Published in final edited form as:

Brain Behav Immun. 2008 May ; 22(4): 461–468.

Relationship between Heart Rate Variability, Interleukin-6, and Soluble Tissue Factor in Healthy Subjects

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Abstract

Decreased heart rate variability (HRV) has been associated with an increased risk of atherosclerosis. We hypothesized that a decrease in frequency domains of resting HRV would be associated with elevated plasma levels of interleukin (IL)-6 and soluble tissue factor (sTF) both previously shown to prospectively predict atherothrombotic events in healthy subjects. Subjects were 102 healthy and unmedicated black and white middle-aged men and women. We determined IL-6 and sTF antigen in plasma and HRV measures from surface electrocardiogram data using spectral analysis. All statistical analyses controlled for age, gender, ethnicity, smoking status, blood pressure, and body mass index. Low amounts of low frequency (LF) power ($\beta=-0.31$, $p=0.007$) and high frequency (HF) power ($\beta=-0.36$, $p=0.002$) were associated with increased amounts of IL-6, explaining 7% and 9% of the variance, respectively. Interactions between LF power and IL-6 ($p=0.002$) and between HF power and IL-6 ($p=0.012$) explained 8% and 5%, respectively, of the variance in sTF. Post hoc analyses showed associations between IL-6 and sTF when LF power ($\beta=0.51$, $p<0.001$) and HF power ($\beta=0.48$, $p<0.001$) were low but not when LF power and high HF power were high. The findings suggest that systemic low-grade inflammatory activity is associated with a decrease in HRV. Furthermore, there was a positive relationship between plasma levels of IL-6 and sTF antigen when HRV was low. Inflammation and related hypercoagulability might particularly contribute to atherothrombotic events in a setting of decreased HRV.

Keywords

Cardiovascular disease; coagulation; cytokines; heart rate variability; inflammation; vagus nerve

Introduction

An imbalance in the autonomic nervous system (ANS) as reflected by reduced heart rate variability (HRV) has been associated with atherosclerotic diseases (Thayer and Lane, 2007). Decreased HRV predicted progression of coronary atherosclerosis (Huikuri et al., 1999), increased risk of acute coronary events (Tsuji et al., 1996), and death from coronary artery

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disease (CAD) (Tsuji et al., 1996). Atherosclerosis is predominately an inflammatory disease (Ross, 1999). Because the vagus nerve inhibits production of proinflammatory cytokines in peripheral tissues via a cholinergic anti-inflammatory pathway (Tracey, 2002), one mechanism that could link decreased HRV with atherosclerosis is by kindling low-grade systemic inflammation (Gidron et al., in press; Thayer and Lane, 2007). In accordance with this notion, HRV was inversely associated with circulating levels of the proinflammatory cytokine interleukin (IL)-6 in patients with chronic kidney disease (Psychari et al., 2005), CAD (Janszky et al., 2004), and decompensated chronic heart failure (Aronson et al., 2001). IL-6 plays a central role in the pathogenesis of CAD morbidity and mortality by promoting atherosclerotic plaque proliferation and instability, thereby ultimately increasing the risk of plaque rupture (Lobbes et al., 2006; Ridker et al., 2000). Elevated IL-6 levels predicted future myocardial infarction in healthy individuals (Ridker et al., 2000) and coronary mortality in patients with unstable angina (Koukkunen et al., 2001).

Inflammation and coagulation processes closely intertwine in the development of atherothrombotic diseases (Croce and Libby, 2007; Esmon, 2004). For instance, IL-6 has been associated with soluble tissue factor (sTF) in the circulation of patients with diabetes (Conway et al., 2004) and with chronic atrial fibrillation (Lim et al., 2004). IL-6 may influence the level of sTF by stimulating its release from endothelial cells into the circulation (Szotowski et al., 2005). Blood-borne or sTF is mainly associated with circulating procoagulant cellular microparticles (Eilertson and Osterud, 2004; Rauch and Nemerson, 2000). Soluble TF plays a major role in atherothrombosis (Giesen and Nemerson, 2000; Moons et al., 2002) by virtue of exerting procoagulant activity in the blood and thrombus propagation after rupture of an atherosclerotic plaque (Steffel et al., 2006). Accordingly, sTF was increased in patients with acute coronary syndromes (Misumi et al., 1998; Suefuji et al., 1997) and predicted poor prognosis in patients with unstable angina (Soejima et al., 1999) and the risk of future CAD in apparently healthy individuals (Keller et al., 2006).

