

## Effects of variation in posture and respiration on RSA and pre-ejection period

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### Abstract

The extent to which variation in posture and respiration can confound pre-ejection period and respiratory sinus arrhythmia (RSA) as indices of cardiac sympatho-vagal activity was examined. Within-subjects changes in these measures were assessed in 36 subjects during different postures and (paced) respiratory frequencies. Changes from supine to sitting to standing led to reduced RSA values and longer pre-ejection periods, reflecting the known decrease in vagal but not the increase of sympathetic activity. Multilevel path analysis showed that within-subjects changes in sympatho-vagal balance were faithfully reflected by changes in interbeat interval, but imperfectly by changes in RSA and pre-ejection period. It was concluded that pre-ejection period should be stratified for posture and RSA for respiratory frequency to reliably index changes in sympatho-vagal balance when these factors are prone to change (e.g., during 24-h ambulatory recording)

**Descriptors:** Sympatho-vagal balance, Posture, Respiration, Interbeat interval, Pre-ejection period, RSA, CO<sub>2</sub>

In psychophysiology, respiratory sinus arrhythmia (RSA) and pre-ejection period are frequently used as noninvasive measures of within-subjects changes in vagal and sympathetic (beta-adrenergic) influences on the heart. The validity of these measures derives from a large body of controlled experimental studies using exposure to exercise, infusion of beta-adrenergic agonists, mental stressors, and blockade of cardiac muscarinic and adrenergic receptors to manipulate cardiac sympathetic and vagal activity (Allen & Crowell, 1989; Cacioppo, Berntson, et al., 1994; Grossman & Kollai, 1993; Krzeminski et al., 2000; Mezzacappa, Kelsey, & Katkin, 1999; Newlin & Levenson, 1979; Penttila et al., 2001; Sherwood, Allen, Obrist, & Langer, 1986; Sherwood, McFetridge, & Hutcheson, 1998; Smith et al., 1989; Svedenahg, Martinsson, Ekblom, & Hjerdahl, 1986). However, two mechanisms have been identified that may affect the validity of these noninvasive indices of cardiac sympatho-vagal balance outside the controlled laboratory environment. Changes in respiratory frequency influence RSA independent of tonic vagal modulation (Berntson, Cacioppo, & Quigley, 1993; Berntson et al., 1997; Grossman & Kollai, 1993; Houtveen, Rietveld, & de Geus, 2002) whereas posture-related changes in preload (ventricular filling) and afterload (aortic diastolic pressure) may influence

pre-ejection period independent of cardiac inotropic drive (Lewis, Rittogers, Forester, & Boudoulas, 1977; Sherwood et al., 1990).

In a typical psychophysiological study, the subject's posture is fixed, usually sitting upright, whereas changes in respiratory frequency in response to the mental (usually emotional) stimuli are relatively mild, for example, increases of 20% over the spontaneous resting frequency. This is different in ambulatory settings, however, particularly those that include both nighttime and daytime recordings. In such settings repeated postural changes are the rule rather than the exception, and respiratory frequency may be much more variable, for instance, due to physical activity and sleep. This problem has arisen with the advent of high quality instruments to reliably measure pre-ejection period and RSA in ambulatory settings (Cybulski, 2000; de Geus & van Doornen, 1996; Grossman, Wilhelm, & Spoerle, 2004; Keenan, 2004; Sherwood et al., 1998; Willemsen, de Geus, Klaver, van Doornen, & Carroll, 1996). Ambulatory designs that capitalize on these new instruments are prone to confound theoretically relevant psychological effects of real-life activities with changes in posture and respiratory frequency. Typical examples are studies that contrast ambulatory recordings from daytime sitting/standing at (stressful) episodes of work to a resting baseline obtained during nighttime sleep recordings (Riese, van Doornen, Houtman, & de Geus, 2004; Vrijkkotte, van Doornen, & de Geus, 2004). To properly interpret the outcome of such studies, it is important to obtain a better understanding of the independent effects of changes in posture and respiratory frequency, and their interaction, on changes in pre-ejection period and RSA.

