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Increased heart rate and reduced heart-rate variability are associated with subclinical inflammation in middle-aged and elderly subjects with no apparent heart disease

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Aim Elevation of inflammation markers, high heart rate, and reduced heart-rate variability are all strong markers of mortality in a broad spectrum of patients. The association between these markers has not been clarified thoroughly. We investigated the associations between markers of inflammation, heart rate, and heart-rate variability.

Methods and results Six hundred and forty-three healthy men and women between 55 and 75 years of age and with no prior history of cardiovascular disease or stroke were included in the study. The baseline study included a physical examination, fasting laboratory tests, and 24-h ambulatory ECG monitoring. We selected the time-domain components of heart-rate variability for further analyses. C-reactive protein concentration and white blood cell count were selected as markers of inflammation. After identifying parameters related to measures of heart-rate variability, we used regression analyses to evaluate independent associations. Heart-rate variability, as measured by the standard deviation of the time between normal-to-normal complexes or the standard deviation of the average of normal-to-normal intervals for each 5-min period, was negatively associated with smoking, C-reactive protein, white blood cell count, blood sugar and triglyceride concentration, female gender, and diabetes. In contrast, physical activity was strongly associated with higher heart-rate variability. In multivariate regression analyses, increased heart-rate and reduced heart-rate variability were significantly and independently related to white blood cell count or C-reactive protein concentration.

Conclusion Increased heart rate and reduced heart-rate variability are associated with subclinical inflammation in healthy middle-aged and elderly subjects. The increased mortality that has been reported in these settings may thus have a common

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aetiology. An autonomic imbalance in favour of the sympathetic system may interact with inflammatory processes to play a more important role in the process of atherosclerosis than previously thought.

Inflammation plays a significant role in the pathogenesis and progression of atherosclerosis.[1] Subclinical inflammation and the concentration of inflammatory markers have been shown in many studies to correlate strongly to cardiovascular mortality and morbidity in both healthy subjects and in subjects with known coronary artery disease.[2–4] However, what really triggers inflammation is not known. Reduced heart-rate variability is a marker of sympatho-vagal imbalance. Like inflammation, reduced heart-rate variability, both in time- and frequency-domain analyses, has been found to be associated with cardiovascular mortality and morbidity in both healthy subjects and subjects with known coronary artery disease.[5,6] Furthermore, in prospective studies reduced heart-rate variability has been shown to be the strongest independent predictor of the progression of focal coronary atherosclerosis.[7] Similarly, higher heart rate, another marker of relative sympathetic dominance, is an independent marker of mortality in a wide spectrum of conditions.[8] It is biologically plausible that altered autonomic balance can trigger inflammation. We hypothesised that in apparently healthy subjects reduced heart-rate variability and high heart rate are associated with subclinical inflammation and evaluated this hypothesis in a population-based study.

Materials and methods

This study is part of the Copenhagen Holter study, which aimed to assess the value of 24-h Holter recording in the risk assessment of middle-aged and elderly men and women with no apparent heart disease, especially in relation to other risk factors. By querying the "Central Person Registration" office, Ministry of Interior, Denmark, all men aged 55 years, and men and women aged 60, 65, 70, and 75 years in two postal regions of the city of Copenhagen were identified ($n = 2969$). Using questionnaire items, a history of previous myocardial infarction, other cardiac diseases, stroke, cancer, and other significant or life-threatening conditions were excluded. All of the other subjects were risk-stratified according to the information on hypertension, diabetes mellitus, smoking habit, familial predisposition to heart disease, obesity (body mass index [BMI] > 30) or hypercholesterolaemia, as diagnosed by a physician. The response to the questionnaire was 68%. Subsequently, all subjects with two or more self-reported risk factors and a random sample consisting of 60% of the subjects with one or no apparent risk factor were invited to take part in the Holter study.

A total of 775 subjects participated in the study. Of these, 643 had an acceptable Holter monitoring for at least 24-h heart-rate variability analyses and constituted the study population of this investigation. All participants underwent an interview, physical examination including anthropometric determinations, fasting laboratory testing and 48-h Holter monitoring.

Blood pressure was measured with a mercury manometer after at least 10 min of rest with the patient in a recumbent position. BMI was calculated as weight in kilograms divided by height in meters, squared. Waist circumference was measured at a midpoint between the lower ribs and iliac crest, and hip circumference was measured at the level of the trochanter. Patients were divided by level of physical activity into two groups: Group 1 with an almost sedentary life-style or a light physical activity level of 2—4 h/week, and Group 2 with a moderate to hard physical activity level of at least 4 h of physical activity or training weekly. Diabetes mellitus was defined as known diabetes or fasting plasma glucose of $\geqslant 7$ mmol/l. Laboratory testing was performed in the morning, after an overnight fast of at least 8 h.

