Ambulatory recording of within- and between-subject variation in autonomic nervous system activity

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Chapter 1

General introduction

Background

Cardiovascular disease (CVD) is currently the major cause of death in Westernized countries, causing over 40.000 deaths per year in the Netherlands. The etiology of CVD is complex, with many different factors (demographical, psychological, lifestyle, physiological) contributing to an increased risk of developing CVD (Brotman *et al.*, 2005). In the past decades, a substantial number of physiological risk factors for CVD have been identified. Among these physiological risk factors are a shift from parasympathetic to sympathetic activity (Curtis & O'Keeffe, 2002) with reduced heart rate variability (Dekker *et al.*, 2000), increased blood pressure (Verdecchia, 2000), and increased heart rate (Fox *et al.*, 2007), which all indicate the involvement of the autonomic nervous system (ANS). Within the population, large individual differences exist in ANS function (Ben Lamine *et al.*, 2004; Berntson *et al.*, 1994; Cacioppo *et al.*, 1994b; Grossman & Kollai, 1993; Salomon *et al.*, 2000). Two main factors causing these individual differences in ANS function are genetics and (chronic) stress. With regard to cardiac autonomic function twin studies have shown substantial heritability for both sympathetic and parasympathetic activity (Kupper *et al.*, 2006; Kupper *et al.*, 2004). Studies using brief (Houtveen *et al.*, 2002; Lucini *et al.*, 2002) as well as prolonged (Riese *et al.*, 2003; Vrijkotte *et al.*, 2004) psychosocial stressors have further shown that stress induces a shift in cardiac autonomic regulation away from parasympathetic control towards increased sympathetic control. These stress-related changes in cardiac autonomic function have been shown to represent a very stable individual characteristic (Burleson *et al.*, 2003; Kasprowicz *et al.*, 1990), and to be partly heritable, such that genetically vulnerable individuals show more autonomic reactivity to stress than less vulnerable subjects (de Geus *et al.*, 2007). Stress may also indirectly influence ANS function by increasing the adoption of unhealthy behaviors such as smoking or failure to exercise regularly. Smoking, for example, increases heart rate and decreases heart rate variability (Hayano *et al.*, 1990), while taking up exercise may lead to the opposite, decreasing heart rate and increasing heart rate variability (Billman, 2002).

Taken the detrimental effects of stress on cardiovascular health (Yusuf *et al.*, 2004) there is a large literature on physiological reactivity to stress (Kamarck & Lovallo, 2003; Lovallo & Gerin, 2003; Schwartz *et al.*, 2003; Treiber *et al.*, 2003). Many of the older studies recorded heart rate and blood pressure only, but more recent studies increasingly try to index (changes) in sympathetic and

parasympathetic activity and in stress-hormonal levels that underlie the outcome at the level of the heart and blood vessels. For instance, about 20% of the 250 studies in the 2005-2007 issues of Psychophysiology and 15% of the 293 studies in Psychosomatic Medicine featured some form of ANS recording. Of note, the majority (82) of the 94 studies on the effects of stress on the ANS were conducted in the laboratory.

Moving outside the laboratory

Laboratory studies generally involve the measurement of ANS parameters during one or more rest periods and during mental and physical challenges, with each period often lasting no more than 10 minutes. Such studies provide valuable information on the mechanisms underlying ANS responses to stress and have been instrumental in establishing the existence of stable individual differences in the ANS response. However, these individual differences in cardiovascular stress responses to standardized laboratory situations do not seem to be readily generalizable to responses to actual real life situations; the association between laboratory and ambulatory measurements has been shown to be moderate at best (Gerin *et al.*, 1994; Kamarck & Lovallo, 2003; van Doornen *et al.*, 1994). It is possible that the psychological and physiological processes induced by laboratory stress are only a poor reflection of the actual processes in everyday real-life stress situations. Perhaps as a consequence, the predictive value of ANS responses to laboratory challenges for later CVD is low, with the response to a challenge hardly contributing to the prediction when basal levels have been taken into account (Barnett *et al.*, 1997; Carroll *et al.*, 1998; Coresh *et al.*, 1992; Kamarck & Lovallo, 2003). One explanation may be that the tasks used and the ANS parameters measured in these follow-up studies were often limited. Usually only heart rate and blood pressure were measured in response to very short-lasting simple stressors, such as the cold pressor test (holding one's hand in ice-cold water for one minute) and mental arithmetic. It is possible that a more thorough reflection of ANS function in combination with more diverse and long-lasting laboratory stressors will increase the prediction of later disease development.

As an alternative to bringing “everyday situations to the laboratory”, researchers have increasingly tried to bring the “laboratory to everyday situations”. This is done by using miniaturized versions of the recording equipment to perform prolonged ambulatory monitoring in naturalistic settings

(Fahrenberg & Myrtek, 1996; Fahrenberg & Myrtek, 2001). The hope is that ambulatory measurement of ANS levels in the natural environment, including responses to stressors at home and at work, will be a better predictor for later CVD. Encouragingly, higher predictive validity for long term health outcomes has already been shown to be the case for blood pressure, where ambulatory levels are better predictors for cardiovascular morbidity and mortality than laboratory or office measurements (Mallion *et al.*, 1999; Palatini & Jullius, 2004; Pickering & Devereux, 1987; Verdecchia *et al.*, 1994; Verdecchia *et al.*, 1998; Verdecchia *et al.*, 2001).

Until fairly recently, ambulatory monitoring was restricted to heart rate, heart rate variability and blood pressure but new technical developments have led to the possibility of measuring additional parameters to better reflect the functioning of the ANS. Examples of ambulatory devices capable of measuring the underlying structure of the ANS are the LifeShirt System (LS, VivoMetrics, Inc., Ventura, CA, USA), the ambulatory impedance monitor (AIM-8; Bio-impedance Technology, Chapel Hill, NC), the AZCG (World Wide Medical Instruments, Dallas, TX) and the Vrije Universiteit Ambulatory Monitoring System (VU-AMS; VU-FPP, Amsterdam) (Nakonezny *et al.*, 2001; Sherwood *et al.*, 1998; Wilhelm *et al.*, 2003; Willemsen *et al.*, 1996). The availability of these new ambulatory devices makes it possible to examine autonomic function measured during normal daily life. Unfortunately, rigorous psychometric testing of the ambulatory assessment of, in particular the impedance cardiogram-derived parameters is scarce. This thesis was inspired to a large extent by a desire to improve this situation. It used the Vrije Universiteit Ambulatory monitoring system (VU-AMS) which has been developed in our department (for more information about the VU-AMS see the website www.psy.vu.nl/vu-ams). The VU-AMS has been successfully applied to assess the influence of genetics and stress on individual differences in sympathetic and parasympathetic nervous activity (Kupper *et al.*, 2005b; Kupper *et al.*, 2006; Kupper *et al.*, 2004; Riese *et al.*, 2003; Riese *et al.*, 2004; Vrijkotte *et al.*, 2000; Vrijkotte *et al.*, 2004; Vrijkotte *et al.*, 2001).

Because sympathetic and parasympathetic nervous system activity are the crucial targets in my research, I will first review the autonomic nervous system below and describe the current strategies to measure its activity.

The autonomic nervous system

The main function of the ANS is coordinating bodily functions to ensure homeostasis and performing adaptive responses when faced with changes in the external and internal environment. The term “autonomic nervous system” was created by John Newport Langley in 1898. Based on anatomical and functional criteria, Langley divided the ANS into three separate components: a sympathetic nervous system including the adrenal medulla, a parasympathetic nervous system and an enteric nervous system. The enteric nervous system consists of a collection of neurons embedded within the wall of the entire gastrointestinal tract. This system controls gastrointestinal motility and secretions. Since this branch of the ANS is not involved in the regulation of the cardiovascular system, it will not be discussed here. The sympathetic branch is better known as the “fight-or-flight” branch of the ANS, meaning that in physical or emotional stressful situations, when the body needs a sudden burst of energy, this branch is activated. This activation is the result of evolutionary processes; think back to those early times when humans were still likely to be confronted by large dangerous animals and had to be ready to fight or run away as fast as possible. This bodily activation includes among others an increase in heart rate, epinephrine, breathing rate, sweat production, blood supply to muscles, and blood pressure. The parasympathetic branch, on the other hand, promotes the normal maintenance of the body by acquiring energy from food and getting rid of wastes. This parasympathetic branch is therefore also called the “rest and digest” branch of the ANS and involves slowing the heart, constricting the pupils, stimulating the gut and salivary glands, and other responses that are not a priority when being "chased by a tiger". Organs are often innervated by both the sympathetic nervous system (SNS) and the parasympathetic nervous system (PNS), which typically exert opposing actions (Figure 1). Some organs are not dually innervated (e.g. sweat glands), however, and even for dually innervated organs, the autonomic branches may have synergistic rather than opposing effects or may otherwise be asymmetrical in their pattern of innervation or action.

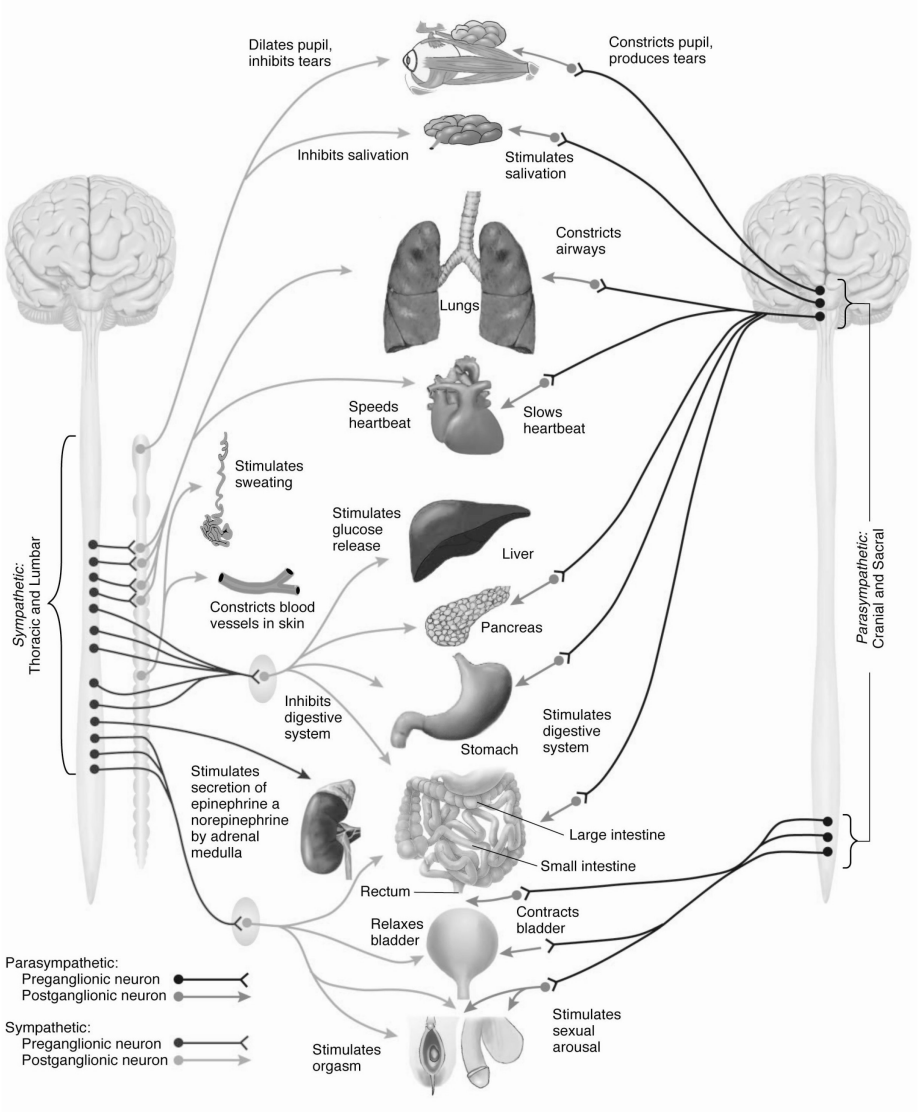

Figure 1 *The autonomic nervous system (from Carlson, NR (2004). Physiology of behavior (8th ed). Boston: Allyn and Bacon).*

Parasympathetic nervous system activity

Physiology

The nerve fibers, also called preganglionic fibers, of the PNS leave from the cell bodies of the motor nuclei of the cranial nerves III, VII, IX and X in the brain stem and from the second, third and fourth sacral segments of the spinal cord. The vagus nerve (or X^{th} cranial nerve) carries fibers to the heart and lungs (as well as other organs) and is the primary parasympathetic innervation of these organs. The preganglionic axons terminate in parasympathetic ganglia, which lie within or very close to the organs innervated by the postganglionic neurons. The preganglionic neurons employ acetylcholine as the primary neurotransmitter, which binds to a nicotine receptor subtype on the postganglionic neurons in the ganglia. Postganglionic parasympathetic fibers also employ acetylcholine as a primary neurotransmitter, although the receptor sub-types on the target organ are commonly muscarinic (Figure 2). The target organs of parasympathetic neurons include among other the heart, lungs, liver, pancreas, bladder and reproductive organs.

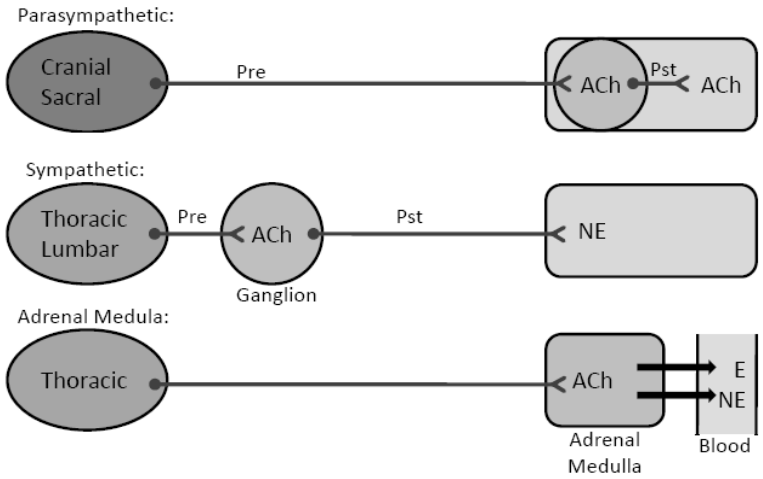

Figure 2 *Pre- and post-ganglionic neurons.*

Measurement

Invasive techniques to measure parasympathetic activity are direct measurement of action potentials in the vagus nerve and vagal cooling, although these measures are too invasive to be used in research with humans. The

measurement of acetylcholine release is not realizable, because survival of the transmitter in the synaptic space and the circulation is so brief because of the speedy action of cholinesterases. One way of examining cardiac parasympathetic control in humans is through pharmacological blockade with for example atropine. Since all parasympathetic postganglionic receptors are muscarinic, high doses of atropine effectively disrupt all parasympathetic influence to the heart. During the blockade, heart period in the absence of all parasympathetic effects can be measured and compared to the unblocked state (Berntson *et al.*, 1994; Martinmaki *et al.*, 2006). Subjects with high parasympathetic activity will show a larger increase in heart rate during blockade than subjects with lower activity.

Non-invasive estimation of parasympathetic cardiac control can further be obtained by measuring time or frequency domain indices of heart rate variability in the respiratory frequency range, also called respiratory sinus arrhythmia (RSA) (Berntson *et al.*, 1994; Cacioppo *et al.*, 1994a; Task Force of the European Society of Cardiology the North American Society of Pacing, 1996). RSA can be derived from the interbeat interval (IBI) time series in the time domain by taking the root mean square of differences between successive interbeat intervals (RMSSD; Penttila *et al.*, 2001) or, in the high frequency domain (HF power) by Fourier analysis (Akselrod *et al.*, 1981; Akselrod *et al.*, 1985) or Wavelet analysis (Pichot *et al.*, 1999; Wiklund *et al.*, 1997) or can be derived by peak-valley estimation (pvRSA; Katona & Jih, 1975) using the time series of IBIs in combination with the respiration signal. Estimates of the pvRSA are obtained by subtracting the shortest IBI during heart rate acceleration in the inspirational phase from the longest IBI during deceleration in the expirational phase. Although all three indices (RMSSD, pvRSA, HF power) seem to reflect parasympathetic activity, few studies have examined the correspondence between these various indices of parasympathetic activity. Under standardized recordings the different time and frequency domain measures are highly correlated with *r*'s > .80 (Grossman *et al.*, 1990; Hayano *et al.*, 1991; Houtveen & Molenaar, 2001; Penttila *et al.*, 2001). The extent to which these three measures of heart rate variability capture the same information across different ambulatory conditions and different subjects has been less well established. For the average 24-hr levels of RMSSD and HF power high test-retest correlations were found after 3 to 65 days in both healthy individuals and cardiac patients (Bigger, Jr. *et al.*, 1992a; Hohnloser *et al.*, 1992; Kleiger *et al.*, 1991; Sinnreich *et al.*, 1998; Stein *et al.*, 1995). Good long-term temporal stability for 24-hr

ambulatory RMSSD has been shown over a period of 7 months (Pitzalis *et al.*, 1996). However, similar temporal stability of ambulatory HF power or pvRSA remains to be established and stability over a period of more than 7 months is currently uncharted for any of these measures.

Sympathetic nervous system activity

Physiology

The preganglionic fibers of the sympathetic branch leave the central nervous system from the thoracic and lumbar regions (region between first thoracic to the second lumbar level) of the spinal cord. Most of the sympathetic ganglia lie close to the spinal cord and form the two chains of ganglia known as the sympathetic trunks (Figure 1). The target organs of sympathetic neurons include among others cardiac and smooth muscle, glandular structures, liver, kidney, bladder, reproductive organs and muscles as well as the skin. The preganglionic neurons employ acetylcholine as the primary neurotransmitter, which binds to a nicotine receptor subtype on the postganglionic neurons in the ganglia. The postganglionic neurons of the sympathetic system employ norepinephrine as the primary neurotransmitter, which can act on α-adrenergic (e.g. in arterioles) or ß-adrenergic receptors (e.g. on the heart). A-adrenergic stimulation causes vasoconstriction by acting on the smooth muscles in the medial layer of the blood vessels. Stimulation of the cardiac ß-adrenergic receptors by norepinephrine released from the cardiac sympathetic nerves increases the pacemaker frequency (i.e. heart rate) as well as contractility of the ventricles. Together vasoconstriction and increased cardiac performance account for the increase in blood pressure seen during sympathetic arousal.

A first exception to the use of norepinephrine as the final effector is found in the sympathetic innervation of eccrine sweat glands, which is cholinergic rather than adrenergic. A second exception is a set of preganglionic neurons that end in a special ganglion, namely the adrenal medulla. Upon activation by preganglionic axons, the adrenal medulla, releases a small amount of norepinephrine into the bloodstream, but most of the released norepinephrine is converted to epinephrine, which is excreted in much larger amounts (Figure 2). Circulating epinephrine preferentially binds to $ß_2$-receptors in the vessels and on the heart, causing vasodilatation (mostly in muscle tissue) and increases in heart rate and contractility.

Measurement

Sympathetic autonomic activity can be assessed invasively by the measurement of regional catecholamine spillover, arterial or venous catecholamine concentration, urinary catecholamine excretion rate or direct microneurographic recordings from nerves innervating the skeletal muscle (Esler *et al.*, 1988; Goldstein *et al.*, 1993; Hagbarth *et al.*, 1972; Hjemdahl, 1993). A different procedure uses pharmacological blockade – either overall (e.g. propranolol) or $ß_1$- (e.g. metoprolol) or $ß_2$-adrenergic (ICI 118-551) receptor specific. Cardiac sympathetic control is estimated by this procedure as the difference between heart period in the unblocked state and during complete blockade of cardiac sympathetic effects.

Sympathetic activity can also be measured non-invasively. Changes in SNS activity modulate the conductance of an applied current to the skin resulting in changes in activity of the sweat glands, or electrodermal activity (Fowles *et al.*, 1981). Sweat gland activation is caused by direct cholinergic stimulation via the preganglionic fibers from the sympathetic nervous system. Blockade studies have shown that atropine strongly reduces sweat gland activity (Foster & Weiner, 1970). Because eccrine sweat glands are at the highest density in palmar and plantar regions, approximately 400/mm^2, most researchers measure skin conductance at these sites. Electrodermal activity increases in response to stress and exercise, in keeping with the increase in sympathetic activity during these conditions (Boucsein, 1992; Critchley, 2002; Dawson *et al.*, 2000). The test-retest reliability coefficients of electrodermal activity over time periods encompassing one day to a year range from .40 to .85 (Ahmed *et al.*, 1994; Freixa i Baque, 1982; Iacono *et al.*, 1984; Schell *et al.*, 1988; Schell *et al.*, 2002; Vossel & Zimmer, 1990).

Thoracic impedance cardiography is a non-invasive technique to measure cardiac sympathetic control (Cacioppo *et al.*, 1994a; Sherwood *et al.*, 1990). In impedance cardiography, the change in the impedance of the enclosed thorax column (dZ) is measured, which is largely a function of aortic blood flow. The impedance cardiogram (ICG) is defined as the first derivative of the pulsatile changes in transthoracic impedance (dZ/dt). From the ICG the pre-ejection period (PEP) can be derived as the time interval between the onset of ventricular depolarization and the opening of the semilunar valves (Sherwood *et al.*, 1990). Changes in PEP index changes in contractility, which in turn depend on changes in ß-adrenergic inotropic effects on the left ventricle. Laboratory studies show

that epinephrine infusion shortens the PEP (Mezzacappa *et al.*, 1999; Schachinger *et al.*, 2001; Svedenhag *et al.*, 1986), whereas ß-blockade prolongs the PEP (Harris *et al.*, 1967; Schachinger *et al.*, 2001; Winzer *et al.*, 1999). During emotional stress, PEP shortens reflecting the sympathetic component of limbic system activation during these conditions (Berntson *et al.*, 1994; Newlin & Levenson, 1979; Sherwood *et al.*, 1986). Also, PEP shortens in a dose-dependent way during bicycle ergometry, reflecting the well-known increases in cardiac sympathetic activity during dynamic exercise (Houtveen *et al.*, 2002; Krzeminski *et al.*, 2000; Miyamoto *et al.*, 1983; Smith *et al.*, 1989a; Svedenhag *et al.*, 1986).

In contrast to the above, postural changes lead to paradoxical responses of the PEP. Head-up tilting from supine to upright systematically prolongs the PEP (Frey & Kenney, 1979; Lewis *et al.*, 1977; Ovadia *et al.*, 1995; Chan *et al.*, 2007) and longer PEPs have also been demonstrated when the subjects goes from supine to sitting to standing (Cacioppo *et al.*, 1994a; Cacioppo *et al.*, 1994b; Houtveen *et al.*, 2005; Sherwood & Turner, 1993; Waldstein *et al.*, 1998). These postural PEP effects would suggest a *decrease* in ß-adrenergic influence on the heart from supine to standing, which is in clear contrast to the known *increase* in sympathetic activity accompanying such changes in posture (Cooke *et al.*, 1999; Esler *et al.*, 1988; Furlan *et al.*, 2000; Laszlo *et al.*, 2001). The failure of the PEP to correctly index changes in sympathetic activity across postures is likely due to the large afterload effects induced by postural changes. Higher afterload will elongate PEP by prolonging the time needed to open the aortic valve, even if contractility is unchanged. Clearly, posture needs to be taken into account when using PEP as a measure of sympathetic activity.

The PEP has been shown to be a stable individual characteristic. In the laboratory, test-retest correlations between .45 and .88 have been found for baseline and stress-task levels of PEP across retest intervals ranging from 28 days to 3 years (Burleson *et al.*, 2003; Matthews *et al.*, 2002; Willemsen *et al.*, 1998). For ambulatory PEP high stability has been found across a few days (Vrijkotte *et al.*, 2004) although no results are available on long-term temporal stability of ambulatory 24-hr measures of PEP. Stability of individual differences may partly arise from genetic factors since substantial heritability for PEP (57%) has been reported (de Geus *et al.*, 2007; Kupper *et al.*, 2006).

It is important to note that the between-subject variance in PEP reflects sympathetic control of cardiac contractility, and not sympathetic activity. Contractility and sympathetic activity may be tightly linked within-subjects

(provided they do not change posture) but between-subjects this need not be true. Chronotropic and inotropic responses to norepinephrine and circulating epinephrine will be modulated by individual differences in the effectiveness of the cardiac $ß_1$- and $ß_2$-adrenergic receptors. Density, affinity and distribution of these receptors may show large individual differences (Liggett, 1995; McCaffery *et al.*, 2002). These individual differences in receptor status may, for instance, lead to a paradoxically long PEP in a subject with high levels of cardiac sympathetic nerve activity when they happen to have very low ventricular ß-receptor densities. In spite of these fears, the scant evidence available does support the idea that *between-subject* differences in absolute PEP reflect differences in cardiac sympathetic activity. Best evidence so far comes from a study in 13 female undergraduate students (Berntson *et al.*, 1994) that showed a high correlation (.82) between absolute PEP and heart period increases in response to sympathetic blockade. In further support, a significant inverse correlation between a subjects' absolute PEP and their plasma epinephrine level was found (Levi *et al.*, 1982).

B-adrenergic inotropic effects on contractility also influence stroke volume (SV), which could be used as a proxy measure for cardiac sympathetic control in addition to the PEP. Stroke volume is the amount of blood pumped through the body per contraction of the left ventricle and can be computed from the impedance cardiogram. Studies on SV have been limited to the laboratory. SV typically increases in response to short lasting stressors (Light *et al.*, 1998; Matthews *et al.*, 2001; Neumann & Waldstein, 2001; Ring *et al.*, 1999). This leaves uncharted how SV changes in response to much longer exposure to stress, such as may occur in the course of a workday, or how it behaves during the ensuing recovery in the evening or during sleep.

A last alternative to index cardiac sympathetic control is low-frequency power (LF power) of heart rate variability. Pagani and coworkers (1986) have advanced the notion that the activity of cardiac sympathetic and parasympathetic nerves is reflected in heart rate variability. A single ratio, spectral power of the heart period time series in the lower frequencies centered around .1 Hz (LF) divided by the power in the higher frequencies centered around the respiratory frequency (HF), is hypothesized to capture the 'sympathovagal' balance. The idea is that during sympathetic activation the resulting tachycardia is usually accompanied by a marked reduction in total power (TP), whereas the reverse occurs during vagal activation. When the

spectral components are expressed in absolute units (ms^2), the changes in TP influence LF and HF in the same direction and prevent the appreciation of the fractional redistribution of the energy. This information is regained when LF and HF are expressed as a ratio, or when LF and HF power are measured in normalized units (nu), which represent the relative value of each power component in proportion to the total power minus the VLF component (Burr, 2007; Malliani *et al.*, 1991). However, the usefulness of the LF/HF ratio (or LFnu) is ultimately determined by its validity, which has been the subject of continued controversy. This controversy is best illustrated by the critical appraisal of sympathovagal balance by dr Eckberg in 1997 (Eckberg, 1997) followed by responses of many equally authorative experts in the field of autonomic nervous system physiology (Malik & Eckberg, 1998; Malliani *et al.*, 1998; Sleight & Bernardi, 1998). For the LF/HF ratio to reflect sympathovagal balance, ideally two assumptions must be met: 1) HF power increases when vagal control over the heart increases; 2) LF power increases when sympathetic control over the heart increases. The former assumption has received a substantial degree of support but the latter assumption, that LF power reflects cardiac sympathetic activity, has proven much more controversial. Surprisingly, direct comparisons of the LF/HF ratio with PEP or electrodermal activity are virtually lacking (see Burgess *et al.*, 2004 for an exception).

Influence of the hypothalamic-pituitary-adrenocortical axis on the effectiveness of sympathetic nervous system activity

Most threats to homeostasis are met by a coordinated neurohumoral response of central limbic and hypothalamic centers that exert combined influences on ANS activity and stress-hormones (Lovallo, 2005). Both adrenal cortex and medulla, for instance, respond to physical and mental stress and metabolic abnormalities (Figure 3). Whereas the adrenal medulla is primarily under SNS control, the adrenal cortex is largely regulated by the hypothalamic-pituitary-adrenocortical (HPA)-axis. A dysfunctional HPA-axis is associated with hypertension (Kelly *et al.*, 1998), the metabolic syndrome (Rosmond & Bjorntorp, 2000), autoimmune processes (Tsigos & Chrousos, 2002), and depression (Holsboer, 2000). The HPA-axis consists of a cascade of physiological reactions that is initiated by the release of corticotrophin-releasing hormone (CRH) from the paraventricular nucleus (PVN) of the hypothalamus. The release of CRH stimulates the release of adrenocorticotrophic hormone (ACTH) from the anterior

pituitary. ACTH in turn stimulates the adrenal gland to release cortisol in the blood stream. The HPA-axis involves a negative feedback cycle; in response to increased cortisol levels, the hypothalamus and pituitary suppress CRH and ACTH production.

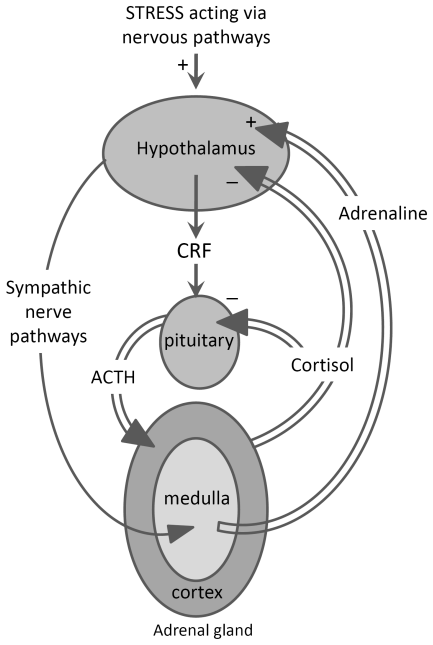

Figure 3 *Interaction between the SNS and the HPA-axis.*

Under influence of the suprachiasmatic nucleus about 10-15 well-defined ACTH driven pulses of cortisol are secreted over 24-hr, resulting in the characteristic cortisol circadian rhythm (Scheer, 2003). Cortisol levels peak early in the morning, prior to awakening, and decrease progressively during the day reaching low levels in the evening. Superimposed on the basal levels of cortisol are stress-induced secretions of cortisol. Cortisol can be measured invasively in blood samples, reflecting the cortisol response over the last hour. As an alternative, cortisol can also be assessed non-invasively in urine or saliva samples. By collecting cortisol from urine, recommended to obtain over 24-hr, the information about the circadian rhythm is lost. In saliva samples, the fraction of unbound cortisol over the last 20-30 minutes is measured. Salivary cortisol is a reliable reflection of plasma or serum cortisol concentrations, with high correlations found between these measures (Goodyer *et al.*, 1996; Harris *et al.*,

1990; Reid *et al.*, 1992; Woodside *et al.*, 1991). Measuring cortisol in saliva has many advantages; it is stress-free, non-invasive, allows frequent and rapid sampling, and sampling can take place at home (Kirschbaum & Hellhammer, 1994). These advantages are balanced by potential errors due to participants' imperfect compliance to the instructed sampling times and unreliability of self-reported awakening time (Kudielka *et al.*, 2003; Kupper *et al.*, 2005).

The cortisol levels measured during the awakening period are partly under control of genetic factors, but cortisol levels throughout the remainder of the day are not heritable (Bartels *et al.*, 2003; Kupper *et al.*, 2005a). Several studies have further shown that main factors influencing cortisol are age, gender, smoking, mood, body composition, use of oral contraceptives, sleep duration, sleep quality and awakening time, although the results are sometimes contradictory (Deuschle *et al.*, 1997; Knutsson *et al.*, 1997; Ukkola *et al.*, 2001; Wust *et al.*, 2000). Besides direct measurement of cortisol, HPA-axis responsivity can also be tested with a dexamethasone suppression test. In healthy subjects dexamethasone causes the pituitary to stop the secretion of ACTH, with a corresponding decrease in cortisol level. The administration of dexamethasone in the evening or midnight ensures that the plasma concentration of dexamethasone is high enough to provide negative feedback at the HPA-axis during the start of the diurnal increase in plasma cortisol concentration that occurs in the early hours of the morning (Sherwood *et al.*, 1990).

The acute response to most physical and psychological stress is dominated by sympathetic nervous system action on the heart and blood vessels, i.e. increased blood pressure and cardiac output, and increased vascular resistance, most prominently in the vessels of non-muscular tissue. Although hypothalamic release of CRH is also immediate, the actual release of cortisol is delayed by many minutes. More importantly, the bulk of steroid effects on tissues (including those of cortisol) is genomic, rather than through membrane-receptor signaling. This means that during exposure to stressors for a period of up to an hour the ongoing cardiovascular response will not be influenced by the stress-induced cortisol rise. However, basal cortisol levels preceding the onset of the stressor do seem to influence the cardiovascular effects of increases in sympathetic activity (Roy *et al.*, 2001). This is called the permissive effect of cortisol (Sapolsky *et al.*, 2000). The term permissive is used because cortisol allows catecholamines to exert their full actions by promoting epinephrine synthesis and inhibiting catecholamine re-uptake (Munck & Naray-Fejes-Toth, 1994). Such time-delayed

permissive effects on cardiovascular reactivity make good evolutionary sense in light of the clear diurnal rhythm in cortisol (Ice *et al.*, 2004). Cortisol levels begin to rise sharply a few hours before awakening suggesting that its augmentation of sympathetic effects is optimal during the day when fight-flight responses can be essential for survival. In spite of the theoretical attractiveness, direct evidence for permissive actions of early morning cortisol levels on sympathetic and cardiovascular stress-reactivity in the course of the day is currently lacking.

Outline of the thesis

This thesis uses data from three different studies to address questions of stability and validity of various measures of ANS activity. Chapter 2, 3 and 4 report on the data obtained from ambulatory recordings in 65 subjects (20 males) with a mean age of 31 years who were measured twice separated by an average time span of 3 years and 4 months. Although ambulatory monitoring provides us with the opportunity to test the effect of stress on ANS in a real life setting, its higher ecological validity is balanced by a lack of experimental control over important confounders of the autonomic nervous system. In comparison to laboratory recording, ambulatory settings are characterized by frequent changes in activity and posture, frequent speech, circadian rhythms, temperature variations and larger variance in emotional state and mental load. In particular the changes in activity and posture are important because sympathetic and parasympathetic activity are known to be very sensitive to these factors (Allen & Crowell, 1989; Houtveen *et al.*, 2005; Kamphuis & Frowein, 1985; Mulder, 1992) and they may explain the largest part of the variance in 24-hr real-life recordings (Grossman *et al.*, 2004). Throughout this thesis, these factors were taken into account by stratifying all analyses for ongoing posture and physical activity, which was detected using repeated diary reports in combination with an inbuilt movement sensor.

In chapter 2, ambulatory recording was used to test the association between three different time and frequency domain measures of RSA in a naturalistic setting: the RMSSD, peak-valley RSA, and HF power. Furthermore, temporal stability was assessed over a 3 year and 4 months period. Using the same data set chapter 3 deals with the temporal stability of ambulatory PEP and stroke volume, as measured by impedance cardiography.

As described above, various non-invasive indicators of sympathetic nervous system activity are in use in psychophysiological studies, including heart rate frequency measures, impedance derived measures, and skin conductance measures. Only few studies have examined the correspondence between these indices of sympathetic activity. In chapters 4 and 5, we tested to what extent these three sympathetic measures are exchangeable in within- and between-subject designs. Chapter 4 first compares PEP to the LF/HF ratio, using the ambulatory data of chapters 2 and 3. Chapter 5 then tests to what extent PEP and electrodermal measures are comparable. For this comparison, data were used of the laboratory study that formed the basis of chapter 6, because ambulatory recording of palmar skin conductance was not considered feasible.

To examine the importance of the interaction between the HPA-axis and the ANS, chapter 6 tested the permissive effects of the early morning cortisol rise on daytime cardiac sympathetic responses to stress. In a double-blind randomized controlled design, 39 subjects were tested twice, once on a placebo day and once on a day on which the early morning cortisol rise was blocked by dexamethasone. Laboratory measurements of ANS function were obtained during exposure to different mental stressors and physical stressors and during subsequent recovery periods. The permissive effect of cortisol was tested in two different ways. First, we tested whether the natural occurring variation in the early morning levels in cortisol could predict sympathetic and cardiovascular reactivity to the stressors. Second, in a within-subject design, sympathetic and cardiovascular reactivity during the placebo day was compared with reactivity during the dexamethasone day.

An important application of ambulatory recording of within- and between-subject variation in the measures used in this thesis is to examine the effect of differences in lifestyles on ANS activity and of experimental intervention on these lifestyles. Based on the vast literature claiming a causal effect of regular exercise on ANS activity, chapter 7 tests the effect of training state on cardiac autonomic control in a naturalistic setting. First, 26 vigorous exercisers were compared to 26 age- and sex-matched sedentary controls who had not engaged in regular exercise during the past year. Next, the 26 vigorous exercisers were subjected to a six week standardized training program to synchronize their training state, after which they were randomized to either 2 weeks of continued training or 2 weeks of detraining.

In the general discussion (chapter 8), the results presented in the previous chapters are summarized and a number of recommendations are made to improve future ambulatory recording of ANS function, based on the results of this thesis.

Chapter 2

Comparison of time and frequency domain measures of RSA in ambulatory recordings

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Abstract

The extent to which various measures of ambulatory respiratory sinus arrhythmia (RSA) capture the same information across conditions in different subjects remains unclear. In this study the root mean square of successive differences (RMSSD), peak valley RSA (pvRSA) and high frequency power (HF power) were assessed during ambulatory recording in 84 subjects of which 64 were retested after about 3 years. We used covariance structure modeling to test the equality of the correlations among 3 RSA measures over 2 test days and 3 conditions (daytime sitting or walking, nighttime sleep) and in groups with low, medium and high mean heart rate (HR), or low, medium and high mean respiration rate (RR). Results showed that ambulatory RMSSD, pvRSA, and HF power are highly correlated and that their correlation is stable across time, ambulatory conditions, and a wide range of resting HR and RR values. RMSSD appears to be the most cost-efficient measure of RSA.

Introduction

Measures of heart rate variability (HRV) provide a window on the modulation of heart rate by the autonomic nervous system, and have broad applications in both human and animal physiology (Berntson *et al.*, 1997; Task Force of the European Society of Cardiology the North American Society of Pacing, 1996). Within-subject studies show that HRV is responsive to changes in psychological state, particularly mental load and emotional stress (Allen & Crowell, 1989; Kamphuis & Frowein, 1985; Langewitz & Ruddel, 1989; Mulder, 1992; Sakakibara *et al.*, 1994), and to changes in posture and physical activity (Hatfield *et al.*, 1998; Houtveen *et al.*, 2002; Houtveen *et al.*, 2005; Tulppo *et al.*, 1996). Between-subjects studies further show that lower levels of HRV independently predict cardiac disease and cardiac mortality (Bigger, Jr. *et al.*, 1993; Dekker *et al.*, 1997; Dekker *et al.*, 2000; Hayano *et al.*, 1991; Lombardi *et al.*, 1987; Nolan *et al.*, 1998; Saul *et al.*, 1988; Singer *et al.*, 1988; Singh *et al.*, 1998; Tsuji *et al.*, 1996).

Most research has focused on HRV in the respiratory frequency range, also known as respiratory sinus arrhythmia (RSA). RSA is the difference in heart period during the inspiratory and expiratory phases of the respiratory cycle. RSA shows virtually no sensitivity to sympathetic nervous system activity but is affected in a dose-response way by muscarinergic blockers in humans (Martinmaki *et al.*, 2006) or vagal cooling in animals (Katona & Jih, 1975). This has led to the use of tonic RSA levels as a proxy for individual differences in vagal cardiac control (Berntson *et al.*, 1997; Task Force of the European Society of Cardiology the North American Society of Pacing, 1996), although not without controversy because of potential confounding by individual differences in sensitivity of chemoreceptor and baroreceptor reflexes (Berntson *et al.*, 1997; Houtveen *et al.*, 2002) and by individual differences in respiratory behavior (Grossman *et al.*, 2004; Grossman & Kollai, 1993; Ritz & Dahme, 2006).

RSA can be derived from the interbeat interval (IBI) time series in the time domain by taking the root mean square of differences between successive interbeat intervals (RMSSD; Penttila *et al.*, 2001) or, in the frequency domain by Fourier- (Akselrod *et al.*, 1981; Akselrod *et al.*, 1985) or Wavelet-analysis (Pichot *et al.*, 1999; Wiklund *et al.*, 1997). RSA can also be derived by peak-valley estimation (pvRSA; Katona & Jih, 1975) using the time series of IBIs in combination with the respiration signal.

An important feature of these time and frequency domain RSA measures is that they can be reliably measured under naturalistic conditions with the use of ambulatory monitoring (de Geus *et al.*, 1995; Houtveen *et al.*, 2005; Wilhelm *et al.*, 2003). In stress research, ambulatory recording is a huge advantage. Different between-subject and within-subject mechanisms may determine cardiovascular reactivity to artificial laboratory stressors than to realistic stressors, encountered repeatedly at home or in the work setting. Generalization of individual differences in cardiovascular stress reactivity from standardized laboratory situations to actual real life situations has indeed been shown to be moderate at best (Gerin *et al.*, 1994; Kamarck *et al.*, 2003; van Doornen *et al.*, 1994).

With regard to potential negative health consequences of stress, ambulatory monitoring may be expected to have higher predictive validity for long-term health outcomes than laboratory measurements. This has already been shown to be the case for blood pressure, where ambulatory levels are better predictors for cardiovascular morbidity and mortality than laboratory or office measurements (Pickering & Devereux, 1987; Verdecchia *et al.*, 1994; Verdecchia *et al.*, 1998; Verdecchia *et al.*, 2001). It is reasonable to assume that prolonged recording of RSA in naturalistic settings will also have added predictive power over short-term recordings. This assumption, however, remains to be tested empirically.

Testing this assumption will require large-scale ambulatory recording in many thousands of subjects and a rational choice between the various RSA measures is direly needed. Unfortunately, the extent to which these measures capture the same information across different ambulatory conditions and different subjects remains unclear. Although time and frequency domain measures are highly correlated under standardized recordings, with *r*'s > .80 (Bigger, Jr. *et al.*, 1992b; Byrne & Porges, 1993; Grossman *et al.*, 1990; Hayano *et al.*, 1991; Houtveen & Molenaar, 2001; Penttila *et al.*, 2001), we may expect them to diverge more strongly under ambulatory recording conditions. In ambulatory recordings, higher ecological validity is balanced by a lack of experimental control over important confounders of RSA. In comparison to laboratory recording, ambulatory settings are characterized by frequent changes in activity and posture, frequent speech, circadian rhythms, temperature variations, and a larger variance in emotional state and mental load. The

differential sensitivity of the various RSA measures to these within-subject factors is currently unknown.

Previous studies have shown that the sharpest changes in RSA levels arise when going from awake to sleep recording and that RSA in awake recordings is most sensitive to changes in physical activity and posture (Feldman & Weidenfeld, 2002; Grossman *et al.*, 2004; Kupper *et al.*, 2004). One design to examine the potential differential sensitivity of various RSA measures to these within-subject factors is to compare the structure of the correlations between ambulatory RSA measures across daytime and nighttime recordings, and, during the daytime part of the recording, across sitting activities and activities involving upright physical activity.

Even within these restricted ambulatory conditions, between-subject variance in the mean and range of respiration rate (RR) and heart rate may still be larger than in laboratory testing. This is problematic because individual differences in RR and heart rate both influence RSA measures and such influence may be independent of cardiac vagal control (Berntson *et al.*, 2005; Grossman *et al.*, 1991; Grossman & Kollai, 1993). The differential sensitivity of the various RSA measures to these between-subject confounders is currently unknown. It can be addressed by comparing the correlation structure of ambulatory RSA measures across groups of subjects selected to have low, medium and high mean heart rate during the ambulatory test day, or across groups with low, medium and high mean RR.

In the present study, we test the correlation between RMSSD, pvRSA, and a frequency domain (HF power) measure of RSA in an ambulatory setting. First, reliability will be assessed for each of the measures by looking at short-term within-day test-retest correlations and correlations between genetically identical twins. Next, temporal stability will be assessed across an average period of three years. Finally, we will use covariance structure modeling to test the equality of the correlations across major changes in ambulatory activity (sleep vs. awake sitting vs. awake standing/walking) and across groups of subjects with low, medium and high mean IBI or low, medium and high mean RR. Based on previous laboratory findings and a previous small-scaled ambulatory study (Vrijkotte *et al.*, 2001) we expect that RMSSD, pvRSA and HF power will show high correlations over time, across ambulatory activity, and across a wide range of mean IBI and RR.

Methods

Participants

Participants were all registered in the Netherlands Twin Register (NTR). They came from families that participated in a linkage study searching for genes influencing personality and cardiovascular disease risk, which is described elsewhere (Boomsma *et al.*, 2000). Out of the 1332 twins and siblings who returned a DNA sample (buccal swabs) for the linkage study, 816 also participated in cardiovascular ambulatory monitoring. Reasons for exclusion were pregnancy, heart transplantation, pacemaker and known ischemic heart disease, congestive heart failure, or diabetic neuropathy. Of these subjects a total of 65 (20 male, 45 female) subjects were tested twice separated by a minimum of 2 years and 1 month and a maximum of 4 years and 8 months (mean 3 years and 4 months). RSA measures could not be reliably obtained in one subject. Age at the first day of testing in the remaining 64 subjects ranged between 18 and 62 years (mean = 30.9, *SD* = 9.7). In addition, 20 randomly selected identical MZ twin pairs (18 male, 22 female) with zygosity confirmed by DNA typing, were also included. Average age of the twins was 27 with a range of 18 to 32. These twins were only tested once. The Ethics Committee of the Vrije Universiteit approved the study protocol and all subjects gave written consent before entering the study. No payment was made for participation, but all subjects received an annotated review of their ambulatory heart rate recordings.

