

RR Interval Variability Is Inversely Related to Inflammatory Markers: The CARDIA Study

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Recent evidence reveals that the immune system is under the direct control of the vagus nerve via the "cholinergic anti-inflammatory pathway." Stimulation of vagus nerve activity significantly inhibits cytokine levels in animal models, and cholinergic agents inhibit cytokine release by human macrophages. Moreover, when vagus nerve activity is decreased or absent, cytokines are overproduced. Atherosclerosis is an inflammatory disease characterized by elevated levels of CRP and IL-6, but the relationship between cardiac vagal activity and cytokine levels in healthy humans is not well understood. Here we measured RR interval variability, an index of cardiac vagal modulation, and CRP and IL-6 in 757 subjects participating in a subset of the year 15 data collection in the CARDIA study of the evolution of risk factors in young adults. Univariate analysis revealed that all indices of RRV were strongly and inversely related to IL-6 (log pg/mL $b = -0.08$ and -0.17 for HF and LF power, $P < 0.001$ respectively) and CRP (log mg/L $b = -0.14$ and -0.26 for HF and LF power, $P < 0.001$ respectively) levels. In the multivariate model including gender, race, age, smoking, physical activity, SBP, BMI, and disease, the inverse relationship between RRV and inflammatory markers, although slightly attenuated, remained significant. These findings are consistent with the hypothesis that diminished descending vagal anti-inflammatory signals can allow cytokine overproduction in humans.

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INTRODUCTION

Advances in understanding the biology of inflammation indicate that an over-expression of cytokines contributes to the development of tissue injury and damage. Excessive production of proinflammatory cytokines mediates the development of autoimmune diseases in humans, including rheumatoid arthritis and inflammatory bowel disease. Therapies that directly target cytokines, for example, TNF and IL-1, can ameliorate the signs and symptoms of disease and have enjoyed widespread clinical use (1–3). Redundant counter-regulatory mechanisms normally control the magnitude of the cytokine response and function to prevent the development of tissue damage. These anti-inflammatory mecha-

nisms include the pituitary adrenocortical axis, the release of soluble cytokine receptors, and the production of anti-inflammatory mediators that inhibit inflammation.

Recent work has implicated the central nervous system in the direct counter-regulation of cytokine release. The cholinergic anti-inflammatory pathway has been defined on the basis that signals transmitted along the vagus nerve into the organs of the reticuloendothelial system inhibit cytokine release and prevent disease. A large body of evidence in animal studies indicates that acetylcholine released following vagus nerve stimulation significantly inhibits cytokine release via a mechanism that requires the expression of the $\alpha 7$ subunit

of the nicotinic acetylcholine receptor (4). Activation of the cholinergic anti-inflammatory pathway with either vagus nerve stimulation or $\alpha 7$ agonists is efficacious in animal models of endotoxemia, sepsis, subcutaneous inflammation, and experimental arthritis. Vagotomy enhances the cytokine response to invasive stimuli, indicating that the vagus nerve is hardwired to tonically regulate the magnitude of the cytokine response under basal and stimulated conditions. The cholinergic anti-inflammatory pathway is the efferent arm of an "Inflammatory Reflex," which can be activated by inflammatory mediators in peripheral tissues that activate firing of afferent signals in the vagus nerve which, in effect, "notify" the central nervous system about the presence of inflammation in the body. This, in turn, activates an opposing, efferent response via the cholinergic anti-inflammatory pathway, which serves to inhibit inflammation and prevent damage (5,6). A growing body of evidence

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has implicated excessive inflammation in the pathogenesis of atherosclerosis. Atherosclerotic plaques are infiltrated by inflammatory cells that mediate the progression of damage to the vessel wall. Serum levels of CRP and IL-6, two circulating markers of inflammation, are correlated with the progression of atherosclerosis. Accordingly, we tested the hypothesis that indices of vagus nerve activity derived from RR interval variability (RRV) are inversely related to levels of IL-6 and CRP using data from 757 participants in the CARDIA study of the evolution of risk factors in young adults.

