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Low vagal tone is associated with impaired post stress recovery of cardiovascular, endocrine, and immune markers

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Abstract Reduced heart rate variability (HRV) and delayed blood pressure recovery are associated with increased cardiovascular risk. Besides this evident link, the vagus is thought to play an inhibitory role in the regulation of other allostatic systems, including inflammation and the hypothalamic–pituitary–adrenal (HPA) axis. However, human evidence is scarce. To further explore these associations and with special regard to the postulated mediating role of the vagus, we hypothesised that subjects with low vagal tone as indexed by reduced resting HRV would show impaired post-stress recovery of cardiovascular, endocrine and immune system markers involved in cardiovascular pathology. 44 healthy men underwent a standardised mental stress test. Besides continuous measurement of systolic and diastolic blood pressure (SBP, DBP), heart rate (HR), and HRV serum cortisol, tumour necrosis factor-alpha (TNF-alpha), and interleukin-6 (IL-6) were measured before, after, 20, and 60 min after stress. Low versus high HRV groups was defined by median split on resting HRV (RMSSD). The task elicited significant time effects for SBP, DBP, HR, HRV, cortisol, and TNF-alpha. Subjects with low baseline HRV showed almost no modulation of HRV coupled with overall reduced HRV levels, and impaired recovery of DBP, cortisol, and TNF-alpha. Confirming our hypothesis, low vagal tone was associated with impaired recovery of cardiovascular, endocrine, and immune markers in healthy males. The data support an inhibitory role of the vagus in the regulation of allostatic systems as described in the neurovisceral integration model. We posit reduced resting HRV as a risk marker for future cardiovascular and other stress-related disease.

Keywords Heart rate variability · Stress · Recovery · Blood pressure · Cortisol · TNF-alpha

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Introduction

Heart rate variability (HRV) is a non-invasive tool to measure autonomic nervous system activity of the heart by power spectral analysis of the heart rate (HR) time series (Akselrod et al. 1981). Autonomic imbalance implying sympathetic overactivity and parasympathetic underactivity play a

central role in the development and maintenance of essential hypertension (Brook and Julius 2003). Decreased HRV is associated with increased risk of hypertension (Liao et al. 1996), coronary artery sclerosis (Huikuri et al. 1999), acute cardiac events (Tsuji et al. 1996), death from coronary artery disease (Tsuji et al. 1996) and all-cause mortality (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology 1996).

Moreover, decreased vagal activity is thought to be linked to other systems associated with physiological regulation and allostasis such as the hypothalamic–pituitary–adrenal cortex (HPA) axis and inflammation (Thayer and Sternberg 2006). Chronic allostatic load, a term for the burden of chronic stress and accompanying changes in personal behaviours, may lead to disease in the long run mediated via autonomic, neuroendocrine, immune, or central nervous system activity (McEwen 1998). According to the allostatic load model, healthy organisms are able to mount an appropriate response to stressors but return to basal levels after the stressor has ended. Those organisms that show continued stress responses after the stressor is over are at increased risk for poor health outcomes. Thus, besides excess cortisol and pro-inflammatory cytokines, delayed post-stress recovery may be one manifestation and act as a mediating link between stress and cardiovascular disease (Brosschot et al. 2005). For example, delayed recovery of blood pressure and HRV following brief laboratory-induced psychosocial stress predicted 3-year increases in blood pressure (Steptoe and Marmot 2005, 2006).

Cortisol, the key stress hormone of the hypothalamus–pituitary–adrenal cortex (HPA) axis, interacts with central, sympathetic, and renal mechanisms and plays a potent role in blood pressure regulation. Literature suggests that persons at risk for the development of hypertension, e.g. borderline hypertensives, salt-sensitive males, and those with a family history of hypertension, show elevated levels of plasma cortisol (al'Absi et al. 1994; al'Absi and Arnett 2000; Walker et al. 1998; Watt et al. 1992; Weber et al. 2008). Furthermore, an inverse relationship between cortisol and HRV was observed in healthy adults (Thayer et al. 2006) which could reflect the inhibitory influence of the prefrontal cortex (PFC) on the amygdala reported in both animal and human studies (Thayer 2006; Thayer and Brosschot 2005). Elevated levels of cortisol, e.g. induced by stress, were postulated to disturb the functional connectivity between the PFC and the amygdala via altered serotonin levels. The inhibitory control that the PFC exerts on the amygdala is thought to be at least partially vagally mediated and thus is consistent with a neurovisceral integration model which stresses the importance of the parasympathetic nervous system in providing negative feedback on sympathoexcitatory stress responses (Thayer and Sternberg 2006; Thayer and Lane 2009).

