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ORIGINAL ARTICLE

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ABSTRACT

Background: Heart rate (HR), heart rate variability (HRV), and inflammation are all associated with cardiovascular morbidity and mortality. The aim of this study was to assess potential inter-relationships between these parameters in a young and healthy population.

Methods: Healthy individuals aged 25–41 years were included in a prospective population-based study. All participants underwent 24-h electrocardiography using a validated device. The standard deviation of all normal RR intervals (SDNN) was pre-defined as the main HRV outcome variable. High-sensitivity C-reactive protein (hs-CRP), total leukocyte (LC) count and LC subtypes were obtained from venous blood samples.

Results: A total of 2064 participants (47% men, 37 years) were included in this analysis. In multivariable linear regression analyses using SDNN as the outcome variable, β-coefficients (95% confidence intervals) per 1 standard deviation (SD) increase on the log-scale were −0.11 (−0.16; −0.07), p < .0001 for hs-CRP, −0.13 (−0.17; −0.09), p < .0001 for total LC count, −0.12 (−0.16; −0.08), p < .0001 for neutrophils, −0.04 (−0.09; 0.00), p = .05 for lymphocytes and −0.08 (−0.09; −0.02), p = .005 for monocytes. There were positive relationships between resting and ambulatory HR and inflammatory biomarkers, except for lymphocytes.

Conclusion: In this large cohort of young and healthy adults, inflammatory parameters were strongly associated with increased HR and decreased HRV, suggesting an important interaction between inflammatory pathways and the autonomic nervous system.

KEY MESSAGE

- Inflammatory biomarkers, such as high-sensitivity C-reactive protein and leukocyte cell count with its subtypes were inversely associated with HRV and positively associated with HR.
- Our findings suggest important interrelationships between inflammatory pathways and the ANS.

Introduction

Inflammation plays a key role in the pathogenesis and progression of atherosclerosis (1). Accordingly, several inflammatory biomarkers have been prospectively associated with all-cause mortality (2–4) and a broad set of cardiovascular outcomes including atrial fibrillation, stroke, and myocardial infarction (5–9).

The autonomic nervous system (ANS) is strongly involved in different mechanisms of the cardiovascular system. Heart rate variability (HRV) has become a validated marker of the autonomic function (10,11).

A reduced HRV is associated with an increased risk for cardiovascular and all-cause mortality (12,13). Interestingly, experimental studies have suggested direct relationships between inflammation and the ANS (14,15). Thus, autonomic dysfunction might be one mechanism why individuals with elevated inflammatory biomarkers have an increased cardiovascular risk. Up to now, some studies in rather small populations have evaluated the relationship between inflammation and HRV, and mainly inverse relationships have been described (16–20). However, information on

inflammatory biomarkers other than high-sensitivity C-reactive protein (hs-CRP) is scarce (21). In addition, inflammatory biomarkers have recently been associated with heart rate (HR) (22), which by itself is an independent risk factor for cardiovascular outcomes (23). Given the tight link between HRV and HR (24), it is currently unclear whether HRV contains any additional information over and above HR.

In order to get a deeper understanding of these interrelationships between inflammation and the ANS, we assessed the relationships of 24-h HR and HRV with several inflammatory biomarkers in a large population-based cohort of young and healthy adults from the general population.

Methods

All inhabitants of the Principality of Liechtenstein aged between 25 and 41 years, namely 5898 individuals, were invited to participate in the *Genotypic and Phenotypic Determinants of Blood Pressure and other Cardiovascular Risk Factors (GAPP)* study, a prospective population-based cohort study. Of them, 61% (3605) refused study participation, 66 (1%) women were pregnant and 57 (1%) individuals had at least one exclusion criteria, leaving 2170 (37%) participants that were enrolled in this study. Study design and methodology have been published previously (25). Main exclusion criteria of the GAPP-study were established cardiovascular disease, arrhythmias, known renal failure, a body mass index (BMI) $> 35 kg/m^2$, current intake of insulin or antidiabetic drugs, daily intake of nonsteroidal anti-inflammatory drugs, regular intake of steroids, or other severe diseases. For the present study, we excluded 106 participants for the following reasons: missing 24-h ECG or recording time $<80\%$ of the target time (n $=$ 39), regular intake of beta blockers (n $=$ 2), missing laboratory values (n $=$ 10), and other missing covariates (n $=$ 55), leaving 2064 participants for the current analysis. The study protocol was approved by the local ethics committee and all study participants have signed a written informed consent.