Although the effect of respiration on RSA has been extensively studied before, parallel respiration effects on pre-ejection period are unknown. Postural effects on pre-ejection period and

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RSA have been examined simultaneously in only one study (Caio, Berntson, et al., 1994) but, unfortunately, this careful study did not include a supine position, which is the most relevant posture from the perspective of an ambulatory study that includes sleep recordings. In the current study, we capitalized on an experimental protocol originally designed to validate impedance cardiography as a means to measure tidal volume (Houtveen, Groot, & de Geus, 2005). This design combined postural changes from supine to sitting to standing with paced breathing at three different frequencies. This allowed us to examine the main and interactive effects of changes in posture (including a supine position) and changes in respiratory frequency (and the related changes in respiratory depth and the arterial partial pressure of CO<sub>2</sub>) on RSA, pre-ejection period, and heart period.

## Methods

### Participants

The participants of the study were 38 healthy subjects aged 18–50. Due to technical problems, only partial data were available for 2 participants, resulting in 36 complete data sets, 18 men (age  $M = 30.1$  years,  $SD = 10.5$ ) and 18 women (age  $M = 29.8$  years,  $SD = 8.8$ ). The study was presented as an investigation of breathing patterns. The participants received 10 euros after the experiment. All participants gave written informed consent.

### Posture and Paced Breathing Conditions

The experiment consisted of 12 different breathing conditions, each of them of 2 min duration. In three different postures (supine, sitting, and standing), the order of which was randomized, subjects breathed at four paced frequencies, the order of which was fixed: (a) spontaneous breathing without pacing, (b) paced breathing at a rate of 25 cpm without pause, (c) paced breathing at a rate of 15 cpm combined with 0.4 s. pause, and (d) paced breathing at a rate of 5 cpm combined with 1 s pause. A visual pacing signal was presented on a 15-in. monitor, positioned at 80 cm in front of them, and consisted of a respiratory-like sinusoid signal with adjustable amplitudes (highest during slow breathing, lowest during fast breathing).

### Physiological Recordings

*General.* Because capnometry was performed after 30 and 90 s of each 2-min breathing condition, the intermediate segment (60 s) was retained as artifact-free heart period and respiratory data period. The mean interbeat interval, RSA, pre-ejection period, and respiratory frequency and amplitude (depth) values were pooled over these 60-s segments for each breathing condition. End-tidal CO<sub>2</sub> (PetCO<sub>2</sub>) values were pooled over the two observations for each condition.

*Interbeat interval, RSA, and pre-ejection period.* The electrocardiogram (ECG) and the impedance cardiogram (ICG) were recorded continuously with the Vrije Universiteit Ambulatory Monitoring System (VU-AMS version 4.3; de Geus, Willemsen, Klaver, & van Doornen, 1995; Willemsen et al., 1996). This system uses six disposable, pregelled Ag/AgCl electrodes. The first ECG/ICG electrode was placed on the sternum over the first rib between the two collarbones. The second ECG electrode was placed at the apex of the heart over the ninth rib on the left lateral margin of the chest approximately 3 cm under the left nipple. The second ICG measuring electrode was placed over the tip of the

xiphoid complex of the sternum. The ICG current electrodes were placed on the back over cervical vertebra C4 and between thorax vertebrae T8 and T9. A ground electrode was placed at the lower right abdomen. Electrode resistance was kept low by cleaning the skin with alcohol and rubbing.

The interbeat intervals were obtained from the ECG by an on-line automated R-wave peak detector. RSA was estimated by two methods. First, uniformly spaced samples were created and discrete Fourier transformed to compute the high frequency heart period variability spectral power values (high frequency power: 0.125–0.5 Hz). This method does not capture respiratory sinus arrhythmia below the lowest frequency in this band (i.e., 0.125 Hz), whereas our 5 cpm pacing condition causes fluctuations in heart rate at a frequency below this range (0.0833 Hz). Therefore, RSA was also estimated by the peak-to-valley method (RSA<sub>pv</sub>) as the mean difference between the shortest heart period associated with inspiration and the longest heart period associated with expiration for each respiratory cycle (Berntson et al., 1997; Grossman, van Beek, & Wientjes, 1990).