Ambulatory recording

Subjects were invited to participate in the study by letter and all subjects were subsequently phoned by the researchers, who provided additional information on the study, and made an appointment with the subjects for 24-hr ambulatory monitoring. The first ambulatory measurement took place during a representative workday (or a day with representative housekeeping chores for those who were not employed). The second ambulatory measurement day took place during a comparable (work) day for most of the subjects, but 17 subjects would only participate if the repeated measurement was scheduled on a leisure day. On the day preceding monitoring and on the monitoring day itself subjects were asked to refrain from leisure time exercise or heavy physical work. Subjects were visited at home between 7:00 –10:00 a.m. and were fitted with the Vrije Universiteit Ambulatory Monitoring System (VU-AMS46; de Geus *et al.*, 1995; Riese *et al.*, 2003; Willemsen *et al.*, 1996). The VU-AMS produced an audible

alarm approximately every 30 min (10 min randomized) to prompt the subject to fill out an activity diary. Subjects were instructed to write down their physical activity and bodily postures during the last 30-min period in chronological order. Diary prompting was disabled during sleep.

The ECG and changes in the thorax impedance (dZ) were recorded continuously using six disposable, pregelled Ag/AgCl electrodes. The first ECG/dZ 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 third ECG electrode is a ground electrode and was placed at the lower right abdomen. A second dZ measuring electrode was placed over the tip of the xiphoid complex of the sternum. The dZ current electrodes were placed on the back over cervical vertebra C4 and between thorax vertebras T8-T9. Electrode resistance was kept low (below 10 kΩ) by cleaning the skin with alcohol and rubbing.

Ambulatory signal scoring

Using the activity diary entries in combination with a visual display of the output of an inbuilt vertical accelerometer, the entire 24-hr recording was divided into fixed periods. These periods were coded for posture (supine, sitting, standing, walking, bicycling), type of ongoing activity (e.g. deskwork, dinner, meetings, watching TV), and physical activity level (no, light, medium or heavy physical activity). Minimum duration of periods was always 5 min and maximum duration was always 1 hr. If periods with similar activity and posture lasted more than 1 hr (e.g., during sleep), they were divided into multiple periods of maximally 1 hr. All periods were classified as lying asleep, sitting or standing/walking based on the dominant posture reported; the exact timing of changes in posture was verified using the accelerometer signal from the ambulatory device. We then looked at the self-reported activity and physical load to determine whether this period could be classified as sitting with light physical activity (desk work, watching TV, writing, eating, reading, etc) or sitting interspersed with intermediate physical activity (machine operation). The periods interspersed with intermediate activity were discarded. For standing/walking periods we selected only those periods in which the subjects reported no more than light physical load. For each period coded for posture, activity, and physical load we determined the average RMSSD, pvRSA, and HF

power. An average of 25 periods was created per subject. The mean duration of the sleep periods was 56 min (*SD* = 11), of the sitting activities 26 min (*SD* =14) and of the standing/walking condition 19 min (*SD* = 12). This procedure allowed us to test the sensitivity of the correlation structure of the three RSA measures to major changes in posture and activity.

From the ECG and the dZ we obtained the IBI time series and respiration signal according to the procedures detailed elsewhere (de Geus *et al.*, 1995). Artifact pre-processing was performed on the inter-beat-interval (IBI) data. When the IBI deviated more than 3 *SD* from the moving mean of a particular period it was automatically identified as an artifact and accepted or overruled by visual inspection. Since artifacts cannot simply be deleted because the continuity of time would be lost, spuriously short IBIs were summed and missing beats were 'created' by splitting spuriously long IBIs. The mean IBI and RMSSD values were computed from these corrected IBI time series across each of the labeled periods. RMSSD was defined as:

$$RMSSD = \sqrt{\frac{1}{n}\sum \left(IBI_i - IBI_{i-1}\right)^2}$$

Per breath, estimates of pvRSA were obtained by subtracting the shortest IBI during heart rate acceleration in the inspirational phase (which was made to include 750 ms from the following expiration to account for phase shifts) from the longest IBI during deceleration in the expirational phase (including 750 ms from the following expiratory pause/inspirational phase). When no phase-related acceleration or deceleration was found, the breath was assigned a pvRSA score of zero. Automatic scoring of RR and pvRSA was checked by visual inspection of the respiratory signal from the entire recording. Breathing cycles that showed irregularities like gasps, breath holding, coughing etc., were not considered valid and were rejected and removed from further processing. The three percent shortest and longest breaths were automatically removed from the entire recording before averaging pvRSA across all remaining breaths to a single mean pvRSA for each of the labeled periods. We discarded 17.3% of all automatically scored breaths. A total of 10.3% of these breaths occurred in periods in which we could not reliably establish posture and activity or in which signal quality was deemed insufficient during visual inspection. A further 7% were non-plausible

long or short breaths, deviated more than 3 *SD* from the mean or had close to zero amplitude.

Computation of HF power by Fourier analysis, a widely used strategy to asses RSA, assumes that the data show at least weak stationarity (Weber *et al.*, 1992). Stationarity of time series may be interpreted as having a stable mean and variance over time. In ambulatory studies and/or for analysis of relatively long data segments, the assumption of stationarity is likely to be violated. Therefore, we improve on the usual Fourier approach by using a Wavelet decomposition for the computation of HF power which does not have a stationarity assumption (Houtveen & Molenaar, 2001). Additionally, by using Wavelet transformation much longer ambulatory fragments can be selected for cross-method comparison. Uniformly spaced samples were created by interpolation of the IBI data using a Wavelet interpolation algorithm. Next, Discrete Wavelet Transformation (DWT) was performed using a cardinal cubic spline function as base (see Houtveen & Molenaar (2001) for more information regarding this procedure). This method results in identical power values for stationary relatively short coded periods (e.g. 7 min of quiet reading) as compared to Fourier transformation, but it is superior for our relatively longer and non-stationary coded periods (e.g. first hour of sleep). The HF power was computed as the sum of the variances of the .125 - .25 Hz and .25 - .5 Hz windows. Note that the size of a frequency window always doubles after each Wavelet decomposition step. Since the DWT (like Fourier) suffers from aliasing effects at both ends, the first and last 40 data points (2.5 s) of the time series were excluded from the derivation of the variances.

Statistical analyses

Reliability, heritability, and temporal stability

To test the reliability of RMSSD, pvRSA, and HF power, the short-term within-day test-retest correlations, and the correlations between genetically identical twins were assessed. The within-day correlations were computed between the second and the third hour of sleep, and also between two periods of comparable sitting activities during daytime recordings (e.g. reading a magazine or newspaper or watching TV).

The MZ correlations and temporal stabilities were separately computed for three main ambulatory conditions (sleep, awake sitting activities, awake standing/walking). When the MZ correlation is not unity this means that

environmental influences are creating dissimilarity between genetically identical subjects. Measurement error is completely contained within these environmental influences. Hence, the MZ correlation sets an upper limit to measurement error corresponding to $[1\text{-}rMZ]^2$. Temporal stability was computed as the intraclass correlation between the first measurement and the second measurement that took place after an average period of 3 years and 4 months.

Correlations between RMSSD, pvRSA and HF power

We used covariance structure modeling (Bollen, 1989) to test four hypotheses regarding the equality of the correlations among the three measures (RMSSD, pvRSA, and HF power). All modeling was performed in Mplus, version 4 (Muthén & Muthén, 2005). LISREL 8.53 (Jöreskog & Sörbom, 2001) was used to calculate the standardized residuals.

The first hypothesis states that the correlation structure remains stable over time, i.e. across the two test days. To test this hypothesis we first estimated the full correlation matrix between RMSSD, pvRSA, and HF power in all ambulatory conditions on Test Day 1 and Test Day 2. This model, in which all relations between the measures are estimated freely, is saturated in the sense that the number of estimated parameters equals the number of observed statistics. The saturated model therefore fits the data perfectly, that is, had zero degrees of freedom, and a X^2 of exactly zero. Subsequently, the correlations between the three measures (RMSSD, pvRSA, and HF power) collected during sleeping, sitting and standing/walking on Day 1 were constrained to be equal to the symmetric correlations collected on Day 2. In addition, the cross-measure cross-test day correlations were fixed to be equal (that is, the correlations of the measures collected on Test Day 1 with those collected on Test Day 2 were fixed to be equal to the correlations of the measures collected on Test Day 2 with those collected on Test Day 1). Figure 1 illustrates this testing procedure.

Under the saturated baseline model, illustrated in the upper panel of Figure 1, all 153 correlations are estimated freely, as is denoted by all correlations having different indices (i.e., all correlations between measures collected on Test Day 1 have index A, all correlations between measures collected on Test Day 2 have index B, all test-retest correlations have index X, and the cross-measure cross-test day correlations have either index C or D). Under the alternative, more restricted, model illustrated in the lower panel Figure 1, the 36 correlations between the measures collected on Test Day 1 are

constrained to be equal to the 36 correlations between the measures collected on Test Day 2. In the lower panel of Figure 1, these correlations all have index A, while correlations with the same numerical extension are actually constrained to be equal (i.e., correlation A1 on Test Day 1 is set equal to correlation A1 on Test Day 2, etcetera). In addition, the cross-measure cross-test day correlations are set to be equal. Therefore, in the lower panel of Figure 1, the 36 correlations of the measures collected on Test Day 1 with the measures collected on Test Day 2, have the same index C as the 36 correlations of the measures collected on Test Day 2 with the measures collected on Test Day 1. Again, correlations with the same numerical extension are fixed to be equal. All in all, this resulted in an alternative model with 72 constraints, and thus 72 degrees of freedom.

The second hypothesis states that the correlation structure is stable over the three main ambulatory conditions (sleep, awake sitting, awake walking). Here, ambulatory data were available for 84 subjects; the 64 subjects that were tested twice and an additional 20 subjects obtained by randomly selecting one of the twins from the 20 MZ twins pairs that were used to compute the MZ twin correlations. The testing procedure with respect to the effect of ambulatory condition is illustrated in Figure 2. Under the saturated baseline model, illustrated in the upper panel of Figure 2, all 36 correlations are estimated freely, as is denoted by all correlations having different indices. More specifically, all correlations between measures collected in the same ambulatory condition have either index A (sleeping), B (sitting) or C (standing/walking). All test-retest correlations of the same measures collected across different ambulatory conditions have index X (between sleeping and sitting), Y (between sleeping and standing/walking), or Z (between sitting and standing/walking). All cross-measure cross-condition correlations have indices D, E, F, G, H and I, respectively. Under the alternative, more restricted, model illustrated in the lower panel of Figure 2, the correlations between the 3 measures (RMSSD, pvRSA and HF power) are constrained across ambulatory conditions (index A), such that correlations with similar numerical extensions are equal (e.g. all A1s are equal). In addition, the cross-measure cross-condition correlations are constrained (index D), such that correlations with the same numerical extension are equal (e.g. all D1s are equal). This resulted in an alternative, restricted model with 21 constraints, and thus 21 degrees of freedom.

			First measurement									Second measurement								
			Sleep			Sitting			Stand/Walk			Sleep			Sitting			Stand/Walk		
			RMSSD	pvRSA	HF	RMSSD	pvRSA	HF	RMSSD	pvRSA	HF	RMSSD	pvRSA	HF	RMSSD	pvRSA	HF	RMSSD	pvRSA	HF
First measurement	Sleep	RMSSD																		
		pvRSA	A1																	
		HF	A2	A3																
	Sitting	RMSSD	A4	A5	A6															
		pvRSA	A7	A8	A9	A10														
		HF	A11	A12	A13	A14	A15													
	Stand/Walk	RMSSD	A16	A17	A18	A19	A20	A21												
		pvRSA	A22	A23	A24	A25	A26	A27	A28											
		HF	A29	A30	A31	A32	A33	A34	A35	A36										
Second measurement	Sleep	RMSSD	X1	D1	D2	D4	D7	D11	D16	D22	D29									
		pvRSA	C1	X2	D3	D5	D8	D12	D17	D23	D30	B1								
		HF	C2	C3	X3	D6	D9	D13	D18	D24	D31	B2	B3							
	Sitting	RMSSD	C4	C5	C6	X4	D10	D14	D19	D25	D32	B4	B5	B6						
		pvRSA	C7	C8	C9	C10	X5	D15	D20	D26	D33	B7	B8	B9	B10					
		HF	C11	C12	C13	C14	C15	X6	D21	D27	D34	B11	B12	B13	B14	B15				
	Stand/Walk	RMSSD	C16	C17	C18	C19	C20	C21	X7	D28	D35	B16	B17	B18	B19	B20	B21			
		pvRSA	C22	C23	C24	C25	C26	C27	C28	X8	D36	B22	B23	B24	B25	B26	B27	B28		
		HF	C29	C30	C31	C32	C33	C34	C35	C36	X9	B29	B30	B31	B32	B33	B34	B35	B36	
First measurement	Sleep	RMSSD																		
		pvRSA	A1																	
		HF	A2	A3																
	Sitting	RMSSD	A4	A5	A6															
		pvRSA	A7	A8	A9	A10														
		HF	A11	A12	A13	A14	A15													
	Stand/Walk	RMSSD	A16	A17	A18	A19	A20	A21												
		pvRSA	A22	A23	A24	A25	A26	A27	A28											
		HF	A29	A30	A31	A32	A33	A34	A35	A36										
Second measurement	Sleep	RMSSD	X1	C1	C2	C4	C7	C11	C16	C22	C29									
		pvRSA	C1	X2	C3	C5	C8	C12	C17	C23	C30	A1								
		HF	C2	C3	X3	C6	C9	C13	C18	C24	C31	A2	A3							
	Sitting	RMSSD	C4	C5	C6	X4	C10	C14	C19	C25	C32	A4	A5	A6						
		pvRSA	C7	C8	C9	C10	X5	C15	C20	C26	C33	A7	A8	A9	A10					
		HF	C11	C12	C13	C14	C15	X6	C21	C27	C34	A11	A12	A13	A14	A15				
	Stand/Walk	RMSSD	C16	C17	C18	C19	C20	C21	X7	C28	C35	A16	A17	A18	A19	A20	A21			
		pvRSA	C22	C23	C24	C25	C26	C27	C28	X8	C36	A22	A23	A24	A25	A26	A27	A28		
		HF	C29	C30	C31	C32	C33	C34	C35	C36	X9	A29	A30	A31	A32	A33	A34	A35	A36	

Figure 1 *Testing equality of the correlations of the RSA measures across the two test days.*
Note: The upper panel shows the Null model in which all 153 correlations are estimated freely. The lower panel shows the Alternative model in which correlations with the same indices are constrained to be equal.

			First measurement								
			Sleep			Sitting			Standing/Walking		
			RMSSD	pvRSA	HF	RMSSD	pvRSA	HF	RMSSD	pvRSA	HF
First measurement	Sleep	RMSSD									
		pvRSA	A1								
		HF	A2	A3							
	Sitting	RMSSD	X1	E1	E2						
		pvRSA	D1	X2	E3	B1					
		HF	D2	D3	X3	B2	B3				
	Standing/ Walking	RMSSD	Y1	G1	G2	Z1	I1	I2			
		pvRSA	F1	Y2	G3	H1	Z2	I3	C1		
		HF	F2	F3	Y3	H2	H3	Z3	C2	C3	
First measurement	Sleep	RMSSD									
		pvRSA	A1								
		HF	A2	A3							
	Sitting	RMSSD	X1	D1	D2						
		pvRSA	D1	X2	D3	A1					
		HF	D2	D3	X3	A2	A3				
	Standing/ Walking	RMSSD	Y1	D1	D2	Z1	D1	D2			
		pvRSA	D1	Y2	D3	D1	Z2	D3	A1		
		HF	D2	D3	Y3	D2	D3	Z3	A2	A3	

Figure 2 *Testing equality of the correlations of the RSA measures across ambulatory conditions. Note: The upper panel shows the Null model in which all 36 correlations are estimated freely. The lower panel shows the Alternative model in which correlations with the same indices are constrained to be equal.*

The third and fourth hypotheses state that the correlation structure is independent of mean IBI and RR respectively. These hypotheses were tested on the data obtained in the 84 subjects on the first test day. We subdivided this sample first into three IBI and next into three RR groups. To do so, mean IBI- and RR-scores were calculated for each participant across the three ambulatory conditions. Based on these mean scores, we distinguished 'low', 'medium' and 'high' IBI and RR groups, corresponding to the lowest 33%, the medium 33%, and the highest 33% of the sample. To test whether the correlation matrices were equal for the low, medium and high groups, multi-group analyses were conducted, in which the correlations between the nine measures recorded on

Test Day 1 (three measures collected under three ambulatory conditions) were first estimated freely in all groups (saturated model), and then constrained to be equal across the groups. For instance, in the alternative model for the IBI groups, the 36 correlations of the low IBI-group were constrained to be equal to the 36 correlations of the medium IBI-group, and the 36 correlations of the high IBI-group, resulting in a restricted model with 72 degrees of freedom. Note that the restrictions concerned the correlations and not the covariances, so the variances of the measures were allowed to differ across groups.

Fit statistics

In testing hypotheses 1 to 4, the more restricted models are always nested under the saturated model (Bollen, 1989). Normally, the fit of nested models is evaluated by means of a likelihood ratio test. This test is constructed by subtracting the X^2-value of the less restrained model with more freely estimated parameters, from the X^2-value of the more restricted model with fewer free parameters. The difference in X^2 (denoted as X^2_{diff}) between the two models follows a X^2-distribution with the number of degrees of freedom (df) equaling the difference in the number of parameters estimated in the two models. The restricted model is considered tenable if its value of the X^2 goodness of fit statistic is not significantly greater than that of the more lenient model, that is, if the difference in X^2 is not significant. However, Browne, MacCallum, Kim, Andersen, and Glaser (2002) noted that the X^2-statistic can be markedly inflated if some measures in the model are highly correlated, for example, if highly reliable measures are used to measure the same or related characteristics several times. In that case, standardized residuals, which are a function of the differences between the observed covariance matrix **S** and the estimated covariance matrix **Σ**, may be (very) small, indicating close fit of the model to the observed data, while the X^2-statistic, and fit indices based on this statistic, indicate a poorly fitting model.

We expected that many of the correlations between the dependent measures in this study would be larger than .80. While such high correlations are desirable in the sense that they indicate reliable measurement and substantial overlap between measures, the consequence may be that the X^2-statistic of the restricted models may assume large values in the presence of trivial misfit. Comparing the restricted model to the more lenient model using the usual likelihood ratio test, may then result in the rejection of a perfectly acceptable

model. In view of the above, we chose to evaluate the fit of the restricted model in a different way. If the fit of the restricted model was in itself acceptable, we always considered the constraints to be tenable, i.e., the sets of correlations to be equal. Although we will report the X^2-statistic of every tested model to conform to common practice, our main indices of fit were the Comparative Fit Index (CFI) and the standardized residuals (Bollen & Long, 1993; Schermelleh-Engel *et al.*, 2003). The CFI is based on the X^2-statistic, but introduces penalties for every additional parameter estimated (i.e., favors more parsimonious models), and is relatively unaffected by sample size. The CFI ranges between 0 and 1.00, with values below .95 indicating poor fit, values between .95 and .97 indicating acceptable fit, and values between .97 and 1.00 indicating good fit. Standardized residuals are standardized differences between the observed covariance matrix **S** and the estimated covariance matrix **Σ**. Ideally, the standardized residuals should lie between –3 and +3, and show a normal distribution by approximation (this can be evaluated readily using LISREL's stem leaf plots). In case the CFI is below .95 and/or the standardized residuals are outside the acceptable range (-3 and +3), the largest (absolute) standardized residual usually indicates the element that is most poorly fitted by the model. To trace these sources of local misspecification, we planned to use the Modification Indices (MIs) supplied by the Mplus program. MIs are calculated for every fixed parameter in the model, and the value of the MIs represents the expected drop in overall X^2 (i.e., improvement in model fit) if the parameter were to be freely estimated.

Missingness

In the comparison across ambulatory conditions, complete data during sleep on both days were available for 51 subjects only, due to ECG or ICG signal loss during sleep. In the presence of missing data, one can use Full Information Maximum Likelihood (FIML) estimation, which uses all available data. However, this method of estimation should only be applied if data are missing (completely) at random (MAR or MCAR; we refer the reader to Shafer and Graham (2002) for a detailed discussion of mechanisms of missingness). The missingness in our data was therefore first examined with SPSS missing data analysis. When considered across all 18 measures, missingness could be considered completely at random (MCAR), as indicated by the non-significance of Little's MCAR test ($X^2(80) = 99.83$, n.s.). In both Mplus and LISREL, FIML estimation could therefore be used

to accommodate missing data so that all available data were used in the model estimation.

Results

Means

Table 1 presents the untransformed means and standard deviations for RMSSD, pvRSA, HF power, IBI, and RR across all ambulatory conditions, and separately for each ambulatory condition (i.e., sleep, awake sitting, and awake standing/walking). Because the RMSSD, pvRSA, and HF power distributions were skewed, their natural logarithms were used in all further analyses. Repeated measures ANOVA showed a significant effect of ambulatory condition on RMSSD ($F(2,51) = 31.63$, $p < .001$), pvRSA ($F(2,50) = 35.94$, $p < .001$), and HF power ($F(2,51) = 10.48$, $p < .001$), and post hoc testing showed that all three measures decreased significantly from sleep to sitting to standing/walking, all $p < .05$. There were no significant main effects of test day on the means of RMSSD, pvRSA, and HF power, or interaction effects between ambulatory condition and test day.

Table 1 *Means (SD) of RMSSD, pvRSA, and HF power separately for the two test days.*

	N	RMSSD (ms)	pvRSA (ms)	HF (ms^2)	IBI (ms)	RR (bpm)
Full recording						
Test	84	47.60	49.82	786.22	803.99	16.70
		(24.06)	(22.08)	(705.55)	(110.44)	(1.10)
Retest	64	41.71	43.95	618.68	792.80	16.63
		(20.85)	(19.87)	(562.17)	(83.65)	(1.35)
Sleep						
Test	78	65.65	60.86	1109.05	981.49	16.23
		(33.19)	(31.22)	(1097.01)	(126.35)	(2.00)
Retest	57	60.36	51.40	991.11	970.61	16.17
		(36.95)	(25.49)	(1163.15)	(107.32)	(2.15)
Sitting						
Test	84	42.00	50.10	688.50	772.67	17.00
		(24.34)	(23.97)	(686.82)	(109.60)	(1.25)
Retest	64	39.47	46.59	590.30	791.15	16.84
		(22.67)	(24.41)	(622.00)	(87.17)	(1.70)
Standing/Walking						
Test	84	38.04	40.35	590.63	689.31	16.87
		(920.52)	(19.44)	(545.34)	(97.25)	(1.01)
Retest	64	33.43	36.77	444.61	700.33	16.72
		(14.73)	(15.64)	(344.60)	(70.57)	(1.25)

Short-term reliability

Within-day correlations between the second and the third hour of sleep, and between two periods of sitting activities all exceeded .85 (see Table 2) suggesting good to excellent short-term reliability for RMSSD, pvRSA, and HF power alike. For comparison, short-term reliability of IBI and RR is also given.

Table 2 *Within-day correlations (intraclass correlations) between the second and the third hour of sleep, and between two periods of light physical sitting activities.*

	N	RMSSD (ms)	pvRSA (ms)	HF (ms^2)	IBI (ms)	RR (bpm)
Sleep	150	.88**	.86**	.89**	.92**	.93**
Sitting	155	.86**	.85**	.87**	.84**	.64**

*** Correlation is significant at .01 level.*

The intrapair MZ correlations further confirmed good reliability (see Table 3). For all three measures, MZ correlations were highest during sitting activities and lowest during standing/walking. Even at standing/walking, however, the lowest MZ correlation for pvRSA suggests that measurement error cannot account for more than 18% of the variance ($[1 - .58]^2$). Based on the within-day test-retest or MZ twin correlations none of the three measures could be favored as the "best", that is, most reliable, RSA measure.

Table 3 *Intrapair MZ twin correlations.*

	N	RMSSD (ms)	pvRSA (ms)	HF (ms^2)	IBI (ms)	RR (bpm)
Full recording	20	.76**	.75**	.76**	.74**	.61**
Sleep	20	.63**	.69**	.65**	.70**	.84**
Sitting	20	.81**	.80**	.80**	.82**	.42*
Standing/Walking	19	.63**	.58**	.60**	.61**	.72**

** Correlation is significant at .05 level.*
*** Correlation is significant at .01 level.*

Temporal stability

Table 4 displays the correlations across the two test days for the three RSA measures, IBI and RR. Temporal stability for RMSSD, pvRSA, and HF power was good when computed across all ambulatory conditions and separately across awake sitting activities. Temporal stability was moderate during standing/walking, potentially because of the low stability of the respiratory frequency during these periods of physical activity. For all measures, recordings proved most stable during sleep. As with short-term reliability, none of the three

measures seemed to be clearly favored by temporal stability as the best RSA measure.

Table 4 *Temporal stability for an average period of 3 years and 4 months.*

	N	RMSSD (ms)	pvRSA (ms)	HF (ms^2)	IBI (ms)	RR (bpm)
Full recording	64	.71**	.58**	.76**	.58**	.74**
Sleep	52	.73**	.72**	.81**	.65**	.91**
Sitting	64	.70**	.68**	.80**	.66**	.69**
Standing/Walking	64	.44**	.44**	.57**	.61**	.37**

*** Correlation is significant at .01 level.*

Correlations between RMSSD, pvRSA, and HF power

Table 5 presents the full correlation matrix between the three RSA measures in all ambulatory conditions on Test Day 1 (upper left block) and Test Day 2 (lower right block) and across test days (lower left block). As can be seen in Table 5, many of the correlations between the dependent measures in this study were larger than .80, and quite a few exceed .90. This justifies our approach of evaluating the fit of the restricted models directly besides comparing the fit of the restricted models to the fit of the saturated model.

Effect of test day

The first hypothesis states that the correlation structure remains stable over time, i.e. across the two test days. The fit indices of the alternative model representing this hypothesis are shown in Table 6 (Model TD). As expected, the difference in X^2 between the saturated model and the alternative model was significant ($X^2_{diff}(72) = 130.12$, $p < .001$). Yet, both the CFI and the standardized residuals indicated that the fit of the alternative model was good. We therefore conclude that the correlations can, to reasonable approximation, be considered equal across the two test days.

Table 5 *Correlations between RMSSD, pvRSA, and HF power separately for lying during sleep, sitting, and standing/walking.*

			First measurement									Second measurement								
			Sleep			Sitting			Standing/Walking			Sleep			Sitting			Standing/Walking		
			RMSSD	pvRSA	HF	RMSSD	pvRSA	HF	RMSSD	pvRSA	HF	RMSSD	pvRSA	HF	RMSSD	pvRSA	HF	RMSSD	pvRSA	HF
First measurement	Sleep	RMSSD	1.00																	
		pvRSA	.79**	1.00																
		HF	.94**	.83**	1.00															
	Sitting	RMSSD	.73**	.50**	.66**	1.00														
		pvRSA	.63**	.59**	.67**	.87**	1.00													
		HF	.70**	.53**	.73**	.93**	.88**	1.00												
	Standing/ Walking	RMSSD	.55**	.32**	.46**	.87**	.72**	.74**	1.00											
		pvRSA	.52**	.46**	.51**	.76**	.84**	.74**	.76**	1.00										
		HF	.57**	.39**	.57**	.87**	.78**	.86**	.94**	.78**	1.00									
Second measurement	Sleep	RMSSD	.73**	.50**	.71**	.59**	.42**	.57**	.39**	.29*	.40**	1.00								
		pvRSA	.58**	.72**	.61**	.36**	.40**	.39**	.21	.26	.24	.77**	1.00							
		HF	.73**	.60**	.81**	.54**	.51**	.64**	.34*	.36**	.46**	.96**	.77**	1.00						
	Sitting	RMSSD	.53**	.24	.51**	.70**	.65**	.70**	.48**	.46**	.52**	.70**	.53**	.65**	1.00					
		pvRSA	.45**	.42**	.51**	.52**	.68**	.59**	.34**	.48**	.43**	.58**	.71**	.59**	.81**	1.00				
		HF	.58**	.39**	.67**	.67**	.70**	.80**	.42**	.50**	.59**	.73**	.58**	.77**	.94**	.81**	1.00			
	Standing/ Walking	RMSSD	.45**	.18	.45**	.60**	.55**	.59**	.44**	.36**	.47**	.67**	.47**	.61**	.92**	.73**	.86**	1.00		
		pvRSA	.30*	.28*	.37**	.41**	.59**	.47**	.28*	.44**	.35**	.48**	.62**	.50**	.73**	.92**	.71**	.73**	1.00	
		HF	.54**	.34**	.63**	.62**	.64**	.75**	.41**	.45**	.57**	.71**	.52**	.75**	.90**	.74**	.95**	.94**	.70**	1.00

* *Correlation is significant at .05 level.*
** *Correlation is significant at .01 level.*

Effect of ambulatory conditions

The second hypothesis states that the correlation structure is stable over the three main ambulatory conditions (sleep, awake sitting, awake standing/walking). Taken that the effect of test day was negligible, the effect of ambulatory condition on the correlations among RMSSD, pvRSA, and HF power was initially tested on data from the first test day only (Model AMBa in Table 6). As expected, the difference in X^2 between the restricted model and the saturated model was significant ($X^2(21) = 95.53$, $p < .001$). However, the CFI was only just below the critical value of .95 (CFI=.94), and the standardized residuals were clearly within the acceptable range. We repeated the analysis on the data from the second test day (Model AMBb). Again, the difference in X^2 between the restricted model and the saturated model was significant ($X^2(21) = 73.35$, $p < .001$), but the small standardized residuals and high CFI indicated good fit. Taken together, the analyses across the two days suggest invariance of the correlation structure of RMSSD, pvRSA, and HF power correlations across sleep, sitting, and standing/walking activities.

Table 6 *The fit indices of all tested models.*

		X^2_{diff}	*df*	CFI	Stand. residuals	
					Min	max
Test day (TD)						
	TD	130.12	72	.98	-.96	1.65
Ambulatory condition (AMB)						
Test day 1	AMBa	95.53	21	.94	-1.78	1.53
Test day 2	AMBb	73.35	21	.95	-1.00	1.63
Groups						
	IBI	107.54	72	.97	-2.33	2.55
	RR	136.67	72	.95	-1.92	1.93

Effect of mean IBI

Next we tested whether the correlations among RMSSD, pvRSA, and HF power were equal for subjects, with low (707.09 ± 43.90), medium (795.95 ± 21.37), or high (922.71 ± 90.52) mean ambulatory IBI. These three groups differed significantly in their IBI scores ($F(2,81) = 92.77$, $p < .001$). Given that the effect of test day was negligible, the effect of group membership on the correlations among RMSSD, pvRSA, and HF power was tested on the data collected on the first test day only. The 36 correlations within each group were constrained to be equal for the low, medium, and high IBI-groups, yielding a restricted model with 72 degrees of freedom (Model IBI). The fit indices of this restricted model are shown in Table 6. Although the difference in X^2 between the

saturated model and the alternative was significant ($X^2_{diff}(72) = 107.54$, $p < .001$), the CFI and the standardized residuals indicated that the fit of the alternative model was acceptable. We therefore conclude that the correlations between the RMSSD, pvRSA, and HF power measures of RSA can be considered approximately equal across groups distinguished with respect to their mean ambulatory heart rate.

Effect of mean RR

Finally, we tested whether the correlations among RMSSD, pvRSA, and HF power were equal for subjects, with low (15.55 ± .45), medium (16.72 ± .25), or high (17.88 ± .68) mean ambulatory RR. These three groups differed significantly with respect to their RR scores ($F(2,81) = 155.09$, $p < .001$). Again, the effect of group membership on the correlations among RMSSD, pvRSA, and HF power was tested on the data collected on the first test day only as the effect of test day had proven negligible. The constraints imposed on the correlation matrices of the RR-groups were analogous to those imposed on the matrices of the IBI-groups. That is, the 36 correlations within each group were constrained to be equal for the low, medium, and high RR-groups, yielding a restricted model with 72 degrees of freedom (Model RR). The fit indices of this restricted model are shown in Table 6. Although the model fitted significantly worse than the saturated model ($X^2_{diff}(72) = 136.67$, $p < .001$), both the CFI and the standardized residuals indicated good fit. We therefore conclude that the correlations between the RMSSD, pvRSA, and HF power measures can be considered approximately equal across groups distinguished with respect to their mean ambulatory RR.

Discussion

Cardiovascular psychophysiology aimed at identifying individuals at risk for future cardiovascular disease is increasingly relying on ambulatory monitoring under the expectation that this has higher predictive validity for long term health outcomes than laboratory measurements (Feldman & Weidenfeld, 2002; Goldstein *et al.*, 2006; Grossman, 2004). RSA is a promising measure for large scale ambulatory studies because it has been linked, both theoretically and empirically, to activity of the parasympathetic nervous system, that, in turn, is paramount to the electrical stability of the heart (Ando *et al.*, 2005; Hull, Jr. *et al.*, 1990; Levy & Schwartz, 1994; Vanoli *et al.*, 1991).

However, RSA can be assessed in many different ways, which clearly differ in terms of costs and whether they are cumbersome to the study-participants or require labor-intensive data-reduction by the researcher. A full ECG recording (e.g. the Holter monitor), for instance, is only mildly cumbersome to the patients who need to wear electrodes and a small portable recording device on the hip. However, this mode of assessment is labor-intensive to the researcher, who needs to perform repeated Fourier analyses to compute HF power on all stationary 5-min segments, which often need visual inspection of the automatically detected erroneous IBIs. In contrast, computation of the RMSSD from the IBI time series obtained from a wristwatch type HR-recording device together with a single elastic recording band around the chest (e.g. the Polar Sporttester) is much less demanding of both participant and researcher. The researcher still needs to visually inspect the automatically detected erroneous IBIs, but computation of time domain measures like RMSSD is otherwise straightforward. Additional recording of the RR would allow computation of pvRSA, but at the cost of adding another layer of work to the data-reduction phase, and an additional burden on the participant, by requiring the wearing of either additional electrodes or respiratory bands.

In this study, we tested whether the assessment of between-subject differences in RSA was sensitive to the method used, that is, whether 'high tech' pvRSA or HF power were superior to 'low tech' RMSSD. The answer is a resounding *no*. All three RSA measures were highly correlated amongst each other and none of them stood out in terms of short-term reliability or temporal stability over a period of more than 3 years. The present findings with respect to the reliability of ambulatory RSA, either defined as a within-day short-term retest coefficient (.85 - .89) or as the intrapair resemblance in genetically identical twins (.58 - .81), are in line with previous studies that showed similarly high test-retest correlations for the average 24-hr levels of RMSSD (.67 - .89) and HF power (.76 - .92) after 3 to 65 days in both healthy individuals and cardiac patients (Bigger, Jr. *et al.*, 1992a; Hohnloser *et al.*, 1992; Kleiger *et al.*, 1991; Sinnreich *et al.*, 1998; Stein *et al.*, 1995). Likewise, the finding that RMSSD in the full recording is temporally stable (.71) across an average period of 3 years and 4 months, is in agreement with the only previous study that had a prolonged test-retest interval and reported long-term stability of .79 for the 24-hr RMSSD level (Pitzalis *et al.*, 1996). The present results contribute to these previous studies by showing similar stability for HF power and pvRSA. In addition, the present study

shows that the high intercorrelations of the three RSA measures do not change over prolonged periods of time. This suggests that longitudinal studies of RSA can, for instance, use pvRSA at the first wave and RMSSD at the second wave.

In keeping with a large body of literature, the mean values of the three RSA measures increased from standing to sitting and from sitting to sleep (Grossman *et al.*, 2004; Houtveen *et al.*, 2005; Martinmaki *et al.*, 2006). Although temporal stability was good to excellent for sitting and sleep, it was only moderate for standing/walking. One possible explanation might be that it is more difficult to arrive at a reliable measure of RSA during standing/walking because the standing/walking periods are relatively short. The mean duration of the sleep periods was 56 min, whereas the mean duration was 26 min for sitting activities, and 19 min for standing/walking. However, if averaging RSA measures across longer periods would yield higher stability than averages across shorter periods then we would expect the temporal stability of the RSA measures to be highest during sleep. This is not the pattern that is evident from Table 3, which shows that correlations during sleep and sitting are comparably high, even though the mean duration of sitting periods was only half the duration of the sleeping periods. Duration per se, therefore, does not seem to explain why RSA measures recorded during walking/standing are less stable than measures obtained in both other conditions. As an alternative explanation, the temporal stability of RSA during standing/walking activities may be lower than that of sitting and sleep, because respiratory behavior in this condition is much more variable. This is directly supported by the lower temporal stability of the respiratory frequency during these periods of physical activity. Based on extensive ambulatory pvRSA data, Grossman *et al.* (2004) have shown that physical activity needs to be taken into account when interpreting ambulatory RSA and our data underscore their warning.

Independent of ambulatory condition, large differences in mean respiratory frequency and heart rate were found. We were concerned that such differences might distort the correlations between the three RSA measures, because RR and heart rate may both distort their relation to cardiac vagal control (Berntson *et al.*, 2005; Grossman *et al.*, 1991; Grossman & Kollai, 1993). This concern was greatly mitigated by the data. Differences in mean resting heart rate or mean RR did not affect the correlations between the various RSA measures; the RSA measures were as highly correlated in a group with a mean heart rate of 83 bpm as in a group with a mean heart rate of 65 bpm, and as

highly correlated in groups with a mean RR of 15 (range 14-16) versus 18 (range 17-20) times per minute. This contradicts the idea that one of these RSA measures is relatively more sensitive than the others to confounders such as individual differences in RR (Grossman, 1992) or in heart rate (Berntson *et al.*, 2005).

Taken together our results suggest that, at least in healthy subjects, neither pvRSA, nor HF power provides superior measures of RSA compared to RMSSD. This favors the RMSSD measure, as the most cost-efficient measure of RSA, because it is most easily obtained with the least effort on the part of the experimenter, and the lowest burden for the participant. This is particularly true in comparison to pvRSA, as this measure necessitates additional recording of the ambulatory respiration signal, which in turn requires the wearing of additional electrodes or a vest, thereby adding to the discomfort of the subjects. However, an obvious advantage of the respiration signal is that it allows a number of additional parameters to be computed from ambulatory recordings, including respiration depth and frequency (de Geus *et al.*, 1995; de Geus *et al.*, 2005; Grossman, 2004; Wilhelm *et al.*, 2003), which are important parameters in themselves. Indeed Ritz and Dahme (2006) recently argued that RSA can be used as a measure of cardiac vagal control only when taking respiratory behavior into account. Likewise, when Fourier or Wavelet analysis are used to obtain HF power, heart rate variability at a lower frequency (LF, .07-.14 Hz) can be measured, which may potentially index sympathetic nervous system activity (Malliani *et al.*, 1991). Furthermore, heart rate variability at very low frequencies (VLF, .001-.07) can be measured, which has been associated with an increased risk for cardiovascular disease independent of HF power (Hadase *et al.*, 2004; La Rovere *et al.*, 2003).

In this study, we used the Wavelet approach to obtain HF power rather than Fourier analysis. Although Fourier analysis has been the more common approach so far, we preferred the Wavelet approach because, in contrast to Fourier analysis, it is not sensitive to violations of the stationarity assumption. Such violations are likely to occur across longer ambulatory recording periods. To deal with this, many ambulatory studies using Fourier transformation have divided the recording into smaller periods of, for example, 5 or 10 min. This reduces probability of non-stationarity and leads to a convergence of Fourier and Wavelet results (Houtveen & Molenaar, 2001). Cross-method convergence may be lower in cases of longer periods as in the current study. Therefore, it remains to

be demonstrated whether the correlations of HF power to RMSSD and pvRSA also holds when Fourier-based HF estimates are used. Wavelet transformation has the additional advantage of allowing precise localization of particular RSA-events in time by providing a full time-frequency decomposition of the IBI time series (e.g., see Pichot *et al.*, 1999). In the current study, we have not used this additional time-frequency information since no comparable information can be obtained from RMSSD and pvRSA.

A limitation of this study that is worth mentioning is that we examined the relative behavior of the three ambulatory RSA measures by comparing them to each other across time and ambulatory conditions. True validity testing, however, would require comparison of each of the RSA measures to some future cardiovascular disease endpoint, or to an external validation criterion of cardiac vagal control, which would be the most plausible explanation for any cardioprotective effects associated with high levels of RSA. Although a number of studies have shown predictive validity of RMSSD (Dekker *et al.*, 2000; Nolan *et al.*, 1998; Singh *et al.*, 1998; Tsuji *et al.*, 1996) and HF power (Bigger, Jr. *et al.*, 1993; Singh *et al.*, 1998; Tsuji *et al.*, 1996) for cardiovascular disease, none has done so for pvRSA. However in these prospective studies, HF and RMSSD were either measured during short periods (Dekker *et al.*, 2000; Singh *et al.*, 1998; Tsuji *et al.*, 1996), or when full 24-hr ambulatory recording was used, in patient samples (Bigger, Jr. *et al.*, 1993; Nolan *et al.*, 1998). Whether prolonged recording of RSA in naturalistic settings has predictive power in the population at large, remains to be established. Taken the high correlations among the three measures in this study, it is hard to imagine that one of these measures would exceed the others in predictive power.

To test which of these RSA measures is most closely associated with individual differences in cardiac vagal control ambulatory recordings could be made under partial and full parasympathetic blockade. We do not consider such long-term (i.e. 24-hr) pharmacological interventions feasible in a true naturalistic setting. However, a number of studies in controlled experimental settings have shown that RMSSD, pvRSA, and HF power all respond to muscarinergic blockade by showing a graded, almost linear, decrease with increasing dose (Berntson *et al.*, 1997; Cacioppo *et al.*, 1994a; Martinmaki *et al.*, 2006; Task Force of the European Society of Cardiology the North American Society of Pacing, 1996). Combined, these findings and the high correlations among the three measures in a realistic ambulatory setting render it unlikely that

one of these measures would exceed the others in detecting individual differences in daily cardiac vagal control. In summary, we conclude that ambulatory RMSSD, pvRSA, and HF power are highly correlated and that their correlation is stable across time, ambulatory conditions, and a wide range of resting HR and RR values. Since the different RSA measurement strategies have varying specific advantages, for instance, providing additional information on RR or on (very) low frequency power, the choice for a specific measure should be based on the exact research questions. In large-scale research that focuses entirely on individual differences in RSA as correlates or predictors of disease risk, RMSSD appears to be the most cost-efficient measure.

Chapter 3

Temporal stability of ambulatory stroke volume and cardiac output measured by impedance cardiography

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Abstract

Recently, devices have become available that allow non-invasive measurement of stroke volume and cardiac output through ambulatory thorax impedance recording. If such recordings have adequate temporal stability, they offer great potential to further our understanding of how repeated or chronic cardiovascular activation in response to naturalistic events may contribute to cardiovascular disease. In this study, 24-hr ambulatory impedance-derived systolic time intervals, stroke volume, and cardiac output were measured in 65 healthy subjects across an average time span of 3 years and 4 months. Stability was computed separately for sleep and daytime recordings. To avoid confounding by differences in posture and physical activity across measurement days, temporal stability was computed using sitting activities only. During the day intraclass correlations were moderate for stroke volume (.29–.46) and cardiac output (.33–.46) and good for systolic time intervals (.55–.81). When test-retest comparison was limited to two comparable days (two work days or two leisure days), correlations for both SV (.42-.46) and CO (.43-.50) improved. Conclusion: Moderate long-term temporal stability is found for individual differences in ambulatory stroke volume and cardiac output measured by impedance cardiography.

Introduction

Frequent and large increases in blood pressure in reaction to psychological stress is hypothesized to be a risk factor for hypertension (Gerin *et al.*, 2000). Blood pressure reactivity is due to a combination of changes in cardiac output (CO) and total peripheral resistance (TPR). The relative contribution of CO and TPR responses to blood pressure reactivity can vary strongly across different types of mental and emotional challenges (Kasprowicz *et al.*, 1990; Lawler *et al.*, 2001; Lovallo *et al.*, 1993). In addition, within a single type of stressor the relative contribution of the TPR response seems to increase with prolonged duration of the stressors (al'Absi *et al.*, 1997; Allen and Crowell, 1989; Carroll and Roy, 1989; Miller and Ditto, 1989; Miller and Ditto, 1991; Ring *et al.*, 2002). Most importantly, large individual differences are seen in the pattern of CO or TPR responses to psychological stress (Brod *et al.*, 1959; Girdler *et al.*, 1990; Kline *et al.*, 2002; Sherwood *et al.*, 1993). Test-retest reliability of CO and TPR reactivity to various laboratory stressors ranges from high across several weeks (Kamarck *et al.*, 1992) to moderately high across one week (McGrath and O'Brien, 2001) and across 3 years (Matthews *et al.*, 2002). This is comparable to the short term (Kamarck *et al.*, 1993; Llabre *et al.*, 1993; Swain and Suls, 1996) or longer term (Allen *et al.*, 1987; Matthews *et al.*, 2002; Sherwood *et al.*, 1997) reliability of systolic blood pressure (SBP) and heart rate (HR) responses to laboratory stressors.

CO and TPR can be computed from the conjoint measurement of only three parameters: heart rate (HR), blood pressure (BP), and stroke volume (SV) (Sherwood *et al.*, 1990). It is very easy to obtain HR and BP non-invasively by using ECG recordings and arm-cuff auscultatory methods respectively. Non-invasive SV has been more elusive, but at least two techniques are now available (Harms *et al.*, 1999; Sherwood *et al.*, 1991) of which impedance cardiography is most often used. In impedance cardiography, two voltage electrodes, typically bands of aluminium-coated Mylar fastened with adhesive strips around the neck and waist, introduce a high-frequency alternating current to the thorax. Two inner and parallel bands measure the changes in the impedance of the enclosed thorax column (dZ), which is largely a function of aortic blood flow. The impedance cardiogram (ICG) is defined as the first derivative of the pulsatile changes in transthoracic impedance (dZ/dt). From the ICG, two systolic time intervals can be derived, the pre-ejection period (PEP) and left ventricular ejection (LVET). In addition, the blood volume ejection rate of the left ventricle

can be estimated by the forward extrapolation of the maximum early slope of dZ, or the $dZ/dt_{(min)}$ amplitude. Using the most widely used equation for the estimation of SV, the Kubicek equation (Kubicek *et al.*, 1966), SV is computed as the product of the total duration of systolic ejection and the volume ejection rate, after taking into account the individual's resting thorax impedance and the height of the thorax column enclosed by the measuring electrodes.