24-h electrocardiography

Participants underwent 24-h Holter ECG monitoring using a validated three-channel device (Schiller AG, Baar, Switzerland). The 24-h ECG was attached by trained study nurses and started in the morning immediately after the study examination. Participants were advised to perform their normal daily activities. Recordings with a duration of less than 80% of the target time (i.e., <19.2 h) or of low quality were repeated whenever possible. Every 24-h ECG study was post-processed using a dedicated software to remove artefacts and redefine premature ventricular and atrial beats (Medilog Darwin, Schiller AG, Baar, Switzerland). Mean 24-h ambulatory HR was automatically calculated by the software. All heart beats defined as normal were used to calculate HRV. We used the standard deviation of all normal RR intervals (SDNN), total power (TP), low frequency (LF), and high frequency (HF) to quantify HRV. The selection of these HRV variables is based on (1) their strong association with cardiovascular outcomes and (2) their physiologic correlate (12,26). SDNN is measured in milliseconds (ms) whereas TP, LF and HF are measured in ms^2. The low- and high-frequency band is between 0.04–0.15 and 0.15–0.40Hz, respectively. LF and HF were normalized by calculating LF/(TP-VLF) $\times$ 100 and HF/(TP-VLF) $\times$ 100, respectively, to receive values independent of TP. SDNN was pre-defined as the main HRV outcome variable.

Blood sampling

Fasting venous blood samples were collected of every participant and immediately processed. Total number of leukocytes (LC), neutrophils, lymphocytes and monocytes were quantified using a validated method (Sysmex XE 5000, Kobe, Japan). Hs-CRP, creatinine, triglycerides, low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) were assayed on a Roche Cobas 6000 analyzer (Roche, Basel, Switzerland). Glycated hemoglobin A$_{1c}$ (HbA$_{1c}$) was measured using high-performance liquid chromatography (Bio-Rad D-10, Bio-Rad Laboratories AG, Reinach, Switzerland). Endothelin-1 (ET-1) was measured using a frozen EDTA plasma sample (Singulex Inc., Alameda, CA). Estimated glomerular filtration rate (eGFR) was calculated using the chronic kidney disease epidemiology collaboration (CKD-EPI) formula. Prediabetes was defined as an HbA1c between 5.6 and 6.4% (27).

Assessment of other study variables

Information about personal, medical, lifestyle and nutritional factors were self-assessed using standardized questionnaires (25). Smoking status was classified as never, former or current smoking. Highest educational status achieved was divided into three categories: high school, college or university degree. Nutritional factors and dietary habits were assessed using the Swiss health survey questionnaire from 2007, where people have to report the frequency of their fruit and vegetable consumption. Fruit and

vegetable consumption was dichotomized into ≥ 5 and < 5 servings per day. Alcohol consumption was dichotomized into drinkers and non-drinkers, while non-drinkers were defined as individuals never drinking alcohol or drinking less than once a month. Moderate and vigorous physical activity was estimated using the International Physical Activity Questionnaire (IPAQ) (28). Individuals reported the frequency and duration of moderate and vigorous activity performed per week. Regular physical activity was defined as moderate activity ≥ 150 or vigorous activity ≥ 75 min per week, respectively. Height and weight were directly measured in a standardized way. BMI was calculated as weight in kilograms divided by height in meters squared. Body fat was assessed by bioelectrical impedance analysis using a validated device (BIA ego-fit, 2010, Eggstätt, Germany). Conventional blood pressure was measured three times in a sitting position after 5 min of rest. The mean of the second and third measurement was used for the current analysis. Resting heart rate (HR) was obtained using a 12-channel resting ECG (Schiller AG, Baar, Switzerland).

Statistical analysis

Baseline characteristics were stratified by sex. Data are presented as medians (interquartile ranges) for continuous variables and numbers (percentages) for dichotomous variables. Group comparisons were done using Wilcoxon's rank sum tests or Chi-square tests as appropriate. Distribution of continuous variables was checked using skewness, kurtosis, and visual inspection of the histogram.

In order to assess the linearity of the relationships between HRV and inflammatory biomarkers, we evaluated SDNN, TP, HF, and LF measures across quartiles of individual inflammatory biomarkers in separate multivariable models. p Values for trend were calculated using quartile-specific medians. After confirming approximately linear relationships, we entered inflammatory biomarkers as continuous parameters in the multivariable models. Due to the skewed distribution, inflammatory variables were log-transformed for all analyses. To improve comparability across biomarkers, we calculated β-coefficients (95% confidence interval (CI)) per one standard deviation (SD) increase. In order to have a better comparability of the effect sizes, all HRV variables were transformed into z-scores. The multivariable models were adjusted for age, sex, BMI, smoking status, educational status, alcohol consumption, systolic blood pressure, prediabetes, fruit, and vegetable consumption, fish consumption, physical activity, LDL-C, HDL-C, triglycerides, ET-1, eGFR, family

history of cardiovascular disease, and body fat. To assess whether HRV has an incremental effect beyond HR, we additionally adjusted all models for either resting or ambulatory HR. In a separate step, hs-CRP and LC were included in the same multivariable adjusted model, in order to assess whether they remain independently associated with HRV. Finally, we used resting and 24-h HR instead of HRV as the outcome variables to assess its relationship with inflammatory biomarkers.