The impedance signal measured by the ICG was amplified and led to a precision rectifier. The rectified signal was filtered at 72 Hz (low pass) to give thorax impedance  $Z_0$ . Filtering  $Z_0$  at 0.1 Hz (high pass) supplies  $dZ$ , which in turn was filtered at 30.0 Hz (high pass) to obtain  $dZ/dt$ , the high frequent impedance changes due to the ejection of blood into the aorta during systole. The resulting  $dZ/dt$  were transmitted to the A/D converter of the microprocessor and sampled at 250 Hz. The usual approach to  $dZ/dt$  data reduction—ensemble averaging—was implemented in the VU-AMS device at the hardware level. Ensemble averaging involves digitizing the  $dZ/dt$  only during a short period (512 ms) around each R-wave in a block of 30 s duration. These digitized samples were summed and divided by the number of synchronized beats in the block. This procedure reduced the impact of single beat fluctuations in the impedance signal through respiration and slow thorax movement. The pre-ejection period (in milliseconds) was defined as the interval from the R-wave peak, minus a fixed interval of 48 ms (Willemsen et al., 1996; Sherwood et al., 1990) to the upstroke of the  $dZ/dt$  (“B-point”), which signals opening of the aortic valves.

*Respiration.* Changes in thoracic and abdominal circumference (used to assess respiratory frequency, respiratory depth, and RSA<sub>pv</sub>) were measured with two BioPac TSD201 respiratory effort transducers (see <http://www.biopac.com>). The two respiratory belts were attached over (a) the rib cage at the level of the fifth thoracic vertebrae, and (b) the abdomen at the level of the navel. The transducers have a variable resistance output between 50 and 125 K $\Omega$ . These signals were low-pass filtered (1 Hz), sampled at 25 Hz, and registered by a BIOPAC MP150 system connected to a data-acquisition computer running the acknowledge v3.7.3 software package. Both respiration signals (thoracic and abdominal) were band-pass filtered using a FIR filter 0.05–0.5 Hz (3–30 cpm). Next, interactive scoring of the peaks and troughs in these segments yielded the respiratory frequency, amplitude, and RSA<sub>pv</sub> values on a breath-to-breath basis.

*Capnometry.* The TG-951T CO<sub>2</sub> sensor Kit (Nihon Kohden Corp.) was used to measure the partial pressure of CO<sub>2</sub> at the end of a normal expiration (PetCO<sub>2</sub>). Digitized PetCO<sub>2</sub> values (in millimeters of mercury) from the capnometer sensor kit were sent to the data-acquisition computer through an RS-232 interface cable.

### Procedure

All experimental sessions took place between 10 a.m. and 4 p.m. and lasted approximately 45 min. First, the six ECG/ICG recording electrodes and the two respiratory belts were attached. Subjects then received a general explanation of the experimental protocol and were seated in a sound-shielded and dimly lit cabin. A practice session took place to familiarize the participants with the pacing. Next, the three 8-min (i.e., four times 2 min: normal breathing, 25 cpm, 15 cpm, and 5 cpm) breathing sessions took place in supine, sitting, and standing postures. During each 2-min breathing condition subjects exhaled through the capnometer mouthpiece after 30 and 90 s. Each breathing session was followed by a short break. Throughout, signal quality of the ECG/ICG and respiratory belts were checked, and, if necessary, the electrodes/belts were reattached during these breaks. At the end of the three breathing sessions, all equipment was disconnected and participants were debriefed and paid.

### Statistical Data Analysis

High frequency power, RSA<sub>p</sub>v, and respiratory amplitude values were  $\log^{10}(x + 1)$  transformed to obtain normal distributions. Repeated-measures Posture (3)  $\times$  Pacing (4) ANOVA tests (implemented as MANOVA using SPSS 12) were performed for the observed interbeat interval, pre-ejection period, high frequency power, RSA<sub>p</sub>v, PetCO<sub>2</sub>, respiratory frequency, and respiratory amplitude values.