Besides the autonomic nervous system and the HPA axis, inflammatory processes are involved in the pathogenesis and maintenance of vascular pathology (Ross 1999; Packard and Libby 2008). Elevated blood levels of pro-inflammatory cytokines such as tumour necrosis factor alpha (TNF-alpha) (Ridker et al. 2000a) and interleukin-6 (IL-6) (Ridker et al. 2000b) are associated with an increased risk of acute cardiovascular events.

Importantly, there is recent evidence that the parasympathetic system inhibits the production of pro-inflammatory cytokines by activated monocytes/macrophages and thus decreases local and systemic inflammation (Marsland et al. 2007; Sloan et al. 2007; Thayer and Fischer 2009), as predicted by the "cholinergic anti-inflammatory pathway" (Tracey 2002). Afferent vagal neurons are thought to relay sensory information regarding early inflammatory activity to the brain, resulting in the activation of efferent vagal fibres leading to the suppression of pro-inflammatory cytokine release. However, whereas there is convergent evidence from animal studies (Bernik et al. 2002; Borovikova et al. 2000; Wang et al. 2003) clinical evidence in humans is just starting to become available, e.g. higher HRV-derived estimates of vagal activity were associated with lower production of TNF-alpha and IL-6 in a mixed sample of healthy adults (Marsland et al. 2007), as well as in women with coronary heart disease both time and frequency domain indices of HRV were negatively correlated with IL-6 levels (Janszky et al. 2004).

To date, no studies have examined the time course of stress responses in cardiovascular, endocrine, and immune responses simultaneously in individuals stratified by risk using resting HRV. Therefore, in the context of the above-outlined associations of the autonomic nervous system, HPA axis, and immune system and with special regard to the postulate of a mediating role of the vagus, the present study investigated acute stress and recovery effects of cardiovascular, endocrine, and immune system markers in healthy males. We hypothesised that subjects with low resting vagal tone as indexed by reduced resting HRV would show impaired post-stress recovery of diastolic blood pressure (DBP), cortisol, and the pro-inflammatory cytokines TNF-alpha and IL-6.

Methods

Study population

Subjects were recruited by advertisement on the university and clinic campus. Recruitment and assessments took place over a period of 10 months (between January and October

2006). Inclusion criteria were 20–50 years of age, male, healthy namely absence of hypertension, allergies, asthma, and other acute or chronic conditions, no psychiatric illness or other psychopathology in the past or at present, normal weight (body mass index (BMI) >18.5 and <30 kg/m^2), and no regular medication. Subjects were examined by an internist to verify the above inclusion criteria. Further, a complete blood count was performed on each subject on the day of the experiment prior to the stress task to ensure absence of acute or chronic inflammation or any other abnormalities such as a dysbalance in the hydration status.

Procedure

Subjects were invited individually for psychophysiological testing in the lab at 09:00. They were instructed to go to bed not later than 23:30 the night before, to get up not later than 07:30, to abstain from caffeinated, alcoholic, or any other pharmaceutical substances the night before, and to have a usual light breakfast with no more than two cups of coffee or black tea on the morning of the appointment.

Subjects were seated comfortably in a high-backed chair in a quiet room with an ambient temperature of 20–24°C. After the first instructions, an 18-G cannula was inserted in a right forearm vein. They then had 30 min time to fill out standardised psychological questionnaires on perceived stress (Perceived Stress Questionnaire by Levenstein et al. 1993, German version by Fliege et al. 2005), anger (STAXI by Spielberger 1999, German version by Schwenkmezger and Hodapp 1991), and anxiety (STAI by Spielberger 1983, German adaptation by Laux et al. 1981) (data not shown, Weber et al. in preparation) and get accustomed to the environment.

Thereafter, electrodes and transducers for detection of ECG and blood pressure were attached to the chest, non-dominant arm, and hand. SBP and DBP were recorded continuously using the Finapres device (Ohmeda 2300, Louisville, CO, USA) attached to the middle digit of the nondominant hand. HR was continuously measured by electrocardiography.

The physiological assessment started with a 5-min resting phase (R1). After R1, an initial blood sample was taken ("before stress"). Thereafter, the stress test began with the manometer test, with a duration of circa 8 min, of which only the first 5 min were used for the analyses, followed by a 5-min mental arithmetic task (both detailed below). Immediately after, the second blood sample was taken ("after stress"), followed by a 5-min recovery phase termed resting phase 2 (R2). 20 and 60 min after stress, third and fourth blood samples were taken. In the recovery period, subjects remained seated, completed post-stress questionnaires (not shown, see above), and were allowed to read a light entertaining magazine (comic). Blood samples

were centrifuged immediately, aliquoted, and kept frozen at −80°C until assayed. Subjects were informed of the experimental risks and signed an informed consent document prior to the investigation. The study protocol including the process of obtaining informed consent was approved by the local ethics committee.