A 48-h Holter recording was made with two-channel SpaceLabs tape recorders (9025, SpaceLabs, Inc., Redwood, WA). The analysis of heart-rate variability was carried out on an FT3000 Medical Analysis and Review Station. From the 48-h Holter recording, the first 24 h were selected for analyses. In all cases, the analysis started at the beginning of the second hours after the start of Holter recording and continued for the next 24 h (from hour 2 to hour 25). This eliminated start-time noise. Analysis was performed by trained personal at the Holter laboratory of Copenhagen University Hospital, Hvidovre. Time-domain components of heart-rate variability were measured and the following parameters were identified: mean and standard deviation for the time between normal-to-normal complexes (MEANNN and SDNN) and standard deviation of the average normal-to-normal intervals for each 5-min period (SDANN), and the percentage of adjacent cycles >50 ms apart (pNN50). In these terms MEANN represents the average 24-h heart rate (60,000/MEANNN = average 24-h heart rate in beats/min). SDNN represents the standard deviation of the circadian sinus node cycle length. SDANN represents the standard deviation of the 5-min mean cycle lengths over the entire 24-h recording. It provides an index of the variability of the average of 5-min intervals over 24 h, but no information about short-term variability. pNN50 stands for very short heart-rate variability. It is independent of diurnal or other long-term trends and reflects almost wholly alterations in autonomic tone that are predominantly vagally mediated.

Night-time analyses

To evaluate the associations between heart rate, measures of heart-rate variability, and inflammatory markers during a standardised sequence when the possible interference of physical and mental activity is minimised, we analysed the MEANNN and SDNN during a 15-min sequence between 2:00 am and 2:15 am. At this time the participants are assumed to be sleeping.

Ethics

Before inclusion, all participants gave their written informed consent. The study was approved by the regional ethics committee for the cities of Copenhagen and Frederiksberg. The Helsinki declaration was complied with.

Statistical analysis

Statistical analyses were performed using the SAS statistical software program (version 8.2). For normally distributed variables, mean and SD are presented. Otherwise, median values are given. The associations were studied in two steps, first using the inflammatory markers as dependent variables and then using heart-rate variability measures as dependent variables. The univariate association between heart-rate variability and other parameters was evaluated with the Spearman or Pearson coefficients, Student *t* test, or Kruskal–Wallis test. To evaluate independent associations, parameters associated with a p value <0.05 were identified and examined in multivariate linear regression or multivariate logistic regression models with forced entry of age and sex. In forward selection models, the p value for inclusion of the variables was set at <0.05. Skewed variables were logarithmically transformed when appropriate, or dichotomized into the upper third versus the lower two thirds.

Results

Of the 775 subjects enrolled in the Holter study, 22 were excluded because one or more exclusion criteria were detected at the time of study-start (7 cases of cancer, 9 of apparent heart disease, and 7 of other significant or life-threatening diseases). One hundred and ten were excluded because the Holter recording was unacceptable for one of the following reasons: technical problems $(n = 64)$, long periods of atrial fibrillation $(n = 13)$, an excessive number of supraventricular premature beats $(n = 10)$, an excessive number of ventricular premature beats $(n = 15)$, and other irregularities $(n = 8)$. A total of 643 subjects were included in the study.

C-reactive protein, heart-rate variability, and other baseline variables

Table 1 demonstrates baseline characteristics of the study population and their distribution among subjects with high versus low concentrations of C-reactive protein (upper third versus lower two thirds). In a logistic multivariate model with age, sex, and all the other variables that were related to C-reactive protein concentration in the univariate model, we found that MEANNN (OR = 0.997, 95% CI [0.994; 0.999], $p = 0.004$) and SDNN (OR = 0.993, 95% CI [0.986; 0.999], $p = 0.03$) were predictors of C-reactive protein concentration. To find the best predictors of C-reactive protein, a forward selection logistic regression analysis was performed with forced entry of age and sex (Table 2).

White blood cell count, heart-rate variability, and other baseline variables

White blood cell count (a normally distributed variable) was associated with MEANNN ($r = -0.18$, $p < 0.001$), SDNN ($r = -0.18$, $p < 0.001$), SDANN ($r = -0.17$, $p < 0.001$), HDL ($r = -0.18$, $p < 0.01$), triglycerides ($r = -0.22$, $p < 0.001$), physical activity level ($6.2 \pm 1.7 \times 10^9$/l in subjects with a high level of physical activity versus $7.0 \pm 2.1 \times 10^9$/l in subjects with a low level, $p < 0.001$)

Table 1 Baseline characteristics of the study population and of subjects with high versus low CRP concentration

