**<.001**
RSA[b]		−.024	.015	.10		**−.063**	**.015**	**<.001**

HR = heart rate; RSA = respiratory sinus arrhythmia; CRP = C-reactive protein; IL-6 = interleukin 6.

Boldface indicates significant results.

[a] GEE analyses were adjusted for age, sex, education, physical activity, alcohol use, smoking, number of chronic diseases, current psychopathology, use of antidepressants, heart medication, anti-inflammatory agents, and statins, and time. RSA was additionally adjusted for respiration rate.

[b] Adjustment[a] + additionally adjusted for BMI.

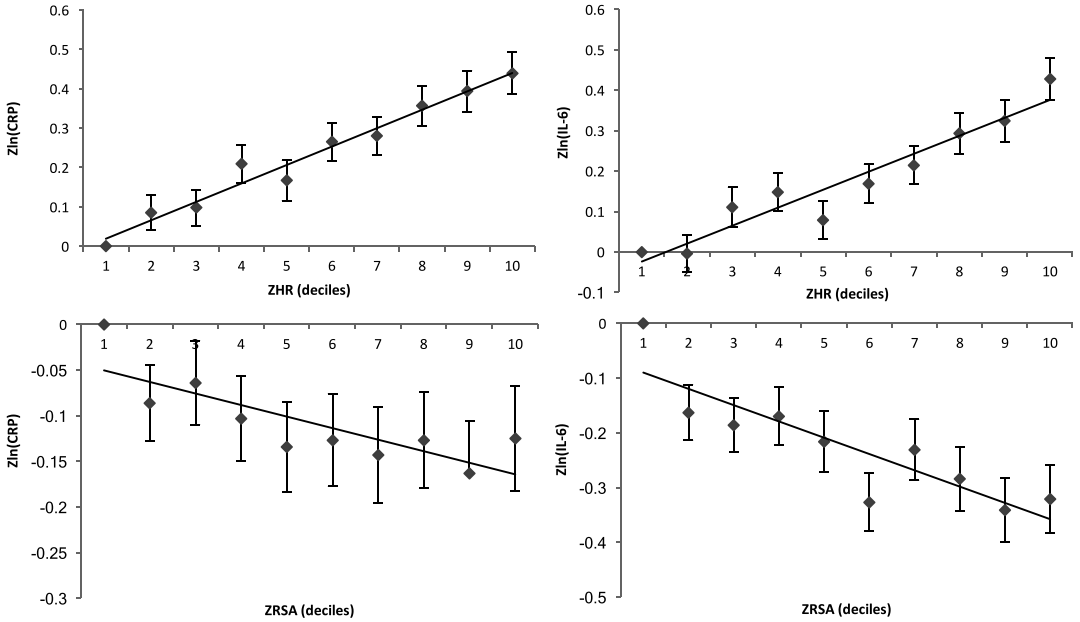

FIGURE 1. Cross-sectional GEE associations between ANS values and inflammatory markers across waves in NESDA. HR and RSA were divided into deciles. Deciles HR (beats per minute): 1 = 41.6–60.4; 2 = 60.4–64.2; 3 = 64.2–67.0; 4 = 67.0–69.4; 5 = 69.4–71.6; 6 = 71.6–74.0; 7 = 74.0–76.4; 8 = 76.4–79.7; 9 = 79.7–84.4; 10 = 84.4–118.5. Deciles RSA (millisecond): 1 = 2.95–18.4; 2 = 18.4–24.1; 3 = 24.1–28.9; 4 = 28.9–33.7; 5 = 33.7–38.7; 6 = 38.7–44.2; 7 = 44.2–50.5; 8 = 50.5–59.8; 9 = 59.8–74.2; 10 = 74.2–265.0.

relationships between ANS values and inflammatory markers are illustrated in Figure 1.

When repeating the analyses with an interaction term between ANS variables and time, we found that time significantly modulated the relationship between HR and CRP and between RSA and IL-6. We therefore stratified these analyses per wave and found that the strength of associations differed somewhat between waves but were all significant in the fully adjusted models (for HR and CRP: $B = .151$; $B = .155$; $B = .126$, for RSA and IL-6: $B = -.046$; $B = -.100$; $B = -.060$, for baseline, 2-, and 6-year follow-up, respectively).