Next, change scores were computed for each measure and subject across the nine paced breathing conditions by subtracting the value of the sitting, 15-cpm breathing condition. These change scores were Z-transformed across subjects and conditions. A multilevel path-analysis (using Mplus V3.0) was performed on the Z-transformed change scores to test the interrelationships between these measures (using RSA<sub>p</sub>v), separately at the between-subjects level and at the within-subjects level. Because within-subjects polynomial contrast tests unambiguously indicated linear effects of the posture conditions on the physiological measures, the posture variable was included in this model as -1, 0, 1 for, respectively, supine, sitting, and standing.

### Results

#### Repeated-Measures Analysis of Variance Tests

Table 1 shows the mean scores for all the experimental conditions. In analyses of interbeat interval, a main effect of posture was found,  $F(2,34) = 66.18$ ,  $p < .001$ . Within-subjects contrast tests indicated significant differences between all three posture conditions, all  $p < .001$ . Changes from supine to sitting to standing postures led to a linear shortening of interbeat interval,  $p < .001$ . Changes in pacing were also reflected in interbeat interval,  $F(3,33) = 19.40$ ,  $p < .001$ , but this effect interacted with posture (Posture  $\times$  Pacing:  $F[6,30] = 12.80$ ,  $p < .001$ ). Post hoc decomposition of this interaction showed that the interbeat interval was increased in the 5 cpm condition during sitting and standing, but not during the supine position,  $p < .01$ .

In analyses of pre-ejection period, a main effect of posture was found,  $F(2,34) = 40.89$ ,  $p < .001$ . Within-subjects contrast tests indicated significant differences between all three posture conditions, all  $p < .001$ . Changes from supine to sitting to standing postures led to a linear effect in the lengthening of pre-ejection period,  $p < .001$ . No main effect of pacing and no interaction effect of Posture  $\times$  Pacing were found for pre-ejection period.

Main effects of posture were found for high frequency power and for RSA<sub>p</sub>v,  $F(2,34) = 25.66$ ,  $p < .001$ ;  $F(2,34) = 37.78$ ,  $p < .001$ . For both measures, within-subjects contrast tests indicated significant differences between all three posture conditions, all  $p < .01$ . Changes from supine to sitting to standing postures led to a linear decrease of high frequency power and RSA<sub>p</sub>v,  $p < .01$ . Main effects of pacing were also found for high frequency power,  $F(3,33) = 38.11$ ,  $p < .001$ , and for RSA<sub>p</sub>v,  $F(3,33) = 397.41$ ,  $p < .001$ . For both measures, within-subjects contrast tests indicated significant differences between all pacing conditions, all  $p < .001$ . A decrease in pacing frequency led to a linear increase of RSA,  $p < .001$ . Interaction effects of Posture  $\times$  Pacing were also found for high frequency power,  $F(6,30) = 6.00$ ,  $p < .001$ , and for RSA<sub>p</sub>v,  $F(6,30) = 8.77$ ,  $p < .001$ . For high frequency power, a ceiling effect was found at 5-cpm breathing in the supine position. In the 15- and 25-cpm breathing conditions a significant difference,  $p < .001$ , was found between sitting and supine postures,