Covariates	Low CRP (lower two thirds) $n = 434$	High CRP(upper third) $n = 209$	All	p-Value
Age (year)	64.3 ± 6.8	64.6 ± 6.6	64.4 ± 6.8	0.6
Women	41%	45%	42%	0.31
Current smoker (%)	44%	52%	47%	0.06
Diabetes (%)	10.1%	13.9%	11.4%	0.16
Hypertension (%)	34%	41%	36%	0.08
Low level of physical activity	22.6%	33.6%	26%	0.003
Body mass index (kg/m^2)	25.6 ± 4.6	27.1 ± 4.0	26.1 ± 4.2	0.0001
Systolic blood pressure (mm Hg)	155.8 ± 25.4	159.4 ± 22.0	156.9 ± 24.5	0.06
Diastolic blood pressure (mm Hg)	90.7 ± 11.4	92.1 ± 10.4	91.2 ± 11.1	0.15
Cholesterol (mmol/l)	6.0 ± 1.0	6.2 + 1.1	6.1 ± 1.1	0.03
High-density lipoprotein (mmol/l)	1.5 ± 0.4	1.4 ± 0.5	1.5 ± 0.5	0.002
Triglycerides (mmol/l)	1.5 ± 1.3	1.7 ± 0.	1.6 ± 1.7	0.004
Blood glucose (mmol/l)	5.8 ± 1.8	5.9 ± 1.9	5.9 ± 1.9	0.5
β-Blocker use	4%	7%	4.8%	0.055
Calcium-antagonist use	6%	12%	8.4%	0.01
NSAID use	17%	23%	19%	0.06
Statin use	2.5%	1.9%	2.3%	0.6
MEANNN[a] (ms)	815 ± 103	778 ± 94	803 ± 102	0.0001
SDNN[b] (ms)	130 ± 35	115 ± 33	124 ± 35	0.0001
SDANN[c] (ms)	118 ± 34	105 ± 32	114 ± 34	0.0001
pNN5O[d]	4.5 ± 5.9	4.8 ± 8.5	4.6 ± 6.9	0.08

[a] MEANNN, mean value for the time between normal complexes.
[b] SDNN, standard deviation of the mean value of time between normal complexes.
[c] SDANN, standard deviation of the average of NN intervals for each 5-min period.
[d] pNN50, the percentage of adjacent cycles >50 ms apart.

Table 2 Results of a forward selection logistic regression analysis demonstrating those factors that best identify high level of CRP and forced entry of age and sex

	CRP OR (95% CI)	*p*-Value
Sex (women)	1.33 (0.88; 2.03)	0.9
Age (year)	1.02 (0.99; 1.05)	0.2
MEANNN (ms)	0.997 (0.995; 0.999)	0.009
SDNN (ms)	0.990 (0.984; 0.997)	0.003
Cholesterol (mmol/l)	1.27 (1.06; 1.53)	0.01
Body mass index (kg/m^2)	1.07 (1.03; 1.13)	0.003
HDL (mmol/l)	0.46 (0.29; 0.73)	0.004
Calcium antagonist-usage (%)	2.4 (1.2; 4.5)	0.008

In addition to the variables shown triglycerides, HDL-cholesterol, physical activity level, smoking, β-blocker usage and use of nonsteroid anti-inflammatory agents were also included in the model.