This study had two aims. First, we sought to replicate in a sample of reasonably healthy subjects an inverse relationship between decreased HRV and IL-6 levels previously observed in patients with CAD (Janszky et al., 2004). We postulated that this relationship would be independent of a variety of atherosclerotic risk factors known to be associated with HRV. Specifically, decreased HRV has been associated with older age, male sex, hypertension, elevated blood glucose and cholesterol levels, smoking, physical inactivity, and overweight (Snieder et al., 2007; Thayer and Lane, 2007). Ethnic differences have also been found with white individuals generally showing lower HRV than their black counterparts (Liao et al., 1995; Wang et al., 2005). These associations could reflect that HRV is mostly driven by breathing induced changes in blood pressure (BP) where sympathetic tone and parasympathetic tone to the heart primarily reflect baroreflex driven variation in heart rate. In atherosclerotic vessels, even at pre-clinical stages, the baroreceptors do not stretch well and there is little input to the brain to drive the baroreflex such that HRV is relatively depressed (Nasr et al., 2005). Second, we hypothesized a direct relationship between IL-6 and sTF, which would be moderated by HRV. A moderator effect statistically defines under what circumstances two variables are associated with one another (Kraemer et al., 2002). We specifically hypothesized that a significant association between plasma levels of IL-6 and sTF antigen would exist when HRV is low (i.e. greater inflammatory activity and greater associated procoagulability in the presence of reduced vagal tone) but not when HRV is high. To our knowledge, these relationships have not been investigated in healthy populations.

Methods

Study participants

All subjects provided informed written consent to participate in a study on cardiovascular physiology. The study protocol was approved by the University of California San Diego (UCSD) Institutional Review Board. The study design intended to recruit a similar proportion of men and women and black and white subjects. Recruitment of subjects was by advertisement, by word-of-mouth, or by referral from local medical practitioners. Data in terms of demographic and metabolic characteristics, HRV measures and IL-6 and sTF levels were complete in 106 subjects allowing for a full linear regression approach. Four subjects were additionally excluded because their IL-6 and sTF levels were outliers even after log transformation (i.e. mean values $>3SD$ above or below the log transformed mean) such that the final sample reported here comprises 102 subjects.

In addition to major medical problems, the following conditions were specific exclusion criteria: congestive heart failure, symptomatic obstructive pulmonary, CAD, cerebrovascular disease, history of life-threatening arrhythmias, cardiomyopathy, severe asthma, diabetes, fasting glucose >120 mg/dl, cancer, liver or renal disease, creatinine >1.4 mg/dl, bruit on physical examination, known sleep disorders, history of psychosis, current drug or alcohol abuse. Women were excluded if they were postmenopausal, diagnosed with premenstrual syndrome, taking oral contraceptives, or pregnant. These exclusion criteria for women were designed to minimize hormonal influences on inflammation and coagulation. Many “healthy” subjects in industrialized countries have cardiovascular risk factors and, as a consequence, have silent atherosclerosis of their blood vessels or rigid blood vessels. No attempt was made to exclude such subjects with e.g. high normal blood pressure (BP), obesity, and smoking.

Demographic factors

Ethnicity was determined via self-report. Subjects who currently smoked ≥ 1 cigarette per day were termed smokers and all others were termed non-smokers. Resting systolic/diastolic BP was required to be $<180/110$ mm Hg at screening. Screening BP was defined as the average of three seated readings. Mean arterial BP (MAP) was used for statistical analyses. No subjects took any medications on a regular basis. Body mass index (BMI) was computed as the ratio of body weight in kilograms divided by the square of height in metres (kg/m^2). All subjects with $\text{BMI} \geq 40 \text{ kg}/\text{m}^2$ (i.e. those with morbid obesity) were excluded from the present study.

