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# Two separable mechanisms are responsible for mental stress effects on high frequency heart rate variability: An intra-individual approach in a healthy and a diabetic sample

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ABSTRACT

Central withdrawal of parasympathetic cardiac control and increased respiratory frequency represent two important determinants of reduced respiratory-related heart rate variability (HRV). However, studies are missing to disentangle their relative contribution during mental stress. Healthy subjects ($n = 10$) and type 2 diabetic patients ($n = 8$), the latter with evidence of cardiac autonomic neuropathy, participated in this study. Using an intra-individual approach, high-frequency (hf) HRV was assessed for spontaneous (during rest and mental stress) and paced breathing (0.15, 0.2, 0.25, 0.3, 0.35, 0.4 and 0.45 Hz; randomized sequence). Mental stress was induced by a challenging reaction time task. Effects of respiratory frequency on hf HRV were individually predicted by paced breathing data. Mental stress decreased hf HRV ($p < .001$), and increased respiratory frequency ($p = .01$). Individual prediction of hf HRV by stress respiratory frequency resulted in lower values ($p = .02$) than observed during rest, indicating that respiratory stress effects were sufficient to reduce hf HRV. However, observed hf HRV values during stress were even lower ($p < .001$). These results indicate that hf HRV reductions during stress can only partly be explained by concomitant respiratory frequency changes. This effect is detectable in healthy subjects and in patients with evidence of diabetic cardiac autonomic neuropathy.



## 1. Introduction

Normal heart rate (HR) varies with breathing: it increases during inspiration and decreases during expiration (Eckberg, 2003; Grossman and Kollai, 1993; Grossman and Taylor, 2007). This modulation of HR by respiration is known as respiratory sinus arrhythmia, respiratory-related, or high frequency (hf) HR variability (HRV). The coupling of the cardiovascular and respiratory systems at respiratory frequency (RF) has a positive influence on pulmonary gas exchange, because efficient ventilation/perfusion matching guarantees optimal oxygen supply and clearance of carbon dioxide (Hoyer et al., 1998; Yasuma and Hayano, 2004). The synchronization of HR and RF in the high frequency range (above 0.15 Hz) is achieved by the parasympathetic branch of the autonomic nervous system. The sympathetic nervous system does not play a role since adrenergic post-receptor signal cascades are too slow to mediate the coupling of HR to RF, which varies within few seconds (e.g. normal resting RF > 0.15 Hz). Respiratory HR oscillations are mediated by a periodic, respiration-coupled inhibition of parasympathetic outflow (Berntson et al., 1997). Central rhythm generators may contribute to this effect (Koepchen et al., 1981) as well as peripheral structures, such as pulmonary stretch receptors and vascular baroreceptors (Bartsch et al., 2007; Bernardi et al., 2001; Eckberg, 2009; Lackner et al., 2011; Zhang et al., 2010). The fundamental influence of respiration on HRV has been demonstrated in different studies (Badra et al., 2001; Bernardi et al., 2001; Bernardi et al., 2000; Ritz and Dahme, 2006; Sanderson et al., 1996; Wilhelm et al., 2004) showing a strong, inverse relationship: an increase in RF predicted lower hf HRV (Angelone and Coulter, 1964; Berntson et al., 1997; Schachinger et al., 1991).

Indices of hf HRV are often used as measures of parasympathetic tone (Eckberg, 2003; Eckberg et al., 1988; Grossman and Kollai, 1993; Kollai and Mizsei, 1990; Porges, 1995), and were found to represent risk indicators of morbidity and mortality (Chandola et al., 2008; Nolan et al., 1998; Thayer et al., 2010). Indeed, hf HRV is increased in young healthy individuals but reduced with aging and disease, conditions known to be associated with decreased parasympathetic control (Chapleau and Sabharwal, 2011; De Meersman and Stein, 2007; Eckberg, 2003; Grossman and Kollai, 1993; Grossman and Taylor, 2007; Routledge et al., 2002).

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Several emotional stress tests (Shapiro et al., 2000; Sloan et al., 1996), such as mental arithmetic (Ruediger et al., 2004) or free speech tasks (Hughes and Stoney, 2000), reduce hf HRV. Similar results have been reported for physiological stressors, such as the cold pressor test (Hughes and Stoney, 2000) and real life stressors, such as sitting in an exam (Sausen et al., 1992) or public speaking (Johnston et al., 2008; Matthews et al., 1986). The reduction in HRV is correlated with the amount of perceived emotional stress (Dishman et al., 2000; Johnston et al., 2008; Kamarck et al., 2003). Furthermore, HRV changes are associated with work-related stress (Kang et al., 2004), work-related worries (Brosschot et al., 2007), effort-reward imbalance (Vrijkotte et al., 2000) and payment expectancies (Pieper et al., 2007). Stress effects on hf HRV are commonly attributed to withdrawal of central parasympathetic control, e.g. (Hughes and Stoney, 2000; Langewitz et al., 1991; Lucini et al., 1995). However, such an interpretation neglects the importance of respiration: RF changes may contribute to the changes in hf HRV without alterations in the parasympathetic drive to the sine node. Importantly, RF may increase in response to mental and social stresses (Hoshikawa and Yamamoto, 1997; Lackner et al., 2011; Ritz et al., 2011; Wientjes et al., 1998), implying that stress effects on high frequency HRV may be, in part, mediated by changes in RF.