**Table 1.** Means (SD) of the Physiological Values

	IBI	PEP	HF power	RSA <sub>p</sub> v	PetCO <sub>2</sub>	R amplitude	R frequency
Supine							
No pacing	0.999 (0.18)	94.9 (15)	3.14 (0.67)	1.87 (0.30)	34.16 (4.6)	0.454 (0.22)	14.47 (4.3)
25 cpm	0.966 (0.16)	93.8 (16)	2.89 (0.62)	1.61 (0.29)	32.11 (3.9)	0.380 (0.20)	24.57 (0.28)
15 cpm	0.954 (0.15)	93.9 (16)	3.04 (0.61)	1.81 (0.29)	33.31 (5.3)	0.467 (0.24)	15.09 (0.53)
5 cpm	0.948 (0.14)	94.3 (15)	3.09 (0.52)	2.27 (0.27)	36.69 (5.4)	0.648 (0.26)	5.23 (0.31)
Sitting							
No pacing	0.902 (0.13)	102.9 (21)	2.89 (0.64)	1.79 (0.33)	34.39 (4.0)	0.409 (0.21)	14.73 (3.5)
25 cpm	0.880 (0.12)	103.2 (19)	2.64 (0.58)	1.56 (0.25)	30.52 (4.5)	0.346 (0.16)	24.60 (0.25)
15 cpm	0.865 (0.12)	103.2 (19)	2.84 (0.54)	1.72 (0.29)	31.05 (5.3)	0.419 (0.18)	15.01 (0.18)
5 cpm	0.886 (0.12)	103.1 (19)	3.09 (0.49)	2.37 (0.29)	34.72 (6.4)	0.630 (0.22)	5.15 (0.40)
Standing							
No pacing	0.798 (0.12)	107.9 (21)	2.56 (0.52)	1.63 (0.29)	33.23 (4.8)	0.476 (0.23)	14.42 (3.5)
25 cpm	0.769 (0.11)	108.9 (21)	2.32 (0.51)	1.32 (0.19)	29.66 (4.6)	0.401 (0.18)	24.56 (0.22)
15 cpm	0.750 (0.11)	109.9 (20)	2.55 (0.47)	1.53 (0.25)	29.57 (4.8)	0.469 (0.22)	15.14 (0.36)
5 cpm	0.802 (0.10)	109.5 (20)	3.00 (0.41)	2.26 (0.24)	33.56 (5.5)	0.727 (0.29)	5.49 (0.76)

IBI: interbeat interval (s); PEP: pre-ejection period (ms); HF power: high frequency heart period variability power; RSA<sub>p</sub>v: RSA estimated by the peak-to-valley method. HF power and RSA<sub>p</sub>v are in  $\log^{10}(x+1)$  transformed units of ms<sup>2</sup> and ms; PetCO<sub>2</sub> values are in units of mmHg; respiratory amplitude values are in  $\log^{10}(x+1)$  transformed (arbitrary) voltage units; respiratory frequency values are in units of cpm.

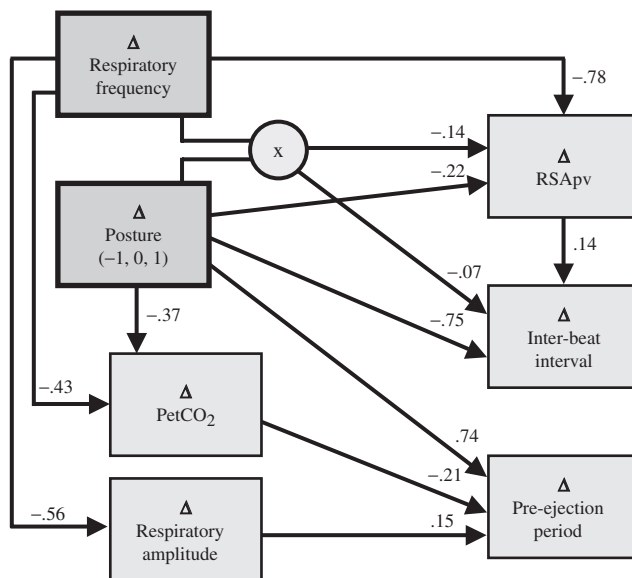
but this difference was no longer significant at 5-cpm breathing. For RSA<sub>pv</sub> no ceiling effect was found at 5 cpm, but the difference between RSA<sub>pv</sub> in the 15- and 5-cpm paced breathing conditions was significantly reduced in the supine position as compared to the standing and sitting positions,  $p < .05$ .

Changes in pacing were also reflected in PetCO<sub>2</sub>,  $F(3,33) = 38.09$ ,  $p < .001$ . The PetCO<sub>2</sub> values were decreased for the 25- and 15-cpm pacing frequencies as compared to no pacing condition,  $p < .001$ . No interaction effect of Posture  $\times$  Pacing was found for PetCO<sub>2</sub>. An unexpected main effect of posture was, however, found for PetCO<sub>2</sub>,  $F(2,34) = 14.86$ ,  $p < .001$ . Within-subjects contrast tests indicated significant differences between all three posture conditions, all  $p < .01$ . Changes from supine to sitting to standing postures led to a linear reduction of PetCO<sub>2</sub>,  $p < .01$ .