Table 3 shows that, in the models without adjustments for BMI, a higher HR at one time point significantly predicted higher inflammatory markers at the next time point (for CRP: $B = .076$; $p < .001$, for IL-6: $B = .056$; $p < .001$). Again, the strength of associations slightly decreased but remained significant after additional

TABLE 3. Prospective Adjusted Association Between Predictor Values at One Time Point (t) With Outcome Values at the Next Time Point ($t + 1$) in NESDA

	Outcome							
	CRP				IL-6			
Predictor	No. Observations	*B*	SE	*p*	No. Observations	*B*	SE	*p*
HR[a]	3538	**.076**	**.017**	**<.001**	3819	**.056**	**.015**	**<.001**
HR[b]		**.065**	**.017**	**<.001**		**.036**	**.015**	**.014**
RSA[a]	3539	−.021	.022	.34	3821	**−.033**	**.017**	**.044**
RSA[b]		−.018	.022	.42		−.024	.017	.15
	HR				RSA			
CRP[a]	3534	−.015	.013	.24	3533	**−.036**	**.012**	**.002**
CRP[b]		−.021	.013	.12		**−.024**	**.012**	**.048**
IL-6[a]	3696	−.020	.012	.10	3696	−4.3E-4	.016	.98
IL-6[b]		−.021	.012	.081		.004	.015	.77

HR = heart rate; RSA = respiratory sinus arrhythmia; CRP = C-reactive protein; IL-6 = interleukin 6.

Boldface indicates significant results.

[a] GEE analyses were adjusted for basal values of the outcome, age, sex, education, physical activity, alcohol use, smoking, number of chronic diseases, current psychopathology, use of antidepressants, heart medication, anti-inflammatory agents, and statins, and time. RSA was additionally adjusted for respiration rate.

[b] Adjustment[a] + additionally adjusted for BMI.

adjustment for BMI (for CRP: $B = .065$; $p < .001$, for IL-6: $B = .036$; $p = .014$). A higher RSA at one time point predicted lower levels of IL-6 only in the model without adjustment for BMI ($B = -.033$; $p = .044$).

The addition of an interaction term between ANS variables and time showed that time modulated the relationship between HR at one time point and CRP at the next time point. Stratified analyses showed that the association was stronger for a higher HR at baseline predicting higher CRP level at 2-year follow-up ($B = .092$; $p < .001$) than for HR at 2-year follow-up predicting CRP at 6-year follow-up ($B = .027$; $p = .27$).

When testing the reverse relationship (Table 3), analyses showed that higher levels of CRP significantly predicted a lower RSA at the next time point, without ($B = -.036$; $p = .002$) and with adjustment for BMI ($B = -.024$; $p = .048$).

To investigate whether these results were independent from disease status, sensitivity analyses were performed that excluded persons with diabetes or cardiovascular disease. These results showed that only the prospective association between CRP and RSA rendered nonsignificant after exclusion. However, in general, there was little change in strength of associations after exclusion, suggesting that our results were not driven by diabetes or cardiovascular disease.

Additional information on the underlying data structure is presented in Table S1, Supplemental Digital Content 1, http://links.lww.com/PSYMED/A472, showing that changes in ANS are correlated with changes in inflammation over time.

TABLE 4. Prospective Association Between ANS Values at Baseline With Inflammatory Markers 4.9 Years Later in NTR Study 1

	Outcome							
	CRP				IL-6			
Predictor	*n*	*B*	SE	*p*	*n*	*B*	SE	*p*
HR sleep[a]	322	**.132**	**.053**	**.013**	330	**.158**	**.063**	**.012**
HR sleep[b]		**.127**	**.051**	**.013**		**.146**	**.061**	**.017**
HR leisure[a]	320	**.126**	**.053**	**.017**	327	.100	.063	.11
HR leisure[b]		**.112**	**.051**	**.027**		.087	.063	.17
HR work[a]	175	.048	.072	.50	175	**.171**	**.087**	**.048**
HR work[b]		.035	.071	.61		.157	.088	.073
RSA sleep[a]	322	.028	.060	.64	330	−.008	.070	.97
RSA sleep[b]		−.012	.058	.83		−.042	.069	.55
RSA leisure[a]	320	−.030	.063	.64	327	.028	.067	.67
RSA leisure[b]		.002	.061	.97		.056	.064	.38
RSA work[a]	175	−.046	.083	.58	175	−.137	.098	.16
RSA work[b]		−.031	.084	.71		−.120	.100	.23

HR = heart rate; RSA = respiratory sinus arrhythmia; CRP = C-reactive protein; IL-6 = interleukin 6; SE = standard error.

Boldface indicates significant results.

[a] GEE analyses were adjusted for age, sex, level of education, and smoking. RSA was additionally adjusted for respiration rate.

[b] Adjustment[a] + additionally adjusted for BMI.