Until recently impedance cardiographic studies have been limited to the laboratory where SV and CO responses are measured in response to short lasting stressors (Light *et al.*, 1998; Matthews *et al.*, 2001; Neumann and Waldstein, 2001; Ring *et al.*, 1999). This leaves uncharted how SV and CO change in response to much longer exposure to stress, such as may occur in the course of a workday. It also remains to be established how SV and CO may change from daytime periods with high sympathetic activation to nighttime periods when sympathetic activation is strongly reduced (Burgess *et al.*, 1997; Lechin *et al.*, 2004; Trinder *et al.*, 2001; van Eekelen *et al.*, 2004). The study of this more prolonged SV dynamics in naturalistic settings requires ambulatory monitoring.

Recently, various systems have become available that allow the ambulatory monitoring of SV through impedance cardiography (Cybulski, 2000; Nakonezny *et al.*, 2001; Sherwood *et al.*, 1998; Willemsen *et al.*, 1996). A number of studies have demonstrated the validity of measuring systolic time intervals, SV and CO with this approach (Riese *et al.*, 2003; Vrijkotte *et al.*, 2004; Willemsen *et al.*, 1996). The temporal stability of individual differences in impedance-derived ambulatory SV and CO remains to be established. In doing so, an important source of confounding will be the potential difference in (physical) activity patterns during the first and the second measurement day. Shifts in posture and physical activity strongly affect cardiac sympathetic drive as well as cardiac afterload and preload which all have an impact on SV (Cacioppo *et al.*, 1994; Sherwood and Turner, 1993). In addition, postural changes are expected to alter the relative position of measuring and current electrodes, the exact shape of the enclosed thorax column, and the resulting basal thorax impedance (Z_0) (Laszlo *et al.*, 2001; Mohapatra, 1981; Toska and Walloe, 2002). Both the electrode distance and the basal thorax impedance are important parameters in the Kubicek equation (Kubicek *et al.*, 1966). It is crucial, therefore, to base test-retest comparisons of SV values on carefully selected periods with unchanged posture and physical activity.

A previous study on ambulatory ICG recordings (Riese *et al.*, 2003) showed that, in a small number of subjects, reliable detection of the B-point in the first derivative of thoracic impedance signal ($dZ/dt_{(min)}$) can be difficult. This point corresponds to the opening of the aortic valve and is used to define the PEP but also to compute the $dZ/dt_{(min)}$, a crucial parameter in SV computation. On theoretical grounds it is more appropriate to measure $dZ/dt_{(min)}$ in relation to this B point (SV_B) (Debski *et al.*, 1993; Doerr *et al.*, 1981; Mohapatra, 1981), but $dZ/dt_{(min)}$ can alternatively be measured in relation to the dZ/dt = 0 baseline (SV_0) (Sherwood *et al.*, 1991). Since the latter can be more reliably established in all subjects, it is prudent to establish temporal stability for ambulatory SV using $dZ/dt_{(min)}$ both in relation to dZ/dt = 0 (SV_0) and to the dZ/dt B-point (SV_B).

The present study reports on ambulatory SV and CO measured by impedance cardiography in 65 subjects who were tested twice across an average time span of 3 years and 4 months. We established long-term temporal stability of individual differences in 24-hr ambulatory SV_0, SV_B, CO_0, and CO_B, while accounting for differences in posture and physical activity on the two measurement occasions.

Methods

Subjects

Participants were all registered with the Netherlands Twin Register (NTR). They came from families that participated in a linkage study searching for genes influencing personality and cardiovascular disease risk, which is described elsewhere (Boomsma *et al.*, 2000). Out of the 1332 twins and siblings who returned a DNA sample (buccal swabs) for the linkage study, 816 were also willing to participate in cardiovascular ambulatory monitoring. Reasons for exclusion were pregnancy, heart transplantation, pacemaker and known ischemic heart disease, congestive heart failure, or diabetic neuropathy. Of these subjects a total of 65 (20 male, 45 female) were tested twice separated by a minimum of 2 years and 1 month and a maximum of 4 years and 8 months (mean 3 years and 4 months). At the first test day the age ranged from 18-62 year (mean = 30.7, S.D. = 9.7). The Ethics Committee of the Vrije Universiteit approved of the study protocol and all subjects gave written consent before entering the study. No payment was made for participation, but all subjects received an annotated review of their ambulatory heart rate and blood pressure recordings.

Ambulatory recording

Subjects were invited to participate in the study by letter and subsequently phoned by the researchers to receive additional information on the study, and to make an appointment for 24-hr ambulatory monitoring. The first ambulatory measurement took place during a representative workday (or a day with representative housekeeping chores for those who were not employed). The second ambulatory measurement day took place during a comparable (work) day for most of the subjects, but 17 subjects would only participate if the repeated measurement was scheduled on a leisure day. On the day preceding monitoring and on the monitoring day itself subjects were asked to refrain from leisure time exercise or heavy physical work. Subjects were visited at home between 7.00 – 10.00 a.m. and fitted with the Vrije Universiteit Ambulatory Monitoring System (de Geus *et al.*, 1995; VU-AMS46, Riese *et al.*, 2003; Willemsen *et al.*, 1996). They received detailed instructions to regularly check the 'all clear' signal of the device (a small blinking light on the side of the device), and how to proceed in case of suspected device malfunction. The VU-AMS produced an audible alarm approximately every 30 minutes (±10 minutes randomized) to prompt the subject to fill out an activity diary. They were instructed to write down their physical activity and bodily postures during the last 30 minutes period in chronological order. Diary prompting was disabled during sleep, but regular beat-to-beat recording of the ICG was maintained throughout the night. The following day the participants were visited again to collect the equipment.

The ECG and ICG were recorded continuously during a 24-hr period (daytime and sleep) using 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 third ECG electrode is a ground electrode and was placed at the lower right abdomen. A 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 vertebras T8-T9 (see Figure 1). Electrode resistance was kept low by cleaning the skin with alcohol and rubbing.

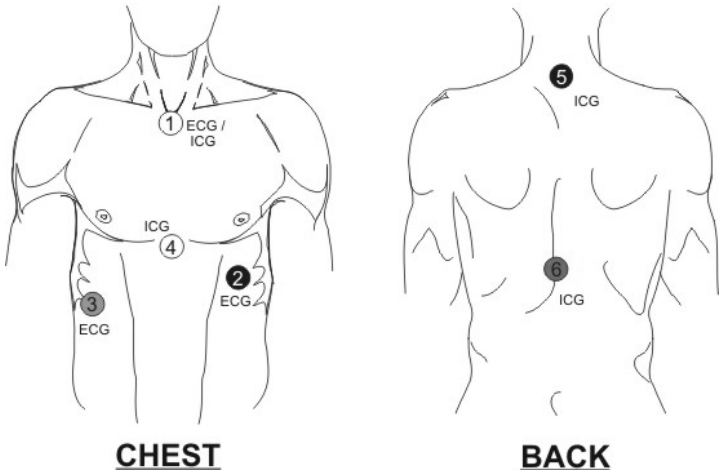


Figure 1 *Location of the six ECG and ICG electrodes.*

Ambulatory signal scoring

The amount of 60-s ensemble averaged ICG waveforms collected during a typical 24-hr ambulatory recording can be up to 1440 complexes for a single subject for a single measurement day. To score 24-hr ambulatory SV in large samples, for instance in our 130 (65 times 2) recordings, this is a very laborious procedure. Unless unlimited resources are available, 60-s ensemble averaging of the ambulatory ICG can be effectively disqualified as a feasible approach in large population studies. To solve this problem, we used the large-scale ensemble average (LSEA) strategy outlined by Riese *et al*. (2003). This strategy hinges on the idea that most ambulatory studies will ultimately average the results obtained on the smaller time scale (e.g. 60-s averages) over much larger time periods. Consecutive fragments of ambulatory recording are identified in which no significant change occurs in the hypothesized causes of intra-individual variance in impedance-derived variables, for instance similar posture, type of activity, physical load, social situation, location or the level of self-experienced mental or emotional strain. Signals within these periods are averaged and scored.

Using the activity diary entries in combination with a visual display of the vertical accelerometer signal, the entire 24-hr recording was divided into fixed periods coded for posture (lying, sitting, standing, walking, bicycling), type of ongoing activity (e.g. desk work, dinner, meetings, watching TV), social situation (e.g. alone, with significant other, with colleagues, with friends), and location (e.g. at work, at home, with family). If fixed periods lasted more than 1 hr (e.g.,

during sleep), they were divided into multiple periods of maximally 1 hr. This procedure allowed us to compute temporal stability for specific postures and across comparable levels of physical activity. Based on the reported times of lunch, diner, bedtime, and awakening we further aggregated the data into four periods of day: morning, afternoon, evening, and nighttime sleep. For the subjects of which the exact time of dinner, lunch, awakening, or bedtime could not be extracted from either diary or body movement, the missing time was imputed with the use of the mean times of these events in the rest of the sample.

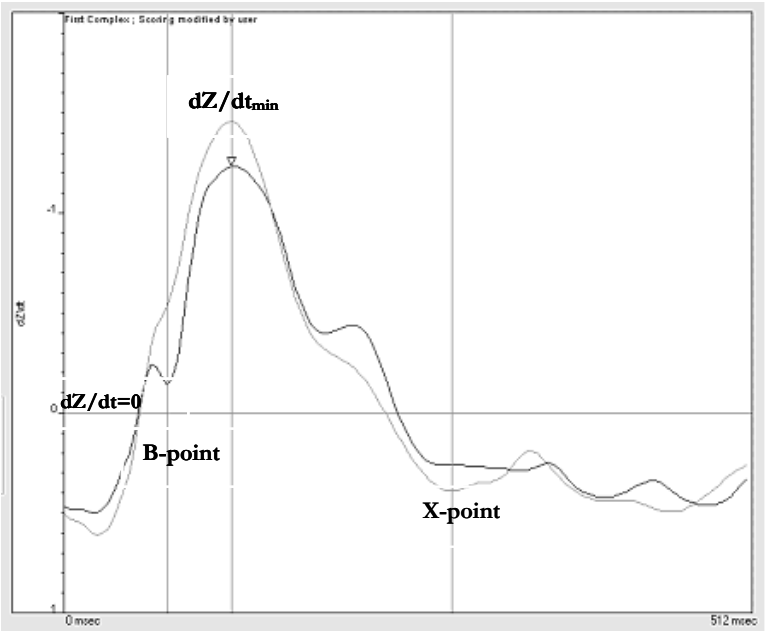


Figure 2 *Graph of a 60-s ensemble averaged ambulatory ICG signal (dark grey), overlaid with the corresponding large-scale ensemble average (light grey). The three vertical bars indicate the B-point, dZ/dt$_{(min)}$, and X-point scored on the large-scale ensemble average.*

Figure 2 plots a typical large-scale ensemble average over a period of 512 ms. The graph shows three vertical lines, representing: (1) upstroke or B-point, (2) dZ/dt$_{(min)}$ and (3) incisura or X-point. The PEP (in ms) is defined as the interval from the R-wave peak, minus a fixed interval of 48 ms (Dailey & Westfall, 1978; Sherwood *et al.*, 1990) to the B-point, which signals opening of the aortic valves. The LVET (in ms) is defined by the interval between the B-point and X-point, which signals the closure of the aortic valves. DZ/dt$_{(min)}$ (Ω/s)

is the difference in amplitude of the dZ/dt waveform at its peak compared to the B-point. Detection of B-point, X-point and $dZ/dt_{(min)}$ was done automatically, but automatic scoring was always followed by interactive visual inspection of all large-scale ensemble averages (see Figure 2). Scoring of the ICG signals on test and retest data was always done by the same rater (first author).

To compute the SV, the VU-AMS relies on Kubicek's equation (Kubicek *et al.*, 1966):

$$SV = \rho * (L_0 / Z_0)^2 * LVET * dZ / dt_{\underline{\min}}$$

In this formula, ρ is the blood resistivity, which was fixed here at 135 Ω·cm, L_0 is the average distance between the electrodes (cm) and Z_0 is the basal thoracic impedance (Ω). The SV is multiplied by the average HR obtained from the R-to-R-wave time series to yield the CO. As mentioned earlier, we used 2 different measures for $dZ/dt_{(min)}$ resulting in two different SV and CO measures, namely SV_B and CO_B based on the $dZ/dt_{(min)}$ computed from the dZ/dt amplitude at the B-point to the peak amplitude, and SV_0 and CO_0 computed from the dZ/dt amplitude at the dZ/dt=0 line to the peak amplitude.

Statistical analyses

Repeated measures ANOVA in SPSS first tested for posture effects (sitting, standing, walking). Next, repeated measures ANOVA was used to test for main effects of measurement day (test, retest) and periods of day (morning, afternoon, evening, sleep). Finally, temporal stability was assessed by intraclass correlation. Intraclass correlations were computed separately for each period of day

Results

The average values for age were 30.7 years (S.D. = 9.7) at the first test day and 34.0 years (S.D. = 9.8) at retesting. Across this time period BMI increased significantly from 24.1 (S.D. = 4.4) to 24.7 (S.D. = 4.8). Repeated measures ANOVA further showed a significant main effect of test/retest day on the average values of L_0 (19.7 - 17.5) and Z_0 (10.5 - 9.6) across the measurement days, but basal impedance corrected for the front electrode distance (L_0^2/ Z_0^2) was comparable at test and retest days (3.9 - 4.2).

During the daytime recordings, we expected posture to be an important source of variance, specific to ambulatory recording. Conform this expectation, the 3 postures (sitting, standing, walking) showed a significant effect on HR, SV, CO, PEP and LVET. As expected, the HR increased significantly from sitting to standing to walking. In parallel, we found a significant linear decrease in PEP and LVET, from sitting to standing to walking. SV_0 was lower during standing than during either sitting or walking. For SV_B the only significant post-hoc contrast was an increase in SV from standing to walking. CO_0 and CO_b increased significantly from sitting to walking and from standing to walking. To avoid confounding by differences in posture and physical activity across measurement days, the ensuing analyses were performed on the averaged periods with sitting activities only. Across both days, an average of 36% of the total awake recording time was spent in sitting activities.

Test -retest differences across the four periods of the day

Complete data during sitting activities for all daily periods and during sleep on both days was available for 51 subjects. Main source of missing data was signal loss during one of the four periods, mostly at night (12 subjects). Six subjects did not perform any sitting activities during the morning or afternoon. Across both test days, daily periods had a significant main effect on most of the cardiovascular measures (Table 1) with the exception of $(dZ/dt_{min}*LVET)_0$, a crucial component of SV_0 calculation.

Post-hoc testing of the Period main effect revealed that SV_0 was significantly higher during the evening and sleep than during the day. SV_B was significantly higher only during sleep. HR was at comparable levels during the morning and afternoon, but decreased mildly during the evening and strongly at night (on average 20 bpm lower than during daytime recording). CO_0 increased significant from morning to afternoon, stayed at a comparable level during the evening and significantly decreased during sleep. For CO_B the only significant post-hoc contrast was between awake periods (morning, afternoon, evening) and sleep. PEP and LVET decreased significantly from morning to afternoon, increased again during the evening and further increased during the sleep.

A main effect of test/retest day was found only on the average 24-hr values of CO_B, although SV_B also showed a trend in the same direction. The values were significantly lower on the retest day compared to the first test day. No interactive effects were found between measurement day and daily periods.

Table 1 *Means (SDs) for each of the cardiovascular variables, measured at four daily periods, on the test and retest days*

Measures		Periods of Day				F_{period}	F_{retest}
		Morning	Afternoon	Evening	Sleep		
		(n= 51)	(n = 51)	(n = 51)	(n=51)		
$dZ/dt_{(min)}$ (Ω/s)	Test	-1.16(.35)	-1.19 (.34)	-1.13 (.33)	-1.04(.30)	**23.18**	2.65
	Retest	-1.13(.47)	-1.12 (.45)	-1.08 (.39)	-.90 (.32)		
$dZ/dt_{(min)}$ from B (Ω/s)	Test	-1.03(.45)	-1.00 (.42)	-.92 (.40)	-.93 (.37)	**12.52**	4.22
	Retest	-.96 (.50)	-.90 (.47)	-.85 (.43)	-.73 (.34)		
L_0^2/Z_0^2 (cm/Ω)	Test	3.47(1.16)	3.66 (1.39)	3.79 (1.45)	3.81 (1.68)	**4.52**	2.39
	Retest	3.32(1.63)	3.89 (3.19)	4.24 (3.05)	5.08 (4.70)		
SV_0 (ml)	Test	149.9(44.6)	160.2(47.4)	166.5(52.3)	170.6(49.8)	**16.72**	3.55
	Retest	131.2(37.9)	140.9(46.5)	158.0(60.3)	161.9(55.5)		
SV_B (ml)	Test	121.0(47.8)	121.7(47.6)	121.1(46.4)	141.9(56.7)	**12.72**	6.45
	Retest	101.1(41.9)	102.3(47.8)	114.1(56.3)	120.0(52.7)		
HR (bpm)	Test	85.4 (12.1)	86.9 (11.5)	82.0 (9.9)	64.2 (7.74)	**247.34**	2.91
	Retest	82.7 (8.8)	84.6 (9.6)	79.8 (11.3)	64.3 (7.49)		
CO_0 (L/min)	Test	12.8 (4.1)	13.9 (4.6)	13.7 (4.8)	11.0 (3.87)	**23.15**	5.78
	Retest	10.8 (3.5)	11.9 (4.0)	12.5 (5.1)	10.3 (3.56)		
CO_B (L/min)	Test	10.4 (4.7)	10.6 (4.5)	9.92 (4.08)	9.19 (4.25)	**7.98**	**7.89**
	Retest	8.4 (3.9)	8.7 (4.3)	9.09 (4.89)	7.64 (3.47)		
PEP (ms)	Test	98.5 (12.8)	96.8 (12.5)	98.4 (11.5)	109.7(11.9)	**138.75**	.69
	Retest	98.5 (13.8)	97.4 (13.0)	98.9 (13.0)	112.0(12.9)		
LVET (ms)	Test	278.6(27.6)	274.5(28.0)	285.9(28.7)	327.6(27.2)	**212.69**	2.78
	Retest	287.4(30.0)	278.9(27.5)	293.4 31.9)	324.5(26.3)		
$(dZ/dt_{(min)} * LVET)_0$ (Ω)	Test	-.34 (.09)	-.34 (.09)	-.34 (.10)	-.36 (.09)	.16	1.20
	Retest	-.34 (.14)	-.33 (.12)	-.34 (.12)	-.31 (.10)		
$(dZ/dt_{(min)} * LVET)_B$ (Ω)	Test	-.28 (.11)	-.27 (.11)	-.26 (.11)	-.30 (.12)	**7.09**	3.47
	Retest	-.27 (.14)	-24 (.12)	-.25 (.12)	-.24 (.11)		

Significant main effects of period or retest (p<.01) are in bold.

Temporal stability

Table 2 displays the intraclass correlations for the cardiovascular variables per periods of day.

Table 2 *Intra-class correlation for the cardiovascular variables across the four daily periods.*

Measures	**Periods of Day**			
	Morning	Afternoon	Evening	Sleep
	(n=61/45)	(n=62/46)	(n=65/48)	(n=53/39)
$dZ/dt_{(min)}$ (Ω/s)	**.44/.57**	**.50/.62**	**.52/.59**	**.63/.72**
$dZ/dt_{(min)}$ from B (Ω/s)	**.37/.48**	**.45/.53**	**.58/.61**	**.58/.66**
L_0^2 / Z_0^2 (cm/Ω)	**.56/.62**	**.36/.37**	**.37/.36**	.05/.14
SV_0 (ml)	**.33/.46**	**.37/.46**	**.41/.36**	.11/.24
SV_B (ml)	**.29/.42**	**.39/.46**	**.46/.42**	**.41/.46**
HR (bpm)	**.50/.57**	**.50/.54**	**.52/.53**	**.68/.76**
CO_0 (L/min)	**.29/.39**	**.31/.37**	**.38/.38**	.17/.26
CO_B (L/min)	**.34/.45**	**.33/.43**	**.46/.48**	**.43/.50**
PEP (ms)	**.80/.81**	**.81/.83**	**.71/.77**	**.66/.79**
LVET(ms)	**.62/.52**	**.76/.77**	**.55/.45**	**.70/.67**
$(dZ/dt_{(min)} * LVET)_0$ (Ω)	**.46/.56**	**.53/.62**	**.42/.48**	**.54/.64**
$(dZ/dt_{(min)} * LVET)_B$ (Ω)	**.32/.44**	**.44/.52**	**.50/.53**	**.54/.62**

Correlations are given for the entire sample and after excluding subjects with one measurement on a workday and one on a leisure day. The column before the "/" reports on the entire sample. The column after the "/"on subjects with either two workdays (n=45) or two leisure days (n=3). Correlations that are significant at p < .05 are in bold.

Temporal stability of SV was moderate. Because stability of HR was good, intraclass correlations for CO were slightly higher than for SV. The largest source of instability in SV was the L_0/Z_0 ratio. $DZ/dt_{(min)}$ and LVET proved to be more stable, the LVET even more so than HR. From the impedance-derived measures, best performance was obtained for PEP, with temporal stability across the average period of 3 years and 4 months above .71 during the daytime and .66 at night.

In 17 subjects, retesting was on a different type of day than testing on the first day (i.e. a workday and a leisure day). To test whether this affected temporal stability, we repeated the analyses after excluding those 17 subjects. The temporal stability of PEP and LVET was largely unchanged. In contrast, an increase was found for the SV and CO measures in the morning and afternoon. In addition, differences of the averaged values across test-retest day were no longer significant.

In contrast to our expectation, SV and CO calculated from the B-point, which at times was ambiguous in visual scoring, led to better results than SV and

CO calculated by using the dZ/dt baseline, particularly in the evening and during sleep. This suggests that the theoretically more sound measure of SV is also the most useful measure in repeated measurement designs. We proceeded with values based on the B-point only in two further analyses that looked at 1) the effect of the duration of the test- retest interval on the temporal stability and 2) the use of relative changes in SV and CO rather than absolute levels.

Table 3 displays separate intraclass correlations for a shorter and a longer time interval. By effectively halving the sample size, significance of the correlations is compromised in comparison to Table 2, but the point estimates of temporal stability appear very comparable for shorter and longer test intervals. We repeated these analyses with three intervals using 2.8 and 3.5-year intervals as cut-points. Again, no evidence was found for a reduction in temporal stability across longer test-retest intervals.

Table 3 *Intra-class correlation for the cardiovascular variables across the four daily periods.*

Measures	Period between test and retest day	**Periods of Day**			
		Morning	Afternoon	Evening	Sleep
Number of subjects	2-3.2 years	32	33	34	28
	> 3.2 years	29	29	31	25
SV_B (ml)	2-3.2 years	**.33**	.28	**.44**	**.52**
	> 3.2 years	.25	**.48**	**.48**	**.35**
HR (bpm)	2-3.2 years	**.63**	**.61**	**.56**	**.73**
	> 3.2 years	**.39**	**.39**	**.47**	**.59**
CO_B (L/min)	2-3.2 years	**.33**	.23	**.44**	**.50**
	> 3.2 years	**.35**	**.41**	**.48**	**.39**
PEP (ms)	2-3.2 years	**.75**	**.76**	**.63**	**.65**
	> 3.2 years	**.89**	**.91**	**.82**	**.68**
LVET (ms)	2-3.2 years	**.70**	**.83**	**.41**	**.73**
	> 3.2 years	**.56**	**.71**	**.68**	**.62**

Separate correlations are given for short (2-3.2 years) and long (>3.2 years) test-retest intervals. Correlations that are significant at p < .05 are in bold.

It has been suggested that absolute SV values obtained from impedance cardiography are less reliable than the within-person changes in SV. Therefore, we also computed percentual change scores for each individual on test and retest days, using the awake periods as the "active" state and sleep levels as the resting state. This approach was previously used by Vrijkotte *et al.* (2000) to show substantial short-term reliability for ambulatory PEP. At all three daytime

periods temporal stability of the within-subject changes was comparable to the temporal stability of the absolute levels (see Table 4).

Table 4 *Intra-class correlation for the change scores for the cardiovascular variables across the three daytime periods.*

Measures	Periods of Day		
	Morning	Afternoon	Evening
	(n=50/36)	(n=51/37)	(n=53/39)
SV_B (ml)	.16/.19	**.40/.45**	**.27**/.24
HR (bpm)	**.39/.35**	.16 /.28	**.32/.35**
CO_B (ml)	.12/.14	**.33/.45**	**.31/.31**
PEP (ms)	**.32/.49**	**.38/.54**	**.33/.50**
LVET(ms)	**.29/.28**	**.30/.43**	.03/.00

Correlations are given for the entire sample and after excluding subjects with one measurement on a workday and one on a leisure day. Correlations that are significant at p < .05 are in bold.

Discussion

The present study tested the temporal stability of ambulatory SV and CO measured with impedance cardiography across an average time span of 3 years and 4 months. The pattern of SV and CO values obtained across the 24-hr ambulatory recording period generally confirm the validity of this method. During daytime, CO increased from sitting to standing to walking with a parallel decrease in PEP. Cardiac output fell mildly below its daytime levels due to the strong bradycardia at night. The net increase in PEP during sleep repeats a similar finding in previous studies (Burgess *et al.*, 1997; Lechin *et al.*, 2004; Trinder *et al.*, 2001; van Eekelen *et al.*, 2004). As expected, there was a significant increase in SV during supine sleep. Changes in posture affected both SV and CO during the daytime, which may reflect the complex balance of the effects of increased cardiac sympathetic activity paired to opposing effects from changes in preload and afterload. We reduced our analyses of temporal stability to these periods to avoid confounding by differences in posture and physical activity across the two measurement days.

For SV the most reliable and stable measure was SV_B, i.e. SV calculated with $dZ/dt_{(min)}$ relative to the B-point, as suggested by Doerr *et al.* (1981), Mohapatra (1981) and Debski *et al.* (1993). By taking the dZ/dt magnitude from the B point instead of the zero baseline, the respiratory influences on dZ/dt waveform, confounding the absolute value of $dZ/dt_{(min)}$, are eliminated (Debski *et al.*, 1993; Doerr *et al.*, 1981; Mohapatra, 1981). In a previous comparison of

interactive scoring by seven different raters, it was found in a few subjects that interrater agreement on the location of the B-point was very low (Riese *et al.*, 2003). As an alternative point for $dZ/dt_{(min)}$ we scored $dZ/dt_{(min)}$ in relation to the dZ/dt = 0 baseline (Sherwood *et al.*, 1991). The lower temporal stability of this alternative SV_0 measure, particularly at night, however, argued against its further use in ambulatory designs.

Intraclass correlations, computed separately for sleep and daytime waking recordings, were acceptable for SV_B (.29 – .46) and CO_B (.33 – .46) measured during sitting activities during the daytime. These findings did improve when we excluded subjects who were measured on a different type of day (leisure vs. work) on the second occasion. The intraclass correlation for SV_B and CO_B generally increased, most strongly during the morning and afternoon. That retesting across a work and a leisure day yields lower stability than retesting across two similar days probably reflects the effects of emotional and mental stressors, which may be more frequent on a workday than on a leisure day.

Our findings are comparable to those found in laboratory studies across a similar time span, e.g. Mathews *et al.* (2002) reported correlations for composite task change scores of .36 for SV and .40 for CO across 3 years. Barnes *et al.* (2004) found in his ambulatory study of 35 adolescent African Americans, who were measured twice across a time span of 2 months, test-retest correlations for SV and CO ranging from .45 to .56 over 24-hr. In a previous study Barnes *et al.* (2002) found test-retest correlation across a time span of 4 months of .52 for daytime CO and .47 for nighttime measures of CO. The slightly higher test-retest correlations most likely reflect the shorter time span, i.e. 2 to 4 months in their study versus 2 to 4 years in the present study.

At time intervals longer than 2 years temporal stability seems to stabilize. We did not find a reduction in intraclass correlations for SV and CO as a function of the test-retest interval, although the power of these analyses, which were done in smaller subsamples, may have been too low to detect subtle differences. Inadequate power also precluded a test of whether stability differed across sexes. Since there were only 20 males with valid retest data (17 at night), we could not meaningfully examine sex differences. In view of the evidence of sex differences in many cardiovascular signals and the potential impact of fluctuations within the menstrual cycle on stability (Girdler *et al.*, 1990; Girdler *et al.*, 1993), this issue remains to be addressed by future studies.

A main source of measurement error was introduced by the L_0/Z_0 ratio. The same fixed anatomical points were used for electrode placement on both of the measurement days but we did not attempt to completely standardize L_0 or Z_0 because we felt that temporal stability of SV should be robust to realistic variation in the exact electrode position. Only minor changes in front electrode distance and basal impedance were found. The unreliability of the L_0/Z_0 ratio, therefore, may reflect two fundamental limitations inherent in our spot electrode approach. The first is that the original Kubicek SV equation applies to a band electrode configuration. Its application to signals recorded using spot electrodes, where Z_0 values are typically much lower and electrode-skin-resistance is more critical, may lead to wider distribution of SV values (Sherwood *et al.*, 1990). A second, related limitation is that we positioned the upper recording electrode at the height of the clavicle. Raaijmakers *et al.* (1998) showed that small shifts in the position of the neck electrode resulted in large changes in impedance and SV (127 to 82 ml) when using the Kubicek equation. Because placement at the neck-thorax transition will cause inhomogeneities in the current density and potential distribution, they strongly recommend placement of the upper recording electrodes at least 6 cm above the clavicula (Raaijmakers *et al.*, 1998). Our choice to place spot electrodes relatively low in the neck was completely given in by practical reasons. To be useful in epidemiologically scaled studies, it is essential that normal subjects, not just highly motivated medical students, can tolerate these measurements for prolonged periods. Neither band electrodes nor highly placed visible spot electrodes on the neck are conducive to these goals. A recent alternative to spot electrodes uses a hybrid spot and band configuration with Mylar bands around the base of the neck as recording electrodes but a single spot electrode behind the ear, and two on the abdomen, as current electrodes (Sherwood *et al.*, 1998). The one group to address temporal stability using this alternative strategy found results encouragingly comparable to ours (Barnes *et al.*, 2002; Barnes *et al.*, 2004).

It has been suggested that part of the measurement error in impedance-based SV, introduced e.g. by between subject differences in thorax shape, intrathoracal tissue composition, heart and aorta dimensions and structure, and blood resistivity, may be negated by using relative within subject changes in SV rather than absolute SV values (Sherwood *et al.*, 1990; Willemsen *et al.*, 1996). To obtain change scores we used a strategy employed previously for PEP and HR

(Vrijkotte *et al.*, 2004) computing percentual change scores for each individual, using the awake periods as the "active" state and sleep levels as the resting state. Temporal stability of these change scores was lower compared to those of the corresponding absolute levels. A more focused computation of reactivity scores may be required, for instance by looking at the response to specific work-related stressors. This, however, would require repeated measurement in more homogenous populations that are subjected to comparable stressors at the two measurement days.

Stroke volume is only one of the targets of ambulatory impedance cardiography. This method also yields the systolic time intervals LVET and PEP, which may be used to index beta-adrenergic drive to the heart (Rasmussen *et al.*, 1975; Weissler *et al.*, 1968). High test-retest reliability across a few days was reported before by Vrijkotte *et al.* (2004) for ambulatory PEP. In the present study, intraclass correlations for the PEP across a much longer period were very good (.66 - .83). This is as good as the stability of PEP obtained under standardized laboratory conditions. Test-retest correlations from .45 to .88. were found for baseline and stress-task levels of PEP across retest intervals ranging from 28 days to 3 years (Burleson *et al.*, 2003; Matthews *et al.*, 2002; Willemsen *et al.*, 1998).

Ambulatory recording of hemodynamic regulation can further our understanding of how repeated or chronic cardiovascular activation in response to naturalistic events can contribute to cardiovascular disease processes. Although the temporal stability across a time span of more than 3 years was only moderate, it must be kept in mind that tracking coefficients for BP itself are also not much higher than .5 (Hottenga *et al.*, 2005; Palti *et al.*, 1988; Woelk, 1994). With this in mind, 24-hr ambulatory SV and CO measured by impedance cardiography can be a meaningful addition to the research on blood pressure regulation.

Chapter 4

Comparing low frequency heart rate variability and pre-ejection period: two sides of a different coin

Goedhart AD, Willemsen G, Houtveen JH, Boomsma DI, De Geus EJC (accepted). Comparing low frequency heart rate variability and pre-ejection period: two sides of a different coin. *Psychophysiology*.

Abstract

The notion has been advanced that a single ratio, spectral power of the heart period time series in the lower frequencies centered around .1 Hz (LF) divided by the power in the higher frequencies centered around the respiratory frequency (HF), may capture variation in cardiac sympathetic control. Here we tested in 24-hr ambulatory recordings whether the LF/HF ratio was correlated with the pre-ejection period (PEP), an established measure of cardiac sympathetic control. The LF/HF ratio did not show the expected correlation to PEP, neither within- nor between-subjects. The average within-subject correlation across an average of 56 separate periods during the ambulatory recording was -.11. Between-subject correlations for LF/HF and PEP, computed separately for sleep, sitting and physical activity, were not significant. In contrast, LF power showed high within- (mean $r > .73$) and between- ($.81 < r < .90$) subject correlation to HF power. We conclude that the evidence to support the LF/HF ratio as a potential marker of cardiac sympathetic control in epidemiology-scaled research may be currently insufficient.

Introduction

Activity of the sympathetic nervous system may be paramount to the detrimental effects of stress on cardiovascular health (Hjemdahl, 1990; Kamarck & Lovallo, 2003; Palatini & Jullius, 2004; Schwartz *et al.*, 1992). As a consequence, cardiovascular psychophysiologists need reliable and valid strategies to measure sympathetic nervous system activity in humans. The golden standard is the direct recording of action potentials from superficial sympathetic nerves in the muscles and the skin (Wallin *et al.*, 1975; Wallin, 1981). Apart from direct recording of nerve activity, measurement of spillover of the post-ganglionic neurotransmitter norepinephrine using radioactive tracers (Esler *et al.*, 1988) can be used as an alternative index of sympathetic nerve activity. The advantage of norepinephrine spillover is that it can be measured on an organ to organ basis, which allows separate measurement of, for instance, renal, lung or cardiac sympathetic activity (Esler, 2000). Less invasive measurements of norepinephrine in arterial and venous blood are also possible, as are measurements of the excretion of norepinephrine and its metabolites in urine, but concerns have been raised about differences in intraneuronal vesicular storage and leakage, re-uptake, extraneuronal clearance and urinary filtration/secretion that may (severely) distort the relation between actual sympathetic nervous system activity and plasma and urine norepinephrine concentrations (Eisenhofer *et al.*, 2004; Esler *et al.*, 1990; Goldstein *et al.*, 1983; Goldstein, 1995; Hjemdahl, 1990).

Invasive measures are extremely valuable for basic research on physiological and pathophysiological mechanisms involving the sympathetic nervous system. When research moves to an epidemiological scale the expense and invasiveness of these methods becomes prohibitive. Furthermore, invasive measures like muscle sympathetic nerve activity or regional norepinephrine spillover restrict research to the confines of a hospital or laboratory setting. This precludes examination of individual differences in sympathetic activity in a natural setting, for instance during sleep or during job-related activities with a substantial mental and emotional load. Nonetheless, it is autonomic control during these naturalistic conditions that may have the largest clinical relevance. It would be extremely valuable, therefore, to have non-invasive, unobtrusive measures of sympathetic nervous system activity. In response to this need, Pagani and coworkers have advanced the notion that a single ratio, spectral power of the heart period time series in the lower frequencies centered around

.1 Hz (LF) divided by the power in the higher frequencies centered around the respiratory frequency (HF), may capture changes in sympathovagal balance: the ratio of sympathetic to vagus nerve traffic to the heart (Pagani *et al.*, 1986; Pagani *et al.*, 1991; Pagani *et al.*, 1997; Pagani & Malliani, 2000 ; Malliani *et al.*, 1991; Malliani *et al.*, 1998; Montano *et al.*, 1994).

The idea behind the LF/HF ratio is that the LF power is sensitive to both vagal and sympathetic activity, whereas the HF power is sensitive to vagal activity only. Increases in sympathetic activity should increase the absolute LF power, but because these are often paired to reciprocal decreases in vagal activity, an actual decrease in LF power may be observed. The LF/HF ratio aims to correct this by taking parallel decreases in vagal activity into account. Although it is accepted that the ratio is sensitive to both vagal and sympathetic activity, it is considered to be relatively more sensitive to the latter. Hence it may be used as an index of cardiac sympathetic control, even if imperfect. Since inter beat intervals require nothing more complicated than a three-lead ECG recording, spectral power derived LF/HF ratio's can be obtained in ambulatory paradigms in huge numbers of subjects at very modest costs. By this virtue alone, LF/HF ratio would enable psychophysiological examination of cardiac autonomic control in large-scale samples, including those of the sizes needed for genetic research (Lander & Kruglyak, 1995; Welcome Trust Case Control Consortium, 2007). This is a very attractive perspective, but the usefulness of the LF/HF ratio is ultimately determined by its validity, which has been the subject of continued controversy.

This controversy is best illustrated by the critical appraisal of the LF/HF ratio by dr Eckberg in 1997 (Eckberg, 1997) followed by responses of many equally authorative experts in the field of autonomic nervous system physiology (Malik & Eckberg, 1998; Malliani *et al.*, 1998; Sleight & Bernardi, 1998). As testified by the Point: Counterpoint section in a 2006 issue of the Journal of Applied Physiology this controversy has continued unabated for 10 years (Bernardi & Sleight, 2006; Billman, 2006; Burnley *et al.*, 2006; Cerutti, 2006; Cohen & Tan, 2006; Eckberg, 2006; Elstad & Toska, 2006; Evans, 2006; Julien, 2006; Laude, 2006; Malliani, 2006; Parati *et al.*, 2006; Piepoli, 2006; Taylor & Studinger, 2006). The strongest concern about the validity of the LF/HF ratio is provided by the studies that directly compare it against invasive measures of sympathetic activity, like peroneal muscle nerve activity or cardiac norepinephrine spillover. Although some studies did report a correlation of these

measures to the LF/HF ratio (Pagani *et al.*, 1997), most studies did not find this correlation across a range of clinical contexts, as reviewed in (Grassi & Esler, 1999). A typical example is provided by a study of Kingwell and colleagues (1994). In 52 healthy subjects, no significant correlation of LF (absolute or normalized) was found at rest to either peroneal sympathetic nerve activity or to cardiac norepinephrine spillover, arguably the closest thing to a gold standard for cardiac sympathetic nerve activity.

It must be noted that the studies above suffered from two major shortcomings, as a natural consequence of the invasive procedures required. First, they were often performed in artificial laboratory conditions with little ecological validity, e.g. within-subject variance in sympathetic activity was usually induced by infusion of nitroprusside or phenylephrine (Pagani *et al.*, 1997; Saul *et al.*, 1990) and secondly they were mostly performed on small sample sizes that required the correlations to be in the .60 - .80 range to be considered "significant". It is unlikely, however, to assume that LF/HF reflects cardiac sympathetic control that closely. Whereas HF power relatively purely reflects cardiac vagal control over the heart (Task Force of the European Society of Cardiology the North American Society of Pacing, 1996) it is fully acknowledged that LF power is influenced by *both* sympathetic and vagal activity. Hence the LF/HF ratio is unlikely to yield a perfect indicator of cardiac sympathetic control, but it may still retain sufficient explanatory and predictive power to be useful in epidemiology scaled research.

Here we compare the LF/HF ratio to an alternative measure of cardiac sympathetic control, the pre-ejection period (PEP) which can be non-invasively obtained by thoracic impedance cardiography (Cacioppo *et al.*, 1994a; Sherwood *et al.*, 1990). Changes in PEP reliably index changes in ß-adrenergic inotropic drive to the left ventricle as shown in laboratory studies manipulating ß-adrenergic tone by epinephrine infusion (Mezzacappa *et al.*, 1999; Schachinger *et al.*, 2001; Svedenhag *et al.*, 1986), amyl nitrite inhalation (Nelesen *et al.*, 1999), adrenoceptor blockade (Harris *et al.*, 1967; Schachinger *et al.*, 2001; Winzer *et al.*, 1999), exercise (Krzeminski *et al.*, 2000; Miyamoto *et al.*, 1983; Smith *et al.*, 1989a), or emotional stress (Berntson *et al.*, 1994; Newlin & Levenson, 1979; Sherwood *et al.*, 1986). Since the PEP can be non-invasively obtained by thoracic impedance cardiography (Cacioppo *et al.*, 1994a; Sherwood *et al.*, 1990) a number of devices are now available for ambulatory recording of the PEP (Cybulski, 2000; Nakonezny *et al.*, 2001; Sherwood *et al.*, 1998; Willemsen *et al.*,

1996). Previous ambulatory recordings have shown daytime and sleep PEP values to be reliable characteristics with high test-retest correlations (r = .90) across a few days (Vrijkotte *et al.*, 2004) as well as good temporal stability (r = .75) over a period of 3.3 years (Goedhart *et al.*, 2006) and substantial heritability (57%) (Kupper *et al.*, 2006).

To support the LF/HF ratio as a measure of cardiac sympathetic control, it should show a negative correlation to the PEP such that longer PEPs are associated with lower LF/HF ratios. Under the assumption that most homeostatic challenges lead to reciprocal changes in sympathetic and vagal activity, more specific predictions are that PEP is negatively correlated to LF power and positively to HF power. Since we appreciate that the LF/HF ratio is at best an imperfect measure of cardiac sympathetic control, data were collected in a relatively large sample and by ambulatory recordings in naturalistic settings, which yields a large number of repeated measures within-subjects. This way the study was sufficiently powered to detect systematic within- and between-subject correlations between PEP and the HRV measures, even when these correlations would be of low to moderate strength.

Methods

Subjects

Participants were all registered with the Netherlands Twin Register (NTR). They came from families that participated in a linkage study searching for genes influencing personality and cardiovascular disease risk, which is described elsewhere (Boomsma *et al.*, 2000). Out of the 1332 twins and siblings who returned a DNA sample (buccal swabs) for the linkage study, 816 were also willing to participate in cardiovascular ambulatory monitoring. Reasons for exclusion were pregnancy, heart transplantation, pacemaker and known ischemic heart disease, congestive heart failure, or diabetic neuropathy. Data in this study come from 65 of these subjects (20 male, 45 female) that were tested twice separated by a minimum of 2 years and 1 month and a maximum of 4 years and 8 months (mean 3 years and 4 months). At the first test day the age ranged from 18-62 year (mean = 30.7, S.D. = 9.7). The Ethics Committee of the Vrije Universiteit approved of the study protocol and all subjects gave written consent before entering the study. No payment was made for participation, but all subjects received an annotated review of their ambulatory heart rate and blood pressure recordings.

Procedure

Subjects were invited to participate in the study by letter and subsequently phoned by the researchers to receive additional information on the study, and to make an appointment for 24-hr ambulatory monitoring. The first ambulatory measurement took place during a representative workday (or a day with representative housekeeping chores for those who were not employed). The second ambulatory measurement day took place during a comparable (work) day for most of the subjects, but 17 subjects would only participate if the repeated measurement was scheduled on a leisure day. On the day preceding monitoring and on the monitoring day itself subjects were asked to refrain from vigorous leisure time exercise or heavy physical work. Subjects were visited at home between 7.00 –10.00 a.m. and fitted with the Vrije Universiteit Ambulatory Monitoring System (de Geus *et al.*, 1995; Riese *et al.*, 2003; Willemsen *et al.*, 1996). The VU-AMS produced an audible alarm approximately every 30 min (± 10 min randomized) to prompt the subject to fill out an activity diary. Participants were instructed to write down a chronological account of posture, physical activity, physical load, location and social situation during the last 30 min period. Diary prompting was disabled during sleep. The following day the participants were visited again between 7.00 –10.00 a.m. to detach and collect the equipment. Recording continued during sleep but this failed in 12 nights (4 in males, 8 in females; 6 on the first test day, 6 on the second test day).

Ambulatory recording

The VU-AMS recorded the ECG and the impedance cardiogram (ICG) continuously during a 24-hr period (daytime and sleep) through six disposable, pregelled Ag/AgCl electrodes. Using the activity diary entries in combination with a visual display of an inbuilt vertical accelerometer signal, the entire 24-hr recording was divided into fixed periods. These periods were coded for posture (e.g. lying, sitting, standing), ongoing activity (e.g. desk work, eating/drinking, meetings, watching TV), physical activity (no, light, medium and heavy), location (e.g. work, home, outside) and social situation (e.g. alone, with colleagues, with friends). The full coding scheme is provided in the appendix. The coded periods were never shorter than 5 min or longer than 1 hr. If periods lasted more than 1 hr (as during sleep), they were divided into multiple periods of maximally 1 hr (e.g. sleep1, sleep2, etc). An average of 27 coded periods was created per

subject with an average duration of 30 min. The 15 most frequently used coded periods across all subjects are shown in Table 1.