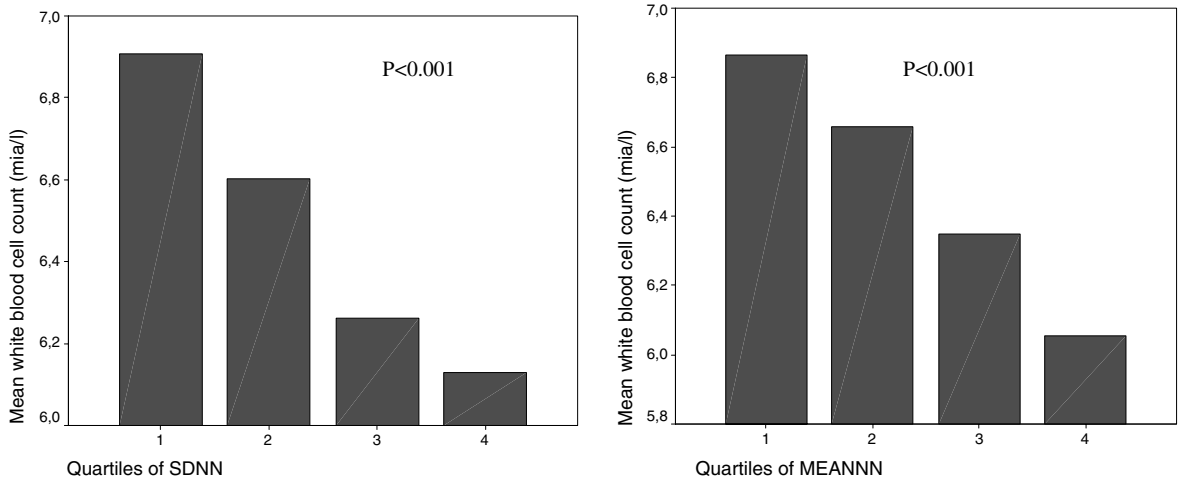

Fig. 1 White blood cell count in relation to heart rate and heart-rate variability. SDNN, standard deviation for the mean value of time between normal complexes (lowest quartile as 1 and highest as 4); MEANNN, mean value for the time between normal complexes (Quartile 1, average heart rate $\geqslant$82; Quartile 2, average heart rate = 75.82; Quartile 3, average heart rate = 70.74; Quartile 4: average heart rate < 70).

and smoking (smokers 7.2×10^9 and non-smokers 5.8×10^9/l, $p < 0.001$) in univariate analyses. Fig. 1 illustrates associations between white blood cell counts and quartiles of SDNN and MEANNN. In a forward selection linear regression analysis model with age, sex, MEANNN, SDNN, HDL-cholesterol, triglycerides, smoking, physical activity level, use of non-steroid anti-inflammatory drugs, β-blockers and calcium antagonists, the best predictors of white blood cell count were smoking (β-value = 1.2, 95% CI [0.9; 1.5], $p < 0.0001$), HDL-cholesterol (β-value = −0.6, 95% CI [−0.9; −0.3], $p < 0.0001$), SDNN (β-value = −0.006, 95% CI [−0.01; −0.002], $p < 0.001$), and physical activity level (β-value = 0.4, 95% CI [0.7; 0.9], $p < 0.01$).

Heart rate and heart-rate variability measures as dependent variables

The univariate correlation of heart rate (MEANNN) and each of the three heart-rate variability measures and clinical parameters of interest are shown in Table 3. Besides systolic blood pressure, "known hypertension" was also analysed in relation to heart-rate variability measures and no association with heart-rate variability measures was found. Table 4 shows the results of linear regression analyses for each of the heart-rate variability measures as dependent variables and other relevant parameters.

Discussion

The major new finding of this study is the association between increased heart rate, decreased SDNN and SDANN, and measures of inflammation in middle-aged and elderly subjects with no apparent heart disease. To our knowledge this is the first investigation to address this issue in healthy middle-aged and elderly subjects. Furthermore, this is the largest study of long-term heart-rate variability in subjects with no apparent heart disease. The association between markers of inflammation and MEANNN, SDNN, and SDANN remained significant after correction for major risk factors and possible confounding factors like age, heart rate, smoking, use of alcohol, physical activity level, hypertension and diabetes, use of β-blockers or non-steroid anti-inflammatory agents.

Table 3 Univariate associations between clinical parameters of interest and measures of heart-rate variability

	MEANNN (ms)	SDNN (ms)	SDANN (ms)	Ln(pNN50)	Night-time MEANNN (ms)	Night-time SDNN (ms)
Continuous variables						
Age (year)	0.13***	—	—	0.09*	—	—
Systolic blood pressure (mm Hg)	0.13***	—	—	—	—	—
Plasma glucose (mmol/l)	—	−0.11**	−010*	−0.11**	—	—
Total cholesterol (mmol/l)	—	—	—	—	—	—
HDL cholesterol (mmol/l)	—	—	—	—	—	—
Triglyceride (mmol/l)	−0.13***	−0.16***	−0.13***	−0.15***	−0.14**	0.11**
White blood cell count (10^9/l)	−0.18***	−0.18***	−0.17***	—	−0.19***	—
Categorial variables						
CRP (μg/ml)						
High (highest tertile)	778 ± 94***	115 ± 35***	105 ± 32***	0.65 ± 1.4	897 ± 131***	41 ± 24
Low (lowest two third)	816 ± 103	130 ± 33	118 ± 34	0.78 ± 1.3	948 ± 144	44 ± 22
Gender						
Men (372)	809 ± 107	128 ± 37**	1116 ± 35	0.76 ± 1.3	941 ± 149*	46 ± 24**
Women (271)	796 ± 93	121 ± 32	1110 ± 31	0.71 ± 1.3	916 ± 130	37 ± 20
Smokers (301)	781 ± 100***	118 ± 33***	108 ± 32***	0.6 ± 1.3*	898 ± 139***	42 ± 23
Nonsmokers (342)	822 ± 98	131 ± 36	118 ± 35	0.8 ± 1.4	960 ± 139	45 ± 22
Physical activity						
Low (167)	788 ± 90***	114 ± 34***	103 ± 32***	0.59 ± 1.4	895 ± 130***	41 ± 22*
High (470)	809 ± 104	129 ± 35	117 ± 34	0.79 ± 1.3	945 ± 143	45 ± 23
Diabetes						
Yes (73)	792 ± 101	115 ± 34***	105 ± 34*	0.78 ± 1.3*	911 ± 143	40 ± 24
No (570)	804 ± 102	126 ± 34	115 ± 34	0.41 ± 1.5	934 ± 142	44 ± 23
β-Blocker usage						
Yes (31)	886 ± 105***	106 ± 27***	94 ± 27***	0.70 ± 1.2	979 ± 138*	43 ± 22
No (643)	799 ± 100	126 ± 35	115 ± 34	0.74 ± 1.3	929 ± 141	43 ± 22
Calcium-antagonist usage						
Yes (54)	839 ± 108*	126 ± 35	116 ± 33	0.55 ± 1.4	969 ± 143*	43 ± 27
No (586)	800 ± 100	125 ± 35	113 ± 34	0.76 ± 1.3	928 ± 141	43 ± 22
NSAID usage						
Yes (121)	811 ± 92	123 ± 40	113 ± 34	0.76 ± 1.3	937.1 ± 144	41 ± 21
No (522)	802 ± 104	125 ± 35	114 ± 34	0.72 ± 1.3	927.6 ± 135	42 ± 23