Heart rate variability

Subjects were tested between 9:00 am and 11:00 am. They were equipped with an electrocardiogram (ECG) (model 78352C; Hewlett-Packard, Andover, MA) that relayed ECG data to an analogue-to-digital converter (DT2801; Data Translation Inc., Marlboro, MA) sampling at 1 kHz. After they sat quietly for 30 minutes, 3-min data were collected during resting seated baseline condition using Global Lab software (Data Translation). Data were stored in a computer for subsequent review, artifact rejection, and calculation. A previously developed program was used to perform review and calculation of data (Nagel et al., 1993). Spectral power analyses were performed in accordance with previously published standards yielding the two frequency domain measures low frequency (LF) power (0.04–0.15 Hz) and high frequency (HF) power (>0.15 Hz) of HRV (Task Force, 1996). Power frequency (Hz) was converted to msec^2 by fast fourier transformation applying customized software (Nagel et al., 1993). Subjects were excluded from the analysis a priori if values were $\geq 40 \text{ msec}^2$ for LF power and $\geq 25 \text{ msec}^2$ for HF power because both values indicated clinical outliers to our subject population. We also calculated the ratio of LF to HF power.

Biochemical measures

Fasting venous blood samples were drawn at 6:00 am and preserved with 3.8% sodium citrate (ratio 9:1) for the analysis of sTF or with EDTA for the analysis of IL-6. Samples were spun in a refrigerated centrifuge. Obtained plasma was immediately frozen in polypropylene tubes at -80°C until further analyzed. Plasma levels of sTF antigen (Imubind Tissue Factor, American Diagnostica, Stamford, CT) and of high-sensitive IL-6 (Quantikine, R&D Systems, Minneapolis, MN) were determined by commercial enzyme-linked immunosorbent assays. The sTF antigen assay recognizes Apo-TF, TF, and TF/factor VII complexes and is not interfered with by other coagulation factors or inhibitors of procoagulant activity. All inter- and intra-assay coefficients of variation were $<10\%$.

Statistical analyses

Data were analyzed using SPSS 13.0 for Windows (SPSS Inc., Chicago, IL) and are presented as means $\pm$ SE or as percentages in tables and as geometric means $\pm$ SE in the text. The level of significance was set at $p\leq 0.05$ (two-tailed). Before analyses, raw values of all variables were examined for deviations from normality by the Kolmogorov-Smirnov test. The distribution of values for BMI, the three HRV measures, IL-6, and sTF deviated from normality and were log-transformed yielding normal distributions. All analyses were performed using the log transformed data. Pearson correlation coefficients were estimated to compute bivariate relationships between variables.

We computed multiple linear regression analyses using forced entry to investigate whether a) HRV measures would predict IL-6 levels, and b) whether the interaction between HRV measures and IL-6 levels would predict sTF antigen levels, independent of demographic covariates. In order to reduce problems with multicollinearity, all independent variables were centered to the mean, and centered values of HRV measures and of IL-6 levels were multiplied to obtain interaction terms (Kraemer et al., 2002). A significant interaction would mean that the slope of the relationship between IL-6 and sTF is different with high HRV (mean centered HRV measure minus one SD) compared to low HRV (mean centered HRV measure plus one SD) (Kraemer et al., 2002). Holmbeck's method was applied for post-hoc analyses on interaction terms to determine the significance of the relationship between IL-6 and sTF for high and low HRV (Holmbeck, 2002). This test particularly allows an assessment of significance within groups (e.g. in a group with lower levels of HRV vs. in a group with higher levels of HRV) and is not solely an indication of the presence or absence of statistical interaction. Leverage statistics identified no cases which influenced these regression models more than others.