NTR

Compared with NESDA, the NTR sample was smaller (study 1: $n = 344$; study 2: $n = 454$) and younger at baseline (study 1: 31.7 [SD = 10.5] years; study 2: 31.8 [SD = 5.4] years). Study 1 consisted of 58.4% females and study 2 of 61.2% females (Table S2, Supplemental Digital Content 2, http://links.lww.com/PSYMED/A473).

Prospective analyses between ANS values and inflammatory markers in study 1 (Table 4) showed that a higher HR during sleep was significantly associated with higher levels of CRP ($B = .132$; $p = .013$) and IL-6 ($B = .158$; $p = .012$) 4.9 years later in the models without adjustment for BMI. When additionally adjusting for BMI, the strength of associations decreased but remained significant (for CRP: $B = .127$; $p = .013$, for IL-6: $B = .146$; $p = .017$). Higher HR during leisure time was also significantly associated with higher CRP, without ($B = .126$; $p = .017$) and with adjustment for BMI ($B = .112$; $p = .027$). Higher HR during work was associated with higher IL-6 levels in the model without adjustment for BMI ($B = .171$; $p = .048$). No significant associations were found between RSA and inflammatory markers at follow-up.

When investigating the reverse associations in study 2 (Table 5), analyses showed that higher IL-6 was associated with higher levels of HR during sleep ($B = .125$; $p = .044$, and $B = .122$; $p = .007$, without and with adjustment for BMI, respectively), leisure time ($B = .102$; $p = .026$, and $B = .103$; $p = .030$, without and with adjustment for BMI, respectively), and work ($B = .133$; $p = .017$, and $B = .128$; $p = .027$, without and with adjustment for BMI, respectively) 5.4 years later. Higher IL-6 was also associated with lower RSA during leisure time ($B = -.097$; $p = .022$, and $B = -.107$; $p = .016$, without and with adjustment for BMI, respectively).

Sensitivity analyses excluding people who showed a ceiling effect for RSA during sleep did not change our results.

We additionally checked for sex interactions in both NESDA and NTR analyses. Within NTR, no significant sex interactions were found. Within NESDA, a higher HR and lower RSA were stronger associated with a higher CRP level in women than in men. However, because no consistent sex interactions were found, these findings might have been due to chance.

DISCUSSION

The current study showed that both higher HR and lower RSA were cross-sectionally associated with higher inflammation levels, as measured by CRP and IL-6. We aimed to unravel directionality by testing associations in both directions in two independent cohorts: NESDA and NTR. In both studies, higher HR at baseline was associated with higher levels of CRP and IL-6 at follow-up. Inflammatory markers at baseline were also associated with ANS activity at follow-up. However, evidence for these reverse associations was less consistent.

The current results contribute to existing evidence that vagal activity is coupled with the inflammatory system (6–14). Longitudinal studies focusing on the relationship between these two biological systems are scarce. One study found that higher CRP predicted increase in vagal activity at follow-up (21), and another suggested that higher vagal activity predicted lower levels of CRP (20). Both studies were rather small in sample size (<200 participants). Our study found consistent evidence for HR predicting subsequent levels of inflammatory markers. Because resting HR has been suggested to be predominantly governed by cardiac vagal control (30), and because RSA, a direct measure of cardiac vagal

TABLE 5. Prospective Association Between Inflammatory Markers at Baseline With ANS Values 5.4 Years Later in NTR Study 2

	Outcome																							
	HR Sleep				HR Leisure				HR Work				RSA Sleep				RSA Leisure				RSA Work			
Predictor	*n*	*B*	SE	*p*	*n*	*B*	SE	*p*	*n*	*B*	SE	*p*	*n*	*B*	SE	*p*	*n*	*B*	SE	*p*	*n*	*B*	SE	*p*
CRP[a]	435	.067	.045	.14	406	.066	.049	.18	247	.107	.061	.081	435	.051	.047	.28	406	−.020	.050	.69	247	−.054	.055	.32
CRP[b]		.063	.048	.19		.070	.054	.19		.103	.065	.11		.008	.050	.88		−.030	.056	.59		−.034	.056	.55
IL-6[a]	446	**.125**	**.044**	**.004**	416	**.102**	**.046**	**.026**	251	**.133**	**.056**	**.017**	446	−.037	.041	.37	416	**−.097**	**.042**	**.022**	251	−.037	.050	.47
IL-6[b]		**.122**	**.046**	**.007**		**.103**	**.048**	**.030**		**.128**	**.058**	**.027**		−.066	.045	.14		**−.107**	**.045**	**.016**		−.016	.051	.75

CRP = C-reactive protein; IL-6 = interleukin 6; HR = heart rate; RSA = respiratory sinus arrhythmia.