MEANNN, mean value for the time between normal complexes; SDNN, standard deviation for the mean value of time between normal complexes; SDANN, the standard deviation of the average of NN intervals for each 5-min period; pNN50, The percentage of adjacent cycles that are >50 ms apart.

* $p \leqslant 0.05$.

** $p \leqslant 0.01$.

*** $p \leqslant 0.001$.

Heart rate and its variability are under sympatho-vagal influence, and reduced heart-rate variability and increased heart rate are thought to be a result of autonomic imbalance. Situations with sympathetic overdrive, like stress and tobacco smoking, reduce heart-rate variability.[9,10] Transthoracic sympathectomy has been shown to significantly increase heart-rate variability measures like SDANN.[11]

Among time-domain measures of heart-rate variability, SDNN and SDANN reflect both sympathetic and parasympathetic modulation of heart rate and reduced SDANN and SDNN values usually indicate relative sympathetic dominance.[12] pNN50, on the other hand, is a marker of parasympathetic activity.[13] pNN50 was not associated with markers of inflammation in this study. This may indicate that in subjects with increased concentrations of inflammatory markers the reduction in heart-rate variability observed may be due mainly to increased sympathetic activity rather than to reduced vagal tone.

To obtain a standardised sequence and eliminate the interference of physical and mental activity, a 15-min sequence of night-time SDNN and MEANN was analysed against the concentration of inflammatory markers. Night-time MEANNN showed absolutely the same tendency as 24-h MEANNN and was independently associated with C-reactive protein concentration, while this was not the case for night-time SDNN (Tables 3 and 4). Night-time heart-rate variability is mostly vagally mediated[14] and thus may underestimate the normal sympathetic influence on heart-rate variability. A Spearman correlation analysis with both C-reactive protein concentration and night-time SDNN showed a significant association ($r = 0.12$, $p = 0.003$) although it did not remain significant after correction for other covariates.

Both reduced heart-rate variability and measures of inflammation like C-reactive protein concentration have been identified as risk factors for cardiovascular mortality and morbidity in a broad spectrum of conditions ranging from the healthy and general population to

Table 4 Linear regression analyses (full models): the association of parameters of interest with measures of heart-rate variability (β-values and 95% confidence interval for the β-values)

	MEANNN (ms)	SDNN (ms)	SDANN (ms)	Ln (pNN50)	Night-time MEANNN (ms)	Night-time SDNN (ms)
Systolic blood pressure (mm Hg)	0.4 (0.1; 0.7)†	—	—	—	—	—
Physical activity (low versus high)	ns	8.4 (3.2; 13.7)‡	8.8 (3.4; 14.2)‡	ns	36.7 (12.3; 61.1)‡	ns
Triglyceride (mmol/l)	−6.3 (−12.3; −0.4)*	—	ns	ns	−8.7 (−17.2; −0.3)*	—
Current smoker	−31 (−47; −16)‡	−5.8 (−10.6; −1.1)*	ns	ns	−54.2 (−75.8; −32.6)‡	ns
MEANNN (ms)	—	0.17 (0.15; 0.20)‡	0.14 (0.11; 0.16)‡	0.006 (0.005; 0.007)‡	—	0.07 (0.05; 0.08)‡
CRP (μg/ml)	−36 (−52; −19)‡	−5.5 (−10.5; −0.4)*	−5.4 (−10.1; −0.2)*	ns	−42.8 (−65.8; −19.9)‡	—
NSID-usage (%)	ns	ns	ns	ns	—	ns
β-blocker usage (%)	86 (50; 122)‡	−32 (−43; −21)‡	−30 (−42; −19)‡	−0.2 (−0.9; −0.1)*	56.2 (6.5; 105.9)*	ns
Sex (men versus women)	29 (12; 45)‡	ns	ns	ns	42.1 (19.2; 64.9)‡	5 (1.1; 8.1)†
Age (year)	1.6 (0.4; 2.9)†	−0.4 (−0.7; −0.06)	ns	ns	2.0 (0.3; 3.7)†	ns
Blood glucose (mmol/l)	—	ns	ns	ns	—	
Diabetes mellitus	—	ns	ns	ns	—	
R^2 for the model	0.13	0.33	0.25	0.24	0.11	0.16