Mental stress test

Manometer test

The manometer test is a well-established standardised computer-based information processing task performed under time pressure (Buchholz et al. 2003; Deter et al. 2001; Weber et al. 2007, 2008; Zimmermann-Viehoff et al. 2008). All tests and instructions were given on a computer monitor. Subjects were presented with an increasing number of three to eleven clocks and had to rapidly decide whether one or more hands of the clocks deviated by more than 90° from the direction of an arrow shown on the top of the screen. Responses were performed by manipulating a mouse with the dominant arm. Wrong answers were indicated by a loud acoustic signal. During the course of the test, the time allowed for the decision was subsequently shortened by 30% until the participant made a mistake. After a mistake, the time allowed for the decision was extended by 30%. This algorithm maintained an individually adapted workload.

Mental arithmetic test

Subjects were then presented via computer with an increasingly difficult series of mental calculations involving addition, subtraction, and division with feedback on their performance (provided by Dr. Immo Curio, Bonn, FRG).

Cardiovascular data analyses

All cardiovascular signals were continuously recorded at 1,000 Hz with DOS-Software (Turbolab, Stemmer, Puchheim, FRG). The electrocardiographic and finger blood pressure curves (recorded with the Finapres) were analysed off-line under visual artefact control by ALYS software (version 1.18, T. Sudhop and H. Schaechinger, Basel, Switzerland). The Finapres has shown good accuracy, reproducibility, and validity in a wide range of situations (Imholz et al. 1993). However, it is known to overestimate absolute blood pressure levels. As we are interested in changes over time this is not a concern for the present investigation.

Continuous monitoring of cardiovascular parameters began with R1, followed by manometer test, mental

arithmetic test, and R2. For each of the 5-min phases, means of SBP, DBP, HR, and HRV were computed to be used for the statistics. Therefore, cardiovascular parameters reflect an integrated value over a complete measurement period.

Heart rate variability

Power spectral density of HR was analysed by Fourier transformation according to published standards (Akselrod et al. 1981; Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology 1996). We chose the root mean square of the successive differences (RMSSD, in ms) in R-R intervals as an index of vagally mediated HRV. In the present sample, RMSSD was significantly correlated with the high-frequency band (0.15–0.40 Hz) of the spectral-derived HRV measure ($r = 0.90$, $P < 0.0001$).

Biochemical analyses

Cortisol

Serum cortisol was measured using a solid-phase ^{125}I radioimmunoassay (Coat-A-Count® Cortisol RIA, DPC Biermann GmbH, Bad Nauheim, Germany) counted in a 1277 Gammamaster Automatic Gamma Counter (Perkin Elmer, Rodgau, Germany). The intraassay coefficient of variation of this RIA was 5.1% or less in seven trials ($n = 20$, mean concentration 85.5–938 nmol/L), and the interassay coefficient of variation was 6.4% or less in 3 trials ($n = 20$, mean concentration 91.0–993 nmol/L). All samples were measured in duplicate.

TNF-alpha and IL-6

The cytokines were measured using a high-sensitive solid-phase ELISA (High-Sensitive Tumor Necrosis Factor alpha Human Biotrak and High-Sensitive Interleukin 6 Human Biotrak, Amersham Biosciences Europe GmbH, Freiburg, Germany). Optical densities were read on a standard micro-titer plate reader (Dynex MRX, Dynex Technologies, Berlin, Germany). All samples were measured in duplicate. TNF-alpha standards are calibrated to WHO reference lot 87/650, whereas 1 pg of assay standard refers to 3 pg of WHO standard (~ 0.12 WHO units). IL-6 standards are calibrated to the NIBSC reference lot 89/548, whereas 1 pg of assay standard refers to 3 pg of NIBSC standard (0.3 NIBSC units). Within-assay precision and between-assay precision were <10% in both assays. The detection limit for both assays is denoted by the manufacturer to be 0.1 pg/ml.

Statistical analyses

Data were analysed by the SPSS statistical software package (SPSS Inc., Chicago, Illinois, USA). Complete data sets with all four time points were available for $n = 43$ subjects for the cardiovascular data, $n = 42$ for cortisol, and $n = 33$ for the cytokines. Missing data were due to technical errors.

For the cardiovascular variables a two groups (high versus low resting HRV) by four time periods (R1, manometer, mental arithmetic, R2) ANOVA was performed to determine time, group, and interaction effects (multivariate tests, Roy's Greatest Characteristic Root) (Vasey and Thayer 1987). For the endocrine and immune measures also a two groups (high versus low HRV) by four time points (pre-stress, immediately after stress, 20 and 60 min after stress) ANOVA was performed. Age was entered as a covariate to check for significant group or interaction effects (time × age, group × age). T tests for paired samples were used to check for differences of SBP, DBP, and HR between individual time points (pre-planned). Further, t tests for independent samples or univariate ANOVA were used to check for differences between high versus low HRV groups at individual time points. Pearson correlations (two-tailed) were used to check for associations between variables. Data are presented as mean ± standard deviation. Effect sizes are reported as point-biserial correlations (r_{pb}). Significance was set at $P < 0.05$.