METHODS

Study Population

The Coronary Artery Risk Development in Young Adults (CARDIA) study is a biethnic, prospective, multicenter epidemiological study of the evolution of cardiovascular risk development in young adulthood. In 1985-1986, 5155 black and white men and women, aged 18 to 30 years, were recruited at Birmingham, AL; Chicago, IL; Minneapolis, MN; and Oakland, CA, to achieve a balance at each site by race (black, white), gender, education (high school degree or less, more than high school), and age (18-24 years, 25-30 years) (7). Participants were examined at study entry and years 2, 5, 7, 10, and 15 with re-examination rates among surviving cohort members of 90.5%, 85.7%, 80.6%, 78.5%, and 73.5%, respectively. Comparisons of CARDIA subjects who participated in the year 15 exam with those who did not indicated that the latter participants were more likely to be African-American, younger, less educated and smokers (data not shown). Site institutional review committee approval and informed consent were obtained for each examination.

At the year 15 exam, subjects seen at the Oakland, CA and Chicago, IL sites (and living within 50 miles of the clinic; N = 721 and 615 respectively) were asked to participate in the RRV substudy of socioeconomic status and development of biological risk, including assessments

of RRV. Of the 1,336 subjects who were eligible for the substudy, 789 (59%) agreed to participate in the RRV substudy. Comparisons of those who did and did not participate in the substudy revealed that participants tended to be of somewhat lower education and income and had somewhat higher BMI, diastolic and systolic blood pressure.

Data Collection

Sub-Study Assessments

Participants arrived at the clinic having eaten a light breakfast but abstaining from caffeinated beverages that morning. Study protocols were explained and written consent was obtained. The RRV protocol was explained and ECG electrodes and a single respiration-monitoring band were attached. Subjects rested quietly in the seated position for a 2 min period after which data were collected for 10 min. Subjects were asked to sit quietly without moving or talking.

Measurement of RR Interval Variability.

Analog ECG signals were collected for 10 min while subjects were resting quietly in the seated position. Signals were digitized at 500 Hz by a National Instruments A/D board and stored on a microcomputer. The ECG waveform was submitted to an R-wave detection routine, resulting in a time series of RR intervals (RRI). In cases in which 5 min epochs of data were compromised by electronic artifact, subject movement, or ectopic beat, identification of all R waves was impossible. RR intervals associated with these artifacts were fixed using established procedures if possible (8). If not, the epoch was excluded from spectral analysis.

Mean heart rate (HR) and the standard deviation of all RRIs (SDRR) were computed for all subjects. Spectral power in the low (0.04-0.15 Hz (LF)) and high (0.15-0.50 Hz (HF)) frequency bands was computed based on 300-second epochs using an interval method for computing Fourier transforms similar to that described by DeBoer, Karemaker, and Strackee (9). Prior to computing Fourier transforms, the mean of

the RR interval series was subtracted from each value in the series and the residual series then was filtered using a Hanning window (10) and the spectral power, i.e., variance (in msec²), over the LF and HF bands was summed. Estimates of spectral power were adjusted to account for attenuation produced by this filter (10).

Measurement of Inflammatory Markers.

C-reactive protein (CRP) was measured using the BNII nephelometer from Dade Behring utilizing a particle enhanced immunonephelometric assay. The assay range is 0.175-1100 mg/L. Expected values for CRP in normal, healthy individuals are < 3 mg/L. Intra-assay CVs range from 2.3-4.4% and inter-assay CVs range from 2.1-5.7%. IL-6 was measured by ultra-sensitive ELISA (R&D Systems, Minneapolis, MN). The lower detection limit is < 0.10 pg/mL, with a detection range of 0.156-10.0 pg/mL and a routine CV in the lab of 6.3%.

Covariates. Measures of systolic blood pressure (SBP), body-mass index (BMI), physical activity, and smoking from the year 15 exam were examined. Selection of these covariates was based on their known associations with both SES and RRV. SBP was measured during seated rest, the average of three measurements with a random zero sphygmomanometer. Physical activity was measured as self-reported participation in heavy and moderate intensity activities and quantified as previously described (11). Smoking was measured as self-reported current smoking (non-smoker, ex-smoker, and current smoker). Analyses examined these factors as potential mediators of the relationship between SES and RRV.

Participants were defined as having diabetes if glucose > 126 mg/dL or if they reported taking diabetes medication. Similarly, hypertension was defined as systolic blood pressure \geq 140 mmHg, diastolic blood pressure \geq 90 mmHg or if they reported taking anti-hypertensive medications. Participants also were asked if they took medication for asthma or high cholesterol.