The expected main effects of pacing were found on respiratory amplitude,  $F(3,33) = 67.03$ ,  $p < .001$ , and respiratory frequency,  $F(3,33) = 25760.36$ ,  $p < .001$ . For both measures, within-subjects contrast tests indicated significant differences between all three pacing frequencies, all  $p < .001$ . The no pacing condition, however, did not significantly differ from the 15-cpm condition. This simply reflects the closeness of this pacing frequency to the average spontaneous breathing frequency of this group ( $M = 14.87$ ,  $SEM = 0.15$  cpm). No main effect of posture and no interaction effect of Posture  $\times$  Pacing were found for either respiratory amplitude or frequency.

### Exploratory Multilevel Path Analysis

More insight into the relationships between these physiological measures can be obtained from the results of the exploratory multilevel (between- and within-subjects) path analysis. The final



**Figure 1.** Standardized within-subjects path diagram. Only significant path coefficients (estimate/ $SE$  estimate  $> 1.96$ ) are shown. The dark gray boxes signal the experimental manipulations; the direction of causation is fixed for arrows flowing from these boxes. Other arrows reflect correlations and causality could, in principle, have been bidirectional. The linear change in interbeat interval, respiratory amplitude, and PetCO<sub>2</sub> with our manipulations, however, suggests that the direction of the arrows is as in our model. The interaction of Posture  $\times$  Respiratory frequency is created by multiplication of both variables and added to the model as an additional variable ("x" in circle).

selected model resulted in an acceptable goodness of fit,  $\chi^2(12) = 12.27$ ,  $p = .42$ . In this model, no significant variance was accounted for at the between-subjects level. The within-subjects model explained 60% of the variance in interbeat interval change, 67% of the variance in RSA<sub>pv</sub> change, 51% of the variance in pre-ejection period change, 35% of the variance in respiratory amplitude change, and 31% of the variance in PetCO<sub>2</sub> change. Figure 1 shows the corresponding significant standardized beta values.

Although most beta values replicate the effects of posture and pacing found in the repeated-measures analysis of variance tests (see above), four remarkable additional results were found. First, no significant path (beta =  $-0.12$ ) was found from changes in pre-ejection period to changes in interbeat interval and an unexpected low beta value ( $.14$ ) was found in the link between changes in RSA<sub>pv</sub> and interbeat interval. This illustrates how, under the conditions of the current study, changes in pre-ejection period and RSA fail to reflect changed cardiac sympathetic and parasympathetic activity. Second, PetCO<sub>2</sub> was not only influenced by changes in respiratory frequency but also by postural change. Third, pacing-induced changes in respiratory amplitude (beta =  $-0.03$ ) and PetCO<sub>2</sub> (beta =  $.04$ ) did not have significant paths to RSA<sub>pv</sub>. Finally, although the pacing manipulation did not influence pre-ejection period overall, pacing-induced changes in respiratory amplitude and PetCO<sub>2</sub> did have significant effects on pre-ejection period: An increase in PetCO<sub>2</sub> decreased pre-ejection period and an increase in respiratory amplitude increased pre-ejection period.

### Discussion

The purpose of the current study was to estimate the influence of systematically manipulated changes in posture and respiratory frequency on within-subjects changes in two frequently used noninvasive measures of cardiac sympathetic and vagal activity, the pre-ejection period and RSA, respectively. Changes from supine to sitting to standing postures led to a shortening of interbeat interval and a decrease in RSA, reflecting the expected decrease in cardiac vagal activity that has been well documented in studies using either passive head-up tilting or active standing (Cooke et al., 1999; Furlan et al., 2000; Matsushima, Tanaka, & Tamai, 2004; Mukai & Hayano, 1995; Siebert, Drabik, Lango, & Szyndler, 2004).