Results

Sample characteristics

The final study sample consisted of 44 Caucasian men with a mean age of 30 ± 7 years and a BMI of 23 ± 3 kg/m^2. 77% ($n = 34$) were students and 21% ($n = 9$) academic professionals. Most of them were involved in moderate sports activities (with a mean of 2.5 ± 1.5 h of physical exercise per week); none of them smoked, and none of the subjects showed any signs of drug abuse or addiction (as it was part of the inclusion criteria). Further, none of the subjects showed any signs of dehydration, acute or chronic inflammation, other illness or pathological condition. The physical examination by an internist confirmed an intact physical status of all participants.

Two groups were defined based on a median split on resting RMSSD (median = 35.5 ms, high versus low RMSSD). This value is nearly identical to the value that we have found for patients in previous investigations and may be a useful value for risk assessment (Melzig et al. 2009; Kraemer et al. 2001). These groups differed significantly in

RMSSD as expected (low HRV group: 25.8 ± 6.5 ms; high HRV group: 51.7 ± 13.9 ms, $F[1,41] = 64.0$, $P < 0.0001$, $r_{pb} = 0.78$). Groups did not differ with regard to BMI, resting HR, SBP, or DBP (all $P > 0.05$). However, the high HRV subjects were significantly younger (27 ± 5 vs. 32 ± 8 years; $F[1,41] = 5.142$; $P = 0.029$, $r_{pb} = 0.33$). Therefore, age was entered as a covariate in all analyses (detailed below).

Cardiovascular effects

Whole sample

The combined stress task elicited significant cardiovascular responses as indicated by significant time effects for SBP (mm Hg; R1: 134.8 ± 16.7; manometer: 146.8 ± 19.9; mental arithmetic: 148.2 ± 18.8; R2: 137.7 ± 18.0; $F[3,39] = 25.930$; $P < 0.0001$), DBP (mm Hg; R1: 83.1 ± 11.5; manometer: 90.1 ± 13.3; mental arithmetic: 91.8 ± 13.2; R2: 86.1 ± 14.4; $F[3,39] = 26.459$; $P < 0.0001$), HR (beats/min; R1: 70.7 ± 8.9; manometer: 73.2 ± 11.5; mental arithmetic: 76.1 ± 12.1; R2: 69.8 ± 9.7; $F[3,39] = 21.296$; $P < 0.0001$), and HRV (RMSSD in ms; R1: 37.9 ± 16.7; manometer: 31.1 ± 14.2; mental arithmetic: 32.5 ± 14.8; R2: 40.0 ± 22.7; $F[3,39] = 13.313$; $P < 0.0001$). Whereas blood pressure and HR significantly increased under the stress HRV decreased.

Comparison of high versus low HRV subjects

Table 1 presents the means and standard deviations of each dependent variable (SBP, DBP, HR, HRV) by HRV group.

High versus low HRV groups did not significantly differ in SBP, DBP, or HR at individual time points (all $P > 0.05$). However, there was a significant time $\times$ group interaction for DBP ($F[3,39] = 4.0$; $P = 0.014$), referring to a higher and ongoing DBP increase under the stress combined with a lack of post-stress recovery in the low HRV subjects (see Fig. 1).

Heart rate variability

There was a significant group effect for HRV ($F[1,41] = 33.122$; $P < 0.0001$), referring to higher overall HRV levels in the high HRV subjects which accords with the grouping by median split upon baseline HRV. Further, there was a significant time $\times$ group interaction ($F[3,39] = 5.650$; $P = 0.003$). Whereas HRV significantly decreased under the stress in the high HRV subjects (from R1 to manometer test; $t(19) = 3.565$; $P = 0.002$; $r_{pb} = 0.63$) and increased with recovery (from mental arithmetic to R2; $t(19) = -3.024$; $P = 0.007$; $r_{pb} = 0.57$), the low HRV group showed no significant changes in HRV across the four phases of the experiment (see Fig. 2).

Biochemical data

Table 2 presents the means and standard deviations of each dependent variable (cortisol, TNF-alpha, IL-6) by HRV group.