Prior to analyses, CRP, IL-6, HF, and LF were log-transformed to approximate a normal distribution. To control for the influence of respiratory rate on HF power, we regressed HF power on respiratory rate and used the residual instead of unadjusted HF in all subsequent analyses. Linear regression was used to assess the relationship of the 3 RRV parameters (HF, LF, and SDRR) and HR, to each of the inflammatory markers, first alone (Table 2) and then with covariates added (Table 3): ethnicity (African-American), gender (female), age, education (more than high school), smoking status (current and former separately), physical activity score, and body-mass index. Finally, self-reported medical condition and medication usage were added to the model (Table 4). Standardized parameters were calculated using SAS version 8.02, PROC REG.

RESULTS

Of the 789 subjects who participated in the study, 757 subjects (96%) had technically adequate data for RRV analysis. Of these, 734 and 678 had acceptable measurement of IL-6 and CRP respectively. 44% were White and 42% were men. Table 1 presents general characteristics of the cohort.

Univariate analysis revealed that all indices of RRV were strongly and inversely correlated to IL-6 and CRP levels (Table 2). In the multivariate model, the relationship between RRV and inflammatory markers remained significant although slightly attenuated (Table 3). Figure 1 displays these data.

Previous studies have suggested that RRV is reduced in patients with hypertension and diabetes (12–14). We therefore added self-reported presence of

Table 1. Characteristics of the Cohort

Characteristics	Subjects with RRV data		
	N	Mean or %	SD
Calculated age at year 15	757	40.0	3.7
Sex, % of female ^a	757	57.6	
Ethnicity, % of Caucasian	757	44.4	
Sex/ethnicity breakdown			
% of male, Caucasian	156	20.6	
% of male, African American	165	21.8	
% of female, Caucasian	180	23.8	
% of female, African American	256	33.8	
SES			
Education, years	757	14.9	2.5
≤ HS	164	21.7	
> HS to college	448	59.2	
Post-college	145	19.2	
Annual Income in \$	746		
< \$42.5K	152	20.4	
\$42.5K to < \$87.5K	279	37.4	
\$87.5K	315	42.2	
BMI (kg/m ²)	754	29.3	7.3
Diastolic blood pressure (mmHg)	757	75.5	10.6
Systolic blood pressure (mmHg)	757	114.1	14.1
RR Interval Variability			
Heart Rate (HR) (bpm)	757	72.6	11.6
Low Frequency (LF) (msec ²)	757	826.7	1106.7
High Frequency (HF) (msec ²)	757	771.7	1272.9
SDRR (msec)	757	46.3	21.8
Diabetes mellitus	45	6.0	
Hypertension	133	17.6	
Smoking status	756		
Non-smoker	486	64.3	
Ex-smoker	128	16.9	
Current smoker	142	18.8	

^aTwo subjects had a sex change operation after year 10, and their sex is coded as 3 at year 15. Thus, they were not included in this table.

these diseases or medication use for these two conditions and asthma or high cholesterol to the multivariate model. As shown in Table 4, the inverse relationships between RRV and IL-6 and CRP remained statistically significant with the

exception of the HF power-IL-6 relationship. Considered together, these results are consistent with an inverse relationship between vagus nerve activity and serum levels of inflammatory markers in healthy subjects.

Table 2. Univariate Relationships between Inflammatory Markers and RRV

Inflammatory Markers	HF (msec ²)		LF (msec ²)		SD (msec)		HR (bpm)	
	b	p	b	p	b	p	b	p
Ln (CRP mg/L)	-0.143	< 0.0001	-0.2646	< 0.0001	-0.009	< 0.0001	0.022	< 0.0001
Ln (IL6 pg/mL)	-0.0764	0.0003	-0.1755	< 0.0001	-0.0058	< 0.0001	0.0121	< 0.0001

Table 3. Multivariable Models of RRV Predictors for Inflammatory Outcomes Including Covariates