Subgroup analyses included multivariable linear regression analyses stratified for sex, prediabetes, and BMI (< 25, 25–29.9 and $\geq 30\,kg/m^2$). p Value for interaction was calculated using a multiplicative interaction term. Other sex-stratified analyses were performed for the relationships between resting and 24-h HR and inflammatory biomarkers. A p value $< .05$ was predefined to indicate statistical significance. Statistical analyses were done using SAS 9.4 (SAS Institute Inc, Cary, NC).

Results

Baseline characteristics stratified by sex are presented in Table 1. The median age of the population was 36.9

Table 1. Baseline characteristics stratified by sex.

n = 2064	Men n = 979 (46.7%)	Women n = 1117 (53.3%)	p value*
Age, years	36.9 (31.6; 40.5)	36.7 (30.9; 40.1)	.19
BMI, kg/m^2	25.6 (23.6; 27.9)	22.5 (20.6; 25.2)	<.0001
Smoking, %			.0003
Current	246 (25.1)	213 (19.1)	
Former	240 (24.5)	250 (22.4)	
Never	493 (50.4)	654 (58.6)	
Systolic BP, mmHg	127 (121; 135)	113 (107; 120)	<.0001
Diastolic BP, mmHg	82.0 (77.5; 87.5)	74.5 (69.5; 79.5)	<.0001
LDL-C, mmol/l	3.2 (2.7; 3.8)	2.6 (2.2; 3.1)	<.0001
HDL-C, mmol/l	1.3 (1.1; 1.5)	1.7 (1.5; 1.9)	<.0001
HbA1c, %	5.4 (5.2; 5.7)	5.4 (5.2; 5.6)	.001
Education, %			<.0001
Basic	64 (6.5)	108 (9.7)	
Middle	489 (50.0)	651 (58.3)	
University	426 (43.5)	358 (32.0)	
Hs-CRP, mg/l	0.9 (0.5; 1.8)	0.9 (0.5; 2.1)	.39
Leukocytes, G/l	5.3 (4.6; 6.2)	5.3 (4.5; 6.1)	.38
Neutrophils	2.8 (2.3; 3.4)	2.9 (2.3; 3.6)	.02
Lymphocytes	1.8 (1.5; 2.1)	1.7 (1.4; 2.1)	.004
Monocytes	0.5 (0.4; 0.6)	0.4 (0.3; 0.5)	<.0001
SDNN, ms	156 (132; 185)	145 (121; 168)	<.0001
Total power, ms^2	4096 (2846; 5901)	3060 (2184; 4241)	<.0001
LF normalized, ms^2	57 (53; 62)	51 (46; 56)	<.0001
HF normalized, ms^2	13 (10; 17)	16 (12; 21)	<.0001
Heart rate resting, bpm	59.7 (53.9; 65.6)	63.2 (57.4; 68.98)	<.0001
Heart rate 24-h, bpm	72.2 (66.6; 78.8)	76.9 (71.9; 82.2)	<.0001

Data are median (interquartile range) or numbers (percentages). BMI: body mass index; BP: blood pressure; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; HbA1c: glycated hemoglobin A1c; hs-CRP: high-sensitivity c-reactive protein; SDNN: standard deviation of all normal RR intervals; LF: low frequency; HF: high frequency.
*p Values are based on Wilcoxon's rank sum tests or chi-square tests, as appropriate.

years and 46.7% were men. Compared to women, men had a significantly higher BMI (median 25.6 versus 22.5 kg/m^2, p < .0001), were more often current smokers (25.1 versus 19.1%, p < .0001), had higher systolic and diastolic blood pressure values, and a worse cholesterol profile (all p values < .0001). There were no significant differences for hs-CRP (0.9 versus 0.9 mg/l, p = .39) and total LC count (5.3 and 5.3 G/l, p = .38). Men had a significantly higher lymphocyte (1.8 versus 1.7 G/l, p = .004) and monocyte count (0.5 versus 0.4 G/l, p < .0001) and women had a higher neutrophil count (2.9 versus 2.8 G/l, p = .02). The SDNN, TP, and normalized LF were significantly higher in men than in women (156 versus 145 ms, 4096 versus 3060 ms^2, and 57 versus 51 ms^2, respectively; all p values < .0001). Normalized HF was significantly higher among women compared to men (16 versus 13 ms^2, p < .0001).

Relationships between heart rate variability and inflammatory biomarkers

Results of the multivariable linear regression analyses comparing SDNN values across quartiles of inflammatory parameters are shown in Table 2. There were significant inverse and linear associations of SDNN with hs-CRP, LC, neutrophils, lymphocytes, and monocytes after multivariable adjustment. Additional adjustment for either resting or ambulatory HR strongly attenuated these effect sizes, but all associations remained statistically significant (Table 2). The association between TP and inflammatory biomarkers was similar, except the not significant relationship with lymphocytes (Table S1). Normalized HF was inversely related to neutrophils and monocytes (Table S2). There was no relationship with hs-CRP and lymphocytes, and the association with total LC count was of borderline significance (p = .08). There were positive linear relationships of normalized LF with total LC count and neutrophils as well as an inverse relationship with hs-CRP (Table S3). The relationships of both LF and HF with inflammatory biomarkers were strongly attenuated after the additional adjustment for resting HR and mainly 24-h HR and most became non-significant (Tables S2 and S3).