Cortisol

There was a significant time effect for cortisol ($F[3,39] = 26.512$; $P < 0.0001$), referring to a stress-associated rise ($t(41) = -1.874$; $P = 0.068$, $r_{pb} = 0.28$)
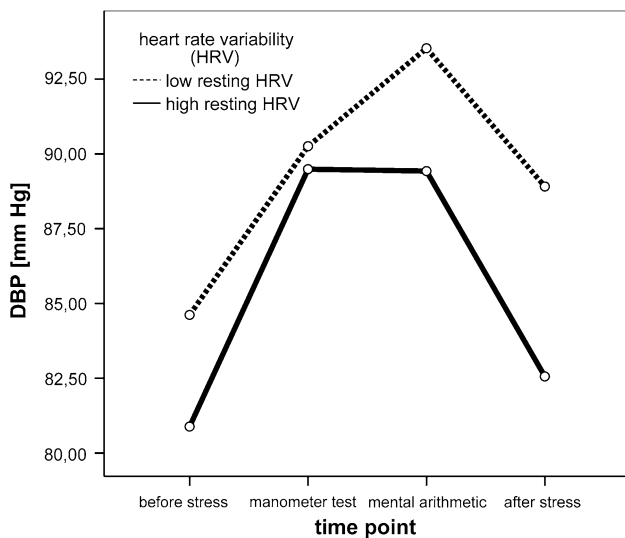
Table 1 Cardiovascular measures (SBP and DBP in mm Hg, HR in beats per min, and HRV (RMSSD in ms)) during the combined mental stress task for low ($n = 23$) versus high ($n = 20$) HRV subjects

	Before stress (R1)	Manometer test	Mental arithmetic	After stress (R2)	Time effect	Group effect	Time × group
SBP							
Low HRV	136.2 ± 17.1	147.6 ± 21.8	149.8 ± 20.4	139.1 ± 20.9	***		
High HRV	132.5 ± 16.7	145.4 ± 18.5	145.8 ± 17.3	135.7 ± 14.7			
DBP							
Low HRV	84.6 ± 11.8	90.3 ± 14.1	93.5 ± 14.8	88.9 ± 17.6	***		*
High HRV	80.9 ± 11.2	89.5 ± 12.8	89.4 ± 11.3	82.6 ± 9.3			
HR							
Low HRV	72.7 ± 8.9	74.2 ± 11.1	77.3 ± 12.1	72.0 ± 9.3	***		
High HRV	69.0 ± 8.6	72.4 ± 12.2	75.4 ± 12.4	67.7 ± 9.8			
RMSSD							
Low HRV	25.8 ± 6.5	23.5 ± 6.5	26.1 ± 8.7	27.2 ± 8.1	***	***	**
High HRV	51.7 ± 13.9	40.0 ± 15.6	39.9 ± 17.0	52.5 ± 26.4			

Shown are means $\pm$ SD of 5-min phases as analysed by repeated measures ANOVA

SBP systolic blood pressure, *DBP* diastolic blood pressure, *HR* heart rate, *HRV* heart rate variability

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Fig. 1 DBP in subjects with high ($n = 20$) versus low HRV ($n = 23$) during the combined stress task. Shown are means of 5-min phases (R1 before stress, manometer test, mental arithmetic, R2 after stress). The low HRV subjects show a higher and ongoing DBP increase under the stress combined with a lack of post-stress recovery (time × group effect; $F[3,39] = 4.004$; $P = 0.014$)
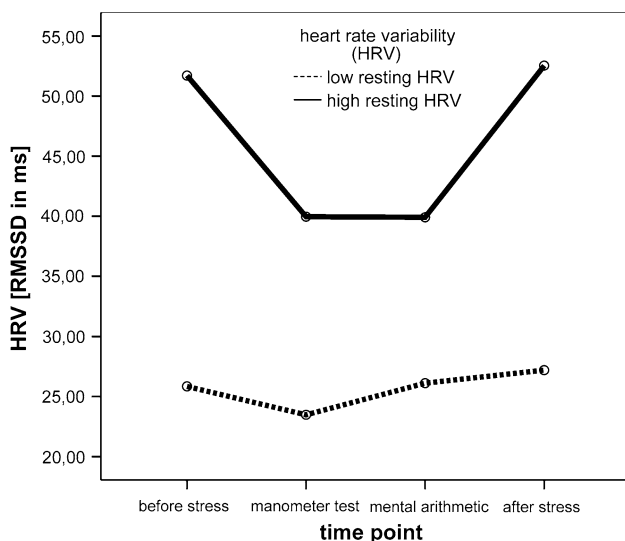

Fig. 2 HRV (RMSSD) in high ($n = 20$) versus low HRV ($n = 23$) subjects during the combined stress task. Shown are means of 5-min phases (R1 before stress, manometer test, mental arithmetic, R2 after stress). The high HRV subjects show higher overall HRV levels ($F[1,41] = 33.122$; $P < 0.0001$) and a significant stress-associated modulation of HRV in contrast to the low HRV subjects (time × group effect; $F[3,39] = 5.650$; $P = 0.003$)

and a significant post-stress decrease regarding the whole sample ($t(41) = 3.713$; $P = 0.001$, $r_{pb} = 0.50$ for cortisol after stress to cortisol at 20 min post-stress; $t(41) = 8.392$; $P < 0.0001$, $r_{pb} = 0.79$ for cortisol at 20 min post-stress to cortisol at 60 min post-stress). However, looking at the two groups separately, only the high HRV subjects showed

significant recovery from stress to 20 min after stress ($t(18) = 4.628$; $P < 0.0001$, $r_{pb} = 0.74$), whereas the low HRV group failed to recover at this time point ($P > 0.05$) (see Fig. 3).