In this study, we aim to clarify whether changes in RF are responsible for changes of hf HRV under stress conditions. If stress induced hf HRV changes were fully explained by RF changes, hf HRV under stress could be predicted by the RF under stress. To test this, hf HRV and RF were measured during rest and stress conditions with normal breathing and during conditions of paced breathing at defined frequencies according to audiotape instructions in each individual. Individual regression models were constructed from hf HRV and RF during paced breathing. These individual models served to predict hf HRV values by subject's RF at rest and stress. Predicted hf HRV were compared with observed hf HRV during rest and stress. This allowed us to test whether stress-induced hf HRV changes are fully attributable to the individual stress-induced RF change. Instead of a between-subject design (e.g. covariance model) or a general population based approach (e.g. a "universal equation"), we chose an intra-individual approach to increase internal validity. Healthy subjects and diabetes patients with evidence of autonomic neuropathy were studied to investigate potential disease related regulation differences.

## 2. Material and methods

### 2.1. Subjects

Ten healthy subjects (6 females, 4 males; age 25.9 ± 1.6 years.; BMI 21.9 ± 1.6 kg/m$^2$) and eight patients (1 female, 7 males; age 49.9 ± 1.1 years, BMI 29.2 ± 1.2 kg/m$^2$) diagnosed with diabetes mellitus type II participated. All healthy subjects had normal findings in routine blood chemistry and hematology, standard electrocardiography and on physical examination. Three patients showed a comorbidity of arterial hypertension, and one patient suffered from a major depression treated with paroxetine (SSRI). Five patients received sulfonylurea drugs, and one patient regularly injected insulin (26 IU/day). All diabetic patients showed lowered baroreflex sensitivity of HR control (4.3 ± 0.4; range: 2.9–5.9 ms/mm Hg), according to (La Rovere et al., 2008).

Prior to participation, subjects signed informed consent. Study procedures were in accordance with the Declaration of Helsinki and IRB approved. All testings were done in the morning between 9:00 and 12:00. The participants were asked to refrain from alcohol, nicotine and caffeine 12 h before the laboratory experiment. Medication intake was delayed by approx. 2 h on the morning of the experiments.

### 2.2. Procedures and data processing

The participants were seated in a comfortable experimental chair and electrodes for ECG-measurement measurement (Tyco Healthcare H34SG Ag/AgCl electrodes) were placed according to a standard lead II configuration. The respiration signal was assessed by a respitrace-like, non-calibrated proprietary system using strain gauge belts positioned on the thorax (level of the xiphoid). A Finapres-cuff (Ohmeda Systems 2300) was attached to the index finger of the left hand which was placed about 15 cm below heart level. The Finapres finger blood pressure device was used instead of a conventional, intermittent cuff blood pressure device so that during the experimental periods the participants were not disturbed by inflation of the blood pressure cuff.

Instructions for the experiments were given, and a 10 minute relaxing period started. Then, each participant underwent nine different experimental conditions (periods) in randomized order. Breathing was paced by auditory tape instructions in seven conditions at defined respiratory frequencies of 0.15, 0.2, 0.25, 0.3, 0.35, 0.4 and 0.45 Hz. Additionally, participants conducted two conditions of spontaneous breathing, one resting period for relaxation, and one stress period. During the stress period, subjects had to perform an adaptive choice-reaction-time task (Schachinger et al., 2003; Szinnai et al., 2005). They were instructed to respond to colored lights which appeared in random sequence by pressing corresponding buttons as accurately and quickly as possible. Using a PC-based control algorithm the inter-stimulus-intervals were shortened or lengthened, thereby modifying task difficulty so that subjects' false-response rate within a continuously moving window approached 50%. Each period lasted 5 min. ECG, continuous Finapres output, and respiratory data were assessed during all periods.

All data were digitized by 12 bit resolution at 1 kHz. ECG data was used to identify QRS complexes. Artifacts were identified and corrected semi-manually using WinCPRS software (WinCPRS 1.6, Absolute Aliens, Oy, Turku, Finland). For spectral analysis of the RR-interval series, WinCPRS software Fast Fourier Transform (FFT) routine was used to calculate power spectra in the frequency range between 0.01 and 0.5 Hz. As described previously (Bertsch et al., 2012), the RR-interval time series was linearly interpolated and resampled with a sampling rate of 5 Hz, the resampled data were tapered using a Hanning window, and the windowed data zero padded to the next power of 2. The FFT spectrum was smoothed using a sliding triangular weighting function in order to increase the number of degrees of freedom and thus improve the statistical relevance of the spectrum. Hf HRV was calculated in the frequency range of 0.15 to 0.45 Hz. RF was calculated from the respiratory belt signal.

### 2.3. Statistical analysis

Hf HRV data were log-transformed (Angelone and Coulter, 1964; Bernardi et al., 2000; Nunan et al., 2010) to control for skewed distribution of the raw data, using $\log_{10}$ logarithm. Linear regression analyses were conducted per participant to calculate the individual regression equation for log-transformed hf HRV data by RF of the paced breathing conditions (0.15 to 0.45 Hz). R-square values are reported to describe the quality of the linear fit.

Individual regression equations were used to predict hf HRV values for RF given at spontaneous resting and stress conditions for each participant. Predicted and observed hf HRV data during rest were compared by Pearson correlation and a paired t-test.

The main study question was tested by a mixed design ANOVA with the dependent variable "hf HRV" and a two-level between-subject factor *group* (healthy vs. diabetes), as well as a three-level within-subject factor *var-type* (observed rest vs. predicted stress vs. observed stress). Three contrasts were constructed a-priori: (A) "predicted stress" against "observed rest", (B) "observed stress" against "observed rest", and (C) "observed stress" against "predicted stress". Contrast A tested whether individual stress effects on RF were sufficient to predict lower hf HRV. Contrast B indicated whether observed hf HRV values were reduced during stress, and contrast C tested whether observed

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