We also computed effect size measure (Cohen's f^2) for the main and interaction effects in regression analysis. By convention, f^2 effect sizes of 0.02, 0.15, and 0.35 are considered small, medium, and large, respectively (Cohen, 1988). We did not correct the p-values by the number of statistical tests performed because the three HRV measures differ in their indicative value of autonomic function (Task Force, 1996). Moreover, the two hypotheses tested, namely that HRV predicts IL-6 and that the level of HRV moderates the relationship between IL-6 and sTF antigen are conceptually different. In this case of specific pre-established hypotheses, some authors do not recommend statistical adjustment for multiple tests as this might render truly important associations insignificant (Perneger, 1998).

Results

Subject characteristics

Table 1 shows the distribution of demographic and medical factors as well as of HRV measures and plasma levels of IL-6 and sTF in the 102 subjects studied. The mean plasma concentrations

of IL-6 and sTF antigen were similar to those measured in other healthy populations applying the same assays (Naumnik et al., 2003; Ridker et al., 2000). Also, mean levels of HRV measures were largely identical with previously obtained results in similarly healthy subjects (Minami et al., 1999).

Bivariate relationships with heart rate variability measures

There was a direct correlation between LF and HF components of HRV ($r=0.57$, $p<0.001$). Table 2 shows that HF power correlated inversely with IL-6 ($p<0.001$) and sTF ($p=0.025$). Also, LF power showed an inverse association with IL-6 ($p<0.001$) and the LF/HF power ratio was directly related to sTF ($p=0.034$). There were also several significant relationships between the HRV measures and virtually all subject characteristics. The directions of the bivariate relationships suggested that a decrease in LF power and/or in HF power were associated with older age, black ethnicity, current smoking, higher MAP, and higher BMI. In addition, the sympathovagal balance (i.e. sympathetic preponderance) was greater in men than in women and in whites than in blacks, respectively. Altogether, these significant bivariate relationships justified adjustment for age, gender, ethnicity, smoking, MBP, and BMI in the subsequent regression equations allowing us to test for an independent relationship between HRV and IL-6 on the one hand and between HRV and IL-6 in determining sTF on the other.

Heart rate variability predicting interleukin-6

In linear regression analyses, we controlled for age, gender, ethnicity, smoking status, MAP and BMI. After these covariates had been entered, LF power independently predicted 7% of the variance in IL-6 (Table 3a) with this association showing a small-to-medium effect size ($f^2=0.07$). HF power was also an independent predictor of IL-6 explaining 9% of the variance in IL-6 (Table 3b) with a small-to-medium effect size ($f^2=0.09$). IL-6 was not independently predicted by the LF/HF power ratio ($p=0.82$).

Smoking status also emerged as an independent predictor of plasma IL-6 levels. As compared to non-smokers, current smokers had significantly higher levels of IL-6 in the model with LF power (Table 3a) and, with borderline significance, in the model with HF power (Table 3b).

Interaction between heart rate variability and interleukin-6 predicting tissue factor

In separate models, we regressed each HRV measure, IL-6 levels, and their interaction on levels of sTF antigen after controlling for age, gender, ethnicity, smoking status, MAP, and BMI. There was a significant interaction between LF power and IL-6 (Table 4a) and between HF power and IL-6 (Table 4b) explaining 8% ($f^2=0.08$) and 5% ($f^2=0.06$), respectively, of the variance in sTF. The interaction between LF/HF power ratio and IL-6 was not significant ($p=0.35$).

These interactions suggested that the slopes of the relationship between IL-6 and sTF would be different with different levels of LF power and HF power. Post hoc analyses controlled for age, gender, ethnicity, smoking status, MAP, and BMI. They revealed a positive association between IL-6 and sTF when LF power was low ($\beta=0.51$, $p<0.001$; Figure 1a) but not when LF power was high ($\beta=0.03$, $p=0.80$; Figure 1b). Also, when HF power was low, there was a positive association between IL-6 and sTF ($\beta=0.48$, $p<0.001$; Figure 2a) that was not observed when HF power was high ($\beta=0.02$, $p=0.86$; Figure 2b).