Cytokines

We saw a significant time effect for TNF-alpha ($F[3,32] = 3.265$; $P = 0.034$), referring to a significant post-stress decrease of TNF-alpha in the high HRV group ($t(17) = 2.584$; $P = 0.019$, $r_{pb} = 0.53$ for the post-stress versus the 60 min post-stress levels), whereas in the low HRV group levels stayed unchanged ($P > 0.05$ for the post-stress to the 20 min and for the post-stress to the 60 min post-stress levels) (see Fig. 4).

For IL-6 there were no significant time, interaction, or group effects at all.

Covariance analyses including age

There were no significant group main effects for age, nor any significant interaction effects (time × age) in any of the cardiovascular (SBP, DBP, HR), endocrine (cortisol), or immune measures (TNF-alpha, IL-6) (multivariate analyses; all $P > 0.05$).

Discussion

The present study investigated acute stress and recovery effects during a combined mental stress test in healthy men. The stress test produced significant cardiovascular responses, as in previous work (Buchholz et al. 2003; Weber et al. 2008). Further, there were significant time effects for cortisol and TNF-alpha, backing the effectiveness of the task in eliciting psychosocial stress (Dickerson and Kemeny 2004). In accordance with our hypothesis, significant differences were observed between subjects with high versus low resting HRV in cardiovascular, endocrine, and immune markers.

Diastolic blood pressure

Subjects with low baseline HRV showed an ongoing and more pronounced DBP rise under the stress and, more importantly, failed to recover during the following 5-min resting phase, as indicated by a significant time × group interaction. Previous research suggests that the observed differences between high and low HRV groups may have implications for the future development of cardiovascular disease. In the ARIC study, hypertensives showed significantly reduced HRV in cross-sectional analyses, as well as

Table 2 Cortisol (nmol/l), TNF-alpha and IL-6 levels (pg/ml) during the combined mental stress task and recovery for low versus high HRV subjects ($n = 22$ vs. $n = 19$ for cortisol; $n = 18$ vs. $n = 18$ for TNF-alpha and IL-6, respectively)

	Before stress (baseline)	Immediately after stress	20 min after stress	60 min after stress	Time effect	Stress versus 20 min post stress	Stress versus 60 min post stress
Cortisol							
Low HRV	358.7 ± 139.5	397.3 ± 140.7	378.5 ± 116.4	289.4 ± 91.5	***		
High HRV	357.3 ± 77.1	388.2 ± 105.1	341.9 ± 113.7	284.0 ± 106.1		***	
TNF-alpha							
Low HRV	0.37 ± .12	0.36 ± 0.12	0.36 ± 0.12	0.36 ± 0.13	*		
High HRV	0.36 ± 0.14	0.36 ± 0.12	0.35 ± 0.11	0.34 ± 0.12			*
IL-6							
Low HRV	4.41 ± 6.32	4.25 ± 6.26	4.30 ± 6.12	4.56 ± 6.30			
High HRV	5.87 ± 10.39	5.75 ± 10.27	5.27 ± 8.71	5.14 ± 8.26			

Shown are means ± SD of 5-min phases, results of repeated measures ANOVA, and t tests to check for recovery effects in high and low groups separately

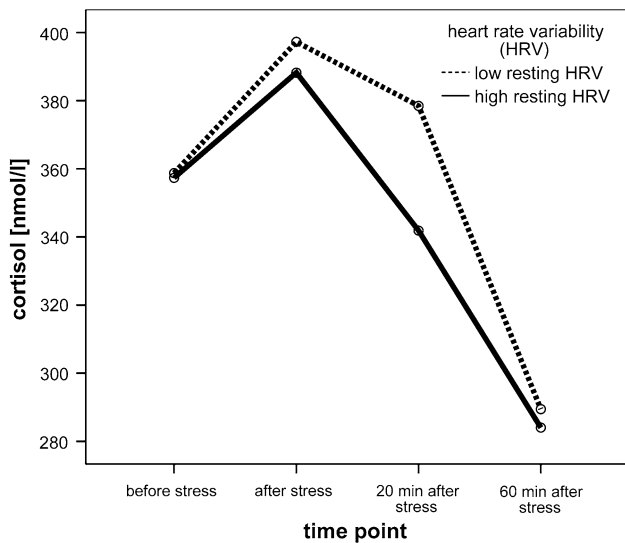$* P < 0.05$, $** P < 0.01$, $*** P < 0.001$