Results of the relationships between HRV variables and continuous inflammatory biomarkers were similar to these results and are presented in Figures 1–3 and S1. Per 1 SD increase in hs-CRP or LC, the SDNN

Table 2. SDNN across quartiles of inflammatory biomarkers.

n = 2064	Quartile 1	Quartile 2	Quartile 3	Quartile 4	p value
High-sensitivity c-reactive protein					
Hs-CRP, mg/l	<0.50	0.50–0.90	0.91–1.83	>1.83	
Age-sex adj.	Ref.	−0.12 (−0.24; −0.008)	−0.18 (−0.31; −0.06)	−0.46 (−0.58; −0.34)	<.0001
Model 1	Ref.	−0.06 (−0.17; 0.05)	−0.03 (−0.15; 0.10)	−0.25 (−0.38; 0.13)	<.0001
Model 2	Ref.	−0.04 (−0.14; 0.06)	−0.04 (−0.15; 0.08)	−0.17 (−0.28; −0.05)	.002
Model 3	Ref.	−0.03 (−0.13; 0.07)	−0.06 (−0.17; 0.05)	−0.17 (−0.27; −0.05)	.001
Leukocytes					
LC, G/l	<4.6	4.6–5.3	5.4–6.1	>6.1	
Age-sex adj.	Ref.	−0.20 (−0.32; −0.09)	−0.29 (−0.40; −0.17)	−0.50 (−0.62; −0.38)	<.0001
Model 1	Ref.	−0.14 (−0.25; −0.03)	−0.16 (−0.28; −0.05)	−0.32 (−0.44; −0.20)	<.0001
Model 2	Ref.	−0.10 (−0.20; −0.003)	−0.15 (−0.25; −0.04)	−0.26 (−0.36; −0.15)	<.0001
Model 3	Ref.	−0.09 (−0.18; 0.01)	−0.08 (−0.18; 0.02)	−0.19 (−0.29; −0.08)	.0008
Neutrophils					
Neutrophils, G/l	<2.4	2.4–2.8	2.9–3.5	>3.5	
Age-sex adj.	Ref.	−0.19 (−0.31; −0.07)	−0.24 (−0.35; −0.12)	−0.47 (−0.58; −0.35)	<.0001
Model 1	Ref.	−0.12 (−0.23; −0.01)	−0.13 (−0.24; −0.02)	−0.30 (−0.41; −0.18)	<.0001
Model 2	Ref.	−0.06 (−0.16; 0.04)	−0.09 (−0.19; 0.009)	−0.18 (−0.28; −0.07)	.0008
Model 3	Ref.	−0.07 (−0.17; 0.03)	−0.06 (−0.15; 0.04)	−0.13 (−0.23; −0.02)	.02
Lymphocytes					
Lymphocytes, G/l	<1.5	1.5–1.7	1.8–2.1	>2.1	
Age-sex adj.	Ref.	−0.10 (−0.22; 0.02)	−0.14 (−0.26; −0.02)	−0.24 (−0.37; −0.12)	.0001
Model 1	Ref.	−0.06 (−0.18; 0.05)	−0.06 (−0.17; 0.06)	−0.09 (−0.21; −0.04)	.21
Model 2	Ref.	−0.10 (−0.21; −0.00)	−0.14 (−0.24; −0.03)	−0.16 (−0.27; −0.05)	.006
Model 3	Ref.	−0.12 (−0.22; −0.02)	−0.13 (−0.23; −0.03)	−0.12 (−0.22; −0.01)	.07
Monocytes					
Monocytes, G/l	<0.4	0.4–0.4	0.5–0.5	>0.5	
Age-sex adj.	Ref.	−0.21 (−0.33; −0.09)	−0.20 (−0.33; −0.08)	−0.33 (−0.46; −0.21)	<.0001
Model 1	Ref.	−0.15 (−0.26; −0.03)	−0.12 (−0.24; −0.001)	−0.21 (−0.33; −0.08)	.004
Model 2	Ref.	−0.13 (−0.23; −0.02)	−0.15 (−0.26; −0.04)	−0.18 (−0.29; −0.07)	.002
Model 3	Ref.	−0.07 (−0.17; 0.03)	−0.07 (−0.18; 0.03)	−0.12 (−0.22; −0.009)	.05