Ethnicity emerged as a significant predictor of sTF with whites having higher geometric mean $\pm$ SE levels of sTF than blacks in the models with LF power (184.1 ± 11.8 vs. 147.6 ± 10.9 pg/mL; $p=0.030$) and HF power (185.8 ± 11.9 vs. 146.2 ± 11.2 pg/mL; $p=0.021$). In a complementary analysis, we regressed the interaction between HRV measures and IL-6 on sTF in the subsamples of black and white subjects to explore whether the interaction would be

similar in the two ethnic groups. The interaction between LF power and IL-6 for sTF was significant in blacks ($\beta=-0.44$, $p=0.025$) and whites ($\beta=-0.30$, $p=0.034$). The interaction between HF power and IL-6 for sTF showed borderline significance in blacks ($\beta=-0.34$, $p=0.071$) but was not significant in whites ($\beta=-0.12$, $p=0.39$). Also, higher MBP significantly predicted higher sTF levels in the model with HF power (Table 4b) and, with borderline significance, in the model with LF power (Table 4a).

Discussion

The HRV literature suggests that one issue associated with the use of HRV as a research tool with regard to cardiovascular disease is that there are a number of variability indexes, and it currently remains unclear which measures may be the best (Task Force, 1996; Thayer and Lane, 2007). We selected three HRV indexes for which there is general consent with regard to their autonomic meaning. The HF component primarily reflects the variability of vagal outflow (i.e. parasympathetic activity) to the heart, whereas the LF component refers to both sympathetic and parasympathetic influences. However, LF power often contains a substantial amount of parasympathetic influence. This may explain why HF power and LF power are frequently directly correlated with each other (such as seen in our subjects) and why decreased levels in HF power and LF power both predict cardiovascular outcome in the same direction (Thayer and Lane, 2007). The LF to HF power ratio quantifies the sympathovagal balance with relatively higher ratios indicating relatively more sympathetic than parasympathetic modulation of the heart rhythm (Task Force, 1996).

We found in a middle-aged sample of reasonably healthy subjects that LF power and HF power were both inversely associated with plasma IL-6 concentration independent of covariates. This finding is in line with previous studies showing that decreased HRV was associated with elevated IL-6 levels in patient populations (Aronson et al., 2001; Janszky et al., 2004; Psychari et al., 2005). Particularly, the bivariate association between LF power and IL-6 levels ($r=-0.35$ vs. $r=-0.27$) and between HF power and IL-6 levels ($r=-0.39$ vs. $r=-0.16$) seemed somewhat stronger in our healthy subjects than in a previous study on women with CAD (Janszky et al., 2004). This suggests that the interplay between depressed HRV and inflammation could be important across a range of preclinical and clinical disease states (Thayer and Lane, 2007).

The association between reduced HF power and increased IL-6 suggests that plasma IL-6 levels could be under tonic vagal control because HF power predominantly denotes parasympathetic activity (Task Force, 1996). This reasoning seems intriguing because it is in agreement with the recently proposed cholinergic anti-inflammatory pathway as mediated by the vagus nerve postulating that vagal withdrawal disinhibits suppression of proinflammatory cytokine production in peripheral tissue (Tracey, 2002). The observation that decreased LF power was also associated with elevated IL-6 could be expected given that the LF and HF component of HRV were so highly correlated. We may assume that parasympathetic modulation of the LF component was substantial because we investigated our subjects at rest when cardiac autonomic balance favors energy conservation through parasympathetic dominance over sympathetic influences (Thayer and Lane, 2007). Therefore, in the context of our analysis, LF power and HF power may reflect similar than different aspects of HRV. The sympathovagal balance – as expressed by the LF to HF power ratio – was unrelated to IL-6 levels. We may speculate that, under resting conditions, the parasympathetic influence on cardiac autonomic control and related HRV indexes (i.e. LF power and HF power) was more important as a predictor of plasma IL-6 levels than sympathetic modulation as reflected by the LF to HF ratio. On the whole, the finding of an inverse relationship between IL-6 and HF power and LF power concurs with previous studies in which decreases in both HF and LF power predicted atherosclerosis progression, incident CAD, and cardiac mortality after myocardial infarction (Bigger et al., 1992; Huikuri et al., 1999; Liao et al., 2002; Tsuji et al., 1996).