Table 1 *The 15 most frequent label combinations during 24-hr monitoring.*

Posture	Activity	Location	Social situation	% of all labels	Time (min)	
					Mean	SD
Lying	Sleep	Home	Significant other	8.3	57	8
Lying	Sleep	Home	Alone	6.6	57	8
Standing/Walking	Active at home/Housekeeping	Home	Alone	4.7	27	16
Sitting	Deskwork	Work	Colleagues	2.9	21	28
Sitting/Standing/Walking	Active at home/Housekeeping	Home	Alone	2.8	28	15
Standing/Walking	Other work	Work	Colleagues	2.2	27	19
Standing/Walking	Active at home/Housekeeping	Home	Significant other	2.0	26	16
Sitting	Watching TV	Home	Alone	1.9	26	18
Sitting	Deskwork	Work	Alone	1.9	22	18
Standing/Walking	Active at home/Housekeeping	Home	Kids	1.6	23	14
Sitting	Active transport	On the road	Alone	1.6	21	14
Sitting/Standing/Walking	Other work	Work	Colleagues	1.6	22	16
Sitting	Deskwork	Home	Alone	1.6	26	17
Sitting	Watching TV	Home	Significant other	1.5	22	17
Sitting	Telephoning /Talking	Work	Colleagues	1.5	20	15

In impedance cardiography, a high frequency alternating current is introduced across the thorax by two electrodes. Two inner and parallel electrodes measure the basal thorax impedance (Z_0) and the change in impedance of the enclosed thorax column (dZ) which is largely a function of aortic blood flow. The ICG signal is obtained as the first derivative of the pulsatile changes in transthoracic impedance (dZ/dt) ensemble averaged with reference to the R-wave (Muzi *et al.*, 1985). In this study, ensemble averaging was done across all beats in an entire coded period. Validity of this large-scale ensemble averaging (LSEA) strategy has been outlined in detail by Riese *et al.* (2003). For every large-scale ensemble average three time points were scored in the ensembled averaged ICG: the upstroke or B-point, the $dZ/dt_{(min)}$ point and the incisura or X-point. The PEP was defined as the interval from the R-wave peak from the ECG, minus a fixed interval of 48 ms to the B-point, which signals the opening of the aortic valves (Lozano *et al.*, 2007; Sherwood *et al.*, 1990; Willemsen *et al.*, 1996). From the ECG and the dZ, we obtained the IBI time series and respiration signal (de Geus *et al.*, 1995; Goedhart *et al.*, 2007). Automatic identification and correction of artifacts in the IBI data was verified by visual inspection of the corrected time series.

In keeping with PEP scoring, LF and HF powers were computed across the entire coded period. To do so we used a Wavelet approach rather than the more common Fourier approach. Computation of IBI powers by Fourier analysis

assumes that the data show at least weak stationarity (Weber *et al.*, 1992). Stationarity of time series may be interpreted as having a stable mean and variance over time. In ambulatory studies and/or for analysis of relatively long data segments (up to 1 hr), the assumption of stationarity is likely to be violated. Wavelet decomposition does not have a stationarity assumption (Houtveen & Molenaar, 2001). Uniformly spaced samples were created by interpolation of the IBI data using a Wavelet interpolation algorithm. Next, Discrete Wavelet Transformation (DWT) was performed using a cardinal cubic spline function as base (see Houtveen & Molenaar, 2001 for more information regarding this procedure). This method results in identical power values for stationary relatively short coded periods (e.g. 7 min of quiet reading) as compared to Fourier transformation, but it is superior for our relatively longer and non-stationary coded periods (e.g. first hour of sleep). Three heart rate variability measures were extracted from the Wavelet analyses. The VLF power was computed as the variance in the .0078125 - .0625 HZ window, LF power as the variance in the .0625 - .125 Hz window, and HF as the variance in the .125 - .5 Hz window. Note that the size of a frequency window always doubles after each Wavelet decomposition step. Since the DWT (like Fourier) suffers from aliasing effects at both ends, the first and last 40 data points (2.5 s) of the time series were excluded from the derivation of the variances.

When increased cardiac sympathetic control is paired to decreased vagal control, the resulting tachycardia is usually accompanied by a marked reduction in total spectral power. When the spectral components are expressed in absolute units (ms^2), the changes in total power influence LF and HF in the same direction and prevent the appreciation of the fractional redistribution of the energy. This information is regained when LF and HF are expressed as a ratio, but alternatively LF and HF can be measured in normalized units (nu), which represent the relative value of each power component in proportion to the total power minus the VLF component (Malliani *et al.*, 1991). Although it has been suggested that LFnu and LF/HF ratio can be considered equivalent carriers of information (Burr, 2007) we will present full data on absolute LF, LFnu, and LF/HF ratio for completeness. To obtain LFnu, the LF power was divided by the sum of the LF and HF power.

Respiration rate was obtained from the thoracic impedance signal (Z) which was passed through a FIR band-pass filter with a low and high cut-off of .1 and .4 Hz (attenuation of the signal in the stop bands of at least 20 dB) to obtain

the dZ changes due to respiration. The continuous dZ signal was visually inspected and segments free of artifacts due to prolonged breath holding or sighing were selected. Interactive scoring of the peaks and troughs in these segments yielded the respiratory frequencies and amplitudes on a breath-to-breath basis. These were averaged to yield a single mean respiratory rate for each coded period. Validity of assessing the respiration by impedance cardiograph in comparison to respiratory belts and spirometry is detailed in Houtveen *et al.* (2005).

Statistical analyses

MIXED ANOVA (SPSS version 14.0) was used to test for mean effects of sex, test day, and ambulatory condition on PEP and the HRV measures, using age as a covariate. To avoid unbalanced cell sizes, these analyses used the aggregated means over all coded periods that fell within three main ambulatory conditions: nighttime sleep, sitting during the day, and mild physical activity (standing/walking). Temporal stability was assessed by intraclass correlation, computed separately for these ambulatory conditions (sleep, sitting and mild physical activity). The main analyses tested the Pearson correlations between the heart rate variability parameters (LF, HF, LF/HF ratio, and LFnu) and PEP both between-subjects and within-subjects. Within-subject correlations used each of the coded periods separately. Between-subject correlations were computed separately on the aggregated means across the three main ambulatory conditions (sleep, sitting and mild physical activity). Where appropriate, the effects of sex and age were first removed in these between-subject analyses by using partial correlations. In both within-subject and between-subject correlations, RR was regressed on HF to obtain a residual score that indicates the (within- or between-) deviation of the observed RSA taken the observed RR. This score (HFres) was used as an additional measure of high frequency fluctuations that is less dependent on concurrent changes in respiratory behavior.

Results

Table 2 presents the untransformed means and standard deviations for PEP and the HRV measures separately during sleep, awake sitting and mild physical activity. Because the LF/HF ratio and the LF, HF, and LFnu power distributions were skewed, their natural logarithms were used in all further analyses.

Table 2 *Means (SDs) of PEP and HRV measures separately for the two test days in males (N = 20) and females (N = 44).*

Sex	Condition	Day	PEP (ms)	LF (ms^2)	HF (ms^2)	LF/HF	LFnu
Males	Sleep	1	107.54	1452.54	1241.49	1.67	58.54
			(11.15)	(1039.00)	(1239.50)	(.81)	(10.52)
		2	105.83	1500.84	1288.14	1.89	60.29
			(13.94)	(1542.25)	(1655.50)	(1.09)	(13.17)
	Sitting	1	95.71	1125.75	855.18	2.24	64.38
			(11.86)	(744.02)	(1118.91)	(1.14)	(11.34)
		2	97.64	1121.29	837.96	2.11	63.45
			(14.46)	(764.93)	(1010.55)	(1.00)	(9.38)
	Mild physical activity	1	96.00	882.86	631.34	2.07	62.71
			(10.93)	(495.58)	(704.17)	(1.02)	(11.32)
		2	100.03	908.55	584.60	2.05	63.18
			(14.38)	(516.60)	(477.85)	(.85)	(7.84)
Females	Sleep	1	104.42	830.79	1008.25	1.09	48.20
			(11.84)	(592.76)	(944.79)	(.50)	(10.71)
		2	108.31	733.35	723.40	1.22	51.54
			(12.92)	(542.29)	(657.78)	(.49)	(9.65)
	Sitting	1	98.22	649.98	580.84	1.52	57.12
			(12.71)	(387.25)	(435.79)	(.51)	(6.55)
		2	96.88	602.58	481.25	1.60	58.15
			(12.73)	(385.75)	(379.36)	(.54)	(7.91)
	Mild physical activity	1	99.76	601.31	554.95	1.52	56.54
			(12.31)	(317.97)	(477.56)	(.57)	(9.49)
		2	99.02	557.15	380.61	1.87	60.86
			(13.03)	(303.66)	(248.68)	(.76)	(8.26)

Mixed ANOVA showed a significant effect of ambulatory condition on PEP ($F(2,315.2) = 44.66$, $p < .001$), LF power ($F(2,314.9) = 5.13$, $p = .01$), LF/HF ratio ($F(2,313.9) = 17.28$, $p < .001$), LFnu power ($F(2,314.2) = 23.05$, $p < .001$) and HF power ($F(2,314.7) = 19.03$, $p < .001$). Significance derived entirely from the difference between sleep values and daytime values. PEP was significantly longer during sleep than during either sitting or mild physical activity, whereas heart rate variability in all frequencies was largest during sleep. Values did not significantly differ between sitting and mild physical activity. Notably, LF/HF

ratio and LFnu power were also lowest during sleep in keeping with the longer PEP. A significant effect of test day was found on HF power ($F(1,314.8) = 6.62$, $p = .01$) only. HF power was significantly higher during the first test day compared to the second. A significant effect of sex was found on LF power ($F(1,343.2) = 48.11$, $p < .001$), LFnu power ($F(1,318.4) = 24.80$, $p < .001$), HF power ($F(1,357.9) = 11.52$, $p = .00$), and the LF/HF ratio ($F(1,323.8) = 16.15$, $p = .00$). Females had lower power in the entire frequency range tested and significantly lower LF/HF ratios. Absence of sex*condition interactions suggest that this was not specific to any one of the ambulatory conditions but found throughout the entire recording.

Because of the sex effect on the HRV measures, the temporal stability for PEP and all HRV measures is given separately for males and females (Table 3). Good temporal stability for LF, HF and LFnu powers and the LF/HF ratio was found over an average period of 3 years and 4 months during sitting and sleep. Physical activity, which is inherently less comparable across repeated test days, produced lower estimates.

Table 3 *Temporal stability of PEP and HRV measures across a period of 3.3 years.*

Sex	Condition	N	PEP	LF	HF	HFres	LF/HF	LFnu
Males	Sleep	16	**.71**	**.88**	**.87**	**.86**	**.68**	**.68**
	Sitting	20	**.76**	**.83**	**.93**	**.92**	**.78**	**.79**
	Mild physical activity	20	**.67**	**.66**	**.88**	**.86**	**.68**	**.65**
Females	Sleep	36	**.72**	**.74**	**.75**	**.74**	**.57**	**.68**
	Sitting	44	**.82**	**.77**	**.72**	**.72**	**.59**	**.56**
	Mild physical activity	44	**.80**	**.53**	**.43**	**.41**	**.46**	**.38**

Correlations significant at p <.05 are in bold.

Tables 4A (females) and 4B (males) show the within-subject correlations across the two 24-hr measurements of PEP and the HRV measures. Across males and females, the average within-subject correlation between PEP and LF power was .30, between PEP and the LF/HF ratio -.11, and between PEP and HF power .33. For PEP and the LF/HF ratio, only 20 out of 64 subjects showed a significant correlation in the expected (negative) direction. When using normalized LF power, 45 subjects failed to show a significant correlation to PEP in the expected direction. For absolute LF power, the expected negative correlation reached significance only in a single subject (#19).

Table 4A *Within-subject correlations between PEP and HRV measures for females*

Nr	Age	N	PEP-LF	PEP-HF	PEP-HFres	PEP-LF/HF	PEP-LFnu	LF-HF	LF-HFres
1	18.0	54	.37	*.33*	.26	.00	.04	.71	.68
2	18.9	37	.21	*.38*	*.37*	-.20	-.21	.31	.31
3	18.9	61	.02	-.06	.00	.11	.11	.78	.77
4	20.2	69	**.35**	**.60**	**.57**	**-.50**	**-.54**	**.83**	**.83**
5	21.9	56	*.31*	**.48**	**.47**	*-.29*	**-.35**	**.67**	**.67**
6	23.0	59	**.38**	**.46**	**.46**	**-.22**	**-.23**	**.76**	**.74**
7	23.0	59	.11	*.27*	*.27*	-.20	-.20	**.74**	**.69**
8	23.1	45	**.57**	**.43**	**.46**	**-.15**	**-.09**	**.79**	**.79**
9	23.5	48	**.26**	**.25**	**.27**	**-.16**	**-.07**	**.89**	**.89**
10	23.9	61	**.63**	**.62**	**.61**	**-.07**	**-.10**	**.93**	**.93**
11	24.0	56	.18	.14	.10	.13	.14	**.75**	**.73**
12	24.6	61	.19	**.60**	**.47**	**-.41**	**-.38**	.18	.20
13	24.7	72	*.30*	**.51**	**.43**	**-.42**	**-.39**	**.73**	**.72**
14	24.9	60	.19	**.45**	**.42**	**-.49**	**-.50**	**.78**	**.79**
15	25.2	65	**.49**	**.54**	*.51*	*-.26*	-.20	**.86**	**.85**
16	25.6	53	*.27*	.15	.09	.18	.20	**.83**	**.81**
17	26.6	62	*.28*	*.26*	*.31*	.07	.08	**.82**	**.77**
18	26.8	58	-.05	.24	.17	**-.34**	*-.33*	**.58**	**.57**
19	27.1	84	**-.34**	**-.34**	**-.38**	**.01**	**-.07**	**.73**	**.73**
20	27.5	44	**.56**	**.49**	**.47**	**-.01**	**.03**	**.75**	**.73**
21	28.0	58	**.45**	**.51**	**.45**	*-.29*	*-.33*	**.68**	**.67**
22	28.5	53	*.35*	.20	.14	.19	.22	**.80**	**.77**
23	28.5	57	*.33*	**.36**	*.32*	**-.22**	**-.25**	**.92**	**.92**
24	28.6	58	.19	.21	.25	-.05	-.01	**.95**	**.94**
25	29.5	64	-.20	.06	-.15	*-.30*	*-.29*	**.61**	**.66**
26	30.0	54	**.37**	**.44**	**.36**	*-.30*	*-.32*	**.86**	**.85**
27	30.7	57	.20	.07	.17	.16	.19	**.69**	**.63**
28	31.2	59	**.25**	**.55**	**.55**	**-.40**	**-.51**	**.66**	**.65**
29	32.7	67	**.48**	**.65**	**.67**	**-.52**	**-.57**	**.78**	**.77**
30	32.9	54	**.68**	**.75**	**.76**	**-.24**	**-.27**	**.93**	**.92**
31	32.9	43	.29	.11	.04	.12	.10	.15	.11
32	35.3	49	.24	.28	.19	-.04	-.02	**.65**	**.63**
33	36.6	51	*.30*	**.47**	**.48**	**-.25**	**-.27**	**.76**	**.76**
34	40.3	72	.06	-.01	.02	.19	.13	**.89**	**.89**
35	42.1	65	-.13	-.19	-.05	.13	.12	**.81**	**.79**
36	42.1	56	**.49**	**.37**	**.43**	.01	.06	**.51**	**.50**
37	42.7	33	.07	-.10	-.08	.25	*.35*	**.88**	**.89**
38	44.0	53	*.29*	**.47**	**.41**	**-.38**	**-.37**	**.70**	**.70**
39	44.1	57	**.39**	*.28*	**.34**	.12	.14	**.84**	**.84**
40	47.3	60	**.48**	**.53**	**.51**	*-.32*	**-.23**	**.85**	**.84**
41	47.5	61	**.49**	**.47**	**.47**	**.23**	*.28*	**.92**	**.92**
42	48.3	40	*.36*	.17	.13	.25	.30	**.83**	**.81**
43	48.4	56	**.63**	**.58**	**.55**	**-.14**	**-.02**	**.83**	**.81**
44	62.3	52	**.37**	**.54**	**.52**	*-.28*	**-.25**	**.14**	**.15**
Avg.	31.5	57	.29	.33	.31	-.12	-.11	.73	.72

Correlations significant at .01 level are in bold.

Correlations significant at .05 level are in bold italics.

In contrast, 38 subjects showed a significant *positive* correlation between PEP and LF power and 39 subjects showed a significant *positive* correlation between PEP and HF power. Mean showed a significant *positive* correlation between PEP and HF power. Mean within-subject correlations between LF and HF power were unanimously high (LF and HF, r = .73) Here, non-significant correlations were nearly absent (exception are subjects #2, #12, #31 and #44). Correction for within-subject changes in respiration barely influenced this correlation (LF and HFres, r = .72).

Because PEP has a known sensitivity to changes in pre- and afterload, changes in posture across the measurement day (supine, sitting, standing) might have attenuated the correlations between PEP and the LF/HF ratio. When the within-subject correlations were recomputed across coded periods with fixed posture (sitting or standing) correlations remained non-significant for PEP and LF/HF as well as PEP and LFnu.

Table 4B *Within-subject correlations between PEP and HRV measures for males*

Nr	Age	N	PEP-LF	PEP-HF	PEP-HFres	PEP-LF/HF	PEP-LFnu	LF-HF	LF-HFres
45	19.5	48	.04	.15	.24	-.17	-.18	.75	.70
46	19.7	52	.87	.87	.84	*-.29*	*-.34*	.92	.90
47	20.5	49	.01	-.23	-.23	.28	.26	.62	.49
48	22.5	61	.11	.18	.22	-.20	-.22	.92	.90
49	24.7	56	.75	.77	.76	-.20	-.13	.83	.84
50	25.0	45	.04	.16	.16	-.12	-.12	.59	.60
51	25.1	60	.41	.61	.54	-.37	-.39	.73	.76
52	25.3	48	.37	*.31*	.39	.05	.12	.80	.80
53	25.4	32	.81	.63	.68	-.01	.17	.84	.87
54	25.8	46	*.31*	.26	.16	.02	.03	.81	.80
55	27.0	58	.46	.34	*.31*	.16	.17	.84	.80
56	27.8	45	-.18	-.24	-.25	.11	.08	.59	.53
57	28.0	51	.64	.62	.64	.05	.18	.89	.90
58	28.5	58	.47	.57	.51	-.19	-.15	.87	.87
59	31.4	48	.24	.37	*.30*	-.22	-.24	.51	.51
60	33.0	57	.38	.49	.53	-.41	-.37	.91	.89
61	38.6	63	.18	.34	.06	*-.32*	*-.26*	.80	.78
62	45.6	80	.03	-.43	-.41	.57	.57	.63	.64
63	47.1	62	*.32*	.35	.36	-.22	-.11	.48	.45
64	50.8	72	*.26*	.31	.30	-.03	-.03	.46	.43
Avg.	29.6	55	.32	.32	.31	-.08	-.05	.74	.72

Correlations significant at .01 level are in bold.
Correlations significant at .05 level are in bold italics.

Table 5 shows the between-subject correlations separately within each of the three main ambulatory conditions for both test days. Partial correlations were computed controlling for the effect of sex and age. During sleep, none of the correlations between PEP and the HRV measures were significant. During sitting and physical activity on day 2 a significant correlation between PEP and LF power was found, but the direction was opposite to the expectation. Between-subject correlation for LF and HF all exceed .79 in all three conditions on both test days, indicating high overlap between the two measures of HRV. Again, partialling out respiration rate barely affected the LF-HF correlation.

Table 5 *Between-subject correlations between PEP and HRV measures.*

Condition	Day	N	PEP-LF	PEP-HF	PEP-HFres	PEP-LFHF	PEP-LFnu	LF-HF	LF-HFres
Sleep	1	58	.26	.17	.16	.12	.11	**.87**	**.86**
			(.19)	(.10)	(.08)	(.17)	(.16)	(**.89**)	(**.89**)
	2	57	.22	.18	.16	.02	.03	**.89**	**.90**
			(.24)	(.16)	(.13)	(.08)	(.10)	(**.91**)	(**.91**)
Sitting	1	64	.24	.24	.23	-.13	-.09	**.87**	**.84**
			(.25)	(.19)	(.18)	(.-04)	(-.01)	(**.88**)	(**.85**)
	2	64	**.38**	**.35**	**.34**	-.14	-.07	**.90**	**.89**
			(**.39**)	(**.34**)	(**.32**)	(-.12)	(-.05)	(**.90**)	(**.89**)
Mild	1	64	.18	***.29***	***.27***	***-.27***	***-.27***	**.81**	**.79**
physical			(.22)	***(.27)***	(***.25***)	(-.21)	(-.22)	(**.81**)	(**.79**)
activity	2	64	**.35**	**.32**	***.29***	-.13	-.05	**.88**	**.88**
			(**.35**)	(***.31***)	(***.27***)	(-.11)	(-.03)	(**.85**)	(**.84**)

In brackets: correlations after partialling out age and sex. **Correlation significant at .01 level.** ***Correlation significant at .05 level***.

Figure 1 combines the within- and between-subject observations on PEP and LF/HF in a single plot for illustrative purposes. It is clear that the expected negative relation between these variables was not observed.

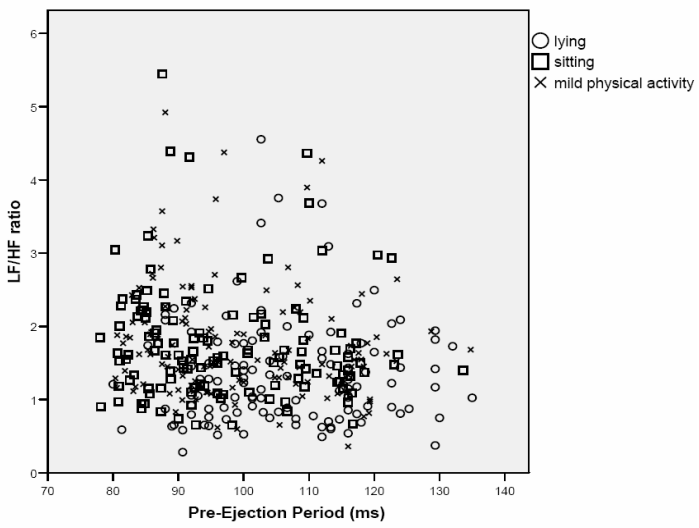


Figure 1 *Scatterplot of the PEP and the LF/HF ratio for all subjects in all conditions.*

Discussion

The notion has been advanced that a single ratio, spectral power of the heart period time series in the lower frequencies centered around .1 Hz (LF) divided by the power in the higher frequencies centered around the respiratory frequency (HF), may capture differences in cardiac sympathetic control (Furlan *et al.*, 2005; Furlan *et al.*, 2006; Pagani *et al.*, 1986; van de Borne *et al.*, 2001). Here we tested in prolonged ambulatory recordings whether the LF/HF ratio was correlated within- and between-subjects with the PEP, a measure of cardiac sympathetic control based on the beta-adrenergic effects on contractility. Increased cardiac sympathetic control is associated with increases in contractility, which is reflected in decreases in PEP. Hence, a negative correlation was expected between PEP and the LF/HF ratio, such that shorter PEPs would be associated with larger LF/HF ratios. This is indeed what was found in the only study that previously addressed the relation between PEP and LF/HF ratio (Burgess *et al.*, 2004). A significant correlation of -.31 was found in nine young males during sleep (no daytime recordings were made).

The results of the present study did not confirm our expectations. The LF/HF ratio did not show the expected negative correlation to PEP. The average within-subject correlation across an average of 56 separate periods during the ambulatory recording was only -.11. Forty-four out of 64 subjects failed to show a significant correlation with PEP in the expected direction. Between-subject correlations for LF/HF and PEP, computed separately for sleep, sitting and physical activity, were not significant. The use of normalized LF power instead of the LF/HF ratio to index cardiac sympathetic control yielded nearly identical results.

The most parsimonious conclusion from these results is that PEP and LF/HF do not measure the same physiological phenomenon; they appear to be "two sides of a different coin". The important question then becomes which of the two actually measures cardiac sympathetic control. A strong argument in favor of the PEP is that it shows the expected reciprocal behavior to HF power, such that longer PEPs are associated with higher HF power. HF power is seen mainly as an index of cardiac vagal control, at least when independent effects of respiratory behavior of HF power are taken into account (Kollai & Mizsei, 1990; Ritz & Dahme, 2006). Although co-activation and co-inhibition can all occur (Berntson *et al.*, 1996; Salomon *et al.*, 2000) the majority of subjects may show reciprocal behavior of vagal and sympathetic cardiac control across the majority of ambulatory conditions, because the bulk of mental, emotional, and physical stressors require the heart rate to speed up and contractility to increase, whereas a return to resting states will be characterized by slower heart rate in parallel to decreased contractility. The LF/HF power failed to show a similar reciprocal behavior to HF as the PEP.

To demonstrate the validity of either LF/HF ratio or PEP as indices of sympathetic control many studies have employed within-subject manipulations known to increase cardiac sympathetic activity like mental stress and exercise. A brief review of this literature supports both LF/HF ratio and PEP as potential measure of cardiac sympathetic control, but PEP is seen to perform better than LF power. Mental stress, for instance, increases the LF power in some studies (Guasti *et al.*, 2005; Langewitz & Ruddel, 1989) but not in all (Hoshikawa & Yamamoto, 1997; Tulen *et al.*, 1999). In contrast, PEP is systematically seen to decrease in response to mental or emotional stress (Berntson *et al.*, 1994; de Geus *et al.*, 2007; Houtveen *et al.*, 2005; Kupper *et al.*, 2006; Newlin & Levenson, 1979; Sherwood *et al.*, 1986). Furthermore, cardiac sympathetic

activation induced by exercise has evoked a decrease in LF power rather than the expected increase, even when correcting for an overall decrease in power by using LF in normalized units (Ahmed *et al.*, 1994; Arai *et al.*, 1989; Dewey *et al.*, 2007; Hayano *et al.*, 1991; Perini *et al.*, 1990). The PEP performs better here, because systematic and dose-dependent shortening of the PEP is seen during exercise (Houtveen *et al.*, 2002; Krzeminski *et al.*, 2000; Miyamoto *et al.*, 1983; Smith *et al.*, 1989a; Svedenhag *et al.*, 1986).

PEP also seems to track decreases in cardiac sympathetic control better than the LF/HF ratio. Acute ß-adrenergic blockade does not give rise to the expected reduction in LF power (Pagani *et al.*, 1986) and may even cause an increase in LF power (Jokkel *et al.*, 1995). Blockade can result in a net decrease in the LF/HF ratio, but this appears mainly due to an increase in HF power (Cogliati *et al.*, 2004). Sympathetic blockade by segmental thoracic epidural anesthesia during rest or tilt had no effect on absolute or normalized LF (Hopf *et al.*, 1995). In keeping with the idea that LF power is also strongly influenced by vagal activity, several studies have shown that cholinergic blockade by atropine causes a substantial reduction or even elimination of both LF and HF fluctuations (Akselrod *et al.*, 1981; Alcalay *et al.*, 1992; Jokkel *et al.*, 1995; Koh *et al.*, 1994; Pomeranz *et al.*, 1985) which makes the behavior of the LF/HF ratio rather unpredictable. In contrast, acute ß-receptor blockade always prolongs PEP (Cacioppo *et al.*, 1994a; Harris *et al.*, 1967; Schachinger *et al.*, 2001; Winzer *et al.*, 1999), whereas PEP is hardly affected by atropine (Cacioppo *et al.*, 1994a; Martinsson *et al.*, 1991).

The only condition where LF power is more well-behaved than the PEP is during orthostatic challenge. Head up tilting is accompanied by an increase in muscle sympathetic nerve activity (Cooke *et al.*, 1999; Furlan *et al.*, 2000; Saito *et al.*, 1997; Shoemaker *et al.*, 2001) and noradrenergic spillover (Esler *et al.*, 1988; Furlan *et al.*, 2000; Laszlo *et al.*, 2001). Head-up tilt systematically increases the LF/HF ratio (Furlan *et al.*, 2000; Kamiya *et al.*, 2005; Montano *et al.*, 1994; Mukai & Hayano, 1995; Pagani *et al.*, 1986; Pagani *et al.*, 1991) although these effects of tilt seem to be mainly driven by a decrease in HF power rather than an increase in LF power (Montano *et al.*, 1994; Mukai & Hayano, 1995). In contrast, a paradoxical lengthening of PEP is seen when subjects go from supine to standing (Houtveen *et al.*, 2005) and head-up tilting from supine to upright is also known to systematically increase PEP (Chan *et al.*, 2007; Frey & Kenney, 1979; Lewis *et al.*, 1977; Ovadia *et al.*, 1995). The failure of the PEP to

correctly index changes in sympathetic activity across postural change is due to the large effects on pre- and afterload effects induced by these postural changes, as explained in detail in Houtveen *et al.* (2005). Remarkably, PEP was still seen to be systematically prolonged during sleep compared to standing activities (see Table 1), which confirms similar findings in other studies (Kupper *et al.*, 2006; Vrijkotte *et al.*, 2004). This suggests that the decrease in sympathetic activity during sleep is strong enough to overcome confounding of the PEP by pre- and afterload. Nonetheless, during postural changes LF/HF ratio is clearly more indicative of the expected changes in cardiac sympathetic control than the PEP. Taken the differential effects of posture on PEP and the LF/HF ratio, the absence of a significant within-subject correlation between these measures could be simply due to the confounding effects of posture. However, when we recalculated the correlations between PEP and the LF/HF ratio separately for supine, sitting, and standing activities, they were still all non-significant.

In defense of the LF/HF ratio, it must be noted that, perhaps as a side effect of its ease of measurement, it has received more rigorous testing as a sympathetic index than the PEP. We cannot rule out that similar scrutiny of the PEP would detect many occasions where it fails to index changes in cardiac sympathetic control. For instance, we found no studies that compared changes in PEP with changes in MSNA or spillover. A second limitation of the present study is that it only compared PEP to the LF/HF ratio in heart rate variability. It is well known that the two oscillatory components observed in the heart rate can also be detected in mean arterial blood pressure and in peroneal MSNA (Cogliati *et al.*, 2004; Pagani & Malliani, 2000). It remains possible that PEP is associated with the LF/HF ratio's in these measures such that lowered sympathetic cardiac control (longer PEP) is associated with decreases in the LF power of blood pressure or even muscle nerve activity. These measures are very hard to measure in an ambulatory setting, however, which would compromise their practical use in large-scale studies.

In conclusion, we find that in ambulatory data the PEP and LF/HF ratio are uncorrelated between-subjects and correlated in the 'wrong' direction within-subjects. Of these two measures, only PEP shows the expected reciprocal relation to HF power, a proxy measure of cardiac vagal control, and it covaries more systematically with the expected changes in cardiac sympathetic control in response to mental and physical stressors, at least when the analyses are

stratified for posture. The predictive power of LF and HF power for cardiovascular disease is beyond question (Bigger *et al.*, 1993; Dekker *et al.*, 2000; Tsuji *et al.*, 1996) as is the usefulness of HRV recordings. However, the evidence to support ambulatory LF power, either absolute or normalized, as a potential measure of cardiac sympathetic control may be insufficient.

Appendix *Label configuration file*

Posture/physical	Type of activity	Location	Social situation
Lying	Desk work	Work	Alone
Sitting	Administrative work	Home	With significant other
Standing	General work-related activities	Outside	With own children
Walking	Household activities	Friends house	With friends
Lying Sitting	Active transportation	On the road	With colleagues
Sitting Standing	Passive transportation	Public building	With (other) family
Sitting Standing Walking	Professional conversation	Doctor/dentist/etc	With others
Standing Walking	Social conversation	House of family	Unknown
Bicycling	Reading, recreative PC use		
Moderate physical activity	Eating/drinking		
Heavy physical activity	Watching TV		
Unknown	Sleeping		

Chapter 5

Sympathetic nervous system activity in the heart and the skin: are they comparable?

Goedhart AD, Willemsen G, De Geus EJC (2008). In M. Kaneko (Ed.), Sympathetic Nervous System Research Developments. New York: Nova Science Publishers.

Abstract

In this study, we investigated whether pre-ejection period (PEP), number of nonspecific skin conductance responses (ns.SCRs) and skin conductance level (SCL) quantify sympathetic nervous system (SNS) activity in a comparable way. Physiological data were obtained from 39 human subjects (23 males) with a mean age of 22.0 years (SD = 2.3) during exposure to seven different mental and physical stressors, and during subsequent recovery periods. Compared to pre-test resting baseline recordings significant decreases in PEP and parallel increases in the number of ns.SCRs and the SCL were found for stressors known to increase SNS activity. The between- and within-subjects correlations between ns.SCRs and SCL were significant and multilevel analysis showed that 43% of the variance in these skin conductance measures overlapped. Between- and within-subjects correlations between PEP and both skin conductance measures were not significant. This suggests that SNS activity is reflected differently by the heart and the skin. We conclude that SNS activity studies, when possible, should include both PEP and skin conductance measurements.

Introduction

Various non-invasive indicators of sympathetic nervous system activity are in use in psychophysiological studies, including heart rate frequency measures, impedance-derived measures and skin conductance measures. Few studies have examined the correspondence between these various indices of sympathetic activity. In the present chapter, we will report on the comparison between pre-ejection period, an index of sympathetic nervous system activity at the heart, and two skin conductance measures indicative of sympathetic nervous system activity at the skin.

Cardiac sympathetic control can be non-invasively obtained by thoracic impedance cardiography (Cacioppo *et al.*, 1994a). In impedance cardiography a high frequency alternating current is introduced across the thorax by two electrodes. Two inner and parallel electrodes measure the changes in the impedance of the enclosed thorax column (dZ), which is largely a function of aortic blood flow. The impedance cardiogram (ICG) is defined as the first derivative of the pulsatile changes in transthoracic impedance (dZ/dt). From the ICG the pre-ejection period (PEP) can be derived as the time interval between the onset of ventricular depolarization and the opening of the semilunar valves. Changes in PEP reliably index ß-adrenergic inotropic drive to the left ventricle as shown in laboratory studies manipulating ß-adrenergic tone by epinephrine infusion (Mezzacappa *et al.*, 1999; Schachinger *et al.*, 2001; Svedenhag *et al.*, 1986), amyl nitrite inhalation (Nelesen *et al.*, 1999), adrenoceptor blockade (Harris *et al.*, 1967; Schachinger *et al.*, 2001; Winzer *et al.*, 1999), exercise (Krzeminski *et al.*, 2000; Miyamoto *et al.*, 1983; Smith *et al.*, 1989a), or emotional stress (Berntson *et al.*, 1994; Newlin & Levenson, 1979; Sherwood *et al.*, 1986). The PEP has been shown to be a stable individual characteristic. The test-retest reliability coefficients generally range between .45 and .83 when measured over a few weeks to 3 year or longer (Burleson *et al.*, 2003; Goedhart *et al.*, 2006; Matthews *et al.*, 2002; Willemsen *et al.*, 1998) and substantial heritability of PEP (57 %) has been reported (de Geus *et al.*, 2007; Kupper *et al.*, 2006).

Skin sympathetic nervous system (SNS) activity can be non-invasively measured by the activity of the sweat glands. The eccrine sweat glands are innervated by efferent neurons from the sympathetic axis of the autonomic nervous system that use acetylcholine as their neurotransmitter. Changes in SNS activity modulate the conductance of an applied current to the skin (mostly

palmar) and are reflected in the resulting changes in electrodermal activity. Blockade studies have shown that a cholinergic antagonist (atropine) strongly reduces sweat gland activity (Foster & Weiner, 1970). The primary function of most eccrine sweat glands is thermoregulation. However, the eccrine glands located on the palms and soles of the feet have been thought of as being more concerned with grasping behavior than with evaporative cooling (Edelberg, 1972), and it has been suggested that these glands are more responsive to emotional stimuli than to thermal stimuli (Dawson *et al.*, 2000). Electrodermal activity incorporates both slow tonic shifts in basal skin conductance level (SCL) and more rapid phasic transient events, that is, skin conductance responses (SCRs), which are also referred to as galvanic skin responses (GSRs) (Boucsein, 1992; Dawson *et al.*, 2000; Fowles, 1986; Venables & Christie, 1980). The frequency of the nonspecific SCRs (ns.SCRs) reflects an important psychophysiological trait which is termed electrodermal lability (Dawson *et al.*, 2000; Lacey & Lacey, 1958; Mundy-Castle & McKiever, 1953). Both SCL and ns.SCRs have been shown to be influenced by emotional stress (Boucsein, 1992; Dawson *et al.*, 2000). The test-retest reliability coefficients over time periods encompassing one day to a year for SCL levels (both during rest and during periods of stimulation) ranged from .40 to .85 and for ns.SCRs correlations ranged from .40 to .76 (Freixa i Baque, 1982; Iacono *et al.*, 1984; Schell *et al.*, 1988; Schell *et al.*, 2002; Vossel & Zimmer, 1990). Moderate heritability estimates between .40 and .50 have been found for electrodermal lability (Crider *et al.*, 2004; Lykken *et al.*, 1988).

Based on the physiological underpinnings above, both PEP and skin conductance are widely used as non-invasive measures of within and between subject differences of SNS activity in psychophysiology (Cacioppo *et al.*, 1994a; Jacobs *et al.*, 1994; Kronholm *et al.*, 1996; Popma *et al.*, 2006; Salomon *et al.*, 2000; Sherwood *et al.*, 1990). However, only very few studies have empirically tested whether PEP and skin conductance are correlated across subjects or how PEP and skin conductance covary within subjects during exposure to different stressors engaging the sympathetic nervous system. A study by Kelsey (1991) found a significant relation between electrodermal lability and PEP reactivity to stress. The subjects with a high frequency of ns.SCRs exhibited greater myocardial reactivity than did the subjects with low frequencies of such responses. However, this relation reflected a comparison between frequency of ns.SCRs at baseline and PEP reactivity during the presence of a stressor. No

mention was made of the relation between ns.SCRs and PEP levels at rest or during the tasks. Tomaka, Blascovich and Swart (1994) measured reactivity (compared to the last minute of the rest period preceding each task) of both ns.SCRs and PEP to a mental arithmetic task performed under two different conditions. They found higher SCR reactivity during the reading aloud condition while PEP reactivity was higher during the silent condition. In this case, PEP and SCR did not seem to follow the same pattern.

The current study was designed to allow a more extended comparison of PEP, ns.SCRs and SCL at rest, during different types of mental and physical stress tasks, and during subsequent recovery. The questions we want to address are whether SNS activity shows the same pattern in the heart and in the skin and to what extent PEP, ns.SCRs and SCL are exchangeable. We expected a positive correlation between ns.SCRs and SCL and a significant negative correlation between PEP and these two skin conductance measures, both between- and within-subjects.

Methods

Subjects

Thirty-nine university students (23 males) between 18 and 28 years (mean = 22.0 years, SD = 2.4) were recruited, who had no overt somatic or psychiatric disease, did not taking cardio-active or psychotropic medication and were not severely obese (BMI <30). The study was approved by an ethics committee, and all subjects provided written informed consent. At the end of the second test day participant received 40 euro.

Protocol

This study is part of a double-blind randomized controlled trial testing the effects of dexamethasone versus placebo on cardiovascular reactivity. Here we only report on the data collected after the administration of a placebo. All women not taking OC (n = 2) were tested in the follicular phase (days 1-11) of their menstrual cycle according to self-report. Women taking OC were not restricted to a specific phase of the menstrual cycle.

Procedure

The subjects were asked to refrain from alcohol- or caffeine-containing beverages the evening before the test day and in the morning before coming to

the laboratory. Testing always took place exactly 3 hours after spontaneous awakening. The experimental session was conducted in a dimly lighted, sound-attenuated cabin, with the subjects facing a video screen at 90 cm. Subject were attached to the electrocardiogram (ECG), the impedance cardiogram (ICG) and the EDA recording devices of the BioPac data-acquisition system (BioPac systems Inc., Santa Barbara, CA). This will be discussed in more detail later on.

The various experimental conditions were explained to the subject and the mental and physical stress tasks were briefly practiced. The actual experiment started by asking the subjects to sit quietly and relax for a pre-test 10 min resting baseline. Next, the following conditions were presented in a fixed order: Stroop color word task (4 min), recovery1 (3 min), tone avoidance task (4 min), recovery2 (3 min), lying (2 min), standing (2 min), recovery3 (2 min), hand grip test (2 min), cold pressure test (1 min), recovery4 (3 min), and the step test (2 min). After the step test a final post-stress resting condition of 13 min concluded the physiological recordings.

Mental and physical stress tasks

Stroop color word (SCW). Subjects were presented with one slide per second on a computer screen which had the name of a color printed in a contrasting colored ink. Participants were requested to verbally identify as fast as possible the color of the ink, not the name of the color.

Tone avoidance task (TA). An 'x' was shown briefly (500 ms) in one of the corners on the screen and the subjects were asked to respond as fast as they possibly could by pressing the button opposite to this corner on a four-button response panel (e.g. 'x' shown in the top left-hand corner, press the bottom right-hand button). Incorrect or too slow responses were punished with a loud noise burst (1000 Hz, 85 dB) that lasted 500 ms. Reaction time had to be shorter than a maximal response period, that was initially set to 550 ms, and was thereafter continuously adapted to the performance of the subject (Willemsen *et al.*, 1996).

Postures. Subjects were asked to lie down for 2 min, followed by standing for 2 min.

Hand grip (HG). During the practice part of the experiment, maximum grip strength in the dominant hand was established with a hand grip dynamometer. During the actual hand grip test subject squeezed at 30% of their maximum voluntary contraction for a period of 2 min.

Cold pressor (CP). The subjects were asked to submerge their dominant hand up to the wrist joint in a bucket of ice water of 3–5 ˚C and to hold the fingers in a relaxed position. After exactly 60 s, the hand was removed from the bucket.
Harvard step test (ST). Subjects were asked to stand comfortably upright before a standard gym bench of exactly 45 cm height. They were asked to step up the bench every 2 s for 2 min (60 steps). Timed verbal commands ensured that the appropriate step frequency was maintained.

Physiological assessments

The ECG and ICG were recorded using seven pregelled Ag/AgCl spot electrodes (UltraTrace, ConMed, USA) in a configuration shown in Figure 1. The electrodes were connected to the ECG100C and NICO100C BioPac modules using extension leads. For each experimental condition the PEP (in ms) was scored as the interval from the R-wave peak, minus a fixed interval of 48 ms (Lozano *et al.*, 2007; Sherwood *et al.*, 1990; Willemsen *et al.*, 1996) to the B-point, which signals opening of the aortic valves.

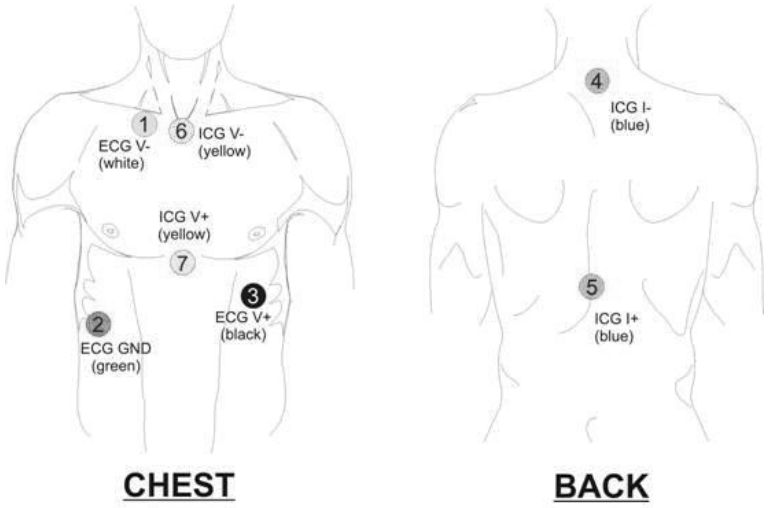


Figure 1 *Location of the seven ECG and ICG electrodes.*

Skin conductance information was collected using a pair of Ag/AgCl (unpolarizable) electrodes (Ø = 6 mm). To ensure sufficient electrode–skin contact, isotonic electrode paste was used (.5% saline in a neutral base). The electrodes were attached with a Velcro strap to the distal phalanx of the index and middle finger (Scerbo *et al.*, 1992) of the non-dominant hand with the leads

connected to the GSR100C BioPac module (Figure 2). The skin conductance is measured with the .5 V constant voltage method. The fluctuating current conducted through the skin of the subject represents the conductance signal. Recorded data was processed to obtain ns.SCRs and SCLs for every period of interest. The SCL was defined as the mean level of skin conductance and the ns.SCRs as the number of phasic increases in conductance of at least .05 μmho per minute.

Figure 2 *Location of the two skin conductance electrodes.*

Statistical analyses

With an independent-samples t-test in SPSS 13.0 (SPSS Inc., Chicago, USA) we first tested whether sex differences influenced baseline PEP, ns.SCRs and SCL scores. We also examined the effect of age on PEP, ns.SCRs and SCL by Pearson correlation analysis. Repeated measures ANOVA was used to test for the effect of condition (rest 1, SCW, recovery1, TA, recovery2, lying, standing, recovery3, HG, CP, recovery4, ST, and rest 2) on PEP, ns.SCRs and SCL. To test for significant reactivity, pre-planned contrast compared the pre-test resting baseline levels of PEP, ns.SCRs and SCL to the levels obtained in the stress conditions. Finally, Pearson correlations were computed between PEP, ns.SCRs and SCL separately for each of the conditions (between-subject correlations) and separately for each of the subjects across all conditions (within-subject correlations).

If a significant relationship between two variables was found, we used multilevel analysis to examine the effect of individual differences on the intercept and slope of the regression between the variables. Multilevel analysis is a general method of analyzing data with a hierarchical or clustered structure

(Snijders & Bosker, 1999). In our data the different stress tasks are clustered within subjects. For this reason, we used multilevel analysis to examine the relationship between PEP on ns.SCRs and SCL, allowing us to investigate the effect of individual differences on the intercept and slope of the regression between the variables. Models with random slope and/or intercept (restricted models) were compared to the unrestricted model. An unrestricted model (also called null model) is one that contains a dependent variable and a level-1 random intercept. We compared the models on the basis of their explained variance and their fit. A model describes the data better than a previous model when it explains more variance in the dependent variable *and* when the fit is significant better. Explained variance was computed with the following formula suggested by Kreft and de Leeuw (1998): (unrestricted error – restricted error)/ unrestricted error. The deviance fit test, or likelihood ratio test, was used to compare the fit of two models. This test is based on the difference between the deviance statistics of the two models, which has a chi-square distribution with degrees of freedom equal to the difference in the number of parameters estimated in the models being compared. Finally, sex and age were added as potential predictors in the level 2 model to see whether these variables could account for the variance of the random intercept and slope.

Results

For the baseline, the average PEP score was 119.49 ms (*SD* = 12.22), the average number of ns.SCRs was 1.64 (*SD* = 1.37), and the average SCL was 11.43 µmho (*SD* = 3.92). An independent-samples *t* test was conducted to see if there were any differences between sexes. No significant sex differences were found (PEP, t (37) = -.29, p = .77; ns.SCRs, t (37) = .99, p = .33; SCL, t (37) = .34, p = .74). The computed correlation of PEP, ns.SCRs and SCL with age revealed no significant effect of age on the three measures (r = -.03, r = -.02, and r = -.05, respectively).