Fig. 3 Cortisol levels in high ($n = 19$) versus low HRV ($n = 22$) groups during the combined stress task (before, after, 20 and 60 min after stress). Only the high HRV subjects show an immediate post-stress recovery ($t(18) = 4.628$; $P < 0.0001$ for the decrease from stress to 20 min post stress)
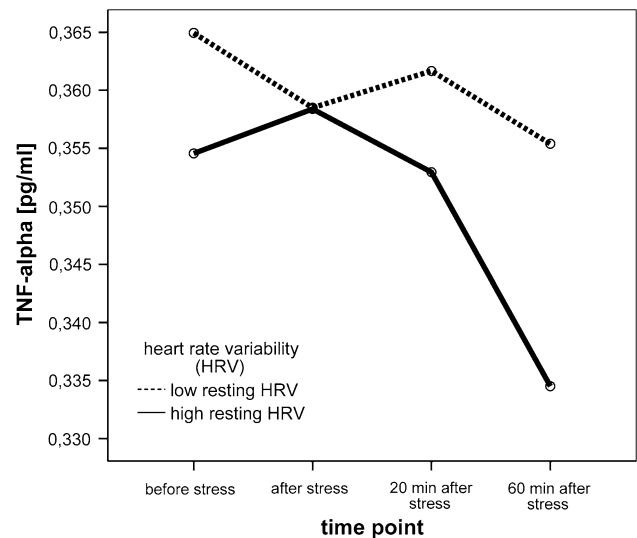

Fig. 4 TNF-alpha levels in high ($n = 18$) versus low HRV ($n = 18$) groups during the combined stress task (before, after, 20 and 60 min after stress). Only the high HRV subjects show a significant post-stress recovery ($t(17) = 2.584$; $P = 0.019$ for the post-stress versus the 60 min post-stress levels)

reduced HRV predicting development of hypertension at follow-up after 3 years (Liao et al. 1996). In subjects with no history of cardiovascular disease exposed to acute psychosocial stress, 3-year increases in SBP were predicted by impaired post-stress recovery of SBP, DBP, and total peripheral resistance, and 3-year increases in DBP were predicted by impaired recovery of SBP, DBP, and HRV during tasks, independently of covariates (Steptoe and Marmot 2005). Hence, impaired post-stress recovery as well as reduced baseline HRV may index disturbances in the regulation of cardiovascular stress responses that contribute to longitudinal changes in blood pressure.

Heart rate variability

The high HRV individuals showed the expected stress-associated decrease of HRV during the stress tasks and return to baseline levels during recovery (Weber et al. 2008). In contrast, the low HRV subjects stayed at significantly lower HRV levels, unaffected by the stage of the task, with no post-stress recovery. Decreased vagal function as indexed by reduced HRV is associated with the known risk factors for cardiovascular disease and all-cause mortality (Thayer and Lane 2007). Our data further suggest that subjects with low resting HRV do not show context-appropriate responses to environmental challenges. This

may predispose them to sustained perturbations of sympathovagal balance with subsequent development of cardiovascular disease. Importantly, autonomic imbalance implying decreased vagal tone, and a failure in the adaptation to stressors, such as seen here, may precede the development of manifest disease and hence identify apparently healthy subjects at risk (Thayer et al. 2009), as it has been shown for diabetes mellitus where decreased HRV preceded its manifestation by standard clinical tests (Ziegler et al. 2001).

Cortisol

Regarding cortisol, all subjects showed almost identical levels before and 60 min after the stress as well as the height of the stress-associated peak did not significantly differ. However, significant differences were observed at 20 min after stress. Whereas cortisol decreased below baseline in the high HRV group, the low HRV subjects maintained their cortisol at nearly the stress level. This may suggest a heightened stress susceptibility of the HPA axis in the low HRV individuals. Excess cortisol leads to sodium retention, hypokalemia, and elevated blood pressure. Corticosteroids enhance vascular contractility by their permissive effects in potentiating vasoactive responses to catecholamines (Yang and Zhang 2004). Thus, the prolonged activation of the HPA axis in the low HRV subjects in response to a brief psychological challenge may promote hypertension in the long run. Furthermore, impaired recovery is regarded as an index of allostatic load (McEwen 1998). Together with the differences observed between groups in the cardiovascular measures, the data support a common pathway for the regulation of allostatic systems (Thayer et al. 2006; Thayer and Sternberg 2006). The prefrontal cortex and the amygdala are important central nervous system structures linked to the regulation of allostatic systems via the vagus nerve. A lack of inhibitory input by the vagus is thought to lead to perturbations in HPA axis regulation, which may reflect as an impaired recovery response to stress, as observed here in the low HRV group.