N in Models Measures of RRV	Inflammatory markers			
	ln (CRP mg/L)		ln (IL6 pg/mL)	
	734 Coeff ^a	p	678 Coeff ^a	P
HF (msec ²)	-0.0889	0.0055	-0.0686	0.0302
Black	0.0249	0.4655	0.0842	0.0128
Female	0.0827	0.0107	0.0101	0.7526
Age (years)	-0.0369	0.2406	0.0079	0.8002
> HS	-0.0441	0.1726	-0.0728	0.0222
Current smoker	0.1192	0.0003	0.0793	0.0149
Ex-smoker	0.0215	0.4997	0.0136	0.668
Physical activity score	-0.0309	0.3387	-0.0631	0.0504
SBP (mmHg)	-0.0067	0.8407	0.0495	0.1352
BMI (kg/m ²)	0.5115	< 0.0001	0.5254	< 0.0001
LF (msec ²)	-0.0954	0.0043	-0.126	0.0001
Black	0.0076	0.8238	0.0642	0.0558
Female	0.0554	0.0933	-0.0222	0.4976
Age (years)	-0.0361	0.2498	0.0007	0.9826
> HS	-0.0449	0.165	-0.0685	0.03
Current smoker	0.1221	0.0002	0.077	0.017
Ex-smoker	0.0244	0.4429	0.014	0.6559
Physical activity score	-0.0303	0.3483	-0.0585	0.0678
SBP (mmHg)	-0.0083	0.805	0.0442	0.1789
BMI (kg/m ²)	0.5076	< 0.0001	0.5162	< 0.0001
SD (msec)	-0.0729	0.0238	-0.0868	0.0065
Black	0.0146	0.6668	0.0761	0.0237
Female	0.0682	0.0359	-0.0036	0.9119
Age (years)	-0.0365	0.2482	0.003	0.9241
> HS	-0.0474	0.1425	-0.0733	0.0207
Current smoker	0.1217	0.0002	0.0785	0.0156
Ex-smoker	0.0223	0.4847	0.013	0.6801
Physical activity score	-0.0325	0.3155	-0.0609	0.0587
SBP (mmHg)	-0.0061	0.8547	0.0479	0.1476
BMI (kg/m ²)	0.5152	< 0.0001	0.5262	< 0.0001
HR (bpm)	0.0852	0.0085	0.0708	0.0272
Black	0.018	0.5966	0.0796	0.0182
Female	0.0689	0.0336	0.0005	0.9877
Age (years)	-0.0198	0.5428	0.022	0.4784
> HS	-0.0497	0.1232	-0.0762	0.0164
Current smoker	0.1161	0.0005	0.0775	0.0177
Ex-smoker	0.0232	0.4657	0.0145	0.6454
Physical activity score	-0.028	0.3896	-0.0585	0.0712
SBP (mmHg)	-0.0125	0.7109	0.0451	0.176
BMI (kg/m ²)	0.5112	< 0.0001	0.524	< 0.0001

^acoeff: Standardized regression coefficients**DISCUSSION**

The results indicate that measures of vagus nerve activity, HF and LF power, and SDRR, are inversely related to serum levels of two proinflammatory mediators, IL-6 and CRP. HR was positively related to these inflammatory markers. As expected, after controlling for important covariates and disease in multivariate analyses, these relationships were attenuated, but with a single exception they remained statistically significant. To our knowledge, these are the first results demonstrating inverse relationships between inflammatory markers and indices of cardiac autonomic regulation in a large sample of healthy young adults. These findings are consistent with evidence from animal studies indicating that the cholinergic anti-inflammatory pathway counter-regulates inflammation.

Relationships between RRV and inflammation have been reported in several studies, although in most, clinical samples have been studied. In 121 women 17 months after a myocardial infarction or revascularization, Jansky et al. reported inverse relationships between IL-6 and SDNN, very low frequency, and low frequency RRV derived from 24-h ECG recordings (15). In 64 patients with decompensated congestive heart failure, IL-6 but not TNF- α was inversely related with SDNN, total spectral power, and ultra low frequency power also derived from 24-h ECG recordings (16). Another study of CHF patients, however, showed that TNF- α was inversely related not only to SDNN but also to LF and HF power from 24-h recordings (17). A case control study of the metabolic syndrome reported that IL-6 was inversely related to RRV derived from 5-min supine recordings although the RRV indices were not identified (18). In 133 healthy 35-year-old men and women, VLF was significantly and inversely related to leukocyte count (19). Finally, in a community study employing 24-h ECG recording, CRP and leukocyte count were inversely related to SDNN but not to pNN50, an index of high frequency RRV (20).