The means and standard deviations for PEP, ns.SCRs and SCL per condition are presented in Table 1. For all three variables significant effects of condition were found, F (12, 23) = 120.72, p = .00 for PEP, F (12, 25) = 32.22, p = .00 for ns.SCRs, and F (12, 27) = 15.51, p = .00 for SCL. As expected, ns.SCRs and SCL were found to increase significantly over baseline levels during conditions known to increase SNS activity, i.e. Stroop color word task, tone avoidance task, standing up, hand grip, cold pressor and the step test. In the supine condition,

which is expected to decrease SNS activity, ns.SCRs indeed decreased but SCL slightly increased. During the recovery periods both ns.SCRs and SCL decreased in comparison to previous task levels, but in the course of the experiment, and lasting to the post-stress resting condition, a slow increase in ns.SCRs and SCL above the pre-test baseline was seen. PEP significantly decreased in response to the Stroop and tone avoidance task, to standing and to the step test. No changes in PEP were seen during hand grip and cold pressor and unexpectedly, PEP decreased in response to lying down. PEP systematically returned to the baseline level during all recovery periods.

Table 1 *Means (SDs) for PEP, ns.SCRs and SCL separately per condition.*

Conditions	PEP(ms)	ns.SCRs (freq)	SCL (µmho)
Rest 1	119.49 (12.22)	1.64 (1.37)	11.43 (3.92)
Stroop	116.62 (12.53)*	4.85 (2.69)*	13.58 (4.01)*
Recovery 1	119.28 (12.27)	1.35 (1.27)*	11.92 (3.51)*
Tone Avoidance	117.13 (13.20)*	4.69 (2.65)*	13.61 (3.76)*
Recovery 2	120.31 (11.07)	1.30 (1.23)*	11.99 (3.52)*
Lying	107.79 (8.99)*	.86 (1.00)*	12.44 (3.55)*
Standing	116.82 (11.79)*	2.13 (1.31)*	12.55 (3.64)*
Recovery 3	118.97 (11.19)	2.15 (1.71)*	12.76 (3.73)*
Hand grip	118.97 (12.20)	3.50 (2.60)*	14.28 (3.83)*
Cold Pressor	119.49 (12.59)	2.40 (2.10)*	13.97 (3.70)*
Recovery 4	120.72 (10.89)	1.59 (1.32)	12.63 (3.45)*
Step test	70.40 (7.89)*	10.57 (4.06)*	16.20 (3.24)*
Rest 2	117.54 (10.73)	2.21 (1.78)*	12.88 (3.71)*

** Significant difference at $p < .05$ level with rest 1.*

The Figures 3, 4 and 5 display the scatterplots between PEP, ns.SCRs and SCL for all data points. Note that these figures contain both within- and between-subject variance. PEP and ns.SCRs (r = -.52), PEP and SCL (r = -.30), and ns.SCRs and SCL (r = .42) were significantly correlated, but the correlations between PEP and the two measures of skin conductance can be largely ascribed to the step test condition, where the shortest PEP and highest ns.SCRs and SCL co-occur.

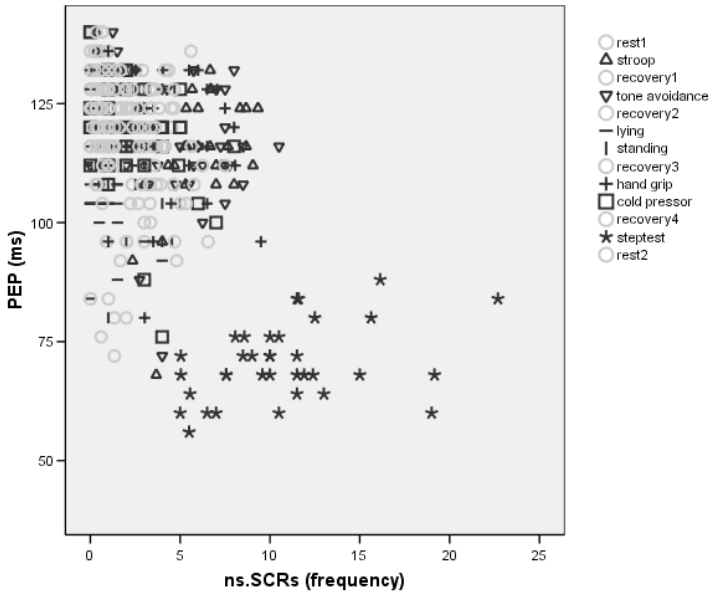


Figure 3 Scatterplot of the number of ns.SCRs per minute and the PEP for all subjects in all conditions.

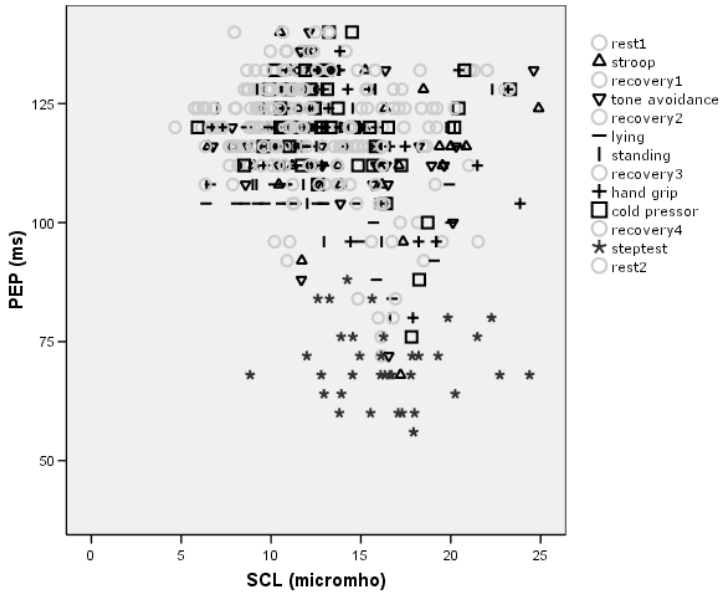


Figure 4 Scatterplot of the SCL and the PEP for all subjects in all conditions.

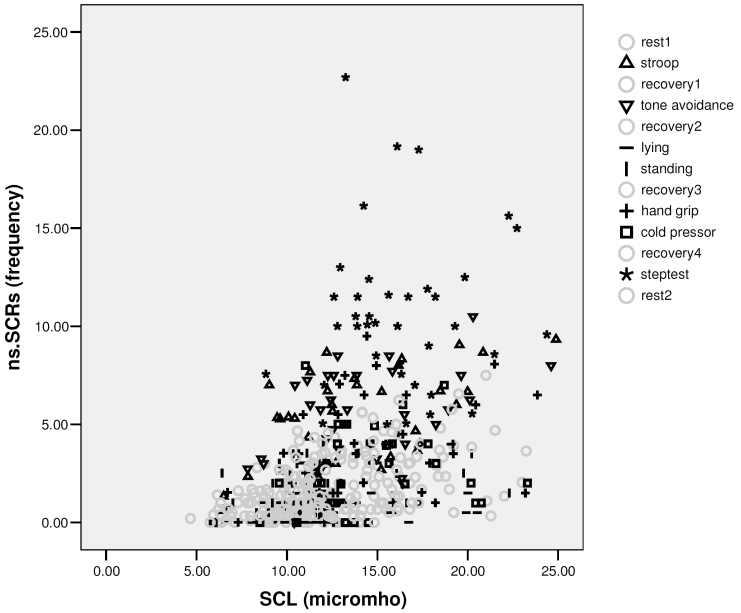


Figure 5 Scatterplot of the number of ns.SCRs per minute and the SCL for all subjects in all conditions.

To separate the between- and within-subject components of the covariance in our measures we first computed between subject correlations separately within each of the 13 experimental conditions. This is shown in Table 2. Moderate correlations ($0.34 < r < 0.55$) were found between the two measures of skin conductance, except during lying, cold pressor and the step test. However, PEP was largely uncorrelated with ns.SCRs and SCL.

Table 2 *Between-subject correlations between PEP, ns.SCRs, and SCL separately per condition.*

Conditions	$r_{PEP\text{-}ns.SCRs}$	*p*	$r_{PEP\text{-}SCL}$	*p*	$r_{ns.SCRs\text{-}SCL}$	*p*
Rest 1	-.12	.49	-.16	.34	**.51**	.00
Stroop	-.03	.85	-.14	.41	**.55**	.00
Recovery 1	-.21	.20	-.15	.37	**.43**	.01
Tone Avoidance	-.15	.38	-.15	.37	**.49**	.00
Recovery 2	-.29	.08	-.24	.14	**.34**	.04
Lying	-.27	.10	-.27	.09	.24	.14
Standing	-.16	.33	-.13	.44	**.36**	.02
Recovery 3	-.27	.10	-.24	.14	**.53**	.00
Hand grip	-.15	.37	**-.36**	.03	.33	.05
Cold Pressor	**-.43**	.01	-.23	.17	.24	.14
Recovery 4	-.19	.26	-.25	.12	**.41**	.01
Step test	**.39**	.02	-.07	.69	.05	.79
Rest 2	-.22	.17	-.28	.08	**.53**	.00

Correlations significant at p < .05 level are bold.

Within-subject correlations across the 13 experimental conditions are shown in Table 3. The mean within-subject correlation between ns.SCRs and SCL was .72, between PEP and ns.SCRs -.63, and between PEP and SCL -.43. Though within-subject correlations were generally high between ns.SCRs and SCL, large individual differences (r ranged from -.96 to .75) were found in the within-subject correlation between PEP and ns.SCRs and between PEP and SCL. Figure 3 and Figure 4, however, strongly suggest that the relation between PEP and ns.SCRs and between PEP and SCL entirely depended on the exercise condition. When we recomputed the within-subject correlations after exclusion of the step test data, only two significant correlations remained between PEP and ns.SCRs, and only one between PEP and SCL (Table 4). The correlation between the two measures of skin conductance also decreased but remained significant overall (mean within-subject r = .59).

Table 3 *Within-subject correlations between PEP, ns.SCRs, and SCL for all conditions.*

Subject	$r_{PEP\text{-}ns.SCRs}$	*p*	$r_{PEP\text{-}SCL}$	*p*	$r_{ns.SCRs\text{-}SCL}$	*p*
1	-.26	.39	-.11	.72	**.56**	.05
3	.20	.53	.51	.09	**.77**	.00
5	**-.77**	.00	**-.64**	.02	**.87**	.00
6	**-.74**	.00	-.54	.06	**.91**	.00
7	**-.62**	.02	-.47	.11	**.66**	.02
8	**-.73**	.01	**-.62**	.02	**.89**	.00
9	-.37	.24	-.29	.35	**.89**	.00
10	**-.92**	.00	**-.73**	.00	**.82**	.00
11	**-.85**	.00	**-.70**	.01	**.74**	.00
12	**-.82**	.00	-.44	.13	**.73**	.01
13	**-.86**	.00	-.56	.05	**.67**	.02
14	**-.88**	.00	**-.71**	.01	**.59**	.03
15	**-.81**	.00	-.46	.11	**.69**	.01
16	-.92	.00	-.35	.24	.39	.19
17	**-.86**	.00	**-.65**	.02	**.87**	.00
18	-.50	.08	-.38	.20	**.91**	.00
19	**-.94**	.00	-.36	.23	**.40**	.18
20	-.48	.10	**-.72**	.01	**.87**	.00
21	-.51	.08	-.23	.46	**.78**	.00
22	**-.68**	.01	-.43	.15	**.75**	.00
23	**-.61**	.03	**-.66**	.01	**.78**	.00
24	**-.91**	.00	**-.81**	.00	**.83**	.00
25	**-.84**	.00	**-.73**	.00	**.78**	.00
26	**-.87**	.00	**-.66**	.01	**.65**	.02
28	**.75**	.01	.41	.18	**.67**	.01
29	**-.96**	.00	-.46	.11	**.57**	.04
30	-.31	.30	-.15	.63	**.91**	.00
31	-.23	.44	-.29	.34	**.64**	.02
32	**-.83**	.00	.49	.09	-.34	.26
33	**-.75**	.00	-.08	.80	**.64**	.02
34	**-.81**	.00	**-.79**	.00	**.96**	.00
35	**-.70**	.01	**-.77**	.00	**.82**	.00
36	**-.68**	.01	**-.59**	.03	**.74**	.00
41	-.55	.07	-.41	.19	**.80**	.00
42	-.41	.16	-.55	.05	**.83**	.00
43	**-.75**	.00	-.02	.96	.49	.09
45	**-.62**	.02	**-.61**	.03	**.90**	.00
47	**-.80**	.00	**-.84**	.00	**.80**	.00
48	-.46	.12	-.49	.09	**.93**	.00

Correlations significant at p < .05 level are bold.

Table 4 *Within-subject correlations between PEP, ns.SCRs, and SCL without step test data.*

Subject	$r_{PEP\text{-}ns.SCRs}$	*p*	$r_{PEP\text{-}SCL}$	*p*	$r_{ns.SCRs\text{-}SCL}$	*p*
1	.37	.24	.46	.14	.49	.10
3	.20	.53	.51	.09	**.67**	.02
5	-.18	.57	-.03	.94	**.76**	.00
6	.53	.07	.45	.14	**.89**	.00
7	.15	.64	.32	.31	.47	.12
8	.02	.96	-.27	.39	**.85**	.00
9	-.37	.24	-.29	.35	**.89**	.00
10	.31	.33	-.16	.72	.32	.32
11	-.35	.26	-.48	.11	.55	.06
12	**-.67**	.02	-.22	.49	**.66**	.02
13	.15	.66	-.10	.76	-.01	.97
14	-.56	.06	**-.73**	.01	.49	.11
15	.11	.74	.25	.44	.49	.10
16	.48	.12	.00	1.00	.18	.58
17	-.28	.39	-.09	.79	**.78**	.00
18	.22	.50	.12	.72	**.90**	.00
19	-.12	.71	-.01	.98	.19	.56
20	-.12	.71	-.16	.62	**.86**	.00
21	.21	.52	.25	.44	**.77**	.00
22	-.12	.72	.14	.66	**.66**	.02
23	.27	.41	-.18	.58	**.58**	.05
24	.02	.95	-.21	.52	.16	.61
25	-.06	.86	-.19	.55	.40	.19
26	.13	.68	-.05	.88	-.05	.89
28	**.75**	.01	.41	.18	**.76**	.00
29	-.45	.14	-.42	.18	**.73**	.01
30	.22	.49	.26	.42	**.91**	.00
31	.17	.61	.01	.98	**.60**	.04
32	-.21	.52	-.22	.50	.39	.21
33	.20	.53	.44	.16	**.84**	.00
34	.04	.89	.01	.98	**.86**	.00
35	-.06	.87	.19	.56	.50	.10
36	-.01	.97	-.05	.88	.54	.07
41	-.55	.07	-.41	.19	.40	.20
42	.13	.68	.35	.27	**.78**	.00
43	-.03	.94	.25	.44	**.71**	.01
45	.09	.77	-.01	.99	**.82**	.00
47	-.10	.77	-.37	.24	.38	.23
48	-.07	.83	-.04	.90	**.91**	.00

Correlations significant at p < .05 level are bold.

To further examine the relationship between ns.SCRs and SCL, we used a multilevel analysis. The regression coefficients of the tested models are shown in Table 5. The random intercept model consisting of ns.SCRs being predicted by SCL explained 37.9 % of variance in ns.SCRs and had a significant better fit than the 'empty' model, $X^2(1) = 159.57$, $p < .001$. The random slope model explained 38.6 % of variance in ns.SCRs and had a significant better fit than the 'empty' model as well, $X^2(1) = 171.80$, $p < .001$. Finally, the extended linear model with a random intercept and a random slope, explained 43.2 % of the total variance in ns.SCRs and had a better fit than both previous models, $X^2(2) = 21.81$, $p < .001$ and $X^2(2) = 9.58$, $p = .008$ respectively. Figure 6 shows the regression lines per individual for the final model. Sex and age could not predict slope or intercept differences. When we reran the multilevel analysis after exclusion of the step test data, comparable results were found. Again, the best fitting model was the model with random intercept and slope, and this model explained 42.5 % of the variance in ns.SCRs.

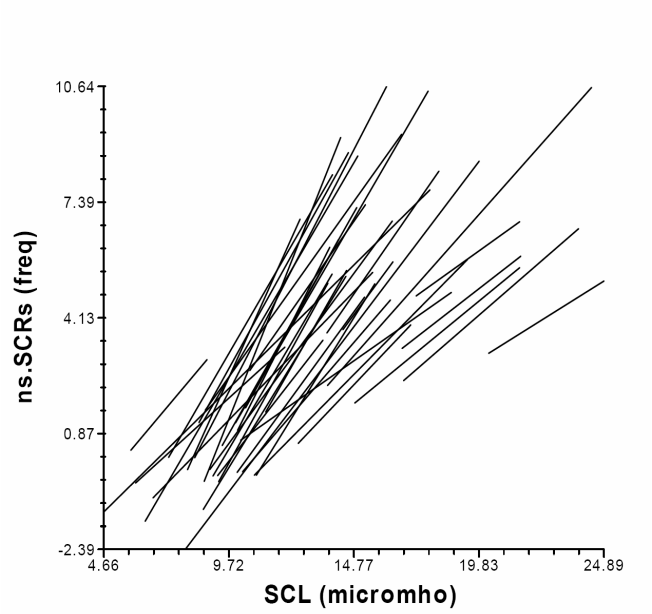


Figure 6 Regression lines per subject for the random intercept and random slope model.

Table 5 *Results of multilevel analysis*

	Empty model		Random intercept		Random slope		Random intercept and slope		Random intercept and slope, sex and age	
	Coefficients(SE)	*p*	*Coefficients(SE)*	*p*	*Coefficients(SE)*	*p*	*Coefficients(SE)*	*p*	*Coefficients(SE)*	*p*
Fixed effects										
Intercept (γ_{00})	3.05 (.21)	.00	-7.93 (0.82)	.00	-7.50 (0.67)	.00	-8.92 (.99)	.00	-5.80 (.85)	.00
SCL (γ_{10})			0.85 (.05)	.00	0.85 (0.06)	.00	.97 (.09)	.00	.56 (.24)	.00
Sex									-.01 (.05)	.82
Age									.00 (.01)	.63
	Variance (SD)	*p*	*Variance (SD)*	*p*	*Variance (SD)*	*p*	*Variance (SD)*	*p*		
Random effects										
Level 1 residual (R_{ij})	9.82 (3.13)	NA[a]	6.10 (2.47)	NA[a]	6.02 (2.45)	NA[a]	5.58 (2.36)	NA[a]	2.13 (1.46)	NA[a]
Level 2 residuals										
Intercept (U_{oj})	0.80 (0.89)	.00	5.67 (2.38)	.00			16.81 (4.10)	.00	14.48 (3.81)	.00
Slope (U_{1j})					0.02 (0.17)	.00	.16 (.40)	.00	.14 (.37)	.00
Deviances	2478.35		2318.78		2306.55		2296.97		1728.31	
Estimated parameters	3		4		4		6		8	
Explained variance			37.9 %		38.6 %		43.2 %		43.3 %	

[a] *The p values are not given because HLM software does not provide a significance test for level 1 residuals.*

Conclusion

Both PEP and skin conductance measures are extensively used as indices of SNS activity. In accordance, we found that PEP generally decreased and ns.SCRs and SCL increased during mental and physical stressors known to engage the SNS. The between- and within-subject correlations between the two measures of skin conductance were overall significant, and results showed that around 43% of the variance in ns.SCRs could be explained by SCL when allowing for individual differences. This suggests that these parameters both reflect SNS activity but that the overlap is imperfect and each skin conductance measure therefore also reflects different components of SNS activity. More surprising results were obtained for the association between PEP and the skin conductance measures. The between-subject correlations between PEP and the two measures of skin conductance were weak or absent during each of the stressful tasks. Within subjects, a significant relation was found between changes in PEP and the two measures of skin conductance, but this was entirely due to a short period of moderately intense exercise, which led to a strong decrease in PEP and a strong increase in ns.SCRs and SCL in most subjects.

The absence of a between-subject correlation between PEP and ns.SCRs and between PEP and SCL was unexpected, though results do correspond with the only other study which compared skin conductance and PEP responses to stress (Tomaka *et al.*, 1994). Between-subject differences in absolute PEP have been shown to closely reflect individual differences in ß-adrenergic inotropic drive (Cacioppo *et al.*, 1994a). In a study of 10 female undergraduate students, a high correlation was found between absolute PEP and heart period increases in response to sympathetic blockade. In further support, a significant inverse correlation between a subjects' absolute PEP and their plasma adrenaline level was found (Levi *et al.*, 1982). At the same time, between-subject differences in electrodermal activity have been shown to correlate to individual differences in the number of sympathetic action potentials in peripheral sympathetic nerves. Within normal ranges of ambient room temperature and subject thermoregulatory states, there was a high correlation between bursts of skin sympathetic nerve activity and SCRs (Wallin, 1981). In addition to measuring SNS activity, between-subject differences in both PEP and skin conductance have been shown to be reliable and stable over time (Goedhart *et al.*, 2006; Schell *et al.*, 2002). So, why did we not find between-subject correlations between these measures?

The most likely explanation is uncorrelated individual differences in the responsiveness of the effector systems. SNS activity changes contractility, and with that PEP, through the effects of noradrenergic fibers acting on β_1- and β_2-receptors on the left ventricle (Kelsey, 1991; Sherwood *et al.*, 1990). Hence, the effect of a fixed amount of cardiac sympathetic activity on contractility (and PEP) depends on the sensitivity of these ß-adrenergic receptors, which is known to vary strongly between individuals (Brodde *et al.*, 2006). Likewise, the effect of a fixed amount of skin sympathetic activity on sweat gland activity (and skin conductance) depends on the number of sweat ducts per area of skin. These also show large individual differences (Sato & Sato, 1983). In other words, a subject with a low ß-receptor sensitivity and a high number of sweat glands may have long PEP and high SCL, whereas another subject with identical SNS activity but high ß-receptor sensitivity and a low number of sweat glands may have a shorter PEP but lower SCL.

More alarming than the absence of a between-subject correlation is the absence of a within-subject correlation. Individual differences in sweat glands or receptor sensitivity should not prevent PEP and skin conductance to correlate across different levels of SNS activation *within* the same individual. Yet, such correlations were not found, although the addition of more powerful engagement of the SNS by the step test did induce a correlation in most subjects. It is unclear why the changes in PEP and ns.SCRs and in PEP and SCL across the other stressors were uncorrelated. One explanation is that the changes in PEP across the stressors are not solely influenced by sympathetic activity, but by changes in preload and afterload effects as well (Lewis *et al.*, 1977). These latter two are usually not that prevalent during experiments where subjects sit down in a lab during the whole experiment (Sherwood *et al.*, 1990) but during conditions that induce a large change in mean arterial pressure or end-diastolic filling, such effects could have influenced PEP independently from true changes in SNS activity. This seems supported by findings for PEP in the supine and cold pressor conditions. Lying down increases preload and reduces afterload. Whereas we would expect SNS activity to be lower in a supine position, PEP in fact became shorter, and this likely reflects decreased afterload. In contrast, SNS activity can be expected to increase during the stressful cold pressor test, but in fact, PEP was unchanged from resting level. This is likely due to the strong increase in mean arterial pressure that is reflexively induced by cold stress. Since ns.SCRs

and SCL are not affected by preload and afterload effects, this may have led to a discrepancy between PEP and skin conductance measures.

The within subject correlation may further have been compromised by a gradual increase in skin conductance level and spontaneous frequencies during the experiment which may not reflect a true increase in SNS activity. Although sweat gland activity (filling of the sweat ducts) appears to be the main determinant of both skin conductance measures (Dawson *et al.*, 2000), sweat on the skin (corneal hydration) is thought to play a role as well, especially for SCL (Boucsein, 1992; Fowles, 1986). This also might explain the proportion if unexplained variance between ns.SCRs and SCL. Sweat duct activity is a volatile process mainly due to recent sympathetic nerve activity, but corneal hydration is a much slower process, probably building up during the experiment independently from changes in sympathetic activation. Since corneal hydration occurs independently from changes in sympathetic activation this may have added to the discrepancy between skin conductance measures and PEP.

Finally, differences in adrenal catecholamine release in response to the various stressors may have acted to reduce the PEP – skin conductance correlation. Ventricular β_1- and β_2-receptors do not respond solely to noradrenaline released from the cardiac sympathetic nerve, they are also highly sensitive to circulating catecholamines. Many of the stressors used are known to increase circulating levels of adrenaline and noradrenaline (Kjaer *et al.*, 1987; Schachinger *et al.*, 2001) which will co-determine PEP responses. In contrast, sweat gland activity is controlled by sympathetic cholinergic fibers acting on muscarinergic type 3 receptors (Kelsey, 1991; Shields *et al.*, 1987). Although these also receive additional hormonal input (Wallin, 1981) they will not be sensitive to circulating noradrenaline or adrenaline.

Apart from the methodological explanations above, the absence of a relation between PEP and skin conductance may also reflect true differences in the activation of the various branches of the SNS during stressful tasks. Such differentiation is known to occur from previous studies using direct recording of skin and muscle sympathetic activity (Wallin, 1981; Grassi & Esler, 1991) that showed that sympathetic activity might not have a uniform effect on all effector organs. Baroreflex engagement may be a powerful source of differences in vascular/cardiac versus skin SNS activity. Unlike cardiac SNS activity , skin SNS activity is not influenced by the baroreflexes (Bini *et al.*, 1981; Vissing *et al.*, 1994; Wallin *et al.*, 1975; Wilson *et al.*, 2001). Task induced changes in

baroreflex activity, therefore, will affect PEP but not SCL and ns.SCR. The well-known individual differences in baroreflex sensitivity (Riese *et al.*, 2006; Tank *et al.*, 2001) may also partly account for the low between-subject correlations.

We conclude that PEP and skin conductance respond to stress tasks in a manner compatible with increased SNS activity but that their response is largely uncorrelated. The heart and the skin, therefore, seem to reflect different aspects of sympathetic nervous system activity. Since all three measures are responsive to SNS activity and have already shown their use in psychophysiological testing we conclude that, whenever possible, PEP, ns.SCR and SCL should all be measured.

Chapter 6

No evidence for permissive effects of the early morning cortisol rise on daytime sympathetic and cardiovascular reactivity to stress

Goedhart AD, Willemsen G, Hoogendijk WJG, Van Weissenbruch MM, De Geus EJC (submitted). No evidence for permissive effects of the early morning cortisol rise on daytime sympathetic and cardiovascular reactivity to stress. *Biological Psychology*.

Abstract

We investigated the permissive effects of the early morning rise in cortisol on sympathetic and cardiovascular reactivity to stress using a double-blind randomized trial design. Thirty-nine students underwent a stress protocol consisting of five mental and physical stressors on a placebo day and on a day on which the early morning cortisol rise was blocked by dexamethasone. Pre-ejection period, skin conductance, blood pressure, heart rate, and respiratory sinus arrhythmia were obtained during the stressors, and subsequent resting periods. For the placebo, no significant association was found between morning cortisol levels and resting sympathetic tone or cardiovascular reactivity. Complete blockade of the normal morning rise in cortisol by dexamethasone gave a mild decrease in resting sympathetic tone but had no effects on sympathetic and cardiovascular reactivity. We conclude that there is currently no evidence for the hypothesized permissive effects of the early morning cortisol rise on daytime reactivity to stress

Introduction

Glucocorticoids (GCs), including cortisol and corticosterone, have powerful actions in a broad range of domains including cardiovascular function, fluid volume and hemorrhage, immunity and inflammation, metabolism, neurobiology, and reproductive physiology (Chrousos & Gold, 1992; de Kloet *et al.*, 1999; Franchimont *et al.*, 2002; e.g. Sapolsky *et al.*, 2000). Originally, GCs were seen to enhance and mediate the response of these various systems to stress (Seyle 1956), but in an authorative review in 1984 their suppressive actions were put to the fore (Munck *et al.*, 1984), and GCs were seen as a means to prevent the stress response from overshooting and threatening homeostasis. More recently, both viewpoints were integrated (Sapolsky *et al.*, 2000). The stimulating, suppressive, and preparative actions all refer to the action of *stress-induced* increases in GC levels that enhance or suppress either the ongoing stressors if it is long-lasting or the acute response of other stress-systems to subsequent stressors. The strength of the effects depends on the magnitude of the stress-induced increase in GC levels. The permissive actions of GCs are unique in that they exert their effects on the acute stress response and occur before and therefore independent of, the stress-induced increase in GC levels.

As an example of such permissive effects, Sapolsky *et al.* (2000) point to the modulation of sympathetic nervous system action on the heart and blood vessels during acute stress i.e. increased blood pressure, cardiac output, and vascular resistance, the latter most prominently in the vessels of non-muscular tissue. The basic idea behind permissive effects of GCs is that tonic increases in GC levels that precede the acute exposure to stressors results in increased sympathetic nervous system reactivity to these stressors (Roy *et al.*, 2001). Clinical evidence from patients with hyposecretion or hypersecretion supports a permissive action of basal cortisol on sympathetic cardiovascular reactivity. Addison patients and adrenalectomized subjects, for instance, are characterized by hypotension and blood pressure underresponsiveness to stress (Lovas & Husebye, 2003; Marik & Zaloga, 2002; Ten *et al.*, 2001). The defect in cortisol production may even elicit life-threatening hypotension, especially under conditions of stress (Oelkers, 1996; Zaloga & Marik, 2001). On the other hand, tonic excess of cortisol can cause pronounced blood pressure reactivity and hypertension (Mantero & Boscaro, 1992; Quinkler & Stewart, 2003).

The modulatory effects of cortisol on acute sympathetic and cardiovascular stress reactivity must by necessity be permissive. Although hypothalamic release

of corticotrophin-releasing hormone (CRH) is immediate during stress, the actual release of cortisol is delayed by many minutes. More importantly, the bulk of steroid effects on tissues (including those of cortisol) is genomic, rather than through membrane-receptor signaling. This means that stress-induced increases in cortisol become noticeable only after minutes to hours, and is, therefore, unlikely to influence the ongoing cardiovascular response. Such time-delayed permissive effects on cardiovascular responsivity make good evolutionary sense in light of the clear diurnal rhythm in cortisol (Burleson *et al.*, 2003). Cortisol levels begin to rise sharply a few hours before awakening suggesting that – taken the genomic delays- its augmentation of sympathetic effects is optimal during the active phase (day in primates, night in nocturnal rodents) when fight-flight responses can be essential for survival.

In spite of the theoretical attractiveness, direct evidence for permissive actions of early morning cortisol levels on human sympathetic and cardiovascular stress-reactivity in the course of the day is currently lacking. Here we tested the permissive effects of cortisol in two different ways. First, we tested whether the natural occurring variation in the early morning levels in cortisol could predict sympathetic and cardiovascular reactivity to a series of standardized mental and physical stressors to which participants were exposed exactly three hours after awakening. This observational design has good ecological validity, but suffers from the shortcoming that it is correlational. Individual differences in psychological disposition may independently affect morning cortisol as well as sympathetic and cardiovascular reactivity. To separate such effects of disposition (or any other third factor) from a direct permissive effect of cortisol on sympathetic and cardiovascular reactivity, we used a double–blind randomized trial. In a within-subject design we compared sympathetic and cardiovascular reactivity during a placebo condition with reactivity during a condition in which the early morning cortisol peak was blocked by dexamethasone (DEX). The synthetic glucocorticoid DEX is a potent and rather selective glucocorticoid receptor (GR) ligand in vivo (Reul *et al.*, 2000). Administration of DEX in the evening causes the pituitary to stop secretion of ACTH, with a corresponding sharp decrease in cortisol secretion at the time of the normal diurnal increase in plasma cortisol in the early hours of the morning (Sherwood *et al.*, 1990).

We hypothesize that under placebo conditions, a larger cortisol level in the morning predicts increased sympathetic and cardiovascular reactivity. Under DEX

suppression conditions, we expect decreases in sympathetic and cardiovascular reactivity compared to the placebo condition.

Methods

Subjects

Thirty-nine university students (23 males) between 18 and 28 years (mean = 22.0 years, SD = 2.4) were recruited, who had no overt somatic or psychiatric disease, did not take cardio-active or psychotropic medication and were not severely obese (body mass index < 30). Two subjects (one male, one female) were excluded from the final analyses due to medication use for asthma or failure to attend the second of the two test days in the study. The study was approved by the ethics review committee of the VU medical department, and all subjects provided written informed consent. After the second test day all participant received 40 euro.

Protocol

Each subject participated in laboratory testing on two separate test days. In a double-blind placebo controlled design, the subjects randomly received either a placebo or .5 mg DEX on the evening before a laboratory test day. In healthy subjects .5 mg DEX suppresses plasma cortisol almost completely (Barton *et al.*, 2002). The color, shape, and weight of the placebo and the DEX tablets were identical. The subjects were instructed to take DEX (or the placebo) at 2200h. The experimenter phoned the subject between 2200-2230h to verify the exact time at which they took the tablet. The order of placebo and DEX test days was randomized. The time between the first and the second test day was approximately 4 weeks. To achieve double-blindness, placebo and DEX tablets were put into non-transparent boxes by an independent agent. They were randomly coded “A” or “B” by that independent agent, and this code was revealed to the researchers only after full completion of the experiment. All women not taking oral contraceptives (n = 2) were tested in the follicular phase (days 1-11) of their menstrual cycle according to self-report. Women taking oral contraceptives were not restricted to a specific phase of the menstrual cycle.

Procedure

The subjects willing to participate received an information folder on the study. Two weeks later, they were phoned to make an appointment for the

testing day in the laboratory. The subjects were asked to refrain from smoking and alcohol- or caffeine-containing beverages the evening before the test day and in the morning before coming to the laboratory. Testing always took place exactly 3 hours after spontaneous awakening. The experimental sessions were conducted in a dimly lighted, sound-attenuated cabin, with the subjects facing a video screen at 90 cm. Subject were attached to the electrocardiogram (ECG), impedance cardiogram (ICG), phonocardiogram, respiration, and electrodermal activity recording devices of the BioPac data-acquisition system (BioPac systems Inc, Santa Barbara, CA) using the appropriate electrodes. The blood pressure was monitored with the Dinamap Pro 100 (Dinamap, Critikon Inc, Tampa, Fla).

The various experimental conditions were explained to the subject and the mental and physical stress tasks were briefly practiced. The actual experiment started by asking the subjects to sit quietly and relax for a pre-stress 10 min resting baseline (rest1). Next, the following conditions were presented, always in the same fixed order: Stroop color word task (4 min), rest2 (3 min), tone avoidance task (4 min), rest3 (3 min), lying (2 min), standing (2 min), rest4 (2 min), hand grip test (2 min), cold pressure test (1 min), rest5 (3 min), and the Harvard step test (2 min). After the step test, a final post-stress resting condition of 13 min concluded the physiological recordings. Because PEP may not be a reliable index of sympathetic control during postural change (Houtveen *et al.*, 2005), the orthostatic manipulations were discarded from the analyses.

Mental and physical stress tasks

Stroop color word. Subjects were presented with one slide per second on a computer screen which had the name of a color printed in a contrasting colored ink. Participants were requested to verbally identify the color of the ink, not the name of the color as fast as possible.

Tone avoidance task. An 'x' was shown briefly (500 ms) in one of the corners on the screen and the subjects were asked to respond as fast as they could by pressing the button opposite to this corner on a four-button response panel (e.g. 'x' shown in the top left-hand corner, press the bottom right-hand button). Incorrect or too slow responses were punished with a loud noise burst (1000 Hz, 85 dB) that lasted 500 ms. Reaction time had to be shorter than a maximal response period, that was initially set to 550 ms, and was thereafter continuously adapted to the performance of the subject. (Willemsen *et al.*, 1996).

Hand grip test. During the practice part of the experiment, maximum grip strength in the dominant hand was established with a hand grip dynamometer. During the actual hand grip test, subjects squeezed at 30% of their maximum voluntary contraction for a period of 2 min.
Cold pressor test. The subjects were asked to submerge their dominant hand up to the wrist joint in a bucket of ice water of 3–5 ˚C and to hold the fingers in a relaxed position. After exactly 60 s, the hand was removed from the bucket.
Harvard step test. Subjects were asked to stand comfortably upright before a standard gym bench of exactly 45 cm height. They were asked to step up the bench every 2 s for 2 min (60 steps). Timed verbal commands ensured that the appropriate step frequency was maintained.

Cortisol sampling

To test the differential effects of placebo and DEX on the cortisol levels during the test day we used the Salivette (Sarstedt, Rommelsdorf, Germany) method. Briefly, subjects were instructed to chew gently on the polyester swab for 60 s to obtain the desired amount of saliva. They were asked to refrain from brushing their teeth and consuming food and drinks from 30 min prior to saliva collection. Sampling of cortisol took place at eight different times. The first four collections were done at home at the time of awakening and 30, 45 and 60 min after awakening, the next two cortisol collections were done during laboratory stress testing at 3 and 5 hours after awakening. Two final samples were taken at home at 2000h and 2230 h.

All home-based samples were kept in a dark cool place by the subjects. Upon arrival in the laboratory the samples were stored frozen at a temperature of –25 °C. Cortisol concentrations were determined in Dresden, Germany, using a commercially available chemiluminescence assay (IBL, Hamburg, Germany). The formula outlined by Pruessner *et al.* (2003) was used to calculate the area under the curve with respect to ground AUC_g for the four morning samples (awake, + 30 min, + 45 min, + 60 min). In addition, we computed the cortisol awakening response (CAR) by subtracting the cortisol level at awakening from the cortisol level 30 min after awakening.

Physiological assessments

The ECG was recorded using three pregelled Ag/AgCl spot electrodes (UltraTrace, ConMed, USA) in a configuration shown in Figure 1. The electrodes were connected to the ECG100C BioPac module using a MEC110C extension lead. Heart period (HP) was quantified as the distance between two R-spikes in the ECG waveform and averaged across all beats in the condition. Mean heart rate (HR) was computed from this as 60000/HP. Cardiac sympathetic reactivity was non-invasively obtained by thoracic impedance cardiography (Cacioppo *et al.*, 1994a). The ICG signals were recorded from four additional pregelled Ag/AgCl spot electrodes, attached to the skin in a configuration as shown in Figure 1. These electrodes were connected (also via an extension) to the NICO100C BioPac module. From the impedance cardiogram (ICG) the Pre-Ejection Period (PEP) was scored as the interval from the R-wave peak, minus a fixed interval of 48 ms (Lozano *et al.*, 2007; Sherwood *et al.*, 1990; Willemsen *et al.*, 1996) to the B-point, which signals opening of the aortic valves. The PEP is used as our first measure of sympathetic tone. Shortening of the PEP reliably indexes increased ß-adrenergic inotropic drive to the left ventricle as shown in laboratory studies manipulating ß-adrenergic tone by epinephrine infusion (Mezzacappa *et al.*, 1999; Schachinger *et al.*, 2001; Svedenhag *et al.*, 1986), adrenoceptor blockade (Harris *et al.*, 1967; Schachinger *et al.*, 2001), exercise (Krzeminski *et al.*, 2000; Miyamoto *et al.*, 1983; Smith *et al.*, 1989a), or emotional stress (Berntson *et al.*, 1994; Newlin & Levenson, 1979; Sherwood *et al.*, 1986).

Continuous skin conductance level (SCL) was measured in microsiemens (µS) from electrodes placed at the distal phalanx of the index and middle finger of the non-dominant hand again using the BioPac system. The skin conductance is measured with the .5 V constant voltage method. The fluctuating current conducted through the skin of the subject represents the conductance signal. The SCL was defined as the mean level of skin conductance and the frequency of spontaneous skin conductance responses (ns.SCRs) as the number of phasic increases in conductance of at least .05 µmho per minute. SCL reflects the activity of the sweat glands, which are innervated by the sympathetic nervous system (SNS). Increases in SNS activity yield increases in SCL as well as increases in the frequency of spontaneous skin conductance responses (Boucsein, 1992; Venables & Christie, 1980). These measures (SCL, ns.SCRs) are therefore used as a second measure of sympathetic tone.

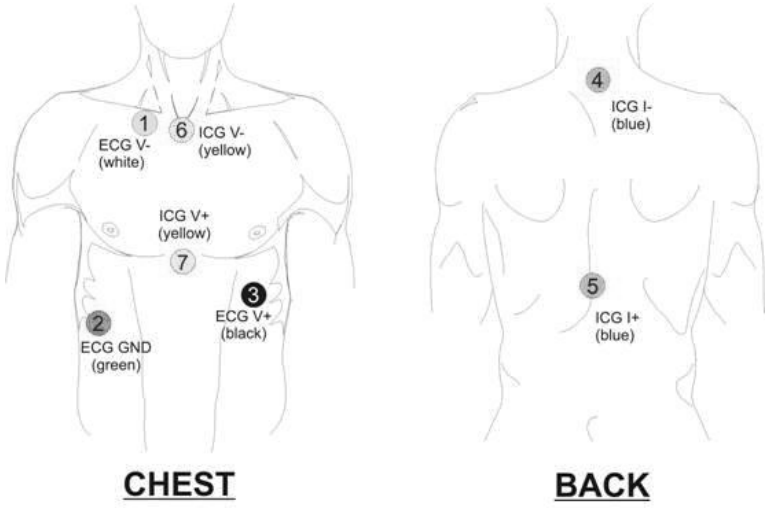


Figure 1 *Location of the seven ECG and ICG electrodes.*

As a measure of parasympathetic tone, respiratory sinus arrhythmia (RSA) was obtained using the peak-to-trough method on the combination of the ECG and a co-registered respiration signal (Grossman *et al.*, 1990). Because the RSA distribution was skewed, the natural logarithm was used in all further analyses. Systolic (SBP) and diastolic (DBP) blood pressure level was measured three times during the final rest, twice during Stroop task, tone avoidance task, the first rest and during all resting periods, and once during all other conditions. The appropriately sized arm cuff of the Dinamap Pro 100 (Dinamap, Critikon Inc, Tampa, Fla) was fastened at the non-dominant arm.

Psychological assessments

Subjects were tested on both test days on depression, state-anxiety, trait-anxiety and neuroticism with the Beck depression inventory (BDI; Beck *et al.*, 1961), the Spielberger anxiety inventory (Spielberger C.D. *et al.*, 1970) and the NEO-personality inventory (Costa & McCrae, 1992). Subjects completed the questionnaires after the final post-stress resting condition.

Statistical analyses

Mean PEP, SCL, ns.SCRs, SBP, DBP, HR and RSA were computed for each experimental condition. In addition, reactivity scores were computed by subtracting the resting period preceding the task from the task level, resulting in 5 reactivity scores per measure (Stroop, tone avoidance, hand grip, cold pressor,

step test). To test for an effect of the early morning cortisol rise on subsequent sympathetic and cardiovascular stress reactivity, a correlational and experimental approach were used. In the correlational approach, the AUC_g and CAR on the placebo day were correlated with all reactivity measures during the various stressors. To experimentally test the effects of cortisol on cardiovascular stress reactivity, a repeated measures ANOVA was performed on the levels of PEP, SCL, nsSCRs, SBP, DBP, HR, and RSA with test day (placebo/DEX) and condition (rest1, Stroop, rest2, tone avoidance, rest4, hand grip, cold pressor, rest5, step test) as within-subject factors. Because no valid PEP data were obtained in 7 subjects during the step test, the repeated measures ANOVA on the levels of PEP was performed separately for the Stroop, tone avoidance, hand grip, cold pressor (and their preceding resting conditions) and for the step test.

Because cortisol effects on reactivity may be confounded by changes in basal sympathetic tone, we started by examining the effects of early morning cortisol rise on resting sympathetic tone. In the correlational approach, the AUCg and CAR on the placebo test day were correlated with the pre- and post-stress resting levels of PEP, SCL and ns.SCR. In the experimental approach, a repeated measures ANOVA was performed on the pre- and post-stress resting levels of PEP, SCL, and ns.SCR with test day (placebo/DEX) and resting condition (pre-stress, post-stress) as within-subject factors.

In all ANOVA's the Greenhouse-Geisser epsilon (ε) was reported when the sphericity assumption was violated (i.e., if the Mauchly test of sphericity was statistically significant at $p < .05$) and partial η^2 (η_p^2) was reported as a measure of effect size. Because our main interest was in the effect of the placebo/DEX manipulation, we limited post-hoc testing to (interaction) effects involving test day. Also, because only a few small sex differences were found, all testing was aggregated across both sexes.

Results

The final sample consisted of 22 males and 15 female subjects. Sample characteristics are shown in Table 1. The sample included one male and one female smoker (number of cigarettes per day $M = 2.7$). Mean state-anxiety and depression levels did not differ between the two test days. Length, weight, and personality were also stable across testing days.

Table 1 *Sample characteristics.*

	Placebo			DEX			
	Mean	*SD*	*Range*	*Mean*	*SD*	*Range*	*p**
Age (yr)	22.1	2.4	19-29	22.1	2.4	19-29	1.0
Length (cm)	178.6	9.4	157-198	178.6	9.4	157-198	.32
Weight (kg)	68.4	10.8	50-91	68.1	10.8	50-90	.06
BMI	21.3	2.1	18-26	21.2	2.1	18-26	.07
BDI depression	.3	.7	0-3	.2	.9	0-5	.57
State anxiety	29.7	5.7	21-46	30.7	6.4	20-53	.34
Trait anxiety	32.4	6.6	22-48	32.1	6.7	22-48	.76
NEO neuroticism	27.1	6.8	14-42	28.5	7.8	15-42	.08

** p-values represent paired t-tests comparing placebo to DEX.*

Diurnal Cortisol

Table 2 shows the mean time of the cortisol sampling on the placebo and the DEX test days. Subjects indicated to have complied well with the instructions and neither awakening nor sampling times were different on the two test days. Figure 2 shows average cortisol levels at the different sampling times during the two test days. To test the effect of the pharmacological manipulation on the cortisol curve, a repeated measures ANOVA was performed on cortisol level with two within-subject factors, test day (placebo/DEX) and sample time (8 samples). This showed a significant interaction effect of test day by sample time ($F(7, 25) = 58.99$, $\varepsilon = 0.46$, *corrected* $p = 0.00$, $\eta_p^2 = .68$) due to a DEX-induced absence of the clear circadian rhythm observed after the administration of the DEX (see Figure 2). Inspection of the individual curves showed that all subjects were suppressors and none showed DEX escape.