MEANNN, mean value for the time between normal complexes; SDNN, standard deviation for the mean value of time between normal complexes; SDANN, the standard deviation of the average of NN intervals for each 5-min period and pNN50, the percentage of adjacent cycles that are >50 ms apart.

‡ $p \leqslant 0.001$.

† $p \leqslant 0.01$.

* $p \leqslant 0.05$.

patients with coronary artery disease and congestive heart failure.[1–8] Interestingly, in almost any clinical situations where increases in inflammatory markers are documented by some studies, reductions in heart-rate variability have been reported by others. Reduced heart-rate variability in *diabetes mellitus* has been known for many years and recent studies report increases in inflammatory markers in diabetes and subclinical inflammation that are now accepted as a part of the insulin resistance syndrome.[15,16] In *hypertension*, activation of inflammation and reduced heart-rate variability have been reported in different studies.[17,18] After *acute myocardial infarction* heart-rate variability diminishes and the number of white blood cell increases.[19,20] In *obesity* the inflammatory system is activated and heart-rate variability is negatively associated with BMI.[21–23] *Cigarette smoking* reduces heart-rate variability and induces an inflammatory reaction.[9,24,25] *Hyperglycaemia* in both diabetic men and non-diabetics is associated with reduced heart-rate variability and activation of inflammation has been observed in the same situation.[26,27] Interestingly, in subjects with *mental depression* disturbances in the immune reaction and inflammatory reaction have been observed; in the same category of patients reduced heart-rate variability is reported and proposed as a link between increased mortality and depression after acute myocardial infarction.[28,29] All these previous findings and observations coincide with the findings in the present study.

In spite of all these findings, the associations between heart-rate variability and inflammation have not been studied properly. In one study, Aronson et al.[30] reported an inverse relation between heart-rate variability and levels of interleukin-6 in patients with congestive heart failure. In another study, Urstad-Jensen et al.[31] reported an association between leukocyte count and heart-rate variability in young healthy men, which is in agreement with our findings. Otherwise, no study has addressed this issue.

The association between inflammation and reduced heart-rate variability/increased heart rate may be explained in two ways: (1) A direct relation may exist between these parameters. Activation of inflammation by autonomic imbalance is biologically plausible as both bone marrow and the lymphoreticular system are innervated by autonomic nerves and are under influence of these systems.[32–34] In addition, sympathectomy, whether surgical or medical, alters and reduces the inflammatory reaction.[35–37] Thus, an imbalance in the autonomic nervous system in favour of the sympathetic system (increased sympathetic activity or reduced parasympathetic activity) could theoretically influence and increase the inflammatory reaction. Inflammation may, in turn, influence the autonomic balance. Interleukin-6 has been found in brain and can influence the autonomic balance

by affecting the hypothalamic-pituitary-adrenal axis at the level of the pituitary and adrenal glands.[37] Other inflammatory substances could theoretically have similar functions.[38] Thus, autonomic imbalance and inflammation may potentiate each other.

(2) Another explanation could be that both reduced heart-rate variability/increased heart rate and inflammation, or at least reduced heart-rate variability/increased heart rate, are epiphenomena of atherosclerosis. Even though the bulk of the evidence supports an independent effect of heart-rate variability on cardiovascular mortality and morbidity, a direct aetiologic relation has not been proven. Even in this case, reduced heart-rate variability will still conserve its significance as a sensitive marker of cardiovascular mortality and morbidity.