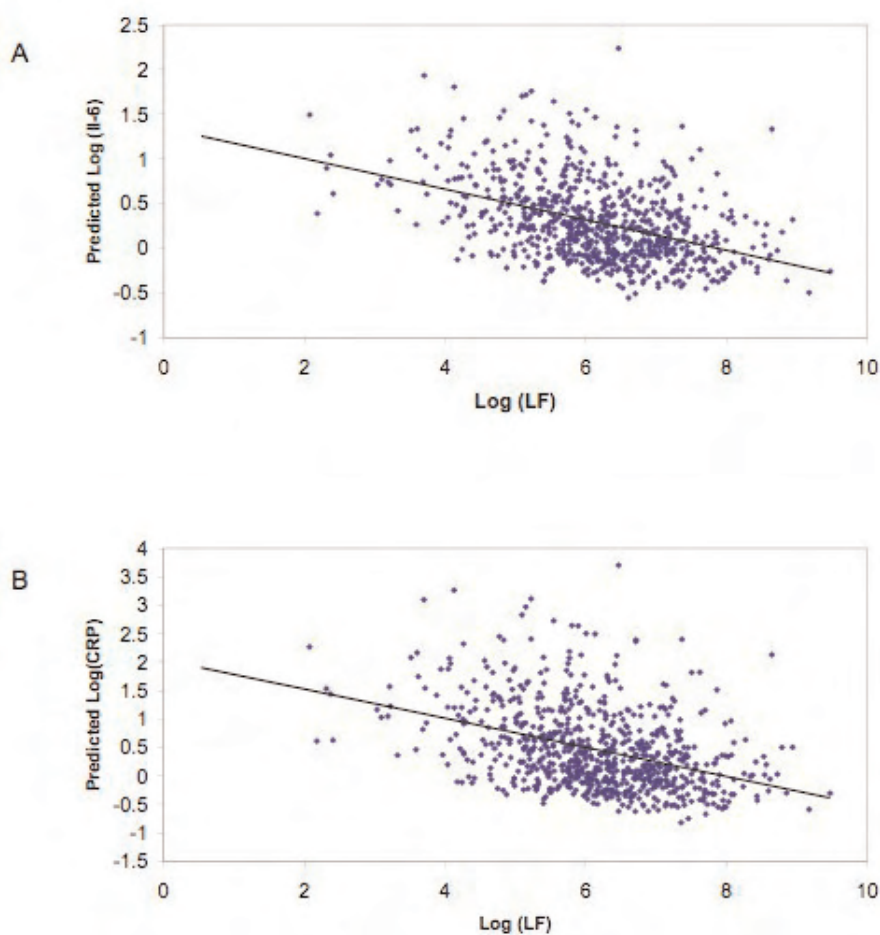


Figure 1. (A) Scatterplot of LF RR interval power (in msec²) vs. in IL-6 based on the regression model; fitted regression line is also plotted. (B) Scatterplot of LF RR interval power (in msec²) vs. in CPR based on the regression model; fitted regression line is also plotted.

With one exception, all of the previous studies to examine relationships between RRV and inflammatory markers found only relationships with measures of RRV < 0.15 Hz or their time domain equivalent, leading to suggestions of sympathetic nervous system activation of inflammation. However, this suggestion is inconsistent with the regularly reported inverse association between RRV and inflammation and thus raises questions about the physiological meaning of LF power.

While there is little question that high frequency RRV reflects cardiac parasympathetic modulation, especially after correction for respiratory rate, the physiolog-

ical significance of LF power is less clear. The best evidence suggests that it reflects both parasympathetic and sympathetic contributions with the latter varying depending upon several factors including posture. In the supine position, atropine eliminates virtually all LF power, indicating that in this position, LF power also principally reflects parasympathetic activity (21,22). In the upright position, however, atropine alone and propranolol alone eliminated approximately 70% of LF power, suggesting that in this position, LF power also may reflect a contribution from the sympathetic system (21).

We measured RRV in the seated position and some data suggest that auto-

nomic differences between the seated and supine positions are relatively small, especially compared with the upright position. Tulen et al. found no difference between the supine and seated positions in HF power but did not measure LF power in the 0.04–0.15 Hz frequency band (23). Taylor et al. demonstrated that atropine eliminated LF power in the 40° tilted position, intermediate between the supine and standing positions, but that atenolol had no effect (22). They also showed that change from the supine to the 40° upright position did not lead to an increase in LF power. Finally, Vybiral et al. demonstrated that administration of the vagomimetic transdermal scopolamine led to a significant increase in LF power in supine subjects (24). These data suggest a substantial degree of similarity in the autonomic profile of the seated and supine positions and that, in both, it appears that LF power principally reflects cardiac parasympathetic modulation.