Table 2 *Mean and range of time of salivary cortisol sampling on the two test days.*

Nr	*Sample*	*Sampling time*						*Time diff#*
		Placebo			**DEX**			
		N	*Mean*	*Range*	*N*	*Mean*	*Range*	*Mean*
1	At awakening	35	0756h	0621-0906h	36	0757h	0629-0905h	00min26s
2	Awake + 0030h	35	0827h	0651-0936h	37	0828h	0659-0932h	00min01s
3	Awake + 0045h	35	0841h	0706 -0951h	36	0844h	0715-0945h	02min28s
4	Awake + 0060h	36	0855h	0721-1005h	35	0901h	0729-1003h	03min08s
5	Awake + 0300h	36	1100h	0927-1210h	36	1104h	0930-1213h	03min51s
6	Awake + 0500h	36	1238h	1100-1400h	36	1239h	1105-1405h	00min56s
7	2000h	35	2004h	1930-2030h	32	2004h	1940-2115h	01min40s
8	2230h	35	2233h	2225-2330h	35	2234h	2209-2330h	01min00s

 # Mean within-subject difference in sampling time between the placebo and DEX test day.

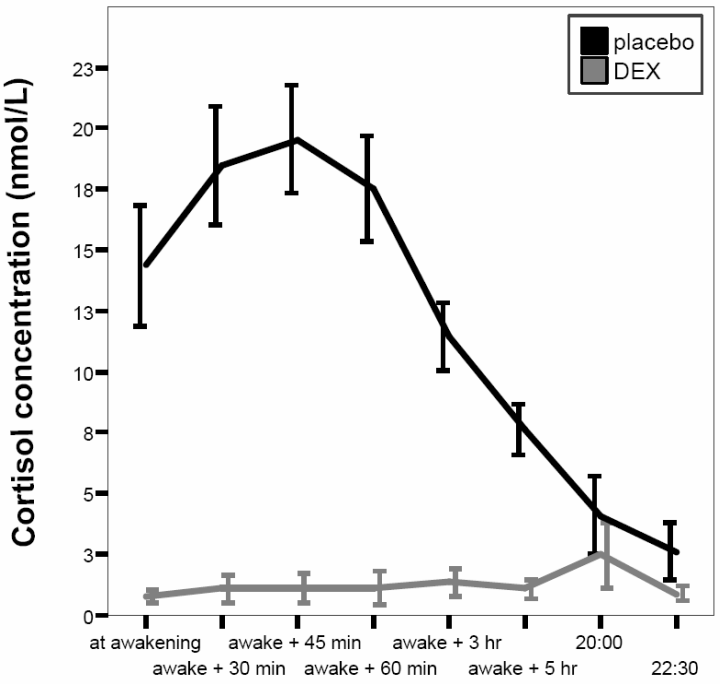


Figure 2 *Cortisol diurnal profiles at the placebo (black) and DEX (grey) test days.*

Effects of morning cortisol levels

Resting sympathetic tone

Table 3 displays the correlations between the two cortisol measures during the morning (placebo condition only) and the resting levels of PEP, SCL and ns.SCR. Out of a total of 12 correlations, no significant findings emerged at $p < .05$. A total of 8 subjects had a slightly negative CAR. Since this may indicate that they had not taken the samples at the appropriate times, we repeated the correlations after excluding these subjects (last column, Table 3). Again, no significant correlations were found.

Table 3 *Correlations between AUC_g and CAR with the sympathetic variables for the pre- and post-stress rest measures.*

Variable	**Condition**	**AUC_g** (n=36)	**CAR** (n=36)	**CAR*** (n=28)
PEP	Pre-stress rest	*.15*	*.22*	**.11**
	Post-stress rest	*.21*	*.25*	**.19**
SCL	Pre-stress rest	*-.07*	*-.17*	**.09**
	Post-stress rest	*.10*	*-.19*	**.24**
ns.SCRs	Pre-stress rest	*.09*	*-.21*	**.07**
	Post-stress rest	*.08*	*-.18*	**.14**

** 8 subjects with negative values were excluded.*

Correlations that are significant at p < .05 are in bold.

Reactivity

Table 4 shows the correlations between the two cortisol measures during the morning (placebo condition only) and the reactivity scores of PEP, SCL, ns.SCRs, SBP, DBP, HR, and RSA. Out of a total of 35 correlations, no significance was found for AUCg at $p < .05$. Out of a total of 35 correlations with CAR, 1 was significant, but not in the expected direction. Results after exclusion of the 8 subjects with a negative CAR yielded 1 significant correlation in the expected direction and 3 in the opposite direction.

Table 4 *Correlations between AUC_g and CAR with the sympathetic and cardiovascular variables for the reactivity measures.*

Variable	Condition	N	AUC_g	CAR	CAR*
PEP reactivity	Stroop	36/28	*.24*	*-.15*	**.33**
	Tone avoidance	36/28	*.19*	*.03*	**.27**
	Hand grip	36/28	*-.20*	*-.18*	**-.20**
	Cold pressor	36/28	*-.18*	*.13*	**-.08**
	Step test	32/24	*.00*	*-.09*	**-.01**
SCL reactivity	Stroop	36/28	*-.30*	*.00*	**-.37**
	Tone avoidance	36/28	*.01*	***-.38***	-.41
	Hand grip	36/28	*.17*	*.23*	**-.10**
	Cold pressor	36/28	*.06*	*.13*	**-.33**
	Step test	36/28	*.24*	*-.06*	**-.19**
ns.SCRs reactivity	Stroop	36/28	*-.24*	*-.01*	**-.31**
	Tone avoidance	36/28	*-.17*	*.24*	**-.37**
	Hand grip	35/27	*.08*	*.28*	**.14**
	Cold pressor	36/28	*-.28*	*.09*	-.41
	Step test	35/27	*.04*	*.32*	.53
SBP reactivity	Stroop	36/28	*-.18*	*-.11*	**-.34**
	Tone avoidance	36/28	*-.18*	*.22*	**.07**
	Hand grip	35/27	*.15*	*.21*	**.22**
	Cold pressor	36/28	*-.15*	*-.30*	**-.37**
	Step test	36/28	*-.08*	*-.07*	**.05**
DBP reactivity	Stroop	36/28	*-.12*	*.11*	**-.19**
	Tone avoidance	36/28	*-.16*	*.15*	**-.05**
	Hand grip	35/27	*.16*	*.22*	**.23**
	Cold pressor	36/28	*-.20*	*-.20*	**-.35**
	Step test	36/28	*-.04*	*-.14*	**-.08**
HR reactivity	Stroop	36/28	*-.15*	*.26*	-.38
	Tone avoidance	36/28	*.13*	*.10*	**.01**
	Hand grip	36/28	*.32*	*.25*	**.32**
	Cold pressor	36/28	*.21*	*-.15*	**-.14**
	Step test	36/28	*.13*	*.25*	**.24**
RSA reactivity	Stroop	36/28	*.00*	*-.04*	**-.02**
	Tone avoidance	36/28	*-.01*	*.01*	**-.29**
	Hand grip	35/27	*-.31*	*-.15*	**-.20**
	Cold pressor	36/28	*.09*	*.19*	**.23**
	Step test	34/26	*-.17*	***-.37***	**-.25**

** Excluding the 8 subjects with negative values.*

Correlations that are significant at p < .05 are in bold.

Effects of DEX suppression

Resting sympathetic tone

Table 5 gives the pre- and post-stress resting values of the sympathetic variables separately for the placebo and the DEX test day. Repeated measures ANOVA showed a significant main effect of test day for PEP ($F(1, 36) = 4.81$, $p = .04$, $\eta_p^2 = .12$), and a significant interaction effect of test day and condition for SCL ($F(1, 36) = 3.23$, $p = .08$, $\eta_p^2 = .08$), and for ns.SCR ($F(1, 36) = 9.00$, $p = .01$, $\eta_p^2 = .20$). Post-hoc testing showed that after administration of DEX the overall PEP in both resting conditions was significantly longer compared to the placebo test day. In the pre- and post-stress resting condition, SCL was significantly lower, and in the post-stress resting condition the spontaneous ns.SCRs were less frequent. All these differences across test days are compatible with a lowered sympathetic tone after the administration of DEX. Importantly, we did not find a significant relationship between resting levels of PEP, SCL, and ns.SCRs and any of the cardiovascular reactivity measures on either placebo or DEX test days, suggesting that effects of DEX on resting sympathetic tone were unlikely to confound DEX effects on cardiovascular reactivity.

Table 5 *Means (SDs) for the pre- and post-stress resting levels separately for the two test days.*

Measures	***Resting level***	***N***	**Cortisol (nmol/L)**	
			Placebo	**DEX**
PEP	pre-stress	37	119.46 (12.51)	123.35 (10.85)
	post-stress	37	117.41 (10.96)	119.89 (11.57)
SCL	pre-stress	37	11.18 (3.86)	10.96 (3.99)
	post-stress	37	12.62 (3.62)	11.41 (3.53)
ns.SCRs	pre-stress	37	1.62 (1.40)	1.87 (1.52)
	post-stress	37	2.26 (1.81)	1.70 (1.45)

Reactivity

The sympathetic and cardiovascular reactivity to the tasks is displayed in Figures 3 (mental stressors) and 4 (step test). The repeated measures ANOVAs on the sympathetic and cardiovascular levels revealed a significant main effect of condition on PEP ($F(8, 22) = 165.16$, $\varepsilon = .30$, *corrected* $p = .00$, $\eta_p^2 = .95$), SCL ($F(8, 29) = 19.26$, $\varepsilon = .43$, *corrected* $p = .00$, $\eta_p^2 = .57$), ns.SCR ($F(8, 27) = 64.45$, $\varepsilon = .28$, *corrected* $p = .00$, $\eta_p^2 = .72$), SBP ($F(8, 28) = 81.94$, $\varepsilon = .40$, *corrected* $p = .00$, $\eta_p^2 = .67$), DBP ($F(8, 28) = 50.57$, $\varepsilon = .51$, *corrected* $p = .00$, $\eta_p^2 = .63$), HR ($F(8, 29) = 216.16$, $\varepsilon = .38$, *corrected* $p = .00$, $\eta_p^2 = .96$), and RSA ($F(8, 24) = 46.26$, $\varepsilon = .48$, *corrected* $p = .00$, $\eta_p^2 = .82$). Post-hoc planned comparisons of task level with the preceding pre-task resting level showed a significant decrease in PEP during the Stroop task and the step test and a significant increase in PEP during the handgrip and the cold pressor tasks. A significant increase in SCL was found for the Stroop task, tone avoidance, handgrip, cold pressor, and the step test. A significant increase in ns.SCRs was found during the Stroop, tone avoidance, handgrip, and the step test, but the cold pressor test did not alter ns.SCRs. SBP and DBP levels significantly increased during all tasks. For HR a significant increase was found for the Stroop task, tone avoidance, handgrip and the step test. RSA significantly decreased in response to the Stroop task and the step test.

The crucial tests for an interactive effect of test day and condition revealed no significant effects on reactivity for PEP (without step test: $F(6,31) = .38$, $\eta_p^2 = .01$; step test: $F(1,29) = 2.87$, $\eta_p^2 = .09$), SCL ($F(8, 29) = 1.10$, $\eta_p^2 = .06$), ns.SCR ($F(8,27) = 1.36$, $\eta_p^2 = .02$), SBP ($F(8, 28) = .48$, $\eta_p^2 = .01$), DBP ($F(8,28) = 1.34$, $\eta_p^2 = .05$), HR ($F(8, 30) = .39$, $\eta_p^2 = .02$) or RSA ($F(8, 25) = .67$, $\eta_p^2 = .03$). Post-hoc testing on the reactivity scores showed that test day did not affect the responses to any of the stressors. Suppression of the morning rise in cortisol by administration of DEX, therefore, did not influence sympathetic and cardiovascular reactivity.

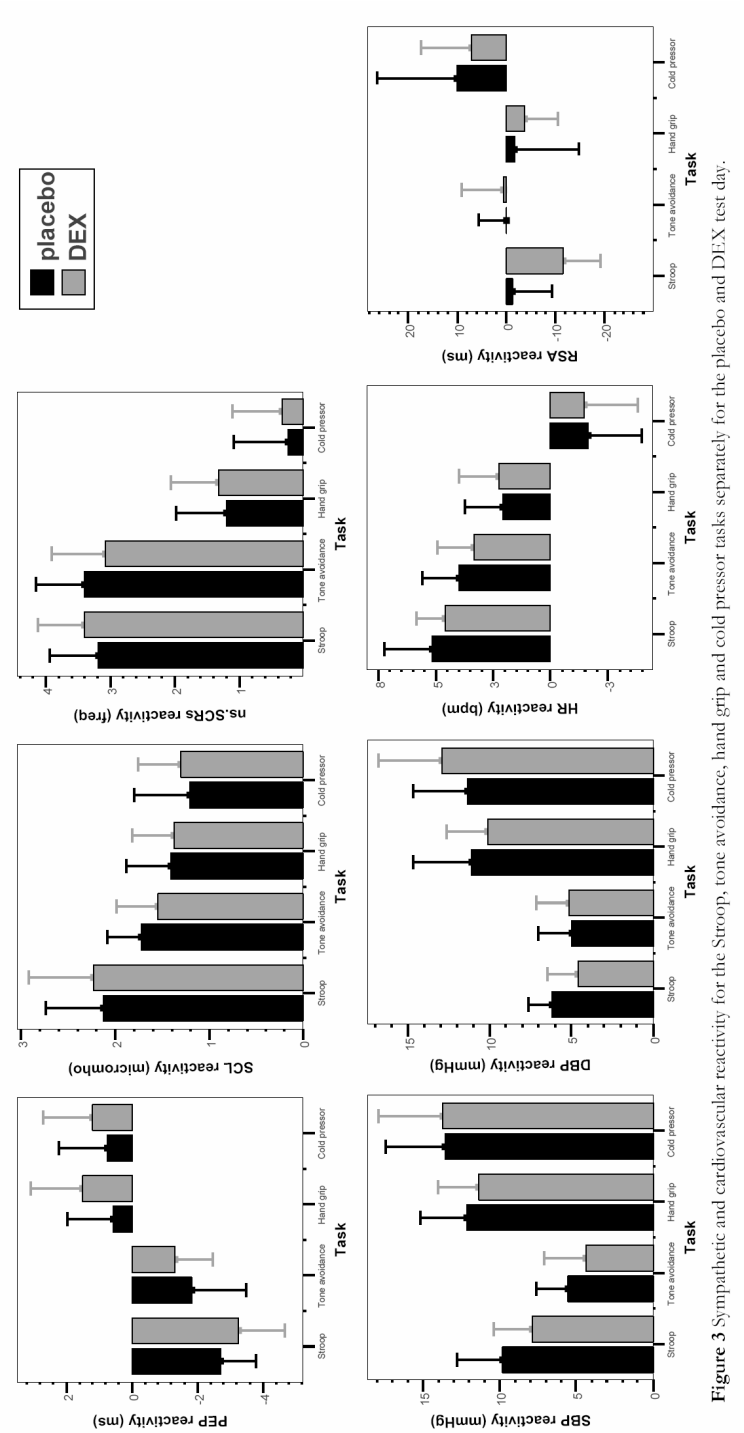


Figure 3 Sympathetic and cardiovascular reactivity for the Stroop, tone avoidance, hand grip and cold pressor tasks separately for the placebo and DEX test day.

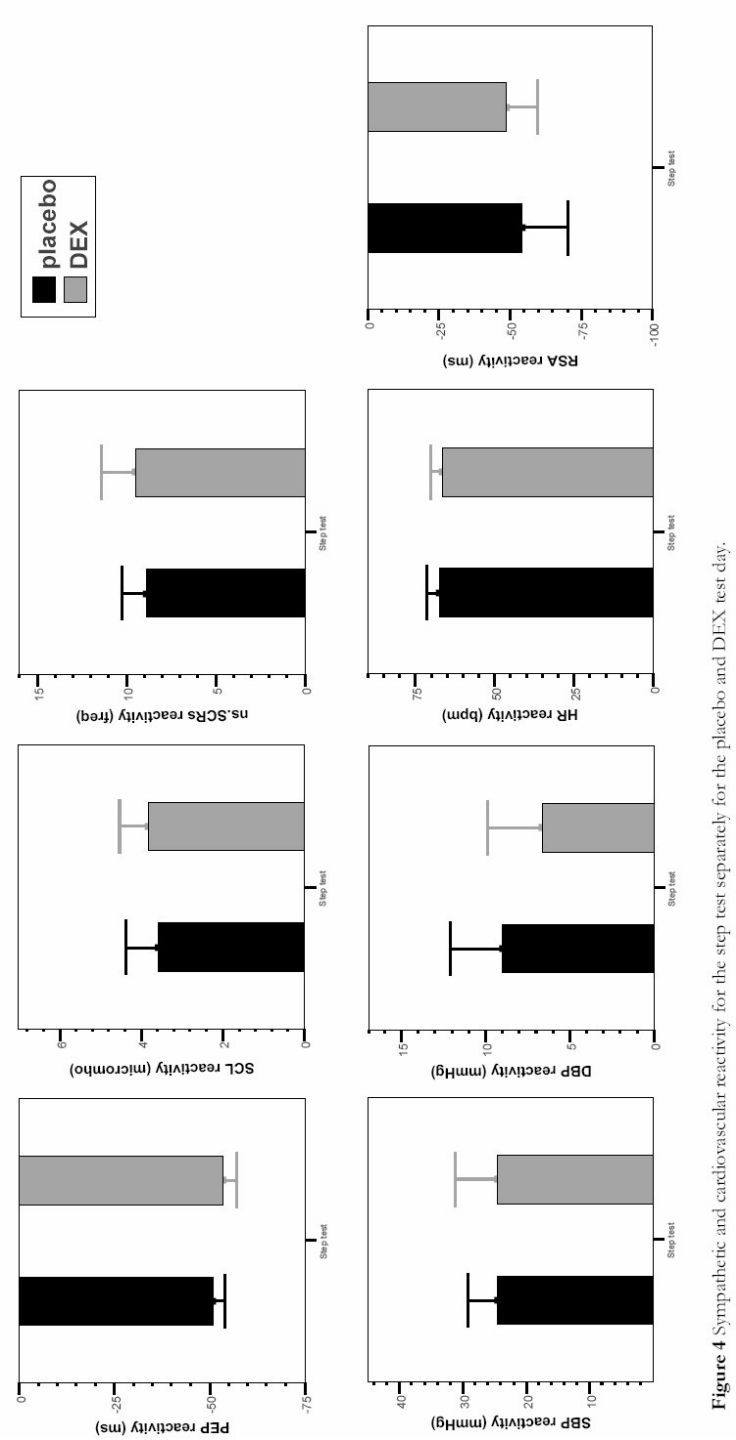


Figure 4 Sympathetic and cardiovascular reactivity for the step test separately for the placebo and DEX test day.

Discussion

In this study, we aimed to provide evidence for a permissive effect of the early morning rise in cortisol on sympathetic and cardiovascular reactivity to stress. We hypothesized that a larger cortisol awakening response would predict larger sympathetic and cardiovascular reactivity to stress. Under conditions of DEX suppression of the early morning cortisol rise, we expected less sympathetic and cardiovascular reactivity compared to the placebo condition. Our results showed no relation between individual differences in cortisol awakening response and sympathetic or cardiovascular reactivity. Complete blockade of the normal morning rise in cortisol by administration of DEX had no effect on sympathetic and cardiovascular reactivity to any of the mental and physical stressors. Thus, no evidence for permissive effects of the early morning cortisol rise on daytime sympathetic and cardiovascular responses to stress were found. Below, we discuss possible reasons for the absence of the hypothesized permissive effects in the study design used.

In our design, we acted on the firm belief that the permissive effects of basal cortisol on sympathetic and cardiovascular reactivity to stress were partly due to *central* actions of cortisol, in particular the structures involved in the modulation of sympathetic drive, i.e. brain regions such as the paraventricular nucleus (PVN) of the hypothalamus, the central nucleus of the amygdala (CeA) (Davis, 1992), the bed nucleus of the stria terminalis (BNST) (Nijsen *et al.*, 2001), the locus coeruleus (LC), and the nucleus tractus solitarius (NTR) (Herman & Cullinan, 1997; Sapolsky, 2003; Van de Kar & Blair, 1999). Glucocorticoid receptors are present in each of these structures in rodents and in many structures in human (de Kloet *et al.*, 1990). Studies which showed memory consolidation by glucocorticoid influences to depend on noradrenergic activation of the basolateral complex of the amygdala make a compelling case for glucocorticoid-sympathetic interaction in these structures (Roozendaal, 2002). In line with this glucocorticoid-sympathetic interaction, we expected basal cortisol levels to modulate individual differences in sympathetic stress-reactivity at the level of the *central* sympathetic drive generated in the amygdale and associated limbic structures. Such effects would have been entirely blocked by DEX, which nearly completely depletes the brain of glucocorticoid action (de Kloet *et al.*, 1975; Meijer *et al.*, 1998), thereby eliminating the hypothesized permissive effects of cortisol on *central* sympathetic neurotransmission.

It is important to note that the central action of cortisol is not taken over by the intrinsic GC action of DEX itself, at least not in the concentrations attained in this study. The efflux transporter P-glycoprotein expressed at the endothelial cells of the blood-brain barrier (BBB) (Cordon-Cardo *et al.*, 1989) hampers the penetration of DEX into the brain (Meijer *et al.*, 1998; Schinkel *et al.*, 1995). All studies reporting effects of DEX on glucocorticoid targets in the brain used high systemic doses or brain implants of DEX, overcoming its P-glycoprotein mediated exclusion from the brain (Feldman & Weidenfeld, 2002; Imaki *et al.*, 1995; Kovacs & Mezey, 1987; Roozendaal & McGaugh, 1996; Sawchenko, 1987). Dexamethasone in the concentrations used here has been found to produce selective activation of GR in the pituitary, whereas mineralocorticoid receptor (MR) in the pituitary or MR and GR in the central nervous system were unaffected (Cole *et al.*, 2000; de Kloet *et al.*, 1974; de Kloet *et al.*, 1975; Miller *et al.*, 1992).

However, this still leaves the possibility that the permissive effects of cortisol on *peripheral* sympathetic neurotransmission may have been taken over by circulating DEX. The peripheral permissive actions of GCs are brought about mainly by enhancing sinoatrial, cardiomyocyte and vascular responsiveness to adrenaline and noradrenaline (Fritz & Levine, 1951; Grunfeld & Eloy, 1987; Ramey *et al.*, 1951; Schomig *et al.*, 1976; Tanz, 1960). This may arise in a number of ways. GC's increase levels of phenylalanine-N-methyltransferase (PNMT), the rate limiting step in adrenaline synthesis (Betito *et al.*, 1992; Betito *et al.*, 1994; Kennedy & Ziegler, 1991; Munck & Naray-Fejes-Toth, 1994; Wurtman & Axelrod, 1966). They further decrease catecholamine re-uptake and deactivation by COMT and MAO, prolonging catecholamine action in the synapses (Dailey & Westfall, 1978; Gibson, 1981; Kennedy & Ziegler, 1991; Munck & Naray-Fejes-Toth, 1994). In addition to presynaptic enhancement, GCs enhance cardiac and vascular responsivity by upregulation of the adrenoceptors. Binding capacity and affinity of beta-receptors are increased (Collins *et al.*, 1988; Sakaue & Hoffman, 1991) as is the efficiency of G-protein receptor signaling, giving rise to a larger cAMP production (Haigh *et al.*, 1990; Haigh & Jones, 1990; Jazayeri & Meyer, 1988; Liu *et al.*, 1992). Finally, by inhibiting prostaglandin synthesis at basal levels, GCs block their vasodilatory effects (Cacioppo *et al.*, 1994a; Handa *et al.*, 1984). Such peripheral GC effects may be partly taken over by DEX, as has already been shown in rats. Treatment with small amounts of DEX produced a

hypocorticoid state in the brain and at the same time modestly increased glucocorticoid actions in the periphery (Karssen *et al.*, 2005).

If the effects of cortisol on *peripheral* sympathetic neurotransmission are partly taken over by DEX, our double-blind procedure was insufficiently solid to detect permissive effects. To establish (or exclude) the existence of such peripheral permissive effects a more rigorous exclusion of cortisol effects might be necessary. In the future, it would be interesting to repeat this experiment using metyrapone, an 11- ß hydroxylase inhibitor of cortisol synthesis. Since metyrapone is no GC there is no chance that metyrapone takes over the effect of cortisol. We note, however, that peripheral permissive effects on reactivity should still have created larger stress reactivity in subjects with larger cortisol responses in the placebo condition. We found only very circumstantial evidence for this. Of all 105 correlations tested, only 6 scores showed a significant correlation with various measures of the early morning cortisol rise, of which only 1 was in the predicted direction, i.e. larger sympathetic or cardiovascular reactivity in subjects with high early morning cortisol levels. This does not exceed the number of false positives that is to be expected due to multiple testing. Under naturalistic conditions, therefore, no permissive effects of cortisol were evident on sympathetic tone or sympathetic and cardiovascular reactivity.

Our null finding needs to be carefully balanced by some shortcomings of our sample and procedure. The sample was relatively small and included only subjects with no evidence of psychiatric disorder who all showed a clear morning peak in cortisol. Including patient populations with a larger range of morning cortisol values (de Kloet *et al.*, 2006; Pfennig *et al.*, 2005) would have increased the statistical power of the study. Also, we relied on self-report to determine the awakening time and compliance with salivary sampling instructions. Combining a similar diurnal cortisol sampling protocol with ambulatory heart rate recording previously showed that part of the subjects misreport the true awakening time, and as a consequence perform the salivary assessments at wrong times (Kupper *et al.*, 2005b). Imperfect compliance to a timed salivary sampling protocol was also reported in studies using covert electronic recording of true sampling times (Broderick *et al.*, 2004). In our study, we found a negative cortisol awakening response in 8 subjects, suggesting that they misreported their awakening time or did not comply with the sampling instructions. Leaving out these subjects left the results essentially unchanged. Furthermore, the measure of the area under the curve in relation to the ground should have been much less sensitive to such

errors, and yet this measure failed to correlate to sympathetic and cardiovascular reactivity too.

A potential shortcoming of the procedure is that we used relatively mild stressors. Inspection of the placebo day in figure 2 shows that cortisol showed the normal diurnal decrease from the beginning to the end of the experiment, suggesting that the experimental procedure did not result in large cortisol responses itself. It is possible that more salient and/or more prolonged stressors would have yielded an effect of dexamethasone on the reactivity. The exercise test, however, was a clear exception to the 'mildness' of the stressors with an average heart rate reactivity of 67 beats. It should be noted that even during exercise the most 'sympathetic' variables PEP, SCL, ns.SCR and SBP tended to be higher rather than lower under dexamethasone, which is in the opposite direction from the hypothesized permissive effects.

In summary, early morning cortisol levels were unrelated to late morning sympathetic and cardiovascular reactivity and blocking the normal morning rise in cortisol by administration of DEX had no effects on sympathetic and cardiovascular reactivity to any of the mental and physical stressors. We conclude that the permissive effects of cortisol on daytime sympathetic and cardiovascular responses to stress remain to be established.

Chapter 7

No effects of training state on ambulatory measures of cardiac autonomic control

Goedhart AD, Bakker F, De Vries M, Kreft J, De Geus EJC (revision submitted). No effects of two weeks of detraining on ambulatory measures of cardiac autonomic control. *Journal of Psychophysiology*.

Abstract

We examined the effect of training state on cardiac autonomic control in a naturalistic setting. Twenty-six vigorous exercisers were compared to 26 age- and sex-matched sedentary controls. The regular exercisers were subjected to a 6-week training program after which they were randomized to 2 weeks of continued training or 2 weeks of de-training. Cardiac autonomic control was measured over a 24-hr period by ambulatory recording, using the pre-ejection period (PEP) and respiratory sinus arrhythmia (RSA). Non-exercising controls had a significantly higher ambulatory heart rate (HR) compared to the regular exercisers but comparable 24-hr levels of PEP and RSA. In regular exercisers, 2 weeks of detraining did not significantly change the 24-hr levels of HR, PEP, or RSA. We conclude that the bradycardia in healthy regular exercisers is due to a lower intrinsic heart rate rather than a shift in cardiac autonomic balance from sympathetic to vagal control.

Introduction

Prospective studies have repeatedly suggested that regular vigorous exercise in leisure time (e.g. sports, jogging, aerobics) is associated with a reduced risk for myocardial infarction and sudden death (Powell *et al.*, 1987; Williams, 2001). An exercise-induced bradycardia with a shift to less sympathetic and more parasympathetic control over the heart rhythm is one of the mechanisms put forward to explain this reduced risk in exercisers, and evidence in favor of this mechanism has accrued in animal studies and studies in cardiac patients (Billman, 2002; Goldsmith *et al.*, 2000; Gutin *et al.*, 2005; Mueller, 2007; Rosenwinkel *et al.*, 2001). In healthy human subjects, however, the evidence for an exercise-induced shift in cardiac autonomic control is more controversial. To quantify autonomic control in exercisers and non-exercisers various studies have used sympathetic, parasympathetic or dual blockade. Using this pharmacological approach vagal cardiac control has sometimes been found to be higher in well-trained persons (Shi *et al.*, 1995; Smith *et al.*, 1989b), but other studies failed to find such an effect (Katona *et al.*, 1982; Kingwell *et al.*, 1992). Likewise, sympathetic cardiac control was shown to be lower in exercisers than in non-exercisers in one study (Lin & Horvath, 1972), but others could not replicate this (Katona *et al.*, 1982; Lewis *et al.*, 1980). In addition, regional noradrenaline (NA) spillover or direct microneurographic recordings from nerves innervating the skeletal muscle, muscle sympathetic nerve activity (MSNA), do not systematically suggest decreased sympathetic tone in exercisers (Alvarez *et al.*, 2005; Meredith *et al.*, 1991; Ray & Hume, 1998; Svedenhag *et al.*, 1984). Taken together, studies using invasive measures of cardiac autonomic control have not found strong evidence for a favorable exercise-induced shift in sympathovagal balance. In contrast, lowered intrinsic HR in exercisers has emerged as a very consistent finding (Katona *et al.*, 1982; Kingwell *et al.*, 1992; Smith *et al.*, 1989b) and may be a sufficient cause for their resting bradycardia.

A disadvantage of invasive techniques (blockade, NA spillover, MSNA) is that they are confined to laboratory testing and are not readily amenable to recordings in naturalistic settings. This makes it hard to examine effects of exercise on, for instance, autonomic control at night or during job-related activities with a substantial mental and emotional load. Nonetheless, it is autonomic control during these naturalistic conditions that may have the largest clinical relevance, and understanding of exercise effects on this 'real life' autonomic control may be very valuable. It is further possible that more

consistent effects of exercise on autonomic cardiac control emerge in such settings. As an alternative to invasive techniques, cardiac sympathetic control can be indexed non-invasively by analyzing the pre-ejection period (PEP), a reflection of myocardial contractility and parasympathetic cardiac control can be indexed by time- or frequency domain indices of heart rate variability in the respiratory frequency range, also called respiratory sinus arrhythmia (RSA). PEP reflects ß-adrenergic inotropic drive to the left ventricle as shown in laboratory studies manipulating ß-adrenergic tone by epinephrine infusion (Mezzacappa *et al.*, 1999; Schachinger *et al.*, 2001; Svedenhag *et al.*, 1986), adrenoceptor blockade (Harris *et al.*, 1967; Schachinger *et al.*, 2001; Winzer *et al.*, 1999), exercise (Krzeminski *et al.*, 2000; Miyamoto *et al.*, 1983; Smith *et al.*, 1989b), or emotional stress (Berntson *et al.*, 1994; Newlin & Levenson, 1979; Sherwood *et al.*, 1986). RSA shows virtually no sensitivity to sympathetic nervous system activity but is affected in a dose-response way by muscarinergic blockers in humans (Martinmaki *et al.*, 2006) or vagal cooling in animals (Katona & Jih, 1975). This has led to the use of RSA a proxy for vagal cardiac control (Berntson *et al.*, 1997; Task Force of the European Society of Cardiology the North American Society of Pacing, 1996), with a note of caution regarding potential confounding by individual differences in sensitivity of chemoreceptor and baroreceptor reflexes (Berntson *et al.*, 1997) and by individual differences in respiratory behavior (Grossman, 2004; Ritz & Dahme, 2006). Although less precise than invasive measures, the huge advantage of the PEP and RSA measures is that they can be reliably recorded in an ambulatory setting (Goedhart *et al.*, 2006; Goedhart *et al.*, 2007).

The link between exercise and RSA or PEP has been examined cross-sectionally by comparing high fit versus low fit subjects or regular exercisers versus sedentary subjects. It has also been examined longitudinally by comparing sedentary subjects before and after a training program. Taken together these studies suggest that highly fit regular exercisers have higher RSA than low fit non-exercisers, but that the effects of training are less convincing than cross-sectional findings (Aubert *et al.*, 2003; Billman, 2002; Buchheit *et al.*, 2005; de Geus *et al.*, 1996; de Geus *et al.*, 1990; de Meersman, 1993; Dixon *et al.*, 1992; Goldsmith *et al.*, 1992; Goldsmith *et al.*, 1997; Hatfield *et al.*, 1998; Kenney, 1985; Rossy & Thayer, 1998; Sacknoff *et al.*, 1994; Shin *et al.*, 1997). For PEP, neither cross-sectional nor longitudinal studies support a link between exercise and PEP (Light *et al.*, 1987; Svedenhag *et al.*, 1986; Svedenhag *et al.*, 1991)

Combining RSA/PEP based studies with the invasive studies, the evidence for large shifts in autonomic control due to exercise is less compelling in healthy humans than it is in cardiac patients (Rosenwinkel *et al.*, 2001) or animals (Billman & Kukielka, 2006; Mueller, 2007). It is of note, however, that most studies on the link between RSA and exercise behavior used laboratory resting conditions, whereas ambulatory recording was used only in a few studies (Goldsmith *et al.*, 1992; Goldsmith *et al.*, 1997; Loimaala *et al.*, 2000; Schuit *et al.*, 1999; Stahle *et al.*, 1999). No studies so far have addressed the effects of exercise on ambulatory PEP levels. Based on the idea that more consistent effects of exercise on autonomic cardiac control may emerge in ambulatory settings, we here address the link between exercise and 24-hr recordings of RSA and PEP. We will separate the results of nighttime and daytime recordings because previous studies have suggested that training effects on heart rate variability may be confined to the daytime but absent in the whole recording or nighttime levels (Schuit *et al.*, 1999; Stahle *et al.*, 1999). We will also control for possible individual differences in posture and physical activity during the daytime, because these are known to affect PEP independently of cardiac sympathetic activity (Houtveen *et al.*, 2005).

The current study has a cross-sectional part and an experimental longitudinal part. First, we compared ambulatory recordings of HR, PEP, and RSA in regular vigorous exercisers to age- and sex-matched sedentary subjects who had not engaged in regular exercise during the past year. In contrast to most of the studies to date, for the experimental phase we chose a detraining paradigm. Most training studies recruit subjects who were untrained at the start of the study, and preferably have a persistent sedentary lifestyle in general under the assumption that this will maximize training outcome. This assumption appears to have validity at face value, but ignores the possibility that sedentary subjects form a selective sample of the population who may be characterized by attenuated sensitivity to the autonomic effects of exercise. Failure to find training effects in sedentary subjects, therefore, does not preclude the possibility that such effects have occurred in moderate or vigorous exercisers. To avoid a potential selection of 'autonomic non-responders' we here deliberately chose to detrain the group of regular exercisers rather than to train the sedentary subjects. Before de-training the exercisers were first subjected to a six week standardized training program to synchronize their training state, after

which they were randomized to either 2 weeks of continued training or 2 weeks of detraining.

We hypothesized that exercisers would have lower ambulatory levels of HR, longer PEPs and higher levels of RSA than non-exercisers. Two weeks of detraining were expected to lead to a decrease in ambulatory RSA and PEP, signaling decreased parasympathetic and increased sympathetic cardiac control respectively.

Methods

Subjects

Twenty-eight regularly exercising subjects (16 males, 12 females) with a mean age of 38.0 years (*SD* = 12.2 years) were recruited from different ministries in The Hague and a police office in Amsterdam. Subjects were included only if they had been engaged in aerobic training for at least 30 consecutive min a day, three days a week for the past year. Records of this were available because all subjects had been frequenting the same fitness centre through a company based discount program. Twenty-eight sex- and age-matched non-exercising subjects (16 males, mean age = 37.9, *SD* = 13.5) comprised the sedentary control group. These subjects were selected from a larger study in which they underwent a very comparable 24-hr ambulatory recording session as described elsewhere (Goedhart *et al.*, 2007). Subjects were included only if they had indicated not to engage in regular exercise both at the time of ambulatory recording as well as in surveys collected 1 to 2 years earlier. All subjects were white-collar workers, mainly engaged in deskwork, and had no history of hypertension or cardiovascular disease.

At the start of the study, the regular exercisers were randomly divided into two groups, a continued training group and a detraining group. Both groups consisted of 13 subjects, the continued training group consisted of 8 males and 5 females and the detraining group of 7 males and 6 females. Two subjects, one male in the detraining group and one female in the continued training group, were excluded from the final analyses due to illness or failure to attend all test days. The matched controls were also excluded.

Ambulatory measurement protocols were approved by the Ethics Committee of the Vrije Universiteit; the detraining study was additionally examined by the Ethics Committee of the faculty of Human Movement Sciences. All subjects gave written consent before entering the study.

Protocol

The experimental phase encompassed a total of 8 weeks. First, both continued training and detraining groups underwent 6 weeks of supervised training. They trained on average 3.6 hours (*SD* = .9 hr) per week with a minimum of 3 times per week, for at least one hour at a minimal intensity of 70 % of the maximal HR, measured with a Polar A5 HR monitor. Maximal HR was established during an all-out test on a bicycle ergometer (10 min warm-up at 130 bpm followed by two bouts of 60 s bicycling at an increasing resistance until exhaustion). All exercises were done on exercising apparatus specially adapted for conditioning the cardiovascular system (bicycle ergometer, rowing ergometer, crosstrainer, treadmill). A research assistant was present during all exercise sessions to record compliance. This phase was intended to synchronize training state at the start of the actual detraining manipulation. In the next phase, the continued training group, that here acts as the control group, continued the previous training regime for another 2 weeks, whereas the detraining group had to completely sustain from sports activities or other vigorous activities in leisure time. During the 2 weeks detraining period subject's absence in the fitness centre was actively monitored and compliance was further checked by regular email. Subjects were ambulatory monitored for a 24-hr period at the start (0 weeks) and end (6 weeks) of the run-in training phase and at the end of the detraining period (8 weeks). They were instructed to keep physical activity at a minimal level on the ambulatory monitoring day and all recordings took place at least one day after a training session.

Ambulatory measurements

The Vrije Universiteit Ambulatory Monitoring System (VU-AMS) continuously recorded the electrocardiogram (ECG) and the impedance cardiogram (ICG) using six disposable, pregelled Ag/AgCl electrodes (de Geus *et al.*, 1995; Riese *et al.*, 2003; Willemsen *et al.*, 1996). Subjects were instructed to wear the device the entire day and night until awakening the next morning. The VU-AMS produced an audible alarm approximately every 60 min (± 10 min randomized) to prompt the subject to fill out an activity diary. They were instructed to write down their physical activity and bodily postures during the last 60 min in chronological order. Diary prompting was disabled during sleep, but regular beat-to-beat recording of the ECG/ICG was maintained throughout the night. The following day the subjects were visited again to collect the equipment. For the exercisers

enrolled in the detraining study this was repeated three times. The non-exercisers were ambulatory monitored for a single 24-hr period only.

Ambulatory signal scoring

The three target variables were HR, PEP and RSA. Scoring of these variables is described in detail elsewhere (Goedhart *et al.*, 2006; Goedhart *et al.*, 2007). Briefly, from the ECG (sampling rate 1000 Hz) the HR was obtained from the time between two adjacent R waves. PEP was defined from the ECG and ICG as the time interval from the Q-wave onset, the onset of the electromechanical systole, to the B-point (from the ICG), which signals opening of the aortic valves (Sherwood *et al.*, 1990; Willemsen *et al.*, 1996). RSA was obtained from the ECG and respiration signals by subtracting the shortest IBI during HR acceleration in the inspirational phase from the longest IBI during deceleration in the expirational phase. When no phase-related acceleration or deceleration was found, the breath was assigned a RSA score of zero. Automatic scoring of PEP and RSA was checked by visual inspection of the impedance and respiratory signal from the entire recording.

Using the activity diary entries in combination with a visual display of the output of an inbuilt vertical accelerometer, the entire 24-hr recording was divided into fixed periods. These periods were coded for posture (supine, sitting, standing, walking, bicycling), ongoing activity (e.g. desk work, dinner, meetings, watching TV), and physical activity (no, light, medium and heavy). Minimum duration of periods was always 5 min and maximum duration was always 1 hour. If periods with similar activity and posture lasted more than 1 hour (e.g., during sleep), they were divided into multiple periods of maximally 1 hour. All periods were classified into three main ambulatory conditions: 1) lying asleep, 2) sitting during the day, or 3) mild physical activity (standing/walking) based on the dominant posture/activity reported in that period; the exact timing of changes in posture/activity was verified using the accelerometer signal from the ambulatory device. Average HR, PEP and RSA was determined for each of these conditions.

Statistical analysis

For the cross-sectional analyses, mean HR, PEP and RSA of the non-exercising subjects was compared to the mean values on the first measurement in the regular exercisers using a repeated measures ANOVA with group (non-exercisers, exercisers) as a between-subject factor and ambulatory condition

(sleep, sitting, mild physical activity) as a within-subject factor. For the longitudinal analyses, a single omnibus repeated measures ANOVA with group (continued training, detraining) as a between-subject factor and time (0 weeks, 6 weeks, 8 weeks) and ambulatory condition (sleep, sitting, mild physical activity) as within-subject factors was used. To establish that the groups had comparable levels of HR, PEP and RSA after the run-in 6 weeks standardized training program, we tested the group by condition effect at the start of the detraining phase (after 6 weeks). To test for an effect of detraining we examined the interactions of group (continued training, detraining) by time (6 weeks, 8 weeks) and group (continued training, detraining) by time (6 weeks, 8 weeks) by ambulatory condition (sleep, sitting, mild physical activity). The Greenhouse-Geisser epsilon (ε) was reported when the sphericity assumption was violated (i.e., if the Mauchly test of sphericity was statistically significant at $p < 0.05$) and partial η^2 (η_p^2) was reported as a measure of effect size. Because the RSA distributions were skewed, its natural logarithm was used in all further analyses.

Results

Cross-sectional comparison

Table 1 presents means and standard deviations for HR, PEP and RSA separately for the non-exercisers and exercisers.

Table 1 *HR, PEP, and RSA in the non-exercisers (N=26) and exercisers (N=26) in each of the ambulatory conditions.*

Measure	Group	Sleep	Sitting	Mild physical activity
HR (bpm)	non-exercisers	62.4 (5.7)*	77.4 (7.1)*	88.1 (9.9)*
	exercisers	57.6(10.8)	70.2 (11.5)	81.0 (12.3)
PEP (ms)	non-exercisers	108.5 (16.9)	101.6 (19.4)	98.1 (17.5)
	exercisers	110.6 (15.2)	103.0 (15.1)	98.1 (13.6)
RSA (ms)	non-exercisers	53.5 (34.2)	42.4 (18.5)	33.8 (10.3)
	exercisers	52.1 (40.8)	53.4 (36.2)	45.6 (33.7)

** Significant difference (p < .05) between the groups.*

The repeated measures ANOVA showed significant main effects of posture on HR ($F(2, 49) = 190.37$, $\varepsilon = 0.72$, *corrected* $p = 0.00$, $\eta_p^2 = .86$), PEP ($F(2, 49) = 46.36$, $\varepsilon = 0.58$, *corrected* $p = 0.00$, $\eta_p^2 = .40$) and RSA ($F(2, 49) = 22.11$, $\varepsilon = 0.64$, *corrected* $p = 0.00$, $\eta_p^2 = .21$). The HR increased significantly from sleep to sitting to mild physical activity. In parallel, we found a significant linear decrease in

PEP, from sleep to sitting to mild physical activity. RSA was significantly lower during mild physical activity compared to lying during sleep and daytime sitting. These patterns are compatible with the expected increase in sympathetic and decrease in parasympathetic cardiac control when going from sleep to (active) daytime activities (Burgess *et al.*, 1997).

A significant effect involving group (non-exercisers, exercisers) was found for HR ($F(1, 50) = 6.5$, $p = 0.01$, $\eta_p^2 = .12$). There was no interaction with ambulatory condition and post-hoc testing confirmed that exercisers had a significantly lower HR at sleep (57.6 vs. 62.4), during sitting (70.2 vs. 77.4) as well as during mild physical activity (81.0 vs. 88.1). No significant main effects of group were found for PEP and RSA. Also no interaction effects were found between group and ambulatory condition.

Detraining effects

Data for the continued training and detraining groups are presented in Table 2 separately per time point and for the different ambulatory conditions. At the beginning of the detraining manipulation, after the 6 weeks standardized training program, no significant differences in HR, PEP or RSA were found between the continued training and detraining groups in any of the ambulatory conditions. More importantly, repeated measures analyses across the 2 weeks continued training /detraining period showed no group by time (overall ambulatory level) or group by time by ambulatory condition interaction for HR, PEP or RSA. This is depicted in Figure 1 which shows the group differences at the beginning and end of the detraining manipulation in the three ambulatory conditions. The two weeks of continued training versus 2 weeks of detraining did not induce the hypothesized decreases in PEP or RSA that would have been compatible with a shift from vagal to sympathetic cardiac control.