The same postural manipulation led to a stepwise increase in pre-ejection period, which would suggest a decrease in beta-adrenergic influence on the heart from supine to standing. This is, however, incompatible with the known effects of these changes in posture. Head-up tilting is well known to be accompanied by an increase in muscle sympathetic nerve activity (Cooke et al., 1999; Furlan et al., 2000; Saito et al., 1997; Shoemaker, Hogeman, Khan, Kimmerly, & Sinoway, 2001), noradrenergic spillover (Erms et al., 2004; Esler et al., 1988; Furlan et al., 2000; Laszlo, Rossler, & Hinghofer-Szalkay, 2001), and cardiac contractility (Vijayalakshmi, Mohan, & Babu, 2002). Physiologically, this increase in sympathetic activity makes good sense. Assumption of the upright posture leads to a gravity-induced displacement of blood to the lower body and a small reduction in total plasma volume (Chae & Suh, 1993; Frey, Tomaselli, & Hoffer, 1994; Laszlo et al., 2001; Toska & Walloe, 2002). This reduces end-diastolic left ventricular filling, which, according to the Frank-Starling principle, in turn, reduces the contractility of the left

myocard. Indeed, end-diastolic and stroke volume are found to be reduced in the upright position (Boudoulas, Barrington, Olson, Bashore, & Wooley, 1985; Chae & Suh, 1993; Frey et al., 1994; Shannon, Maher, Santinga, Royal, & Wei, 1991; Sherwood & Turner, 1993; Shoemaker et al., 2001; Toska & Walloe, 2002; Waldstein, Neumann, & Merrill, 1998). The decrease in stroke volume is compensated by an increase in heart rate that is secondary to vagal withdrawal and increased cardiac sympathetic activity. Normally, therefore, we should expect the pre-ejection period to shorten in passive tilting, and this decrease should even be enhanced in active standing when afferent muscle feedback serves to further increase sympathetic activity.

The paradoxical increase in pre-ejection period when going from sitting to standing has been demonstrated before (Boudoulas et al., 1985; Cacioppo, Uchino, & Berntson, 1994; Cacioppo, Berntson, et al., 1994; Sherwood & Turner, 1993; Waldstein et al., 1998), and head-up tilting from supine to upright is also known to systematically increase pre-ejection period (Frey & Kenney, 1979; Lewis et al., 1977; Ovadia, Gear, Thoele, & Marcus, 1995). Importantly, the increase in pre-ejection period during head-up tilting is not abolished by beta blockade (Ovadia et al., 1995). The failure of the pre-ejection period to correctly index changes in sympathetic activity across postural change is likely due to the large afterload effects induced by postural changes. Afterload, or end-diastolic aortic pressure, is reflected in mean arterial pressure, which is known to increase when going from supine to upright (Chae & Suh, 1993; Cooke et al., 1999; Frey et al., 1994; Laszlo et al., 2001; Shannon et al., 1991; Sherwood & Turner, 1993; Shoemaker et al., 2001; Toska & Walloe, 2002; Waldstein et al., 1998). Higher afterload will elongate pre-ejection period by prolonging the time needed to open the aortic valve, even if contractility is unchanged. The low (or even reversed) pre-ejection period reactivity found during cold stress is another instance of the impact of afterload on pre-ejection period, where changes in pre-ejection period fail to detect the strong increase in sympathetic nerve activity (van Doornen, Snieder, & Boomsma, 1998; van Eekelen, Houtveen, & Kerkhof, 2004; Frey & Kenney, 1979; Montoya, Brody, Beck, Veit, & Rau, 1997). In contrast to the strong posture effect, changing the respiratory frequency had virtually no effect on the pre-ejection period. However, pacing-induced decreases in respiratory depth and increases in PetCO<sub>2</sub> both mildly decreased pre-ejection period.