Cytokines

There was no immediate stress-associated change in TNF-alpha levels which is concordant with the literature (Altemus et al. 2001; Dugué et al. 1993; Heesen et al. 2002; Miller et al. 2005; Steptoe et al. 2001, 2007). However, our high HRV subjects showed a significant recovery of TNF-alpha at 60 min after stress, with levels falling below baseline, whereas the low HRV group did not show any significant changes. The observed post-stress decrease contrasts with some authors reporting delayed increases of

TNF-alpha, that is, at 45 min post-stress (Altemus et al. 2001; Ellins et al. 2008). Heightened TNF-alpha levels and reduced resting HRV have both been associated with atherogenesis and development of cardiovascular disease (Ridker et al. 2000a). Post-stress recovery may buffer sustained elevations of blood pressure, HR, and other markers of allostatic load (Brosschot et al. 2005; McEwen 1998; Thayer and Sternberg 2006). If this protective mechanism fails under the effect of acute psychosocial stress, such as suggested in our low HRV subjects, an individual may be at increased risk of developing vascular pathology (Kop 2003). Research from animal studies suggests that activation of the efferent vagal pathway plays a role in the tonic inhibitory control of the release of pro-inflammatory mediators, particularly TNF-alpha (Bernik et al. 2002; Borovikova et al. 2000; Wang et al. 2003). Our results go along with these reports and add to the still scarce body of literature in humans (Janszky et al. 2004; Marsland et al. 2007). Vagal regulation of immune reactivity has been proposed as a pathway linking psychosocial factors to risk for inflammatory processes including atherosclerosis (Marsland et al. 2007; Tracey 2002), which we may on the basis of the present data extend to the postulate of a neurovisceral integration model implying other physiological systems as well (Thayer and Sternberg 2006).

Regarding IL-6, there were no significant changes at all which parallels a number of other (Kop et al. 2008; Miller et al. 2005; Dugué et al. 1993; Lutgendorf et al. 2004; Heinz et al. 2003; Heesen et al. 2002) but not all authors (Edwards et al. 2006; von Känel et al. 2006; Brydon et al. 2005; Steptoe et al. 2001).

Clearly, findings need to be interpreted with caution. Circulating cytokine levels are generally low in healthy subjects, which may hinder the detection of changes (Kop et al. 2008; Steptoe et al. 2007). In addition, we measured unstimulated cytokine levels which are naturally lower than stimulated levels, but have the advantage of reflecting undistorted data. We previously reported a decrease of unstimulated TNF-alpha along with reductions of tinnitus disturbance and perception of stress in a sample of chronic tinnitus sufferers as a result of a 10-week stress management intervention and hence postulating TNF-alpha to be a stress marker (Weber et al. 2002).

Regarding the cardiovascular data, differences between high and low HRV subjects were numerically small, too, as to expect in this healthy male population. At the same time, the Finapres device is known to overestimate absolute blood pressure values (Imholz et al. 1993); however, having excluded hypertensive subjects, we were not so much interested in the absolute values as in the differences between different phases. As raised, our study sample was restricted with respect to gender and ethnicity, as well as the small sample size warrants caution when generalising

the results to a wider context. However, the fact that significant differences were detected in this small homogenous sample increases the trustworthiness of the results.

To conclude, marked differences in several biological systems occurred between high versus low HRV subjects during recovery from brief mental stress. Whereas the high HRV group showed significant recovery of DBP, HRV, cortisol, and TNF-alpha, the low HRV subjects showed impaired recovery in all of these measures. Whereas the link of autonomic activity and cardiovascular measures may appear evident, the heart being under tonic inhibitory control by the vagus, our results also lend support to a link with the HPA axis (Thayer et al. 2006), as well as supporting the concept of the "cholinergic anti-inflammatory pathway" in humans (Tracey 2002). Thus, the data support the inhibitory role that the vagus is thought to play in a neurovisceral regulation model of allostatic systems (Thayer and Sternberg 2006). Common parallel pathways of different biological axes have been delineated earlier (Elenkov and Chrousos 2006). However, our data underline the importance of individual differences in resting HRV in healthy men and provide support for the sensitivity of HRV in detecting a dysregulation of allostatic systems (Thayer and Sternberg 2006; Tracey 2002). We, hence, posit that low resting HRV may identify healthy subjects at risk for future disease, be it cardiovascular (in the first place), (auto-) immune or other stress-related condition. Follow-up studies should verify this hypothesis.

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