Data are β-coefficients (95% confidence interval). Model 1 adjusted for age, sex, body mass index, smoking status, educational status, alcohol consumption, systolic blood pressure, prediabetes, fruit and vegetable consumption, fish consumption, physical activity, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglycerides, endothelin-1, estimated glomerular filtration rate, family history of cardiovascular disease, body fat; Model 2 additionally adjusted for resting heart rate. Model 3 additionally adjusted for ambulatory heart rate instead of resting heart rate. LC: Leukocytes; Hs-CRP: high-sensitivity C-reactive protein.
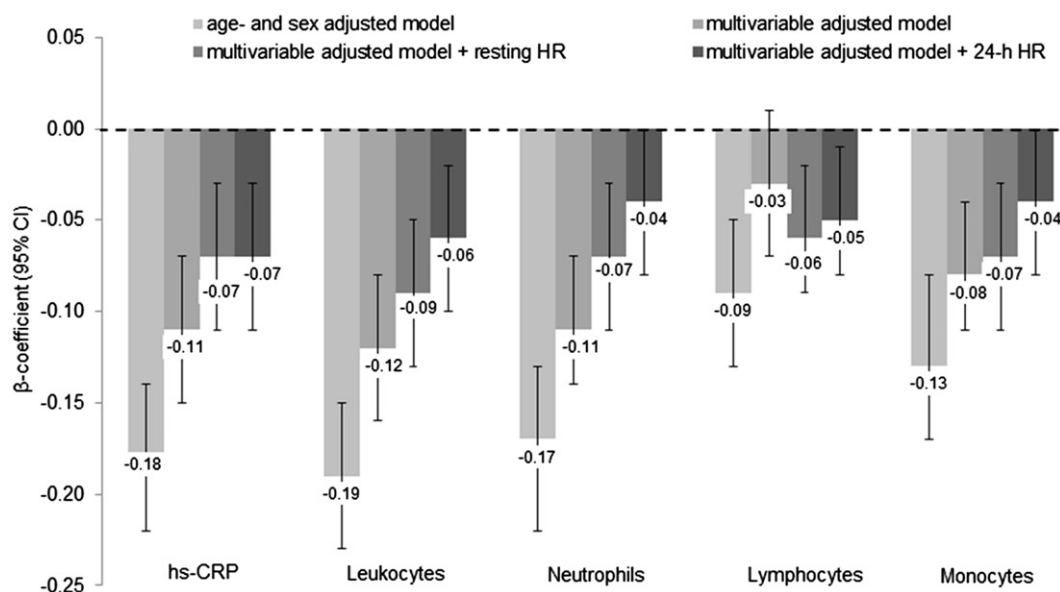
Figure 1. Relationship between SDNN and inflammatory biomarkers. Data are presented as β-coefficients (95% confidence intervals) per 1 standard deviation increase. SDNN: standard deviation of all normal RR intervals; Hs-CRP: high-sensitivity C-reactive protein. Model 1 was adjusted for age, sex. Model 2 was additionally adjusted for body mass index, smoking status, educational status, alcohol consumption, fruit and vegetable consumption, fish consumption, systolic blood pressure, prediabetes, physical activity, low- and high-density lipoprotein cholesterol, triglycerides, endothelin-1, estimated glomerular filtration rate, family history of cardiovascular disease and body fat. Model 3 was additionally adjusted for resting heart rate. Model 4 was additionally adjusted for 24-h heart rate instead of resting HR. n = 2096.