Plasma IL-6 levels were significantly associated with sTF antigen levels independent of covariates, corroborating similar findings from patient populations (Conway et al., 2004; Lim et al., 2004). This observation was also in agreement with previous observations on inflammation processes interacting with thrombogenic changes in general (Croce and Libby, 2007; Esmon, 2004) and with the effect of IL-6 on release of sTF from endothelial cells in particular (Szotowski et al., 2005). However, our finding that HRV was a moderator of the association between IL-6 and sTF antigen levels is novel. IL-6 and sTF were only directly related to one other when HRV was low but not when HRV was high. Again, this was observed for both HF power and LF power but not for the LF to HF power ratio. We therefore propose that the direct relationship between levels of IL-6 and sTF is particularly relevant in a setting of decreased HRV.

Atherosclerotic risk factors related to demographics (age, gender, ethnicity) and metabolic disturbance (MBP, BMI) were variably associated with the HRV measures in similar directions as previously observed such that decreased HRV was generally associated with greater atherosclerotic risk (Snieder et al., 2007; Thayer and Lane, 2007). This may suggest that atherosclerotic risk factors should be controlled for in analyses investigating an independent relationship between HRV and inflammation, even in healthy populations. This seems particularly important in a context of decreased baroreflex sensitivity with greater atherosclerosis lowering HRV (Nasr et al., 2005). Regression analyses yielded smoking status as an independent predictor of IL-6 and MAP as an independent predictor of sTF. Both these associations concur with previous studies (Ridker et al., 2000; Sommeijer et al., 2006). In terms of ethnicity, white subjects had higher sTF antigen levels than blacks. To the best of our knowledge, this finding has previously not been reported and seems contradictory to the much higher CAD risk in African Americans than in whites (Clark, 2005). In contrast and compatible with their increased cardiovascular risk, blacks had lower levels of LF power than whites (Task Force, 1996). However, the moderating effect of LF power for the relationship between IL-6 and sTF was quite similar in blacks and whites. The respective moderating effect of HF power seemed somewhat stronger in whites than in blacks, although it did not reach statistical significance in both groups. Parsimoniously interpreted, our data do not provide a strong argument for ethnic differences in vagal function as a moderator of the association between inflammation and coagulation activity in the circulation.

Under steady state conditions, even in reasonably healthy subjects, decreased HRV in both the LF and HF spectra could reflect a low-grade inflammatory state and related procoagulant activity. Such a mechanism could provide a unifying theory for why decreased HRV and elevated circulating levels of both IL-6 and TF may all predict atherothrombotic events. With regard to the potential clinical consequences of such a theory, it has been shown that decreased HRV and vagal tone are favourably restored by physical exercise and other life style modifications, including stress management (Blumenthal et al., 2005; Thayer and Lane, 2007; Tracey, 2007). It therefore seems warranted to investigate whether increasing HRV by way of behavioural interventions may alter inflammation and associated coagulation processes and ultimately cardiovascular outcome (Edwards et al., in press).

Our study has several limitations. First, its cross-sectional nature does not allow us making an inference on a causal mechanism leading from decreased HRV to elevated IL-6, and from elevated IL-6 to an increase in sTF. Second, we did not control for psychosocial stressors and related changes in hypothalamic-pituitary adrenal (HPA) axis function, all of which could account for some of the associations observed in our study. Psychosocial factors have been associated with reduced HRV in several studies (Hemingway et al., 2001) and generally healthy individuals with low cortisol responsivity showed less flexibility in HRV with acute mental stress compared to cortisol responders (Kunz-Ebrecht et al., 2003). Acute and chronic stressors particularly increase IL-6 and endogenous cortisol surge may dampen this stress-induced IL-6