The mechanisms of activation of inflammation in diabetes mellitus, hypertension, obesity, coronary artery disease, mental depression, and other states are not clear. This study suggests that sympathetic overactivity/dominance could be a mechanism. Hypertriglyceridaemia is seen in many of these situations and is inversely related to heart-rate variability. This may support this theory because hypertriglyceridaemia is a metabolic consequence of sympathetic activity. Autonomic imbalance could be secondary to other conditions like congestive heart failure, acute myocardial infarction, and stress, as well as a primary condition. Interestingly, the heritability of heart-rate variability has been demonstrated previously.[39]

There are some limitations to this study. This is a study of middle-aged and elderly men and women and the results may not be applicable to younger age groups. The population studied is Caucasian and the application and extrapolation of results to other ethnic groups should be done carefully. The findings of this study are cross-sectional associations and not causal relations. From a methodological point of view, a follow-up period and evaluation of the associations between hard end-points and measures of HRV and inflammation in this study may be desirable. However, these were not the objectives of this study and will be dealt with separately. The study was carried out over a period of about a year and thus seasonal variation may have had an effect on heart-rate variability results. More importantly, physical and mental activities are factors that may have had some influence on results. We corrected for physical activity by incorporating both the level of physical activity and mean heart rate into the models where heart-rate variability was evaluated. Acute physical activity increases heart rate and influences heart-rate variability. While subjects with higher levels of physical activity have reduced inflammatory markers, acute physical activity up to moderate severity does not influence inflammatory markers.[40]

Conclusions

Reduced heart-rate variability, measured by SDNN or SDANN, and increased mean heart rate are associated with subclinical inflammation in middle-aged and elderly subjects with no apparent heart disease. The increased mortality that has been reported in these settings may thus have a common aetiology. Autonomic imbalance and inflammation could interact with each other and may play a more important role in relation to atherosclerosis than previously anticipated.

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References

1. Ross T. The pathogenesis of atherosclerosis: a perspective for the 1990s. *Nature* 1993;**362**:801—9.
2. Morrow DR, Rifai N, Antman EM et al. C-reactive protein is a potent predictor of mortality independently of and in combination with troponin T in acute coronary syndromes: a TIMI 11A substudy. *J Am Coll Cardiol* 1998;**31**:1460—5.
3. Phillips AN, Neaton JD, Cook DG et al. Leukocyte count and risk of major coronary heart disease events. *Am J Epidemiol* 1992;**136**(1):59—70.
4. Ridker PM, Cushman M, Stampfer MJ et al. Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. *N Engl J Med* 1997;**336**:973—9.
5. Huikuri HV, Mälkikallio TH, Airaksinen KEJ et al. Power-law relationship of heart rate variability as a predictor of mortality in the elderly. *Circulation* 1998;**97**:2031—6.
6. Tsuji H, Larson MG, Venditti FJ et al. Impact of heart rate variability on risk for cardiac events: the Framingham Heart Study. *Circulation* 1996;**94**:2850—5.
7. Hikuri HV, Jokinen V, Syvänne M et al. for the Lipid Coronary Angioplasty Trial (LOCAT) study Group. Heart rate variability and progression of coronary atherosclerosis. *Atheroscler Thromb Vasc Biol* 1999;**19**:1979—85.
8. Palatini P. Elevated heart rate as a predictor of increased cardiovascular morbidity. *J Hypertens* 1999;**17**:S3—S10.
9. Pope 3rd CA, Eatough DJ, Gold DR et al. Acute exposure to environmental tobacco smoke and heart rate variability. *Environ Health Perspect* 2001;**109**(7):711—6.
10. Hughes JW, Stoney CM. Depressed mood is related to high-frequency heart rate variability during stressors. *Psychosom Med* 2000;**62**:796—803.
11. Tygesen H, Wettervik C, Claes G et al. Long-term effect of endoscopic transthoracic sympathicotomy on heart rate variability and QT dispersion in severe angina pectoris. *Int J Cardiol* 1999;**70**(3):283—92.
12. Lombardi F. Clinical implications of present physiological understanding of HRV components. *Cardiac Electrophysiol Rev* 2002;**6**:245—9.
13. Stein PK, Kleiger RE. Insights from the study of heart rate variability. *Annu Rev Med* 1999;**50**:249—61.
14. Task force of the European society of cardiology and the northern American Society of Pacing and Electrophysiology. Heart rate Variability. Standards of measurement, physiological interpretation, and clinical use. *Circulation* 1996;**93**:1043—65.
15. Festa A, D'Augostino RJ, Howard G et al. Chronic subclinical inflammation as part of the insulin resistance syndrome. The insulin resistance atherosclerosis study (IRAS). *Circulation* 2000;**102**:42—7.
16. Liao D, Cai J, Brancati FL et al. Association of vagal tone with serum insulin, glucose, and diabetes mellitus — the ARIC study. *Diabetes Res Clin Prac* 1995;**30**:211—21.