Figure 2. Relationship between normalized HF and inflammatory biomarkers. Data are presented as β-coefficients (95% confidence intervals) per 1 standard deviation increase. HF: high frequency; Hs-CRP: high-sensitivity C-reactive protein. Model 1 was adjusted for age, sex. Model 2 was additionally adjusted for body mass index, smoking status, educational status, alcohol consumption, fruit and vegetable consumption, fish consumption, systolic blood pressure, prediabetes, physical activity, low- and high-density lipoprotein cholesterol, triglycerides, endothelin-1, estimated glomerular filtration rate, family history of cardiovascular disease and body fat. Model 3 was additionally adjusted for resting heart rate. Model 4 was additionally adjusted for 24-h heart rate instead of resting HR. n = 2096.
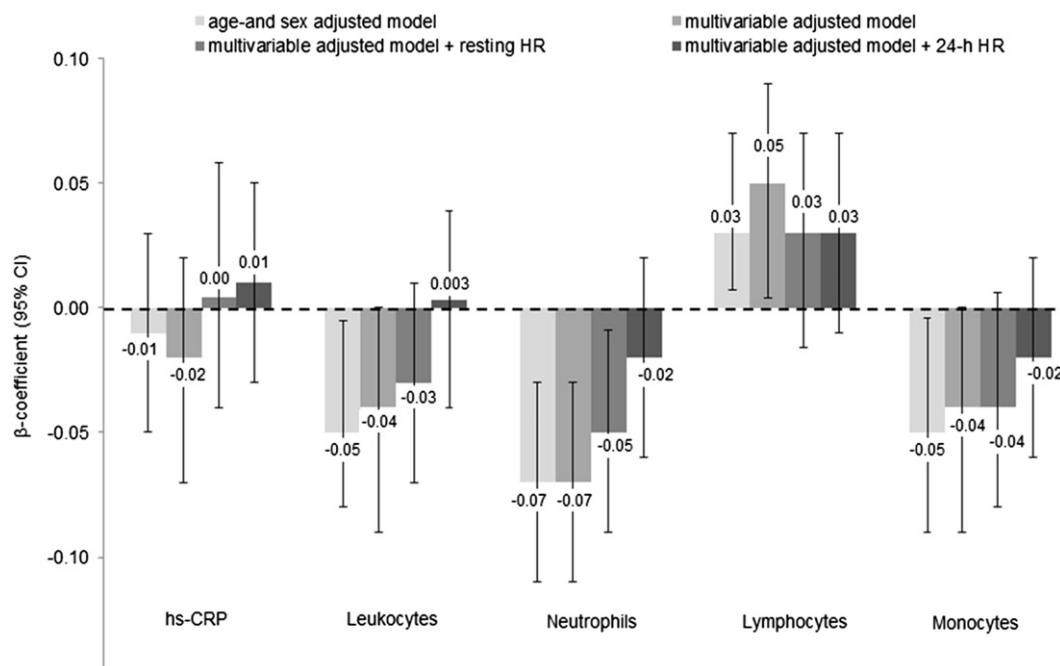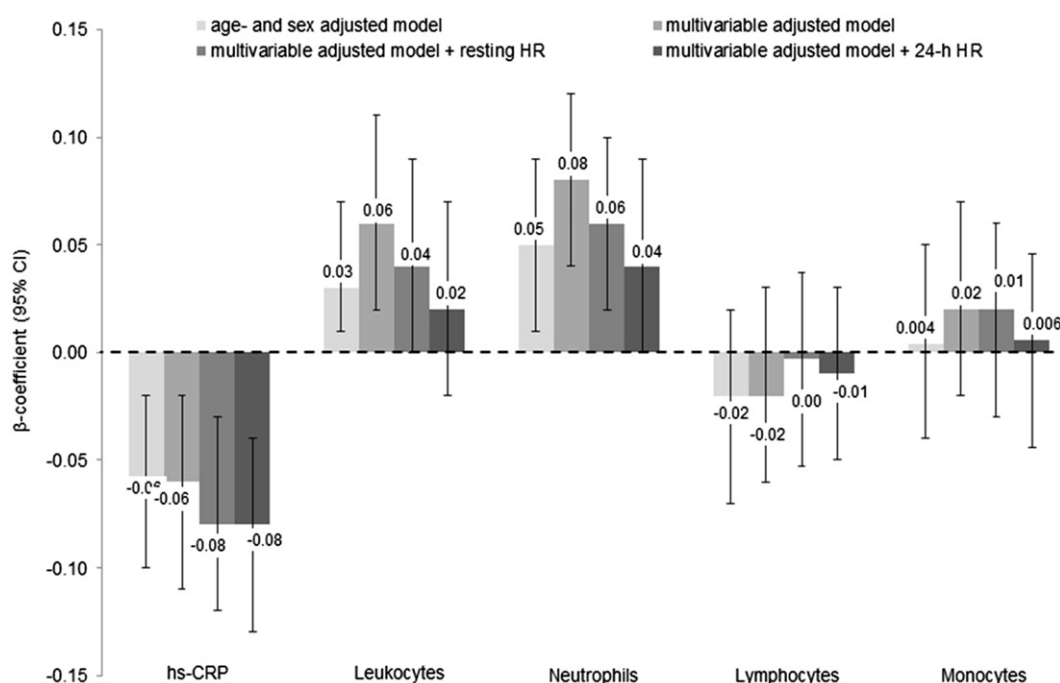
Figure 3. Relationship between normalized LF and inflammatory biomarkers. Data are presented as β-coefficients (95% confidence intervals) per 1 standard deviation increase. LF: low frequency; Hs-CRP: high-sensitivity C-reactive protein. Model 1 was adjusted for age, sex. Model 2 was additionally adjusted for body mass index, smoking status, educational status, alcohol consumption, fruit and vegetable consumption, fish consumption, systolic blood pressure, prediabetes, physical activity, low- and high-density lipoprotein cholesterol, triglycerides, endothelin-1, estimated glomerular filtration rate, family history of cardiovascular disease and body fat. Model 3 was additionally adjusted for resting heart rate. Model 4 was additionally adjusted for 24-h heart rate instead of resting HR. n = 2096.

(z-score) is decreasing by 0.11 (95% CI −0.14; −0.06), p < .0001 and 0.12 (−0.16; −0.08), p < .0001, respectively. After the adjustment for resting HR, the relationships with SDNN attenuated by 36% for hs-CRP, 25% for LC, 36% for neutrophils, 13% for monocytes. The additional adjustment for 24-h HR instead of resting HR attenuated these relationships even stronger (−36% for hs-CRP, −50% for LC, −63% for neutrophils, and −50% for monocytes), although all of them remained significant. The attenuation effect of the correlation between TP and inflammatory biomarkers was even stronger, such that only the relationship with hs-CRP remained significant (Figure S1). The multivariable adjusted β-coefficients for normalized HF and LF are shown in Figures 2 and 3. Again, all relationships were strongly attenuated after the adjustment for 24-h HR and nearly all of them lost level of significance. Including hs-CRP and LC in the same multivariable adjusted model, both of them remain inversely associated with HRV (Table S4).