Establishing causality is impossible in cross-sectional studies, but prior studies of vagus nerve activity and inflammation examined clinical samples in patients with disease (for example, CHF or MI). Because these clinical states promote both inflammation and reduced RRV, it is plausible that reduced RRV is the product of inflammation. However, the same inverse relationships between RRV and inflammation also appear in our data and the other community study of healthy subjects, consistent with the view that low levels of RRV are antecedent to inflammation, although the presence of a third factor responsible for both cannot be excluded. It now appears that in our data from the CARDIA study of heart disease in young adults there is an inverse relationship between low frequency RR interval variability and the inflammatory markers IL-6 and CRP, even after control of relevant covariates and cardioactive medications or hypertension or diabetes, which is consistent with the hypothesis of a cholinergic anti-inflammatory pathway that regulates inflammation in humans.

Table 4. Multivariable Models of RRV Predictors for Inflammatory Markers, Including Covariates and Current Medication Use, Diabetes, and Hypertension

N in Models Measures of RRV	Inflammatory markers			
	ln (CRP mg/L)		ln (IL6 pg/mL)	
	Coeff ^a	p	Coeff ^a	P
HF adjusted for respiratory rate ^b	-0.0866	0.0118	-0.0401	0.2353
Black	0.0248	0.5027	0.0975	0.0076
Female	0.0886	0.0116	-0.0073	0.8321
Age (years)	-0.0428	0.2089	0.0241	0.4734
> HS	-0.0521	0.1348	-0.0884	0.0096
Current smoker	0.1278	0.0004	0.0558	0.1118
Ex-smoker	0.0317	0.3571	-0.0063	0.8524
Physical activity score	-0.0307	0.3781	-0.0643	0.0628
SBP (mmHg)	-0.0272	0.4915	0.0614	0.1119
BMI (kg/m ²)	0.4804	< 0.0001	0.5111	< 0.0001
Have chronic health condition ^c	0.0680	0.0686	0.0001	0.9997
LF (msec ²)	-0.0926	0.0055	-0.1260	0.0001
Black	0.0084	0.8050	0.0643	0.0560
Female	0.0549	0.0956	-0.0223	0.4973
Age (years)	-0.0391	0.2131	0.0006	0.9855
> HS	-0.0436	0.1767	-0.0685	0.0303
Current smoker	0.1219	0.0002	0.0769	0.0173
Ex-smoker	0.0245	0.4419	0.0139	0.6568
Physical activity score	-0.0290	0.3689	-0.0584	0.0683
SBP (mmHg)	-0.0267	0.4533	0.0435	0.2148
BMI (kg/m ²)	0.5007	< 0.0001	0.5160	< 0.0001
Have chronic health condition	0.0525	0.1224	0.0023	0.9461
SD (msec)	-0.0723	0.0247	-0.0868	0.0065
Black	0.0153	0.6526	0.0761	0.0238
Female	0.0671	0.0387	-0.0036	0.9099
Age (years)	-0.0401	0.2058	0.0028	0.9301
> HS	-0.0459	0.1558	-0.0732	0.0211
Current smoker	0.1212	0.0003	0.0783	0.0160
Ex-smoker	0.0223	0.4832	0.0130	0.6817
Physical activity score	-0.0309	0.3403	-0.0608	0.0594
SBP (mmHg)	-0.0263	0.4603	0.0463	0.1883
BMI (kg/m ²)	0.5074	< 0.0001	0.5257	< 0.0001
Have chronic health condition	0.0570	0.0940	0.0044	0.8952
HR (bpm)	0.0825	0.0108	0.0708	0.0276
Black	0.0185	0.5859	0.0796	0.0183
Female	0.0680	0.0356	0.0005	0.9882
Age (years)	-0.0234	0.4546	0.0219	0.4810
> HS	-0.0483	0.1339	-0.0762	0.0166
Current smoker	0.1160	0.0005	0.0775	0.0179
Ex-smoker	0.0233	0.4639	-0.0145	0.6460
Physical activity score	-0.0267	0.4103	-0.0585	0.0716
SBP (mmHg)	-0.0310	0.3863	0.0447	0.2074
BMI (kg/m ²)	0.5042	< 0.0001	0.5239	< 0.0001
Have chronic health condition	-0.0531	0.1184	0.0014	0.9672

^acoeff: Standardized regression coefficients^bHF power regressed on respiratory rate and residual used in analyses^cSelf-reported hypertension or diabetes or cardioactive medication usage

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