Table 2 *HR, PEP, and RSA in the untrained control group and in the continued training (N=13) and detraining (N=13) groups per time point and for the different ambulatory conditions.*

Measure	Group	Time	Phase	Sleep	Sitting	Mild physical activity
HR (bpm)	continued training	0 wks	Start of training	58.5 (10.2)	71.3 (9.3)	81.5 (8.4)
	detraining			56.7 (11.7)	69.2 (13.7)	80.6 (15.7)
	continued training	6 wks	Start of detraining	56.5 (10.1)	69.3 (8.5)	78.2 (7.9)[b]
	detraining			56.5 (12.4)	70.4 (13.6)	80.3 (12.1)
	continued training	8 wks	End of detraining	57.1 (10.7)[a]	72.4 (12.0)	83.4 (11.3)
	detraining			56.1 (9.7)	70.6 (10.3)	79.6 (9.7)
PEP (ms)	continued training	0 wks	Start of training	106.7 (16.3)	100.1 (16.1)	95.9 (14.2)
	detraining			114.5 (13.6)	105.8 (14.0)	100.4 (13.1)
	continued training	6 wks	Start of detraining	114.7 (12.4)	102.9 (10.1)	98.8 (8.6)[b]
	detraining			116.2 (13.4)	105.0 (13.1)	100.2 (10.8)
	continued training	8 wks	End of detraining	115.2 (13.3)[a]	105.5 (10.7)	99.8 (10.9)
	detraining			119.7 (11.8)	107.5 (11.5)	102.4 (9.8)
RSA (ms)	continued training	0 wks	Start of training	64.8 (54.1)	51.3 (30.5)	43.8 (26.9)
	detraining			39.4 (14.1)	55.4 (42.2)	47.4 (40.4)
	continued training	6 wks	Start of detraining	60.6 (41.7)	53.2 (32.8)	43.9 (20.3)[b]
	detraining			47.0 (23.9)	60.0 (45.0)	49.8 (37.7)
	continued training	8 wks	End of detraining	69.7 (55.5)[a]	52.0 (31.4)	38.6 (21.8)
	detraining			41.2 (13.8)	54.8 (36.2)	45.7 (31.5)

** Significant difference ($p < .05$) between non-exercising control group and training/detraining group at the start of the experiment.* [a] *N = 11;* [b] *N=12*

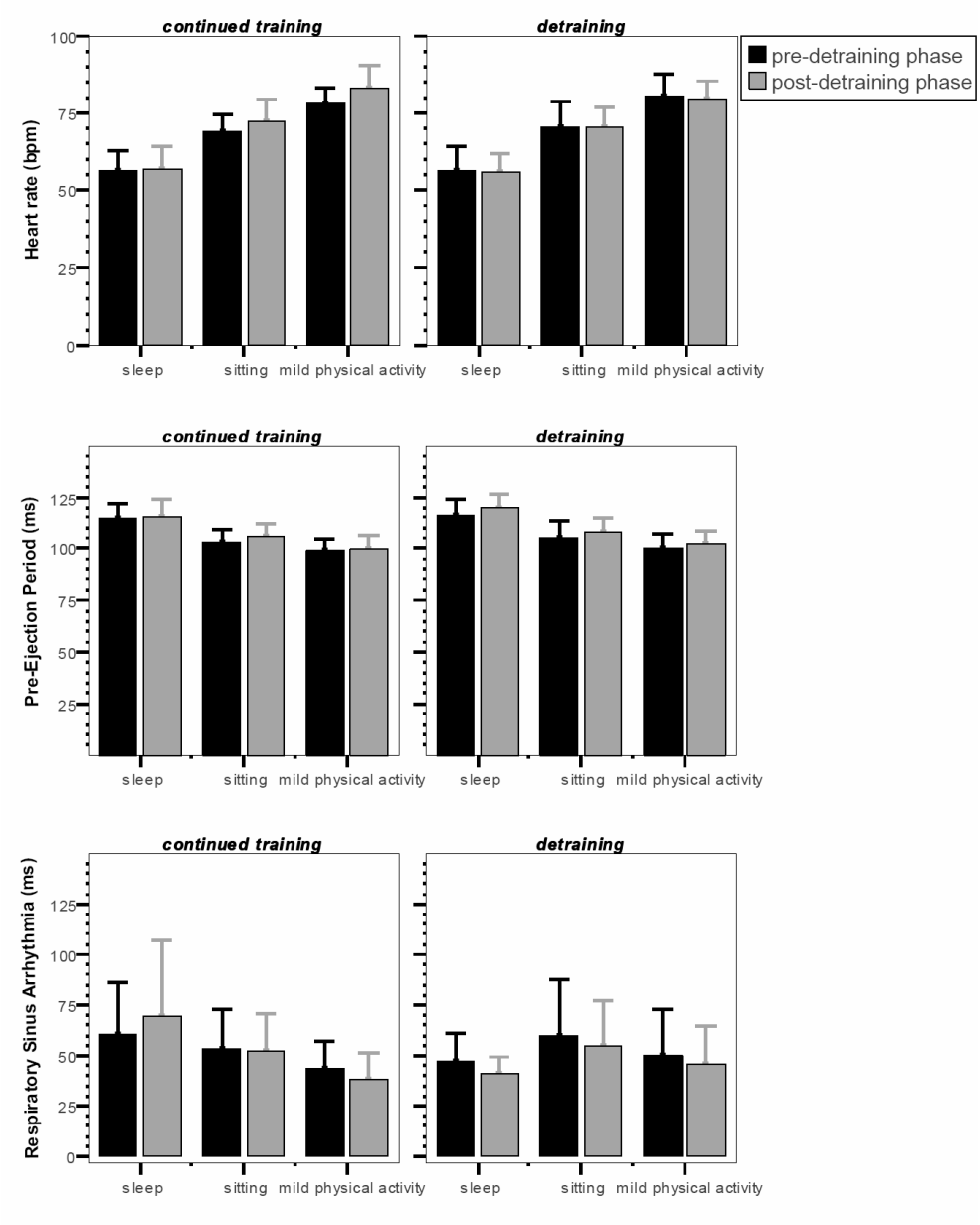


Figure 1 *Bar graphs of the mean heart rate, pre-ejection period and respiratory sinus arrhythmia per ambulatory condition before and after the detraining phase separately for the continued training and detraining group.*

Discussion

This study examined the effect of training state on cardiac autonomic control in a naturalistic setting using PEP and RSA as proxy measures for sympathovagal balance. A cross-sectional comparison of subjects who had been engaged in regular vigorous exercise with persistent sedentary subjects revealed no differences in PEP and RSA throughout a 24-hr recording, although HR was substantially lower at all time points.

Previous studies on the link between exercise and RSA have focused mainly on short-term laboratory recordings. A number of these studies reported higher RSA in regular vigorous exercisers (Buchheit *et al.*, 2005; Dixon *et al.*, 1992; Goldsmith *et al.*, 1992; Shin *et al.*, 1997) but not all studies support this cross-sectional difference (Hatfield *et al.*, 1998) and some even report the opposite finding of lower RSA in exercisers compared to non-exercisers (Sacknoff *et al.*, 1994). Studies assessing aerobic fitness rather than exercise status have reported higher RSA in the more aerobically fit subjects (Kenney, 1985 ; Rossy & Thayer, 1998), even in an ambulatory setting (Goldsmith *et al.*, 1997), but the correlation is not always found (de Geus *et al.*, 1996; de Geus *et al.*, 1990; Hatfield *et al.*, 1998). To date a much smaller number of studies had examined the cross-sectional link between exercise and PEP. Only one study has found longer PEPs signaling decreased sympathetic cardiac control, in exercisers compared to non-exercisers during baseline resting conditions (van Doornen & de Geus, 1989). Others have not found such an effect (Light *et al.*, 1987; Svedenhag *et al.*, 1986; Svedenhag *et al.*, 1991).

Cross-sectional comparisons suffer from the short-coming that they may be confounded by the effects of unmeasured "third variables" like genetics or socio-economic factors. These may either create spurious associations between fitness or exercise behavior and autonomic nervous system activity (Hautala *et al.*, 2003), or act to hide a true association. To establish causality, a number of longitudinal training studies have been performed that have been reviewed by Sandercock et al (2005). Meta-analysis suggested an average increase in RSA during short-term laboratory recordings at rest, but not in ambulatory recordings. From their figure 2 (Sandercock *et al.*, 2005) this meta-analytic result appears to be largely driven by two studies that did not include a comparison control group (Carter *et al.*, 2003; Iwasaki *et al.*, 2003) which makes it hard to separate effects of habituation to the measurement procedures from training effects. The training studies that (randomly) assigned untrained subjects to a

non-exercise control manipulation or a standardized exercise training program have generally failed to find a specific training-induced increase in RSA (Boutcher & Stein, 1995; de Geus *et al.*, 1996; de Geus *et al.*, 1990; Loimaala *et al.*, 2000; Uusitalo *et al.*, 2004). All training studies that added PEP as a measure have failed to find changes in PEP, even after prolonged exercise training (de Geus *et al.*, 1993; de Geus *et al.*, 1990; Sherwood *et al.*, 1989; Svedenhag *et al.*, 1986; Svedenhag *et al.*, 1991).

Although more powerful than cross-sectional studies, training studies also have specific short comings. By necessity, training studies have to select subjects who were untrained at the start of the study, and preferably had a sedentary lifestyle in general. In view of the emerging evidence that there are strong genetic differences in the response to training of parameters like maximal oxygen uptake (VO_{2max}) (Bouchard & Rankinen, 2001) and HR (Rice *et al.*, 2002), sedentary subjects may potentially represent a selected group of autonomic low or non-responders. Low exercise responsiveness may even contribute to sedentary behavior if the same genetic factor that prevents large shifts in autonomic cardiac control also decreases the propensity to engage in regular exercise behavior. Evidence in favor of such an underlying factor was provided by a twin study that showed a significant genetic correlation between RSA and weekly energy expenditure in leisure time exercise (de Geus *et al.*, 1993). To avoid a potential selection of 'autonomic non-responders' whilst still addressing causality, the current study examined the effects of two weeks of detraining on the cardiac autonomic control of regular exercisers, who had been standardized in their training practices in the preceding 6 weeks.

In contrast to our hypothesis, no significant effects of two weeks of detraining were found on PEP, an index of sympathetic cardiac control, or on RSA, an index of parasympathetic cardiac control. Detraining effects were absent throughout the 24-hr recording. i.e. during sleep, during sitting daytime activities, as well as during mild physical activity. Our results are in keeping with findings by Weinstein and colleagues (2007) who used a similar design in which regular exercisers were randomized to continued training or 2 weeks of exercise deprivation. Subjects in neither control nor exercise-withdrawal groups showed alterations in RSA during 10 min of quiet sitting. Exercise-withdrawal also failed to show an effect on resting levels of low frequency heart rate variability (LF), a measure that has been used as an alternative to PEP to index cardiac sympathetic control.

Detraining findings obtained in regular exercisers seem to differ somewhat from the findings in recently trained subjects. Pichot *et al.* (2002) trained 6 sedentary subjects for 13 weeks followed by 7 weeks of recovery. Using nighttime levels, a significant drop in the ratio of LF/HF was found during training, which was interpreted as a shift towards more vagal control over the heart. Seven weeks of detraining did not reverse this shift in LF/HF ratio. De Geus et al (1993; 1996) subjected 12 sedentary subjects to 4 months of training followed by 4 months of detraining and compared these to a non-training control group. Although HR significantly decreased with training and fully returned to baseline levels after detraining, no parallel changes in PEP or RSA were found. Gamelin et al (2007) trained 10 sedentary subjects for 12 weeks followed by 8 weeks of training cessation. Training significantly increased supine LF and tended to increase supine HF, leaving the LF/HF ratio unchanged. The increase in LF was reversed by two weeks of detraining. No effects of training or detraining were found on LF or HF at 60^{o} tilt. Finally, Gutin *et al.* (2000) followed 70 obese 7 to 11 year-old children for 8 months in a randomized cross-over design. Half of the children first trained for 4 months and then detrained for 4 months; the other half acted as a waiting-list control for 4 months followed by 4 months of training. There index of parasympathetic control, the RMSSD, closely followed the training manipulation such that training increased RMSSD by about 6 ms, whereas detraining completely reversed this effect.

With the exception of the obese children, the current evidence does not make a compelling case in favor of short-term shifts from sympathetic to parasympathetic cardiac control, either in regular exercisers or recently trained subjects. Perhaps such training effects are confined to populations characterized by high levels of sympathetic control and low levels of parasympathetic control. Evidence in favor of this idea comes from a study by Roveda and colleagues (2003) who subjected heart failure patients and healthy controls to a supervised 4 month exercise program. At the start of the exercise program, heart failure patients had significantly higher MSNA than age matched healthy controls. After four months of exercise, MSNA in the heart failure patients showed a significant decrease to the level of the healthy controls, whereas MSNA levels in the healthy controls were not influenced by the exercise program at all.

The idea that exercisers differ from non-exercisers in sympathovagal balance is primarily driven by their lower resting HR; a systematic finding across many studies that was reconfirmed in the current study. The exercise

bradycardia is robust to correction for genetic influences and seems to reflect a true causal effect of exercise (de Geus *et al.*, 2003). Training of previously sedentary subjects often leads to a decrease in HR (reviewed in: Fagard & Cornelissen, 2007) and even short periods of detraining can decrease HR to untrained levels in subjects who were only recently trained (Mujika & Padilla, 2000; Wang *et al.*, 1997). A different picture emerges for subjects who have been involved in regular exercise for many years, like the regular exercisers participating in this study. No effects of detraining were noticeable on either basal HR at sleep or HR during the daytime. These findings are in keeping with other studies that found that detraining that lasts 2 to 4 weeks did not result in a change in resting HR in highly trained athletes or in regularly trained individuals (Cullinane *et al.*, 1986; Mujika & Padilla, 2000; Weinstein *et al.*, 2007). Longer term detraining in these subjects, however, does seem to reverse bradycardia not only in recently trained subjects but also in well-trained athletes (Bonaduce *et al.*, 1998; de Geus *et al.*, 1996; Mujika & Padilla, 2001)

We believe that an exercise-induced decrease in intrinsic HR provides a parsimonious explanation for the paradoxical absence of clear cut effects of training and detraining on sympathovagal balance paired to the strong evidence for exercise-induced bradycardia (Bonaduce *et al.*, 1998; de Geus *et al.*, 1996). Dual blockade studies indeed point to a lower intrinsic HR as the most replicated source of resting bradycardia in exercisers (Katona *et al.*, 1982; Kingwell *et al.*, 1992; Lewis *et al.*, 1980; Smith *et al.*, 1989b; Uusitalo *et al.*, 1996) and this is supported by findings in animals (Lin & Horvath, 1972; Negrao *et al.*, 1992). Although the exact physiological mechanism causing a reduction in intrinsic HR remains elusive, it has been hypothesized that it may be caused by a mechanical effect on the pacemaker tissue imposed by cardiac hypertrophy or by an alteration in myocardial cell metabolism (Bhan & Scheuer, 1972; Katona *et al.*, 1982). The combined results from detraining studies suggest that these adaptations apparently take time, but once in place are robust against short periods of detraining but ultimately reversible by longer periods of detraining (Mujika & Padilla, 2001).

There are some limitations of the present study that should be discussed. First, we did not verify the detraining manipulation by maximal performance tests or VO_{2max} recording. We instead ensured compliance by making the importance of adhering to the study design very clear at enrollment. The participants, high-level executives in the ministry or the police office, are

characterized by high conscientiousness. Because they were recruited in the office-based fitness centre, they were well acquainted creating a strong level of social control. In addition, there was active surveillance of the fitness centre itself by research assistants throughout the study. Finally, the exit interview, which recorded their physical activity over the past 2 weeks, suggested that they had not engaged in compensatory physical activity otherwise (gardening, extra commuter bicycling etc). Second, the PEP and RSA measures that were used to index cardiac autonomic control may be imperfect measures of vagal and sympathetic cardiac control in training/detraining studies. Changes in end-diastolic filling and mean arterial pressure can affect PEP without true changes in sympathetic cardiac control and changes in respiratory rate or depth may influence RSA independent of changes in parasympathetic control (Grossman, 2004). Although we did not find evidence for detraining effects on respiration rate and impedance-derived stroke volume (data not shown) no recording of blood pressure or tidal volume was done. Hence, we cannot rule out detraining effects on these measures that might compromise the interpretation of PEP and RSA.

Conclusion

This study shows that regular exercise is not associated with changes in nighttime or daytime levels of PEP or RSA and that 2 weeks of training cessation in regular exercisers does not change their ambulatory PEP or RSA levels. This study is the first study to address the effects of training state and detraining on these measures using prolonged ambulatory recording rather than short-term laboratory testing. In spite of study limitations, the results fit in quite well with those of previous training and detraining studies using PEP, the LF/HF ratio, RSA or more invasive indices of autonomic control. We conclude that in healthy subject populations, training and detraining induced changes in ambulatory heart rate may have to be explained to a large extent by changes in intrinsic heart rate. Changes in cardiac autonomic control seem to play a modest role at best.

Chapter 8

Summary and discussion

The central theme of this thesis was the non-invasive measurement of within- and between-subject variation in autonomic nervous system activity. Reliable and valid non-invasive measures are essential to ambulatory monitoring, which in turn is crucial for the ecologically valid study of the link between biology and behavior. In this final chapter, I first summarize the main results from the laboratory and two ambulatory studies presented in this thesis. Next, I describe the implications of these results for future ambulatory monitoring of ANS function.

Comparability of different measures of cardiac vagal control

Heart rate variability measures within the respiratory frequency range, also called respiratory sinus arrhythmia (RSA), provide us with a window on the modulation of heart rate (HR) by the parasympathetic branch of the autonomic nervous system. (Berntson *et al*, 1997; Task Force of the European Society of Cardiology the North American Society of Pacing, 1996). Cardiac parasympathetic control is paramount to the electrical stability of the heart (Ando *et al.*, 2005; Hull, Jr. *et al.*, 1990; Levy & Schwartz, 1994; Vanoli *et al.*, 1991) and lower levels of RSA have been shown to independently predict cardiac disease and cardiac mortality (Bigger, Jr. *et al.*, 1993; Dekker *et al.*, 1997; Dekker *et al.*, 2000; Hayano *et al.*, 1991; Lombardi *et al.*, 1987; Nolan *et al.*, 1998; Saul *et al.*, 1988; Singer *et al.*, 1988; Singh *et al.*, 1998; Tsuji *et al.*, 1996). The fact that RSA may be readily obtained from noninvasive measurement of the heart period, either with or without simultaneously measuring respiration, makes it a promising measure for large-scale ambulatory studies.

The second chapter of this thesis examined whether the assessment of individual differences in ambulatory assessed RSA is sensitive to the method used or, otherwise formulated, whether “high tech” pvRSA or HF power (labor-intensive) is superior to “low tech” RMSSD (readily obtained). The answer is a resounding no. The correlations between the three measures of RSA were high (r > .80) and these correlations remained stable over time and within different ambulatory conditions (sleep, daytime sitting and daytime standing/walking). This demonstrates that these measures are indications of the same underlying construct. None of the three RSA measures stood out in terms of short-term reliability or temporal stability over a period of more than 3 years. Excellent temporal stability was found for sleep (.72 - .81) and sitting (.68 -. 80) levels over an average period of 3 years and 4 months. However, temporal stability for

periods of standing/walking was rather moderate, ranging from .44 to .57. These results underscore the need to take physical activity into account when interpreting ambulatory RSA measures (Grossman *et al*, 2004).

Independent of ambulatory condition, large differences in mean respiratory frequency and HR were found. We were concerned that such differences might distort the correlations between the three RSA measures, because respiratory frequency is known to affect RSA independent of cardiac vagal control and may do so differently for time- and frequency-based measures. For instance, breathing at a low respiration rate (RR) will tend to make the HF power underestimate the RSA (visible mostly in LF power), whereas the pvRSA and RMSSD are not susceptible to this problem. Recently, Berntson and colleagues have also expressed mathematical concerns about the RMSSD, which they argued to be too sensitive to HR for a reliable measurement of RSA (Berntson *et al.*, 2005). Both theoretical concerns were greatly mitigated by empirical observation. Differences in mean resting HR or mean RR did not affect the correlations between the various RSA measures; the RSA measures were as highly correlated in a group with a mean HR of 83 bpm as in a group with a mean HR of 65 bpm, and as highly correlated in groups with a mean RR of 15 (range 14-16) versus 18 (range 17-20) breaths per minute. This contradicts the idea that one of these RSA measures is relatively more sensitive than the others to confounders such as individual differences in RR or in HR.

Taken together, our results suggest that each of the measures studied – pvRSA, HF power or RMSSD – can be used as a reliable and stable measure of ambulatory RSA. Since the different RSA measurement strategies have varying specific advantages, for instance, providing additional information on RR or on (very) low frequency power, the choice for a specific measure should be based on the exact research questions.

Temporal stability of ambulatory stroke volume and cardiac output

Chapter 3 focused on two cardiovascular measures, stroke volume (SV) and cardiac output (CO), that are routinely derived from impedance cardiography in laboratory settings, but have been used with great reluctance in ambulatory studies. That is unfortunate because these indices offer great potential to further our understanding of the way chronic cardiovascular activation in response to naturalistic events may contribute to cardiovascular disease (Carrasco & Van de Kar, 2003; Davis, 1992; Federenko *et al.*, 2004; Fritz & Levine, 1951; Imaki *et*

al., 1995; Nijsen *et al.*, 2001; Sapolsky, 2003; Sherwood *et al.*, 1986; Van de Kar & Blair, 1999). The reluctance to use ambulatory SV and CO is based on a number of considerations. Ever since the landmark publication of the guidelines committee (Sherwood *et al.*, 1990) there has been a general concern about the validity of impedance-derived SV and CO. A major reason is that the ICG waveform shows large individual variation which may depend on a multitude of factors including variation in electrode placement, anatomical differences in the size and shape of the thorax and the heart and other unknown factors. Amongst others, this can hamper reliable detection of the B-point in the first derivative of thoracic impedance signal which is used to compute the dZ/dt(min), a crucial parameter in SV computation. Doubts about the validity of impedance-derived SV and CO were reinforced by a meta-analysis of all studies comparing them to SV and CO obtained from different invasive methods (Raaijmakers *et al.*, 1999). A total of 154 studies comparing the ICG to a reference method (dye dilution, indirect Fick method, echocardiography) were analyzed. An overall correlation of .53 (95% CI: .43-.62) was found for CO values, which was increased to .67 if repeated measures were averaged. This points to reasonable validity for research on large groups, but for diagnostic interpretation, a correlation of .53 may not be sufficiently accurate.

Additional concerns arise when SV and CO are measured in an ambulatory setting. Shifts in posture and physical activity strongly affect cardiac sympathetic drive as well as cardiac afterload and preload which all have an impact on SV (Cacioppo *et al.*, 1994a; Fallo *et al.*, 1994). In addition, postural changes are expected to alter the relative position of measuring and current electrodes, the exact shape of the enclosed thorax column, and the resulting basal thorax impedance (Z_0) (Grossman & Kollai, 1993; Mohapatra, 1981; Toska & Walloe, 2002) compromising valid use of the Kubicek equation (Raison & Miller, 2003) which assumes these to be stable.

In spite of the concerns above, a number of studies have demonstrated that, at a group level, ambulatory SV and CO are well-behaved across a 24-hr period (Riese *et al.*, 2003; Vrijkotte *et al.*, 2004; Willemsen *et al.*, 1996). SV and CO both increase with physical activity, whereas SV increases during the night to compensate for the lowered HR, leaving CO at comparable or only slightly lower levels to sitting at rest. The crucial question, however, is whether absolute levels or within-day changes in ambulatory SV and CO tap into stable individual differences in cardiac regulation. To address this question long-term temporal

stability of these measures was assessed in chapter 3. We selected data only from periods with fixed posture and low physical activity and we used both the zero-crossing of the ICG and the B-point to compute SV and CO. Even so, only moderate stability was found for SV and CO, in the range of .29 to .46, which is in range with results of previous laboratory and ambulatory studies (Barnes *et al*, 2002; Barnes *et al*, 2004; Matthews *et al*, 2002).

Although the ICG waveforms can differ greatly between subjects they are reasonably stable within a subject. A common feeling, therefore, is that within-subject changes in impedance derived SV and cardiac output are probably reliable, even if absolute values are not. To address this 'feeling', we also computed percentual change scores for each individual on test and retest days, using the awake periods as the "active" state and sleep levels as the resting state. Again, only modest stability of SV and CO reactivity was found (.12 - .45), which, if anything, was lower than that for the absolute SV and CO levels.

A major cause for the low stability of both absolute SV values and change scores was the L_0^2/Z_0^2 ratio. In principle this should be a stable trait, at least within individuals, since the amount of impedance per area body surface is not expected to change that much. In contrast, the product of dZ/dt and LVET can be reasonably expected to change over time, due to true changes in cardiac control. In the actual observations, however, the L_0^2/Z_0^2 ratio proved to be less stable than the product of dZ/dt and LVET, particularly at night.

I conclude that ambulatory SV and CO may capture group differences, for instance when subjects with chronic stress are compared to non-stressed subjects, but are not sufficiently reliable to index individual differences in correlational designs, for instance in genetic studies. Having said this, it must be kept in mind that tracking coefficients for a major CVD risk factor like blood pressure itself are also not much higher than .5 (Hottenga *et al.*, 2005; Palti *et al.*, 1988; Woelk, 1994).

Ambulatory indices of sympathetic nervous activity: heart and skin

In addition to SV and CO, chapter 3 also addressed the temporal stability of ambulatory PEP. PEP is an index of contractility of the left ventricle. Contractility is influenced only by the sympathetic part of the ANS: there is an abundance of functional adrenergic receptors on the ventricle but no acetylcholine receptors. In psychophysiology, the PEP has become the first measure of choice to index cardiac sympathetic control. Its validity in within-

subject designs has been shown in many studies that employed manipulations known to increase cardiac sympathetic activity like epinephrine infusion, amyl nitrite inhalation, mental stress and exercise. These manipulations systematically decrease PEP (Berntson *et al.*, 1994; de Geus *et al.*, 2007; Krzeminski *et al.*, 2000; Kupper *et al.*, 2006; Mezzacappa *et al.*, 1999; Miyamoto *et al.*, 1983; Nelesen *et al.*, 1999; Newlin & Levenson, 1979; Houtveen *et al.*, 2002; Houtveen *et al.*, 2005; Schachinger *et al.*, 2001; Sherwood *et al.*, 1986; Smith *et al.*, 1989; Svedenhag *et al.*, 1986). In addition, pharmacological blockade of cardiac sympathetic effects results in the expected elongation of the PEP (Cacioppo *et al.*, 1994a; Harris *et al.*, 1967; Schachinger *et al.*, 2001; Winzer *et al.*, 1999), whereas PEP is hardly affected by blockade of cardiac vagal effects (Cacioppo *et al.*, 1994a; Martinsson *et al.*, 1991). Between-subject differences in absolute PEP have been shown to closely reflect individual differences in ß-adrenergic inotropic drive (Cacioppo *et al.*, 1994a). In a study of 10 female undergraduate students, a high correlation was found between absolute PEP and heart period increases in response to sympathetic blockade. In further support, a significant inverse correlation between a subjects' absolute PEP and their plasma adrenaline level was found (Levi *et al.*, 1982).

The only caveat in using PEP as an index of cardiac sympathetic control is its sensitivity to preload and afterload effects. Cardiac contractility can increase independently from sympathetic effects when the stretch of the myocardial muscle fibers increases. Thus, when increased preload occurs in the absence of increased sympathetic activity, it decreases the PEP leading to the erroneous suggestion of increased cardiac sympathetic control. The reverse problem occurs when the pressure in the aorta is increased (afterload) in the presence of increased sympathetic activity; because it takes longer for the aortic valves to open, the PEP becomes longer erroneously suggesting *decreased* cardiac control. Preload and afterload effects explain why PEP did not decrease or even increase during the cold pressor test or a hand grip test (chapter 4), both of which should lead to increased cardiac sympathetic control, and why PEP paradoxically increases with head up tilting and in going from supine to standing (Houtveen *et al.*, 2005).

In spite of this caveat PEP can still reliably track decreases in cardiac sympathetic control under conditions of increased preload and reduced afterload. During sleep preload and afterload effects would tend to shorten PEP, but instead we find it to be systematically longer during sleep compared to

daytime activities (see chapters 3 and 4), which confirms similar findings in other studies (Kupper *et al.*, 2006; Vrijkotte *et al.*, 2004). This suggests that the decrease in sympathetic activity during sleep is strong enough to overcome confounding of the PEP by pre- and afterload effects. During daytime recordings, preload and afterload effects on PEP need not be a problem if they are anticipated in the design and analysis strategy of the study. When this is done, very high test-retest correlations (r = .90) across a few days are observed for ambulatory recording of both daytime and sleep PEP (Vrijkotte, van Doornen, & de Geus, 2004). In addition, as demonstrated in chapter 3, very good long-term temporal stability of the 24-hr measurements across the four daily periods is seen (.66-.81), which is, in fact, as good as the stability of PEP obtained under standardized laboratory conditions (Burleson *et al*, 2003; Matthews *et al*, 2002; Willemsen *et al*, 1998). This leads me to conclude that, provided a study-design and a data-analysis strategy that appropriately take into account the independent effects of preload and afterload, ambulatory PEP is a reliable and stable index of cardiac sympathetic control.

Using PEP as the comparison measure, chapter 4 and 5 of this thesis explored two other often used indices of SNS activity; the ratio of LF and HF power in the IBI time series and skin conductance level. Both are non-invasive measures and can, in principle, be assessed in ambulatory designs. Chapter 4 reports on the comparison of PEP with the LF/HF ratio in an ambulatory design. The idea behind the LF/HF ratio is that the LF power is sensitive to both vagal and cardiac sympathetic activity, whereas the HF power is sensitive to vagal activity only. Increases in cardiac sympathetic activity should increase the absolute LF power, but because these are often paired to reciprocal decreases in vagal activity, an actual decrease in LF power may be observed. The LF/HF ratio aims to correct this by taking parallel decreases in vagal activity into account. Although it is accepted that the ratio is sensitive to both vagal and cardiac sympathetic activity, it is considered to be relatively more sensitive to the latter. Hence it may be used as an index of cardiac sympathetic control, even if imperfect.

In spite of its widespread use, the validity of the LF/HF ratio has been the subject of continued controversy (Eckberg, 1997; Malik & Eckberg, 1998; Malliani *et al.*, 1998; Sleight & Bernardi, 1998). The results obtained in chapter 4 have forced me into the camp of the LF/HF skeptics. This is regrettable because a

second non-invasive measure of cardiac sympathetic control would have been most welcome. However, the findings were very clear cut. Most subjects failed to show the expected negative correlation between PEP and the LF/HF ratio across an average of 56 separate periods during the ambulatory recording. The average within-subject correlation was only -.11. Between-subject correlations, computed across the two test days but separately for sleep, sitting and physical activity, were .07, -.13 and -.20 respectively, all non-significant. The use of normalized LF power instead of the LF/HF ratio to index cardiac sympathetic control yielded similarly disappointing results. Furthermore, only PEP, but not the LF/HF ratio showed the expected reciprocal behavior to HF power indicative of the reciprocal vagal and sympathetic cardiac control, which one expects in the majority of subjects and across the majority of ambulatory conditions. I conclude, therefore, that the evidence to support the LF/HF ratio or (normalized) LF power as potential measures of cardiac sympathetic control in epidemiology-scaled research is currently insufficient.

Chapter 5 compared PEP with two skin conductance variables, number of nonspecific skin conductance responses (ns.SCRs) and skin conductance level (SCL), indices of sympathetic activity in the skin (Boucsein, 1992; Dawson *et al.*, 2000; Fowles, 1986; Schell *et al.*, 2002; Venables & Christie, 1980). The underlying idea was that in response to many stressors the SNS acts as a unitary system, such that increased sympathetic activity is seen in multiple effector systems, including the skin and the left ventricle. As expected, during mental and physical stressors known to engage the SNS there was a decrease in PEP and an increase in the two skin conductance indices. For the skin conductance indices, overall significant between-subject correlations ($.34 < r < .55$) and within-subject correlations (mean $r = .72$) were found and about 43% of the variance in these skin conductance indices overlapped. This suggests that these skin conductance parameters both reflect SNS activity but, since the overlap is imperfect, the two skin conductance indices contain partly unique information about ongoing skin SNS activity. Since they can be extracted from the same signal, it seems prudent to assess them in parallel in future studies.

Surprisingly, no solid association was found between either of the two skin conductance indices and PEP. Between-subjects correlation between PEP and ns.SCRs and SCL was non-significant at rest and during each of the stressors. This may partly reflect individual differences at the level of the effectors that act to hide a correlation of SNS activity to the heart and the skin. The β_1- and β_2-

receptor density on the left ventricle is known to vary strongly between individuals (Brodde *et al.*, 2006) as is the number of sweat ducts per area of skin (Sato & Sato, 1983). Yet, these two traits are important determinants of absolute PEP and SCL levels. It is thus possible that a subject with a low ß-receptor sensitivity and a high number of sweat glands may have long PEP and high SCL, whereas another subject with identical SNS activity but high ß-receptor sensitivity and a low number of sweat glands may have a shorter PEP but lower SCL.

More alarming was the ambiguous pattern of within-subject correlations where such individual differences in the heart and skin effector systems cannot play a role. A strong and significant correlation was found between stressor elicited changes in PEP and the two measures of skin conductance *only* when short periods of moderately intense exercise were included in the analyses. These led to a strong decrease in PEP and a strong increase in ns.SCRs and SCL in most subjects. Across all other stressors, the changes in PEP and ns.SCRs and in PEP and SCL, were not significantly correlated. It is unclear why SNS activity was neatly reflected in both heart and skin responses, yet failed to show coherence across these effector systems. A first explanation is that the ß1- and ß2-receptors do not respond solely to noradrenaline released from the cardiac sympathetic nerve. They are also highly sensitive to circulating catecholamines, whereas sweat gland activity is controlled by sympathetic cholinergic fibers acting on muscarinergic receptors that are insensitive to circulating catecholamines. A second explanation is that the changes in PEP across the stressors were strongly influenced by posture and blood pressure changes. Our design was intended to create substantial changes in SNS activity. For this reason we added three conditions that are known to cause increased noradrenergic tone, the cold pressor test, the hand grip test and orthostasis. Clearly, this came at the price of confounding the manipulation of SNS activity with that of changes in preload and afterload effects. In an attempt to deal with this, we recomputed the correlations post-hoc using only across the sitting conditions that did not evoke strong afterload effects (i.e. rest1, stroop, tone avoidance, recovery1-4). Still no significant correlation was found, but now a restriction of range may be at work. Future studies could readdress this issue by using emotional stressors of different intensity (e.g. by varying the financial bonus or the level of ego-threat and deliberate harassment).

Apart from the methodological explanations above, the absence of a relation between PEP and skin conductance may also reflect true differences in the activation of the various branches of the SNS during stressful tasks. That is, the underlying idea that the SNS acts as a unitary system affecting multiple effector systems simultaneously to the same extent may be too naïve. Previous studies have shown that some differentiation is known to occur such that SNS activity does not uniformly increase to all effector organs (Grassi & Esler, 1999; Wallin, 1981). Baroreflex engagement, for instance, may be a powerful source of differences in vascular/cardiac versus skin SNS activity. Unlike cardiac SNS activity, skin SNS activity is not influenced by the baroreflexes (Bini *et al.*, 1981; Vissing *et al.*, 1994; Wallin *et al.*, 1975; Wilson *et al.*, 2001). Task induced changes in baroreflex activity, therefore, will affect PEP but not SCL and ns.SCR. The well-known individual differences in baroreflex sensitivity (Riese *et al.*, 2006; Tank *et al.*, 2001) may also partly account for the low between-subject correlations.

Adding it all up, the results for SCL and ns.SCRs at first sight seem comparable to those obtained for the LF/HF ratio. Neither LF/HF ratio nor SCL measures showed solid correlation to the PEP, either within or between subjects. The interpretation of these two sets of 'zero' correlations, however, is quite different. In contrast to the LF/HF ratio, the SCL measures did systematically change in the expected direction with changes in SNS activity. Adding the exercise condition even induced a strong significant correlation. Electrodermal activity unequivocally shows good correlation to the number of sympathetic action potentials in skin sympathetic nerves (Wallin, 1981), whereas the LF/HF ratio does not correlate to the "golden standard" of cardiac sympathetic activity, cardiac noradrenaline spillover (Alvarenga *et al.*, 2006; Kingwell *et al.*, 1994). Finally, there are very plausible physiological pathways that link sympathetic nerve activity to variation in SCL and ns.SCRs whereas the exact source of LF fluctuations in HR remains to be established. Thus, the non-overlap of skin SNS activity with that of cardiac SNS activity is not a weakness but a strength that can be exploited in future research. I suggest that the *simultaneous measurement of PEP, SCL, ns.SCR* yields a multivariate measure of SNS activity that has more explanatory power than each measure alone.

Permissive effects of cortisol on sympathetic stress reactivity

Glucocorticoids (GCs), including cortisol and corticosterone, have powerful actions on the cardiovascular system. As reviewed by Sapolsky *et al*. (2000) these effects can be permissive, stimulating, suppressive, and preparative. The stimulating, suppressive, and preparative actions all refer to the action of *stress-induced* increases in GC levels. The permissive actions of GCs are unique in that they occur before and therefore independent of, the stress-induced increase in GC levels. Specifically, tonic increases in GC levels that precede the acute exposure to stressors by hours, are hypothesized to modulate sympathetic nervous system effects on the heart and blood vessels during the stressor, such that sympathetic effects on blood pressure, cardiac output, and vascular resistance reactivity are enhanced. Such time-delayed permissive effects on cardiovascular responsivity make good evolutionary sense in light of the clear diurnal rhythm in cortisol (Burleson *et al.*, 2003). Cortisol levels begin to rise sharply a few hours before awakening suggesting that – taken the genomic delays- its augmentation of sympathetic effects is optimal during the active phase (day in primates, night in nocturnal rodents) when fight-flight responses can be essential for survival.

In spite of the theoretical attractiveness, direct evidence for permissive actions of early morning cortisol levels on human sympathetic and cardiovascular stress-reactivity in the course of the day is currently lacking. In chapter 6 we tested the permissive effects of cortisol in two different ways. First, we tested whether the natural occurring variation in the early morning levels in cortisol could predict sympathetic and cardiovascular reactivity to a series of standardized mental and physical stressors to which participants were exposed exactly three hours after awakening. Next, we used a double–blind randomized trial to compare sympathetic and cardiovascular reactivity during a placebo condition with reactivity during a condition in which the early morning cortisol peak was blocked by administration of the synthetic glucocorticoid DEX during the previous evening.

In line with the glucocorticoid-sympathetic interaction predicted by permissive effects we expected basal morning cortisol levels to modulate individual differences in sympathetic stress-reactivity at the level of the central sympathetic drive generated in the amygdale and associated limbic structures. Such effects would have been entirely blocked by DEX, which nearly completely depletes the brain of glucocorticoid action (de Kloet *et al.*, 1975; Meijer *et al.*,

1998), thereby eliminating the hypothesized permissive effects of cortisol on central sympathetic neurotransmission. However, successful blockade of the normal early morning peak in cortisol by administration of DEX had no effect on sympathetic and cardiovascular reactivity to any of the mental and physical stressors. Thus, no evidence for permissive effects of the early morning cortisol rise on daytime sympathetic and cardiovascular responses to stress were found at the level of *central* generators of sympathetic activity. This still leaves the possibility of permissive effects of cortisol on *peripheral* sympathetic neurotransmission which may have been taken over by circulating DEX. Such peripheral permissive actions of GCs can be brought about by enhancing sinoatrial, cardiomyocyte and vascular responsiveness to adrenaline and noradrenaline (Fritz & Levine, 1951; Grunfeld & Eloy, 1987; Ramey *et al.*, 1951; Schomig *et al.*, 1976; Tanz, 1960). We note, however, that peripheral permissive effects on reactivity should have created larger stress reactivity in subjects with larger cortisol responses in the placebo condition. We found only very circumstantial evidence for this. Of all 105 correlations tested, only 6 scores showed a significant correlation with various measures of the early morning cortisol rise, of which only 1 was in the predicted direction, i.e. larger sympathetic or cardiovascular reactivity in subjects with high early morning cortisol levels. This does not exceed the number of false positives that is to be expected due to multiple testing.

I conclude, therefore, that the permissive effects of cortisol on daytime sympathetic and cardiovascular responses to stress remain to be established

Effect training state on ambulatory cardiac autonomic control

An exercise-induced bradycardia with a shift to less sympathetic and more parasympathetic control over the heart rhythm is one of the mechanisms put forward to explain this reduced CVD risk in exercisers, and evidence in favor of this mechanism has accrued in animal studies and studies in cardiac patients (Billman, 2002; Goldsmith *et al.*, 2000; Gutin *et al.*, 2005; Mueller, 2007; Rosenwinkel *et al.*, 2001). As reviewed in chapter 7 the evidence for an exercise-induced shift in cardiac autonomic control is less strong in healthy humans, either in studies using invasive assessments of sympathetic and vagal effects (Alvarez *et al.*, 2005; Katona *et al.*, 1982; Kingwell *et al.*, 1992; Lewis *et al.*, 1980 Meredith *et al.*, 1991; Ray & Hume, 1998; Svedenhag *et al.*, 1984) or in studies using RSA and PEP (Boutcher & Stein, 1995; de Geus *et al.*, 1996; de Geus

et al., 1990; Loimaala *et al.*, 2000; Sherwood *et al.*, 1989; Svedenhag *et al.*, 1986; Svedenhag *et al.*, 1991; Uusitalo *et al.*, 2004). Of note, the bulk of these studies used laboratory resting conditions. Ambulatory recording of RSA was used in a few studies only (Goldsmith *et al.*, 1992; Goldsmith *et al.*, 1997; Loimaala *et al.*, 2000; Schuit *et al.*, 1999; Stahle *et al.*, 1999) and no study addressed the effects of exercise on ambulatory PEP levels. Based on the idea that more consistent effects of exercise on autonomic cardiac control may emerge in ambulatory settings, chapter 7 addressed the link between training state and 24-hr recordings of RSA and PEP.

We first compared RSA and PEP levels from regular vigorous exercisers to those in age- and sex-matched sedentary controls. Next, the regular exercisers were subjected to a standardized training program of 6 weeks in which we attempted to make their training state as comparable as possible. After this run-in standardization phase they were randomized to 2 weeks of continued training or 2 weeks of de-training. We deliberately chose a detraining design to address a specific short coming in training studies. By necessity, training studies have to select subjects who were untrained at the start of the study, and preferably had a sedentary lifestyle in general. In view of the emerging evidence that there are strong genetic differences in the response to training of parameters like maximal oxygen uptake (VO_{2max}) (Bouchard & Rankinen, 2001) and HR (Rice *et al.*, 2002), sedentary subjects may potentially represent a selected group of autonomic low or non-responders. Low exercise responsiveness may even contribute to sedentary behavior if the same genetic factor that prevents large shifts in autonomic cardiac control also decreases the propensity to engage in regular exercise behavior (de Geus *et al.*, 1993). Detraining manipulations avoid this potential selection of 'autonomic non-responders' whilst still addressing causality.

Results showed that non-exercising controls had a significantly higher ambulatory HR compared to the regular exercisers but entirely comparable 24-hr levels of PEP and RSA. In the regular exercisers, 2 weeks of detraining did not significantly change the ambulatory levels of PEP or RSA. We conclude that the bradycardia in healthy regular exercisers paired to the paradoxical absence of clear cut effects of training and detraining on sympathovagal balance is best explained by an exercise-induced decrease in intrinsic HR. Dual blockade studies indeed point to a lower intrinsic HR as the most replicated source of resting bradycardia in exercisers (Katona *et al.*, 1982; Kingwell *et al.*, 1992; Lewis *et al.*,

1980; Smith *et al.*, 1989b; Uusitalo *et al.*, 1996) and this is supported by findings in animals (Lin & Horvath, 1972; Negrao *et al.*, 1992). Although the exact physiological mechanism causing a reduction in intrinsic HR remains elusive, it has been hypothesized that it may be caused by a mechanical effect on the pacemaker tissue imposed by cardiac hypertrophy or by an alteration in myocardial cell metabolism (Bhan & Scheuer, 1972; Katona *et al.*, 1982). The combined results from training and detraining studies reviewed in chapter 7 suggest that these adaptations apparently take time, but once in place are robust against short periods of detraining but ultimately reversible by longer periods of detraining (Mujika & Padilla, 2001).

Unintentionally, this study provided a compelling example of the need to do a full assessment of cardiac autonomic balance in ambulatory studies instead of just measuring HR, even though the latter can be done more cost-efficient and with commercial devices that minimize subject discomfort. Had we relied on HR recordings only as our proxy for sympathovagal balance, we would have probably concluded that exercise indeed shifts autonomic control towards vagal activity, and that the effects were robust to two weeks of detraining! As it stands, I must conclude differently. In healthy subject populations, training and detraining induced changes in ambulatory HR may have to be explained to a large extent by changes in intrinsic heart rate. Changes in cardiac autonomic control seem to play a modest role at best.

Future ambulatory monitoring of ANS function

The results of the studies presented in this thesis have implications for large-scale ambulatory studies with the aim of exploring within- and between-subject variation in ANS function. In fact, part of my PhD thesis mission was to provide scientific guidance for the development of a new version of the Vrije Universiteit Ambulatory Monitoring System (VU-AMS) in close collaboration with the technical department (ITM) of the Faculty of Psychology and Education. This device, the VU-AMS.5fs, has now been thoroughly beta-tested and recently became available for researchers (see appendices I to III). Below, I describe the implications of the results in this thesis for data collection, study design, and data reduction in future ambulatory monitoring of ANS function, and some of the new features that were implemented in the VU-AMS.5fs to address the identified needs.

Data collection: which measures to choose?