increase (Kunz-Ebrecht et al., 2003; Ranjit et al., 2007; von Känel et al., 2006). In turn, low HRV by its own right may lead to HPA axis dysregulation or sympathetic hyperactivity both favoring proinflammatory cytokine production (Thayer and Sternberg, 2006). Third, our assay to determine sTF antigen levels did not specifically assess for a recently detected alternatively spliced form of sTF in plasma that exerts particular prothrombotic potential (Bogdanov et al., 2003). However, previous studies showing that sTF antigen levels were related to CAD risk also employed earlier assay techniques (Misumi et al., 1998; Soejima et al., 1999; Suefuji et al., 1997). There was a time lag of three to five hours between the blood draw and assessment of HRV. Given the diurnal variation in cytokines, this could also have impacted on the results. Fourth, our subjects were reasonably healthy and, therefore, it is unclear whether the relationship between IL-6 and sTF is similarly moderated by HRV in CAD populations. Also, exclusion criteria for women were rather rigorous, thereby limiting inference from our data to the general population of women. For instance, our men and women did not differ in IL-6 levels independent of covariates, whereas recent data suggests that greater IL-6 expression in women than men could be explained by gender differences in HRV (O'Connor et al., 2007). Fifth, although significant with our ample sample size, effect sizes of relationships between HRV, IL-6, and sTF were at best medium. Hence, it remains to be seen whether these associations have prognostic value in terms of future acute coronary syndromes (i.e. unstable angina and myocardial infarction) and death from CAD in populations with and without established CAD.

Taken together, we showed that decreased HRV is independently associated with elevated plasma IL-6 levels in reasonably healthy subjects as has been shown in patient populations (Aronson et al., 2001; Janszky et al., 2004; Psychari et al., 2005). We further found an independent relationship between plasma levels of IL-6 and sTF which have previously predicted an increased risk of future atherothrombotic events in apparently healthy individuals (Keller et al., 2006; Ridker et al., 2000). Most intriguingly, the relationship between elevated IL-6 and sTF was only significant when HRV was lowered but not when it was increased.

Acknowledgements

This study was financially supported by grants HL36005 and RR00827 from the National Institutes of Health.

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Figure 1. Relationship between IL-6 and sTF at different levels of heart rate variability

The partial regression plots depict the independent relationship with fit line between plasma levels of interleukin (IL-6) and soluble tissue factor (sTF) in the four groups of subjects as categorized by different levels of heart rate variability. There were significant relationships between IL-6 and sTF in the group with lower levels of low frequency power (Panel A) and in the group with lower levels of high frequency power (Panel C). IL-6 and sTF were not significantly related to each other in the group with higher levels of low frequency power (Panel B) and in the group with higher levels of high frequency power (Panel D). Adjustment was made for age, gender, ethnicity, smoking status, mean arterial blood pressure, and body mass index. Note the identical scaling of the x- and y-axes. All variables in the partial regression plots are residuals.

Table 1**Characteristics of the 102 subjects studied**

Age [years]	36.1 ± 0.8 (23 – 52)
Gender [men / women]	50% / 50%
Ethnicity [black / white]	43% / 57 %
Current smoker [yes / no]	14% / 86 %
Mean arterial blood pressure [mmHg]	91.5 ± 1.1 (69.4 – 119.6)
Systolic blood pressure [mmHg]	125.2 ± 1.5 (96.0 – 168.7)
Diastolic blood pressure [mmHg]	74.7 ± 1.0 (53.7 – 100.0)
Body mass index [kg/m ²]	26.3 ± 0.5 (18.0 – 39.8)
Interleukin-6 [pg/mL]	1.4 ± 0.1 (0.1 – 9.0)
Soluble tissue factor antigen [pg/mL]	192.0 ± 11.2 (49.3 – 648.0)
Low frequency power [msec ²]	6.1 ± 0.5 (0.4 – 35.4)
High frequency power [msec ²]	4.5 ± 0.4 (0.3 – 16.1)
Low / high frequency power ratio	1.8 ± 0.1 (0.1 – 6.5)