17. Mussalo H, Vanninen E, Ikaheimo R et al. Heart rate variability and its determinants in patients with severe or mild essential hypertension. *Clin Physiol* 2001;**21**:594–604.
18. Bautista LE, López-Jaramillo P, Vera LM et al. Is C-reactive protein an independent risk factor for essential hypertension? *J Hypertens* 2001;**19**:857–61.
19. Dinerman JL, Mehta JL, Saldeen TG et al. Increased neutrophil elastase release in unstable angina pectoris and acute myocardial infarction. *J Am Coll Cardiol* 1990;**15**:1559–63.
20. Casolo GC, Stroder P, Signorini C et al. Heart rate variability during the acute phase of myocardial infarction. *Circulation* 1992;**85**:2073–9.
21. Emdin M, Gastaldelli A, Muscelli E et al. Hyperinsulinemia and autonomic nervous system dysfunction in obesity: effects of weight loss. *Circulation* 2001;**103**:513–9.
22. Karason K, Molgaard H, Wikstrand J et al. Heart rate variability in obesity and the effect of weight loss. *Am J Cardiol* 1999;**83**: 1242–7.
23. Chambers JC, Eda S, Bassett P et al. C-reactive protein, insulin resistance, central obesity, and coronary heart disease risk in Indian Asians from the United Kingdom compared with European whites. *Circulation* 2001;**104**:145–50.
24. Minami J, Ishimitsu T, Matsuoka H. Effects of smoking cessation on blood pressure and heart rate variability in habitual smokers. *Hypertension* 1999;**33**:586–90.
25. Danesh J, Muir J, Wong YK et al. Risk factors for coronary heart disease and acute-phase proteins. A population-based study. *Eur Heart J* 1999;**20**:954–9.
26. Singh JP, Larson MG, O'Donnell CJ et al. Association of hyperglycemia with reduced heart rate variability (The Framingham Heart Study). *Am J Cardiol* 2000;**86**:309–12.
27. Wu T, Dorn JP, Donahue RP et al. Associations of serum C-reactive protein with fasting insulin, glucose, and glycosylated hemoglobin: the Third National Health and Nutrition Examination Survey, 1988–1994. *Am J Epidemiol* 2002;**155**:65–71.
28. Irwin M. Immune correlates of depression. *Adv Exp Med Biol* 1999;**461**:1–24.
29. Carney RM, Blumenthal JA, Stein PK et al. Depression, heart rate variability, and acute myocardial infarction. *Circulation* 2001;**104**:2024–8.
30. Aronson D, Mittleman MA, Burger AJ. Interleukin-6 levels are inversely correlated with heart rate variability in patients with decompensated heart failure. *J Cardiovasc Electrophysiol* 2001;**12**:294–300.
31. Jensen-Urstad M, Jensen-Urstad K, Ericson M et al. Heart rate variability is related to leukocyte count in men and to blood lipoproteins in women in a healthy population of 35-year-old subjects. *J Int Med* 1998;**243**:33–40.
32. Maestroni GJ, Cosentino M, Marino F et al. Neural and endogenous catecholamines in the bone marrow. Circadian association of norepinephrine with hematopoiesis? *Exp Hematol* 1998;**26**:1172–7.
33. Maestroni GJ, Conti A. Noradrenergic modulation of lymphohematopoiesis. *Int J Immunopharmacol* 1994;**16**(2):117–22.
34. Maestroni GJ, Conti A. Modulation of hematopoiesis via alpha 1-adrenergic receptors on bone marrow cells. *Exp Hematol* 1994;**22**(3):313–20.
35. Kasahara K, Tanaka S, Hamashima Y. Suppressed immune response to T-cell dependent antigen in chemically sympathectomized mice. *Res Commun Chem Pathol Pharmacol* 1977;**18**:533–42.
36. Madden KS, Felten SY, Felten DL et al. Sympathetic nervous system modulation of the immune system. II. Induction of lymphocyte proliferation and migration in vivo by chemical sympathectomy. *J Neuroimmunol* 1994;**49**(1–2):67–75.
37. Jüttler E, Tarabin V, Schwaninger M. Interleukin-6: a possible neuromodulator induced by neuronal activity. *Neuroscientist* 2002;**8**:268–75.
38. Huang QH, Takaki A, Arimura A. Central noradrenergic system modulates plasma interleukin-6 production by peripheral interleukin-1. *Am J Physiol* 1999;**273**(2 Pt 2):R731–8.
39. Sigh JP, Larson MG, O'Donnell CJ et al. Heritability of heart rate variability. The Framingham Heart Study. *Circulation* 1999; **99**:2251–4.
40. Gaspardone A, Perino M, Ghini AS et al. Exercise induced myocardial ischaemia does not cause increase in C-reactive protein concentration. *Heart* 2000;**84**(6):668A–9A.