A relationship between within-subjects changes in RSA and changes in respiratory frequency has repeatedly been described and/or demonstrated before (Berntson et al., 1997; Eckberg, 2003; Grossman et al., 2004; Houtveen et al., 2002; Sasano et al., 2002). Our results suggest that the modulation of respiration on the association between RSA and cardiac vagal activity may be posture specific. However, sympathetic influences could have contaminated our RSA<sub>pv</sub> measure, specifically in the 5-cpm breathing conditions and, therefore, could also have been responsible for this interaction effect. At 5 cpm, the typical low frequency sympathetic versus high frequency vagal filter characteristics do not differentiate vagal and sympathetic contributions anymore (see Berntson et al., 1993). Thus, reduced sympathetic activation in the supine position could very well have been responsible for the relatively reduced RSA<sub>pv</sub> values found in the 5-cpm supine condition as compared to the 15-cpm supine condition. Pacing-induced effects of respiratory depth and the arterial partial pressure of CO<sub>2</sub> on RSA have not been demonstrated in the current study. However, our design did not allow us to discriminate between the primary effects of res-

piratory rate, depth, and CO<sub>2</sub> on RSA. These effects may emerge in a (full factorial) experimental design with independent and larger respiratory depth and CO<sub>2</sub> variation.

Under normal—posture controlled—experimental conditions, within-subjects changes in RSA and pre-ejection period can be used as indices of the vagal and sympathetic beta-adrenergic control of the heart period (e.g., see Cacioppo, Berntson, et al., 1994; de Geus & van Doornen, 1996). The results of the current study, however, demonstrate that changes in posture and respiratory frequency (and the related changes in depth and PetCO<sub>2</sub>) exert large effects on these noninvasive sympatho-vagal measures, such that within-subjects changes in heart period can become almost unrelated to changes in RSA and pre-ejection period. The question arises, therefore, whether pre-ejection period and RSA can be used to index changes in cardiac sympathetic and parasympathetic activity under circumstances when no experimental control over posture or respiration is possible, for example, during ambulatory monitoring. We are optimistic that this is still feasible as long as the study design fully appreciates the inherent limitations of these measures demonstrated here. The modulation of the relationship between vagal activity and RSA by respiratory frequency can be explicitly charted in field studies by recording both RSA and respiratory frequency as has been proposed before (Grossman et al., 2004). As long as postural changes are carefully documented, for instance, by inbuilt movement and posture detectors, it is further possible to compare different activities/moods across instances of these activities performed in the same posture.

At first sight, this seems to limit the research questions that can be addressed. Some of the more interesting within-subjects comparisons come with an inherent change in posture, for instance, when daytime work levels of pre-ejection period/RSA are compared to the sleeping baseline value of these measures. Careful interpretation of the results, however, may overcome the potential shortcomings of pre-ejection period and RSA in such naturalistic designs. As an example we point to a recent ambulatory monitoring study that reported *increased* pre-ejection period values during sleep (+6.4 ms) as compared to daytime sitting (Vrijkotte et al., 2004). In the present study, we demonstrated that the change from sitting to supine posture by itself leads to a *decrease* in pre-ejection period (−8 ms). Apparently, the decrease in activity of the beta-adrenergic influence on the heart during sleep is strong enough to overcome the opposing afterload effects on pre-ejection period. The conclusions of Vrijkotte et al. (2004) that there is a significant recovery of cardiac sympathetic activity at night, therefore, seems entirely valid, and is supported by studies showing an increase in pre-ejection period from a presleep supine baseline (Burgess, Penev, Schneider, & van Cauter, 2004; Trinder et al., 2001). However, their finding that subjects with the highest work stress showed the least awake/sleep differences in pre-ejection period should be interpreted with more care. As the authors suggested, this effect may reflect a true work stress effect on wake–sleep changes in cardiac sympathetic effects on the heart, driven by beta-receptor down-regulation. Alternatively, however, it may reflect a work stress effect on posture or sleep-induced changes in afterload (blood pressure “dipping”) that may be entirely unrelated to cardiac sympathetic regulation.

In general, the results of the current study confirm the necessity of stratifying for posture and respiration when using pre-ejection period and RSA to index within-subjects changes in sympatho-vagal balance. Valid interpretation of changes in

pre-ejection period and RSA is entirely feasible in studies that, by necessity, confound measurement conditions with changes in posture and respiration, for instance, when ambulatory recordings from daytime sitting/standing at work are compared with nighttime supine sleep. These studies do, however, require a de-

sign that takes the significant postural and respiration effects on these variables into account. This can be done by careful recording of daily activities through diaries in combination with a movement detector and coregistration of respiratory behavior and ECG.

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