Subgroup analyses were performed for sex, prediabetes and BMI (Table S5 and S6). A significant interaction was found for the relationship between SDNN, TP and neutrophil cell count, showing a stronger inverse association among men compared to women (both p for interaction = .002). None of the other subgroup analyses were significant.

Relationships between heart rate and inflammatory biomarkers

A positive and linear association was found for the association of ambulatory HR with hs-CRP, LC, neutrophils and monocytes (Table S7). Results of the relationships between resting and ambulatory HR with continuous inflammatory biomarkers are presented in Figure 4 and S2. Per one SD increase on the log-scale the multivariable adjusted β-coefficients (95%CI) for ambulatory HR were 0.62 (0.24; 1.01), p = .002 for hs-CRP, 1.10 (0.73; 1.48), p < .0001 for LC, 1.32 (0.95; 1.69), p < .0001 for neutrophils and 0.61 (0.23; 0.99), p = .002 for monocytes (Figure 4). Additional adjustment for SDNN lowered the effect sizes of these relationships by 74% for hs-CRP, 47% for LC, 38% for neutrophils and 4.5% for monocytes, however with the exception of hs-CRP, all of them remained statistically significant. Lymphocyte count was not significantly associated with HR after multivariable adjustment. A significant
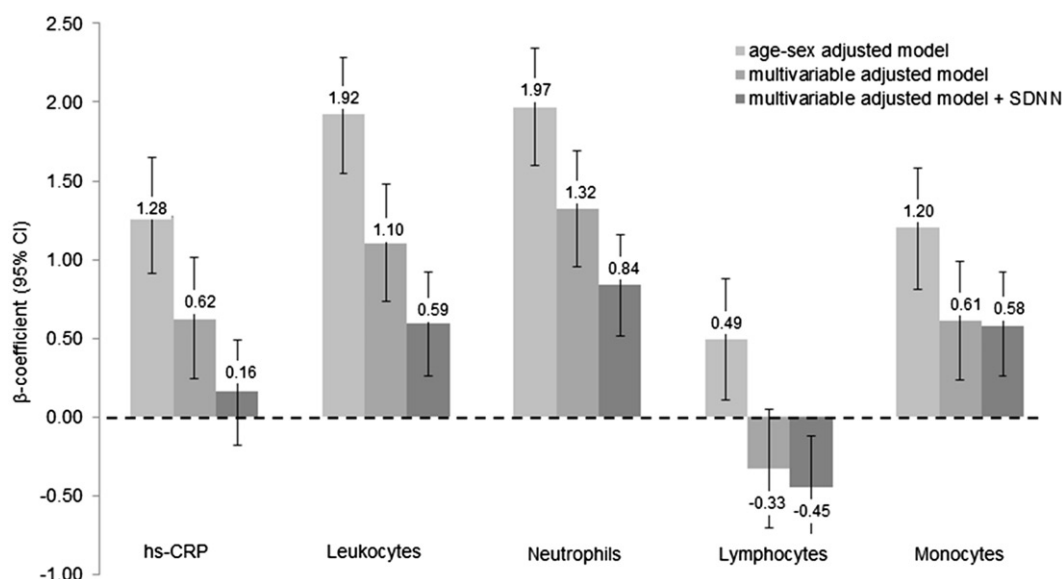
Figure 4. Relationship between ambulatory heart rate and inflammatory biomarkers. Data are presented as β-coefficients (95% confidence intervals) per 1 standard deviation increase. Hs-CRP: high-sensitivity C-reactive protein. Model 1 was adjusted for age, sex. Model 2 was additionally adjusted for body mass index, smoking status, educational status, alcohol consumption, fruit and vegetable consumption, fish consumption, systolic blood pressure, prediabetes, physical activity, low- and high-density lipoprotein cholesterol, triglycerides, endothelin-1, estimated glomerular filtration rate, family history of cardiovascular disease and body fat. Model 3 was additionally adjusted for the standard deviation of all normal RR intervals. n = 2096.

sex-interaction was found for the associations of resting and ambulatory HR with neutrophils (p for interaction .02 and <.0001, respectively) with a stronger relationship among men compared to women (Table S8).

Discussion

In this large population-based study, we found strong and independent relationships of HR and HRV as measured by SDNN with a large set of inflammatory biomarkers, including hs-CRP, total LC count and several LC subpopulations. These relationships persisted after comprehensive multivariable adjustment but were strongly attenuated after additional adjustment for HRV or HR, while ambulatory HR has more strongly attenuated the relationships compared to resting HR. Although these results suggest that HR and HRV carry similar information with regard to their association with inflammatory biomarkers (24), it is important to emphasize that most relationships remained statistically significant after mutual adjustment, suggesting that both parameters carry some incremental information over each other. Up to now, few if any studies have additionally adjusted the relationships for HR, even though it is suggested to take HR into account when investigating HRV (24). Finally, TP and normalized HF and LF did not provide additional information over and above ambulatory HR

alone. Therefore, further studies are needed to more accurately assess the additional information of individual HRV variables over and above resting and ambulatory HR.