Boldface indicates significant results.

[a] GEE analyses were adjusted for age, sex, level of education, and smoking. RSA was additionally adjusted for respiration rate.

[b] Adjustment[a] + additionally adjusted for BMI.

activity (5), also showed evidence of association, we suggest that vagal changes precede inflammatory changes. This hypothesis is further supported by the NTR data showing a stronger relationship between HR and inflammatory markers during sleep, when vagal activity predominates, than during work, when sympathetic activity prevails (31). Effects of vagal activity on IL-6 and CRP over time might reflect the cholinergic anti-inflammatory pathway: the neural mechanism that inhibits the inflammatory response by vagal acetylcholine secretion (4). Alternatively, low cardiac vagal activity may increase the risk for atherosclerosis, e.g., by effects on blood pressure (2,32,33), and atherosclerosis may in turn drive inflammatory responses (34).

Besides the effect of cardiac autonomic activity on future inflammation, we also found reverse associations. The NESDA data showed an association between CRP at baseline and subsequent RSA. The NTR data, in contrast, showed associations between IL-6 at baseline and HR and RSA at follow-up. Although this pattern of results is less consistent than the effect of HR on future proinflammatory state, we cannot rule out reverse effects of CRP or IL-6 on cardiac autonomic regulation. A bidirectional effect of the two systems on each other is biologically plausible, because activation of the inflammatory response has been suggested to stimulate the hypothalamus, which in turn influences autonomic activity (35).

Prospective studies provide a better stab at causality than cross-sectional studies but are still imperfect. Many unmeasured confounders can influence both autonomic activity and inflammation. If they do so at different time points in life, this can create the false impression of causality. Furthermore, repeatedly measuring two systems that show substantial tracking can lead to erroneous conclusions about causality. It has indeed been shown that HR and RSA show substantial tracking over time (24). The found prospective association between IL-6 and RSA/HR, for instance, could therefore have arisen from earlier effects of vagal activity on baseline inflammation levels. A promising next step to further unravel causality is to establish whether genetic variances of one biological system predicts the other, suggesting that shared genetics is in play as a third underlying factor.

The current research is one of few that investigates the relationship between cardiac autonomic activity and inflammatory markers longitudinally and does so within two independent prospective studies. However, some limitations need to be considered. First, although we suspect vagal activity to predict subsequent inflammatory markers, we only found a cross-sectional association between low RSA and high levels of CRP and IL-6. The lack of predictive power of RSA might be due to a higher standard deviation for this measure than for HR, rendering HR as the more powerful measure to discern associations. However, although resting HR is mostly determined by the strongly age-dependent intrinsic HR (4,5) and, particularly during sleep, vagal activity, it is also partly controlled by the sympathetic nervous system. Processes related to intrinsic heart regulation and sympathetic influences may have further contributed to the inflammatory state. Second, there were multiple methodological differences between the NESDA and the NTR sample. For instance, most NESDA samples consist of people with (a history of) depression and/or anxiety. Although we adjusted for psychopathology within NESDA, the sample differences complicate a one-on-one comparison between the two studies. In

contrast to NESDA, the NTR study only measured ANS and inflammatory data at one time point. Therefore, we were unable to correct for these measures at baseline. In addition, there were differences between sensitivity of assays of the inflammatory markers and between measured covariates. Furthermore, the conditions of ANS data collection were not uniform between the two studies, as data for NTR were collected during 24 hours of a regular day and data for NESDA were collected in a laboratory setting. One could argue that the NTR data are more ecologically valid but also more prone to interpersonal variability than the NESDA data. These methodological differences compromise comparability between the studies. However, the combined data of the two studies allowed us to confirm that the pattern of results in the relationship between cardiac autonomic activity and inflammatory markers holds across such diverse settings.

In conclusion, lower cardiac vagal activity is associated with higher levels of inflammation. Coupling between these two biological systems is most evident in cross-sectional data but clearly reflects a prolonged coupling across the time course as shown by longitudinal analyses. Our analyses provide stronger evidence for an effect of cardiac autonomic regulation on inflammatory markers than the other way around. Future studies should further investigate the causal mechanisms underlying this relationship.

Source of Funding and Conflicts of Interest: This work was supported by the Geestkracht program of the Netherlands Organisation for Health Research and Development (Zon-Mw, Grant Number 10-000-1002), by the FP7-Marie Curie Grant (PCIG12-GA-2012-334065 to F.L.), and by participating universities (VU University Medical Center, Leiden University Medical Center, University Medical Center Groningen). The authors report no conflicts of interest.

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