Values are given as means ± SE with range or percentages

Table 2
Correlation matrix for heart rate variability measures

	LF power	HF power	LF / HF power ratio
Age	-0.32 ^c	-0.46 ^c	0.16
Gender	-0.10	0.15	-0.27 ^b
Ethnicity	-0.32 ^c	-0.13	-0.20 ^a
Smoking	-0.09	-0.20 ^a	0.12
Mean arterial blood pressure	-0.23 ^a	-0.31 ^b	0.09
Body mass index	-0.40 ^c	-0.44 ^c	0.05
Interleukin-6	-0.35 ^c	-0.39 ^c	0.05
Soluble tissue factor antigen	-0.03	-0.22 ^a	0.21 ^a

The columns show the Pearson correlation coefficients (r) between subjects' characteristics and heart rate variability measures.

^aSignificance levels of correlations: $p \leq 0.05$

^bSignificance levels of correlations: $p \leq 0.01$

^cSignificance levels of correlations: $p \leq 0.001$

Coding was -0.5 (men) or +0.5 (women) for gender; -0.5 (white) or +0.5 (black) for ethnicity; and -0.5 (not smoking) or +0.5 (smoking) for current smoking status

LF, low frequency; HF, high frequency

Table 3

Table 3a. Regression model for low frequency power predicting interleukin-6

Table 3b. Regression model for high frequency power predicting interleukin-6

Variables entered	Standardized β -coefficient	<i>P</i> -value	Delta R^2
Age	0.03	0.798	0.022
Gender	-0.15	0.160	0.001
Ethnicity	0.09	0.369	0.030
Smoking status	0.20	0.049	0.026
Mean arterial blood pressure	-0.20	0.087	0.012
Body mass index	0.12	0.253	0.038
Low frequency power	-0.31	0.007	0.066

Variables entered	Standardized β -coefficient	<i>P</i> -value	Delta R^2
Age	-0.03	0.783	0.022
Gender	-0.08	0.450	0.001
Ethnicity	0.15	0.135	0.030
Smoking status	0.17	0.087	0.026
Mean arterial blood pressure	-0.18	0.119	0.012
Body mass index	0.08	0.469	0.038
High frequency power	-0.36	0.002	0.085

The entire model accounted for 19.5% of the IL-6 variance ($F=3.26$, $df=7,94$, $P=0.004$)

The entire model accounted for 21.4% of the IL-6 variance ($F=3.66$, $df=7,94$, $P=0.002$)

Table 4

Table 4a. Regression model for LF power and IL-6 predicting soluble tissue factor

Table 4b. Regression model for HF power and IL-6 predicting soluble tissue factor

Variables entered	Standardized β -coefficient	<i>P</i> -value	Delta R^2
Age	0.14	0.187	0.055
Gender	-0.15	0.142	0.055
Ethnicity	-0.22	0.030	0.020
Smoking status	-0.08	0.399	<0.001
Mean arterial blood pressure	0.22	0.058	0.014
Body mass index	0.07	0.509	0.011
Interleukin-6	0.27	0.007	0.061
Low frequency power	0.13	0.233	0.008
LF power X IL-6 interaction	-0.28	0.002	0.075

Variables entered	Standardized β -coefficient	<i>P</i> -value	Delta R^2
Age	0.11	0.336	0.055
Gender	-0.15	0.145	0.055
Ethnicity	-0.23	0.021	0.020
Smoking status	-0.12	0.218	<0.001
Mean arterial blood pressure	0.25	0.039	0.014
Body mass index	0.03	0.803	0.011
Interleukin-6	0.25	0.014	0.061
High frequency power	0.03	0.834	<.001
HF power X IL-6 interaction	-0.25	0.012	0.053

The entire model accounted for 29.7% of the sTF variance ($F=4.32$, $df=9,92$, $P<0.001$)The entire model accounted for 26.8% of the sTF variance ($F=3.74$, $df=9,92$, $P<0.001$)