Our findings confirm and expand prior studies showing negative associations of HRV with mainly hs-CRP (16–19). Importantly, our results were additionally adjusted for HR, providing incremental information. The consistent results across various inflammatory biomarkers found in our study may also indicate a broad interplay between different inflammatory pathways and the ANS. These data are in line with experimental studies showing direct relationships between of the ANS and inflammatory cells (14,15). To our best knowledge this is one of the first studies from the general population showing the relationships of 24-h HRV and HR with not only hs-CRP but also total LC count and its subtypes. Thus, a link between HRV and inflammation may constitute one potential factor that might explain the increased cardiovascular risk previously shown for both entities (2,12). Inflammation is based on complex processes and various pathways are involved. Our findings showed that several inflammatory pathways seem to be involved in the relationship with HRV. Potential underlying mechanisms for these relationships are multifaceted. Norepinephrine, as the main sympathetic neurotransmitter, has been negatively associated with HRV and positively related to inflammatory

parameters (17). Experimental studies have shown expression of adrenoceptors on immune cells, suggesting a direct influence of the immune system by adrenoceptor agonists (29–31). Additionally, other studies showed a reduced inflammatory reaction as a consequence of medical sympathectomy (32,33). Vagal activity seems to have an anti-inflammatory effect via a cholinergic pathway, which results in a down-regulation of pro-inflammatory cytokines (14).

Some of the described mechanisms partly explain and support our findings. HF is mainly modulated by parasympathetic activity (10,34). An inverse relationship of HF with neutrophils and monocytes was found, assuming either an anti-inflammatory effect of vagal activity for those immune cells or an increased vagal activity as a consequence of low neutrophil and monocyte cell counts. In contrast, lymphocytes were positively related to HF. This finding could be supported by the results of a previous study showing a protective effect of lymphocytes and an adverse effect for neutrophils for the occurrence of cardiovascular events (35,36). Others have showed that inflammation could increase lymphocyte apoptosis, which may contribute to unfavorable outcomes (37). SDNN was negatively associated with all inflammatory biomarkers, although the relationship with the lymphocyte cell count was weakest and reached only borderline significance. The association of lymphocyte cell count with different HRV variables should further be investigated to understand the underlying mechanisms of the relationship between lymphocytes and autonomic function.

Based on previous studies, resting and 24-h HR are known to be positively associated with hs-CRP, fibrinogen and interleukin, which is in line with our findings (17–19,22). Additionally, our data showed a positive association with total LC count, neutrophils and monocytes, but similar to HRV, no significant relationship with lymphocytes. Physical activity and other healthy lifestyle habits reduce HR (38) and improve autonomic function, which may in consequence lower inflammatory biomarker levels. Others suggest that the protective effect of physical activity on inflammation is mediated by autonomic function (39). This cross-sectional study cannot prove the above mentioned pathways, but nevertheless highlights the need for future studies to better understand the relationship between inflammation and the ANS.

A significant sex-interaction for the relationship between HRV, HR and neutrophil cell count was found, with stronger inverse associations among men compared to women. This sex-interaction might in part be reasoned by an influence of ovarian hormones on the immune system (40). However, additional studies are needed to better investigate these sex differences. Although autonomic dysbalances might play an important role in the development of type 2 diabetes mellitus (41), prediabetes was not a significant effect modifier in the relationship between HRV and inflammatory biomarkers.

Strengths and limitations

Major strengths of the present study are the population-based study design, the well-characterized study population, the enrollment of young and healthy participants with a relatively short exposure history to environmental factors and the availability of 24-h ECG data to quantify HRV over an individual's normal day. However there are several potential limitations, which should be taken into account for the interpretation of our results. First, this is a cross-sectional analysis such that causality of the relationship between HRV and inflammatory biomarkers cannot be addressed. Second, we enrolled mainly Caucasians in our study and the generalizability of our results to other populations is uncertain. Third, as in any observational study, residual confounding could be an issue despite our comprehensive multivariable adjustment. Fourth, it was aimed to get a deeper understanding of the interrelationship between HRV and inflammatory biomarkers. Based on our analyses, risk prediction for future events is not possible. Fifth, it is unclear if variables, such as body fat mass or BMI are confounders or mediators of the observed relationships. If some mediators should have been included in our models, we would expect that the true relationships between inflammation, HR and HRV would be somewhat stronger. Sixth, since regular intake of statins might have an anti-inflammatory effect and few individuals of our study population are currently treated with statins, our results might be minimally influenced.

Conclusion

In this large population of young and healthy adults from the general population, we found strong and independent relationships of HR and HRV with a broad set of inflammatory biomarkers. Relationships between SDNN and inflammatory biomarkers were weakened but remained significant after additional adjustment for ambulatory HR, suggesting incremental information of HRV over HR. Thus, our findings suggest important interrelationships between inflammatory pathways and the ANS.

Disclosure statement

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