Measuring parasympathetic nervous system activity. To measure cardiac vagal control either RMSSD, pvRSA, or HF power can be used. The results presented in this thesis do not lead me to favor one measure over the others. I do note that accurate assessment of all three measures depends crucially on the quality of the IBI time series from which they are computed. The old VU-AMS system (version 4.6) used a powerful dynamic online R-wave detector, but did not store the full ECG waveform. As such it did not outperform commercial wrist-watch type HR-recording devices, that need only a single elastic recording band around the chest to record the IBI time series. In an estimated 90% of the subjects, IBI time series will be of sufficient quality 90% of the time to extract reliable HR and RMSSD values with automated error checking of the IBI time series and only minimal visual inspection. However, that leaves out 19% of the potential data, and, if this missingness is non-random, may introduce bias. Using Compact Flash memory cards, storage capacity of the new VU-AMS.5fs is now >1 gigabyte which makes it possible to record the full ECG signal recording at a maximum sampling rate of 1000Hz with a 16-bit resolution. The resulting higher quality IBI time series allows for more sophisticated automated IBI detection and it makes visual inspection more informative. This will greatly increase the amount of data that can be salvaged for all three RSA measures, most strongly benefiting Wavelet- or Fourier-analysis which is the most sensitive to missing or incorrectly scored IBIs.

A remaining question is whether the additional recording of the respiration signal needed for pvRSA outweighs the added burden on the study participants that need to wear either additional electrodes or respiratory bands. However, this method has the huge advantage of providing extra information on respiration behavior (Houtveen *et al.*, 2006). Currently, the field is littered with concerns about the use of RSA as a measure of cardiac vagal control, without appropriate control for respiratory behavior (Eckberg, 2003; Grossman *et al.*, 2004; Grossman & Kollai, 1993; Houtveen *et al.*, 2002; Ritz & Dahme, 2006). Whether such concerns are valid in ambulatory recordings that take physical activity into account remains to be established. Analyses in chapter 2 and 5 that explicitly used between-subject variation in RR as a potential modulator of RSA yielded essentially identical results with or without correction for RR. This may reflect the fact that RR did not show dramatic between-subject variation across sitting activities and sleep (range 14 to 20 breaths per minute). However, strong within-subject manipulation of respiratory behavior in a range of 5 to 25 breaths per

minute clearly shows that interpretation of RSA can be compromised by respiratory behavior (Houtveen *et al.*, 2006). Ultimately, the choice for a specific measure must depend on the exact research question and ambulatory design. In very large-scale research that (1) focuses on prediction of disease risk, (2) aims for minimum discomfort to the participants, and (3) wants to avoid labor-intensive data reduction by the researcher, the RMSSD seems the most appropriate measure.

Measuring sympathetic nervous system activity. The present thesis examined four measures that have been used as indicators of sympathetic autonomic control; PEP, LF/HF ratio, SCL, and ns.SCRs. Among these measures, the LF/HF stood out as a very poor measure of SNS activity. In fact, based on these results I concluded that the LF/HF ratio should not currently be used as an index of sympathetic activation. The other three measures, PEP and two skin conductance indices proved valid indicators of sympathetic effects on the heart and the skin respectively, although for PEP strong effects on pre- or afterload need to be dealt with in design or analysis strategy. These three measures reflect non-overlapping information on changes in SNS activity and should, whenever possible, be measured simultaneously to provide the most complete picture of the sympathetic activation in the whole body. To enable future researchers to do so an important change was needed to the VU-AMS.5fs. Previous versions of the VU-AMS recorded either ECG and SCL or ECG and ICG, but simultaneous recording of the PEP and SCL was not feasible. The new device allows full parallel recording of the ECG, thorax impedance (dZ) and skin conductance.

Although technologically feasible, a serious problem with ambulatory skin conductance measurement is that the electrode placement may restrict individuals in their normal daily activities. The most common place to measure skin conductance is at palmar sites, because eccrine sweat glands are at the highest density in these regions. The next best alternative, the soles of the feet, is even less practical. An specific solution has been found by researchers using SC to detect hot flashes in menopausal women (Thurston *et al.*, 1994). Skin conductance electrodes placed on the sternum proved to be the most sensitive and specific physiological measure of hot flashes. However, in contrast to palmar and plantar skin conductance, sternal skin is relatively unresponsive to psychological stimuli (Freedman, 1989). Current studies of the feasibility of using alternative locations of the skin conductance electrodes, e.g. on the back or on

the dorsal side of the hand, will hopefully remove this final restriction on ambulatory SCL recording and allow the measurement of PEP as well as SCL and ns.SCRs in ecologically valid settings.

Study design: factors to take into account

Appropriate technology to collect ANS data is only the first step in ambulatory recording. Interpreting the ANS data in terms of relevant within- and between-subject variation in psychological (e.g. work stress), lifestyle (exercise) or biological (e.g. genetics) factors requires the ANS data collection to be accompanied by the collection of information on (confounding) variables that could influence the ANS independently of the relevant study variables.

Posture and physical load. As ambulatory recording is characterized by frequent changes in posture and activity, a solid strategy is needed to identify significant changes in posture and physical activity to be able to take these factors into account during data analysis. The classical way to collect information on activity and posture is through the participant reports. In the present study we used paper diaries in which participants entered their activities and posture for each half hour period of the day, a simple method which captures a large part of the variation in posture and activity. Still, diary information remains subjective and in particularly the exact timing of changes in behavior may be less accurately presented by the participants. We improved timing of the onset and offset of changes in posture and activity with the use of an inbuilt recorder for body movement. Specifically, a piezoelectric circuit in the VU-AMS device, which is attached by a belt to the hip, measures the vertical acceleration of the subject. Vertical acceleration is very sensitive to postural changes, but it does not discriminate well between bicycling and walking, for instance, and misses all more subtle 'movements in place'. In the new VU-AMS.5fs, we added a measurement of horizontal acceleration, in the hope to further increase the accuracy of the assessment of body movement.

Psychological factors. Before starting a large ambulatory study, one needs to be aware that ANS function may be influenced by both short-term mood changes (e.g. fluctuations in mood as results of a short lasting stressor) and more chronic mood state (e.g. depression). Many different questionnaires are available to measure general mood and stress status, with respect to the measurement day or to a prolonged period of time, e.g. the months preceding the ambulatory measurement. To control for, or study, the more acute mood effects throughout

the day, repeated information needs to be obtained from participants throughout the day. This can be done by asking the subjects to add this information in the diary they are completing, stating at every entry how they felt or how stressed they were at the time of entry. An excellent alternative is provided by the use of electronic devices. For instance, participants may be provided with a small handheld device which prompts the subject to provide all the information needed (Houtveen & van Doornen, 2007). If the handheld device also assesses posture and ongoing activity it can even entirely replace paper diaries. The current omnipresence of mobile phones provides further options for the acquisition of information on daily mood or activities through short automated phone calls or Short Message Service (SMS) based queries. Electronic devices are more intrusive than diaries but they allow a more standardized and complete recording of mood and activity status, and can provide more accurate timing of specific ambulatory events.

Social factors. A neglected area so far is the effect of location (e.g. indoor, outdoor; home, in public, at work, etc.) and social situation (alone, with significant other, with colleagues, etc) on ANS activity. Data collection on these factors can be done through similar strategies as mood, posture and ongoing activity. Using diaries we have systematically collected data on a few social factors, but a careful analysis remains to be done. A first obvious problem that needs to be tackled is that there are very many social situations, which reduces the number of subjects per cell. Appropriate clustering of social situations in meta-categories may require theoretical input from social psychology.

Data reduction: From prolonged continuous recordings to a few core numbers

Ambulatory monitoring creates a huge amount of physiological data. When storing one-minute ensemble averages of the impedance cardiogram, as was done in the present thesis, the measurement over 24-hr would result in 1440 complexes. The newly developed VU-AMS.5fs will even store the impedance complexes continuously on a beat-to-beat basis, which will result 100800 data points over a 24-hr period with an average HR of 70 beats per minute. Steps need to be taken to manage these data adequately. We use two major strategies for data reduction: (1) large scale averaging across periods with a fixed type of activity, and (2) nearly full automated data scoring that feeds directly into SPSS scripts to identify possible artifacts or outliers. This restricts (labor-intensive) visual inspection to suspect signal parts only rather than the entire recording.

For large-scale averaging, first the information from the activity diaries is used in combination with a visual display of the inbuilt vertical accelerometer signal to divide the entire 24-hr recording into fixed periods. These periods are coded for posture (e.g. lying, sitting, standing), type of ongoing activity (e.g. desk work, eating/drinking, meetings, watching TV), physical activity level (no, light, medium or heavy physical activity), location (e.g. work, home, outside) and social situation (e.g. alone, with colleagues, with friends). A full coding scheme is provided in the appendix of chapter 4. The coded periods are never shorter than 5 min or longer than 1 hr. If periods lasted more than 1 hr (as during sleep), they were divided into multiple periods of maximally 1 hr (e.g. sleep1, sleep2, etc). An average of about 30 (typical range 20 to 50) coded periods is thus created per subject with an average duration of 30 min. Scoring of the HR and RMSSD by the AMSGRA program or of the PEP, SV and CO by the AMSIMP program then proceeds on large-scale ensemble averages across these 20 to 50 coded periods. As described by Riese et al (2003) the average value of the PEP obtained on a small time scale (e.g. 60-s averages) is almost perfectly correlated to the PEP obtained from these large-scale ensembles, which substantially reduces interactive scoring of the impedance cardiogram (automated signal scoring of which is known to be very hazardous). Both AMSGRA and AMSIMP programs are freely available at www.psy.vu.nl/vu-ams.

All physiological measures are ultimately averaged across the same coded periods, i.e. per variable we end up with no more than 50 numbers. Depending on the research question we often aggregate this data set even further to arrive at mean values across all sleep periods, all sitting periods, all standing periods and all periods with light or moderate physical activity (heavy physical activity is usually avoided by asking the subjects not to engage in exercise on the measurement day). Alternatively we do this separately across morning, afternoon, and evening periods or, on a work day, across leisure time and work time.

Near-fully automated data scoring is done for the respiration signal by the AMSRES program (www.psy.vu.nl/vu-ams). Output is on a breath-to-breath basis, but a preprogrammed script can rapidly aggregate the data across the coded periods, whilst removing likely outliers or artifacts in the ECG and respiration signals. The overall strategy for VU-AMS data reduction and the accompanying SPSS scripts can be found in appendices IV and V; the scripts are also available on request from vu-ams@psy.vu.nl.

In closing

This thesis shows that ambulatory recording of ECG, ICG and SC provides reliable and stable indices of within- and between-subject variation, which, even if imperfect, are valid indicators of vagal effects on the heart and sympathetic effects on the heart and the skin. This further opens the door for much needed large-scale ambulatory monitoring studies of individual differences in the delicate balance between the two branches of the ANS. A shift from parasympathetic to sympathetic activity is a main risk factor for CVD (Curtis & O'Keeffe, 2002; Dekker *et al.*, 2000; Verdecchia, 2000; Fox *et al.*, 2007). Detection of this shift is increasingly done by ambulatory monitoring under the expectation that this has higher predictive validity for long term health outcomes than laboratory measurements (Feldman & Weidenfeld, 2002; Goldstein *et al.*, 2006; Grossman, 2004). In view of the ongoing improvements in ambulatory technology, an appropriate closing conclusion is that this field is very much 'on the move'.

Samenvatting

Dutch summary

Ambulant gemeten variatie in autonome zenuwstelselactiviteit

Het centrale thema van dit proefschrift is het meten van variatie in autonome zenuwstelselactiviteit. Zowel variatie binnen personen als tussen personen werd behandeld. Het autonome zenuwstelsel heeft als taak het reguleren van de basale processen in de organen die nodig zijn voor het normaal functioneren van het lichaam. Het autonome zenuwstelsel bestaat uit twee takken, het parasympathische zenuwstelsel, ook wel het vagale zenuwstelsel genoemd, en het sympathische zenuwstelsel. Het autonome zenuwstelsel zou vergeleken kunnen worden met de aansturing van een auto; de vagale tak is dan de rem en de sympathische tak het gaspedaal. Dit wil zeggen dat in het algemeen een toename in vagale sturing van het hart een verlaging van de hartslag veroorzaakt, terwijl tegenovergesteld een toename in sympathische hartsturing de hartslag verhoogt. In rustige situaties, zoals tijdens slaap, heeft het parasympathische zenuwstelsel de overhand, terwijl bij fysieke belasting zoals sporten en mentale belasting zoals stress het sympathische zenuwstelsel de touwtjes in handen neemt. Een veelvuldige of zelfs chronische verschuiving van de balans van vagale en sympathische zenuwstelselactiviteit naar meer sympathische en minder parasympathische activiteit heeft grote gevolgen voor de gezondheid, met name wat betreft het risico op hart- en vaatziekten. Het optreden van deze veelvuldige of chronische verschuiving kan het beste worden aangetoond door langdurige metingen (24-uurs metingen) uit te voeren in levensechte situaties, de zogenaamde ambulante metingen. Dit proefschrift beschrijft de belangrijkste resultaten van één laboratoriumstudie en twee ambulante studies waarin de betrouwbaarheid, stabiliteit en validiteit werd getest van een aantal maten die activiteit van het vagale en sympathische zenuwstelsel weergeven én die geschikt zijn voor ambulante meting.

Een maat die voornamelijk beïnvloed wordt door de vagale tak is de periodieke variatie in opeenvolgende hartslagen, ook wel hartslagvariabiliteit genoemd. Een veelgebruikte maat van hartslagvariabiliteit is de respiratoire sinus aritmie (RSA), waarbij alleen gekeken wordt naar de ademhalingsafhankelijke variabiliteit in de hartslag. Tijdens inademing gaat de hartslag sneller, terwijl tijdens uitademing de hartslag iets vertraagt. Aangetoond is dat een lage RSA, dus weinig variatie in de hartslag, een onafhankelijke voorspeller is van het ontstaan van hart- en vaatziekten, alsmede van de eventueel hierop volgende sterfte. Het feit dat de RSA zeer eenvoudig verkregen kan worden uit de hartslag, eventueel door tegelijkertijd de ademhaling te meten, maakt het een

veelbelovende maat voor grootschalige ambulante onderzoeken. Er zijn verschillende methodes om RSA te meten; in hoofdstuk 2 werd gekeken naar de drie meest gebruikte maten, namelijk RMSSD, pvRSA en HF power. Onderzocht werd in hoeverre de betrouwbaarheid en stabiliteit van deze maten verschilt in ambulant onderzoek. De drie maten bleken hoog met elkaar gecorreleerd en deze correlaties bleven stabiel over een gemiddelde periode van ruim 3 jaar. Ook bleven deze correlaties onveranderd binnen verschillende ambulante condities (slaap tijdens de nacht, zitten en staan/lopen gedurende de dag). Er is een groot verschil in de moeite die nodig is om deze maten te verkrijgen, zowel wat betreft de registratie als de verwerking, maar de eenvoudigste maat (RMSSD) gaf een even betrouwbare en stabiele indruk van ambulante RSA als de meest complexe maat (HF power).

Een maat van voornamelijk sympathisch oorsprong, is de samenknijpkracht van het hart, ook wel contractiliteit genoemd. Door zowel het elektrocardiogram als ook het impedantiecardiogram te meten, kan de tijd tussen het begin van de elektrische impuls in de pacemakercellen en het openen van de linkerhartklep bepaald worden. Dit tijdsinterval wordt ook wel de pre-ejectie periode (PEP) genoemd en is de meest gebruikte maat van contractiliteit. In hoofdstuk 3 werd aangetoond dat ambulant gemeten PEP betrouwbaar is en dat verschillen tussen personen stabiel blijven over een periode van meer dan 3 jaar. Belangrijk hierbij is wel dat er rekening wordt gehouden met de effecten van veranderingen in houding en fysieke activiteit. Deze veranderingen kunnen namelijk de PEP deels onafhankelijk van veranderingen in de sympathische aansturing van het hart beïnvloeden. Omdat veranderingen in houding en fysieke activiteit nu eenmaal onvermijdelijk horen bij een levensechte meting, moet men dus een studieopzet en data-analysestrategie kiezen die rekening houdt met deze factoren.

Hoofdstuk 3 behandelde ook het ambulant meten van twee cardiovasculaire maten, slagvolume (SV) en hartminuutvolume (CO), die afgeleid kunnen worden uit het impedantiecardiogram. Ondanks het feit dat beide maten van belang zijn om een beter inzicht te krijgen in de effecten van alledaagse gebeurtenissen op het cardiovasculaire systeem, is er grote terughoudendheid in het gebruik van ambulant gemeten SV en CO. Een belangrijke reden hiervoor is de angst voor de vele denkbare verstorende invloeden op het impedantiesignaal, zoals de exacte plaatsing van de elektrodes, anatomische verschillen in de grootte en vorm van de thorax en de ligging van het hart. Hier bovenop komen weer de invloeden van veranderingen in houding en fysieke activiteit. Niettemin lieten de resultaten in hoofdstuk 3 zien dat wanneer er gedegen rekening wordt

gehouden met deze factoren, verschillen in de ambulant gemeten SV en CO tussen personen toch verrassend stabiel zijn over een periode van ruim 3 jaar.

Een alternatieve maat van sympathische hartaansturing is de ratio van laag frequente (langzame) en hoog frequente (snelle) variatie in de hartslag (LF/HF). Het idee achter deze maat is dat de LF beïnvloed wordt door zowel de sympathische als de parasympathische tak van het autonome zenuwstelsel en de HF alleen door de parasympathische tak. Door LF te delen door HF wordt de parasympathische invloed uitgemiddeld en blijft de sympathische invloed over. Ondanks het feit dat de maat LF/HF zeer veel gebruikt wordt, is er veel discussie over de validiteit van deze maat. De resultaten van hoofdstuk 4, waarin de relatie tussen de PEP en de LF/HF ratio werd getest binnen en tussen personen, lieten zien dat deze discussie volstrekt gegrond is. Gebaseerd op het feit dat beide maten sympathische hartaansturing weerspiegelen verwachten we een relatie tussen deze twee. Echter, zowel binnen een persoon als tussen personen werd geen bewijs gevonden voor een relatie tussen PEP en LF/HF ratio. Alleen de PEP, en niet de LF/HF ratio, liet de verwachte negatieve correlatie met de parasympathische maat HF zien. Vandaar dat geconcludeerd kan worden dat er onvoldoende bewijs is om de ambulant gemeten LF/HF ratio als een valide maat van sympathische hartaansturing te gebruiken.

Sympathische zenuwstelselactiviteit kan ook gemeten worden in de huid. In hoofdstuk 5 werd gekeken of de toename in sympathische activiteit door verschillende mentale en fysieke stressoren in twee verschillende eindorganen, het hart en de huid, te meten was. Inderdaad lieten alle stressoren de verwachte sympathische reactie zien in zowel de PEP (toename in contractiliteit van het hart) als in de huidgeleiding (toename in huidgeleiding). De resultaten in hoofdstuk 5 lieten ook zien dat deze effecten op de samenknijpkracht van het hart en de huidgeleiding onderling, zowel binnen een persoon als tussen personen, weinig samenhang vertonen. Het feit dat we geen correlatie vonden tussen de samenknijpkracht van het hart en de huidgeleiding zou mogelijk kunnen duiden op verschillende effecten van sympathische activiteit tijdens uiteenlopende stresstaken op het hart en de huid. Dat gegeven kan door onderzoekers als een voordeel worden uitgebaat. Door de effecten op het hart en de huid tegelijkertijd te meten, wordt een meer volledig inzicht in sympathische zenuwstelselactiviteit verkregen.

Het vermoeden bestond dat cortisol, ook wel het stresshormoon genoemd, een grote invloed zou hebben op het sympathische zenuwstelsel. In hoofdstuk 6 werd gekeken naar het zogenaamde permissieve effect van cortisol

op sympathische en cardiovasculaire stressreactiviteit tijdens een aantal verschillende mentale en fysieke stresstaken. De term permissief verwijst naar de effecten van basale niveaus van cortisol in plaats van cortisolniveaus tijdens stress. Verwacht werd dat mensen met een hoger cortisolniveau in de ochtend sterker zouden reageren op de stresstaken. Hiervoor werd echter geen bewijs gevonden. In hoofdstuk 6 werd ook gekeken naar de effecten van cortisol op de stressreactiviteit binnen personen. Dit werd gedaan door de proefpersonen twee keer op identieke wijze te meten, een keer na een placebo en een keer na inname van het medicijn dexamethason, welke de productie van cortisol remt. De resultaten lieten zien dat een sterke verlaging van cortisol in de ochtend geen effect had op de sympathische en cardiovasculaire stressreactiviteit. Vooralsnog is er dus nog geen sterk bewijs voor de veronderstelde permissieve effecten van cortisol op het sympathische zenuwstelsel.

Een van de vele concrete vraagstellingen waarbij het ambulant meten van het autonome zenuwstelsel een belangrijke rol kan spelen is de vraag naar de oorzaak van bradycardie (zeer lage hartslag) bij sporters. Deze wordt nu vaak toegeschreven aan een, door sport veroorzaakte, verschuiving naar minder sympathische en meer parasympathische controle over het hartritme. Dit zou een van de mechanismen kunnen zijn die het gereduceerde risico op hart- en vaatziekten bij sporters kan verklaren. In hoofdstuk 7 werd de relatie tussen sportgedrag en ambulante 24-uurs niveaus van RSA en PEP onderzocht. De resultaten lieten zien dat niet-sportende controlepersonen een significant hogere hartslag hadden dan fervente sporters, maar volledig vergelijkbare 24-uurs niveaus van RSA en PEP. Ofwel ondanks dat de hartslag in de niet sportende groep vergeleken met de fervente sporters wel hoger was, vonden we geen verschil in parasympathische en sympathische maten van hartaansturing. Ook het effect van twee weken verplicht niet-sporten (‘de-training’) werd onderzocht in de groep fervente sporters. Het bleek dat het tijdelijke niet-sporten geen noemenswaardig effect had op de RSA en PEP. Bradycardie bij mensen die frequent sporten lijkt dus niet eenduidig te kunnen worden verklaard door effecten van sporten op de parasympathische en sympathische hartaansturing. Eerder zou de verklaring voor de bradycardie gezocht moeten worden in een verandering in het hart zelf. Wat deze studie duidelijk liet zien, is dat het belangrijk is om niet alleen de hartslag te meten, maar ook de aparte bijdrage van parasympathische en sympathische hartaansturing. Als we de resultaten van dit onderzoek alleen zouden baseren op de gegevens van de hartslag zouden we foutief de lagere hartslag in de fervente sporters kunnen interpreteren als een

door sport veroorzaakte verschuiving in de balans richting meer parasympathische aansturing.

In de bijlagen van het proefschrift staan een aantal technische beschrijvingen van de hardware en software die is gebruikt bij de diverse experimenten. Dit proefschrift heeft namelijk als wetenschappelijke leidraad gediend bij de ontwikkeling van een nieuwe versie van het Vrije Universiteit Ambulante MeetSysteem (VU-AMS), in nauwe samenwerking met de instrumentatiedienst van de faculteit Psychologie en Pedagogiek. Zo maakte de oude VU-AMS (versie 4.6) gebruik van een dynamische online R-toppen detector, maar was niet in staat om het volledige ECG op te slaan. Bij sommige personen ging dit ten koste van de betrouwbaarheid en stabiliteit van de PEP en RSA. Door het gebruik van Compact Flash geheugenkaarten is de opslagcapaciteit van de nieuwe VU-AMS (VU-AMS.5fs) aanzienlijk vergroot waardoor het nu mogelijk is om de volledige ECG- en ICG-signalen op te slaan. Een verdere vernieuwing is de mogelijkheid om gelijktijdig het elektrocardiogram, het impedantiecardiogram én de huidgeleiding te registreren, zodat gebruik gemaakt kan worden van de unieke informatie over de activiteit van het sympathische zenuwstelsel die de PEP en de huidgeleiding weerspiegelen.

De VU-AMS.5fs is nu uitgebreid getest en in de afrondingsfase van mijn proefschrift beschikbaar gemaakt voor collega onderzoekers wereldwijd.

Tot slot

Dit proefschrift heeft laten zien dat de meting van het ECG, ICG en de huidgeleiding betrouwbare en stabiele indicatoren oplevert van vagale sturing van het hart en van de sympathische effecten op het hart en de huid. Zoals al eerder gezegd, is een verschuiving in de balans van parasympathische naar sympathische activiteit een belangrijke risicofactor voor hart- en vaatziekten. Daarom is het detecteren van deze verschuiving van groot belang. Dit onderzoek zal in toenemende mate worden gedaan met ambulante metingen in levensechte situaties, omdat deze, vergeleken met metingen in het laboratorium, een grotere voorspellende waarde hebben voor de gezondheid op langere termijn. Verdere ontwikkelingen in de ambulante meettechnologie, waaraan ik in dit proefschrift heb getracht een bescheiden bijdrage te leveren, blijven zeer wenselijk.

References

Ahmed MW, Kadish AH, Parker MA, & Goldberger JJ (1994). Effect of Physiological and Pharmacological Adrenergic-Stimulation on Heart-Rate-Variability. *Journal of the American College of Cardiology* 24: 1082-1090.

Akselrod S, Gordon D, Madwed JB, Snidman NC, Shannon DC, & Cohen RJ (1985). Hemodynamic regulation: investigation by spectral analysis. *AJP - Heart and Circulatory Physiology* 249: H867-H875.

Akselrod S, Gordon D, Ubel FA, Shannon DC, Berger AC, & Cohen RJ (1981). Power spectrum analysis of heart rate fluctuation: a quantitative probe of beat-to-beat cardiovascular control. *Science* 213: 220-222.

al'Absi M, Bongard S, Buchanan T, Pincomb GA, Licinio J, Lovallo WR (1997). Cardiovascular and neuroendocrine adjustment to public speaking and mental arithmetic stressors. *Psychophysiology* 34, 266-275.

Alcalay M, Izraeli S, Wallachkapon R, Tochner Z, Benjamini Y, & Akselrod S (1992). Paradoxical Pharmacodynamic Effect of Atropine on Parasympathetic Control - A Study by Spectral-Analysis of Heart-Rate Fluctuations. *Clinical Pharmacology & Therapeutics* 52: 518-527.

Allen MT & Crowell MD (1989). Patterns of autonomic response during laboratory stressors. *Psychophysiology* 26: 603-614.

Allen MT, Sherwood A, Obrist PA, Crowell MD, & Grange LA (1987). Stability of cardiovascular reactivity to laboratory stressors: a 2 1/2 yr follow-up. *Journal of Psychosomatic Research* 31: 639-645.

Alvarenga ME, Richards JC, Lambert G, Esler MD. (2006). Psychophysiological mechanisms in panic disorder: a correlative analysis of noradrenaline spillover, neuronal noradrenaline reuptake, power spectral analysis of heart rate variability, and psychological variables. *Psychosomatic Medicine*. 68(1):8-16.

Alvarez GE, Halliwill JR, Ballard TP, Beske SD, & Davy KP (2005). Sympathetic neural regulation in endurance-trained humans: fitness vs. fatness. *Journal of Applied Physiology* 98: 498-502.

Ando M, Katare RG, Kakinuma Y, Zhang D, Yamasaki F, Muramoto K, & Sato T (2005). Efferent vagal nerve stimulation protects heart against ischemia-induced arrhythmias by preserving connexin43 protein. *Circulation* 112: 164-170.

Arai Y, Saul JP, Albrecht P, Hartley LH, Lilly LS, Cohen RJ, & Colucci WS (1989). Modulation of Cardiac Autonomic Activity During and Immediately After Exercise. *American Journal of Physiology* 256: H132-H141.

Aubert AE, Seps B, & Beckers F (2003). Heart rate variability in athletes. *Sports Medicine* 33: 889-919.

Barnes VA, Johnson MH, Dekkers JC, & Treiber FA (2002). Reproducibility of ambulatory blood pressure measures in African-American adolescents. *Ethnicity & Disease* 12: S3-S6.

Barnes VA, Johnson MH, & Treiber FA (2004). Temporal stability of twenty-four-hour ambulatory hemodynamic bioimpedance measures in African American adolescents. *Blood Pressure Monitoring* 9: 173-177.

Barnett PA, Spence JD, Manuck SB, & Jennings JR (1997). Psychological stress and the progression of carotid artery disease. *Journal of Hypertension* 15: 49-55.

Bartels M, de Geus EJC, Kirschbaum C, Sluyter F, & Boomsma DI (2003). Heritability of daytime cortisol levels in children. *Behavior Genetics* 33: 421-433.

Barton C, March S, & Wittert GA (2002). The low dose dexamethasone suppression test: effect of time of administration and dose. *Journal of endocrinological investigation* 25: RC10-RC12.

Beck AT, Erbaugh J, Ward CH, Mock J, & Mendelsohn M (1961). An Inventory for Measuring Depression. *Archives of general psychiatry* 4: 561-&.

Ben Lamine S, Calabrese P, Perrault H, Dinh TP, Eberhard A, & Benchetrit G (2004). Individual differences in respiratory sinus arrhythmia. *American Journal of Physiology-Heart and Circulatory Physiology* 286: H2305-H2313.

Bernardi L & Sleight P (2006). Point: Counterpoint: Cardiovascular variability is/is not an index of autonomic control of circulation. *Journal of Applied Physiology* 101: 686.

Berntson GG, Bigger JT, Jr., Eckberg DL, Grossman P, Kaufmann PG, Malik M, Nagaraja HN, Porges SW, Saul JP, Stone PH, & van der Molen MW (1997). Heart rate variability: origins, methods, and interpretive caveats. *Psychophysiology* 34: 623-648.

Berntson GG, Cacioppo JT, Binkley PF, Uchino BN, Quigley KS, & Fieldstone A (1994). Autonomic cardiac control. III. Psychological stress and cardiac response in autonomic space as revealed by pharmacological blockades. *Psychophysiology* 31: 599-608.

Berntson GG, Cacioppo JT, & Fieldstone A (1996). Illusions, arithmetic, and the bidirectional modulation of vagal control of the heart. *Biological Psychology* 44: 1-17.

Berntson GG, Lozano DL, & Chen YJ (2005). Filter properties of root mean square successive difference (RMSSD) for heart rate. *Psychophysiology* 42: 246-252.

Betito K, Diorio J, Meaney MJ, & Boksa P (1992). Adrenal phenylethanolamine N-methyltransferase induction in relation to glucocorticoid receptor dynamics: evidence that acute exposure to high cortisol levels is sufficient to induce the enzyme. *Journal of neurochemistry* 58: 1853-1862.

Betito K, Mitchell JB, Bhatnagar S, Boksa P, & Meaney MJ (1994). Regulation of the adrenomedullary catecholaminergic system after mild, acute stress. *The American journal of physiology* 267: R212-R220.

Bhan AK & Scheuer J (1972). Effects of physical training on cardiac actomyosin adenosine triphosphatase activity. *The American journal of physiology* 223: 1486-1490.

Bigger JT, Jr., Fleiss JL, Rolnitzky LM, & Steinman RC (1992a). Stability over time of heart period variability in patients with previous myocardial infarction and ventricular arrhythmias. The CAPS and ESVEM investigators. *Am J Cardiol*. 69: 718-723.

Bigger JT, Jr., Fleiss JL, Rolnitzky LM, & Steinman RC (1993). Frequency domain measures of heart period variability to assess risk late after myocardial infarction. *J Am Coll.Cardiol*. 21: 729-736.

Bigger JT, Fleiss JL, Rolnitzky LM, & Steinman RC (1993). The Ability of Several Short-Term Measures of Rr Variability to Predict Mortality After Myocardial-Infarction. *Circulation* 88: 927-934.

Bigger JT, Jr., Fleiss JL, Steinman RC, Rolnitzky LM, Kleiger RE, & Rottman JN (1992b). Correlations among time and frequency domain measures of heart period variability two weeks after acute myocardial infarction. *Am J Cardiol*. 69: 891-898.

Billman GE (2002). Aerobic exercise conditioning: a nonpharmacological antiarrhythmic intervention. *Journal of Applied Physiology* 92: 446-454.

Billman GE (2006). Point: Counterpoint: Cardiovascular variability is/is not an index of autonomic control of circulation. *Journal of Applied Physiology* 101: 684-685.

Billman GE & Kukielka M (2006). Effects of endurance exercise training on heart rate variability and susceptibility to sudden cardiac death: protection is not due to enhanced cardiac vagal regulation. *Journal of Applied Physiology* 100: 896-906.

Bini G, Hagbarth KE, & Wallin BG (1981). Cardiac rhythmicity of skin sympathetic activity recorded from peripheral nerves in man. *Journal of the Autonomic Nervous System* 4: 17-24.

Bollen KA & Long JS (1993). *Testing structural equation models*. Newbury Park, CA: Sage.

Bonaduce D, Petretta M, Cavallaro V, Apicella C, Ianniciello A, Romano M, Breglio R, & Marciano F (1998). Intensive training and cardiac autonomic control in high level athletes. *Medicine and Science in Sports and Exercise* 30: 691-696.

Boomsma DI, Beem AL, van den Berg M, Dolan CV, Koopmans JR, Vink JM, de Geus EJ, & Slagboom PE (2000). Netherlands twin family study of anxious depression (NETSAD). *Twin.Res.* 3: 323-334.

Bouchard C & Rankinen T (2001). Individual differences in response to regular physical activity. *Medicine and Science in Sports and Exercise* 33: S446-S451.

Boucsein W (1992). *Electrodermal Activity*. New York: Plenum Press.

Boutcher SH & Stein P (1995). Association Between Heart-Rate-Variability and Training Response in Sedentary Middle-Aged Men. *European Journal of Applied Physiology and Occupational Physiology* 70: 75-80.

Brod, J, Fencl, V, Hejl, Z, Jirka, AJ, 1959. Circulatory changes underlying blood pressure elevation during acute emotional stress (mental arithmetic) in normotensive and hypertensive subjects. *Clinical Science* 18, 269-279.

Brodde OE, Bruck H, & Leineweber K (2006). Cardiac adrenoceptors: Physiological and pathophysiological relevance. *Journal of Pharmacological Sciences* 100: 323-337.

Broderick JE, Arnold D, Kudielka BM, & Kirschbaum C (2004). Salivary cortisol sampling compliance: comparison of patients and healthy volunteers. *Psychoneuroendocrinology* 29: 636-650.

Brotman DJ, Walker E, Lauer MS, & O'Brien RG (2005). In search of fewer independent risk factors. *Archives of Internal Medicine* 165: 138-145.

Browne MW, MacCallum RC, Kim CT, Andersen BL, & Glaser R (2002). When fit indices and residuals are incompatible. *Psychol.Methods* 7: 403-421.

Buchheit M, Simon C, Charloux A, Doutreleau S, Piquard F, & Brandenberger G (2005). Heart rate variability and intensity of habitual physical activity in middle-aged persons. *Medicine and Science in Sports and Exercise* 37: 1530-1534.

Burgess HJ, Penev PD, Schneider R, & Van Cauter E (2004). Estimating cardiac autonomic activity during sleep: impedance cardiography, spectral analysis, and Poincare plots. *Clinical Neurophysiology* 115: 19-28.

Burgess HJ, Trinder J, Kim Y, & Luke D (1997). Sleep and circadian influences on cardiac autonomic nervous system activity. *AJP - Heart and Circulatory Physiology* 273: 1761-1768.

Burleson MH, Poehlmann KM, Hawkley LC, Ernst JM, Berntson GG, Malarkey WB, Kiecolt-Glaser JK, Glaser R, & Cacioppo JT (2003). Neuroendocrine and cardiovascular reactivity to stress in mid-aged and older women: long-term temporal consistency of individual differences. *Psychophysiology* 40: 358-369.

Burnley M, Wilkerson DP, & Jones AM (2006). Point: Counterpoint: Cardiovascular variability is/is not an index of autonomic control of circulation. *Journal of Applied Physiology* 101: 683.

Burr RL (2007). Interpretation of normalized spectral heart rate variability indices in sleep research: A critical review. *Sleep* 30: 913-919.

Buss AH & Durkee A (1957). An inventory for assessing different kinds of hostility. *J Consult Psychol.* 21: 343-349.

Byrne EA & Porges SW (1993). Data-dependent filter characteristics of peak-valley respiratory sinus arrhythmia estimation: a cautionary note. *Psychophysiology* 30: 397-404.

Cacioppo JT, Berntson GG, Binkley PF, Quigley KS, Uchino BN, & Fieldstone A (1994a). Autonomic cardiac control. II. Noninvasive indices and basal response as revealed by autonomic blockades. *Psychophysiology* 31: 586-598.

Cacioppo JT, Uchino BN, & Berntson GG (1994b). Individual differences in the autonomic origins of heart rate reactivity: the psychometrics of respiratory sinus arrhythmia and preejection period. *Psychophysiology* 31: 412-419.

Carrasco GA & Van de Kar LD (2003). Neuroendocrine pharmacology of stress. *European Journal of Pharmacology* 463: 235-272.

Carroll D, Roy M (1989). Cardiovascular activity during prolonged menta arithmetic challenge: Shifts in the haemodynamic control of blood pressure. *Journal of Psychophysiology* 3, 403-408.

Carroll D, Smith GD, Willemsen G, Sheffield D, Sweetnam PM, Gallacher JEJ, & Elwood PC (1998). Blood pressure reactions to the cold pressor test and the prediction of ischaemic heart disease: data from the Caerphilly Study. *Journal of Epidemiology and Community Health* 52: 528-529.

Carter JB, Banister EW, & Blaber AP (2003). Effect of endurance exercise on autonomic control of heart rate. *Sports Medicine* 33: 33-46.

Cerutti S (2006). Point: Counterpoint: Cardiovascular variability is/is not an index of autonomic control of circulation. *Journal of Applied Physiology* 101: 685.

Chan GS, Middleton PM, Celler BG, Wang L, Lovell NH. (2007). Change in pulse transit time and pre-ejection period during head-up tilt-induced progressive central hypovolaemia. *J Clinical Monitoring Computing* 21(5):283-93.

Chrousos GP & Gold PW (1992). The concepts of stress and stress system disorders. Overview of physical and behavioral homeostasis. *JAMA: The Journal of the American Medical Association* 267: 1244-1252.

Cogliati C, Colombo S, Ruscone TG, Gruosso D, Porta A, Montano N, Malliani A, & Furlan R (2004). Acute beta-blockade increases muscle sympathetic activity and modifies its frequency distribution. *Circulation* 110: 2786-2791.

Cohen MA & Tan CO (2006). Point: Counterpoint: Cardiovascular variability is/is not an index of autonomic control of circulation. *Journal of Applied Physiology* 101: 686.

Cole MA, Kim PJ, Kalman BA, & Spencer RL (2000). Dexamethasone suppression of corticosteroid secretion: evaluation of the site of action by receptor measures and functional studies. *Psychoneuroendocrinology* 25: 151-167.

Collins S, Caron MG, & Lefkowitz RJ (1988). Beta-adrenergic receptors in hamster smooth muscle cells are transcriptionally regulated by glucocorticoids. *Journal of Biological Chemistry* 263: 9067-9070.

Cooke WH, Hoag JB, Crossman AA, Kuusela TA, Tahvanainen KUO, & Eckberg DL (1999). Human responses to upright tilt: a window on central autonomic integration. *Journal of Physiology-London* 517: 617-628.

Cordon-Cardo C, O'Brien JP, Casals D, Rittman-Grauer L, Biedler JL, Melamed MR, & Bertino JR (1989). Multidrug-resistance gene (P-glycoprotein) is expressed by endothelial cells at blood-brain barrier sites. *Proceedings of the National Academy of Sciences of the United States of America* 86: 695-698.

Coresh J, Klag MJ, Mead LA, Liang KY, & Whelton PK (1992). Vascular Reactivity in Young-Adults and Cardiovascular-Disease - A Prospective-Study. *Hypertension* 19: 218-223.

Costa P & McCrae R (1992). *Revised NEO Personality Inventory (NEO-PI-R) and NEO Fivefactor Inventory (NEO-FFI): Professional manual*. FL: Psychological Assessment Resources.: Odessa.

Crider A, Kremen WS, Xian H, Jacobson KC, Waterman B, Eisen SA, Tsuang MT, & Lyons MJ (2004). Stability, consistency, and heritability of electrodermal response lability in middle-aged male twins. *Psychophysiology* 41: 501-509.

Critchley HD (2002). Electrodermal Responses: What Happens in the Brain. *The Neuroscientist* 8: 132-142.

Cullinane EM, Sady SP, Vadeboncoeur L, Burke M, & Thompson PD (1986). Cardiac Size and VO2Max do Not Decrease After Short-Term Exercise Cessation. *Medicine and Science in Sports and Exercise* 18: 420-424.

Curtis BM & O'Keeffe JH (2002). Autonomic tone as a cardiovascular risk factor: The dangers of chronic fight or flight. *Mayo Clinic Proceedings* 77: 45-54.

Cybulski G (2000). Ambulatory impedance cardiography: new possibilities. *Journal of Applied Physiology* 88: 1509-1510.

Dailey JW & Westfall TC (1978). Effects of adrenalectomy and adrenal steroids on norepinephrine synthesis and monamine oxidase activity. *European Journal of Pharmacology* 48: 383-391.

Davis M (1992). The Role of the Amygdala in Fear and Anxiety. *Annual Review of Neuroscience* 15: 353-375.

Dawson ME, Schell AM, & Filion DL (2000). The Electrodermal System. In Cacioppo JT, Tassinary, LG, & Berntson, GG (Eds.). *Handbook of Psychophysiology*. New York: Cambridge University Press.

Debski TT, Zhang Y, Jennings JR, Kamarck TW (1993). Stability of cardiac impedance measures: aortic opening (B-point) detection and scoring. *Biological Psychology* 36, 63-74.

De Geus EJ, Posthuma D, Kupper N, van den Berg M, Willemsen G, Beem AL, Slagboom PE, & Boomsma DI (2005). A whole-genome scan for 24-hour respiration rate: a major locus at 10q26 influences respiration during sleep. *Am J Hum.Genet*. 76: 100-111.

De Geus EJ, van Doornen LJ, & Orlebeke JF (1993). Regular exercise and aerobic fitness in relation to psychological make-up and physiological stress reactivity. *Psychosomatic Medicine* 55: 347-363.

De Geus EJ, Willemsen GH, Klaver CH, & van Doornen LJ (1995). Ambulatory measurement of respiratory sinus arrhythmia and respiration rate. *Biol.Psychol*. 41: 205-227.

De Geus EJC, Karsdorp R, Boer B, de Regt G, Orlebeke JF, & van Doornen LJP (1996). Effect of aerobic fitness training on heart rate variability and cardiac baroreflex sensitivity. *Homeostasis* 37: 28-51.

De Geus EJC, Boomsma DI, & Snieder H (2003). Genetic correlation of exercise with heart rate and respiratory sinus arrhythmia. *Medicine and Science in Sports and Exercise* 35: 1287-1295.

De Geus EJC, Doornen LJP, Visser DC, & Orlebeke JF (1990). Existing and Training Induced Differences in Aerobic Fitness: Their Relationship to Physiological Response Patterns During Different Types of Stress. *Psychophysiology* 27: 457-477.

De Geus EJC, Kupper N, Boomsma DI, & Snieder H (2007). Bivariate Genetic Modeling of Cardiovascular Stress Reactivity: Does Stress Uncover Genetic Variance? *Psychosomatic Medicine* 69: 356-364.

De Kloet CS, Vermetten E, Geuze E, Kavelaars A, Heijnen CJ, & Westenberg HGM (2006). Assessment of HPA-axis function in posttraumatic stress disorder: Pharmacological and non-pharmacological challenge tests, a review. *Journal of Psychiatric Research* 40: 550-567.

De Kloet ER, Oitzl MS, & Joels M (1999). Stress and cognition: are corticosteroids good or bad guys? *Trends in neurosciences* 22: 422-426.

De Kloet ER, Reul JMHM, & Sutanto W (1990). Corticosteroids and the brain. *The Journal of steroid biochemistry and molecular biology* 37: 387-394.

De Kloet ER, van d, V, & de Wied D (1974). The site of the suppressive action of dexamethasone on pituitary-adrenal activity. *Endocrinology* 94: 61-73.

De Kloet ER, Vreugdenhil E, Oitzl MS, & Joels M (1998). Brain Corticosteroid Receptor Balance in Health and Disease. *Endocrine Reviews* 19: 269-301.

De Kloet R, Wallach G, & McEwen BS (1975). Differences in corticosterone and dexamethasone binding to rat brain and pituitary. *Endocrinology* 96: 598-609.

De Meersman RE (1993). Heart rate variability and aerobic fitness. *American Heart Journal* 125: 726-731.

Dekker JM, Schouten EG, Klootwijk P, Pool J, Swenne CA, & Kromhout D (1997). Heart rate variability from short electrocardiographic recordings predicts mortality from all causes in middle-aged and elderly men. The Zutphen Study. *Am J Epidemiol*. 145: 899-908.

Dekker JM, Crow RS, Folsom AR, Hannan PJ, Liao D, Swenne CA, & Schouten EG (2000). Low Heart Rate Variability in a 2-Minute Rhythm Strip Predicts Risk of Coronary Heart Disease and Mortality From Several Causes : The ARIC Study. *Circulation* 102: 1239-1244.

Deuschle M, Gotthardt U, Schweiger U, Weber B, Korner A, Schmider J, Standhardt H, Lammers CH, & Heuser I (1997). With aging in humans the activity of the hypothalamus-pituitary-adrenal system increases and its diurnal amplitude flattens. *Life Sciences* 61: 2239-2246.

Dewey FE, Freeman JV, Engel G, Oviedo R, Abrol N, Ahmed N, Myers J, & Froelicher VF (2007). Novel predictor of prognosis from exercise stress testing: Heart rate variability response to the exercise treadmill test. *American Heart Journal* 153: 281-288.

Dickerson SS & Kemeny ME (2004). Acute stressors and cortisol responses: a theoretical integration and synthesis of laboratory research. *Psychol.Bull*. 130: 355-391.

Dixon EM, Kamath MV, Mccartney N, & Fallen EL (1992). Neural Regulation of Heart-Rate-Variability in Endurance Athletes and Sedentary Controls. *Cardiovascular Research* 26: 713-719.

Doerr, BM., Miles, DS, Frey, MA (1981). Influence of respiration on stroke volume determined by impedance cardiography. *Aviation, Space, and Environmental Medicine* 52, 394-398.

Eckberg DL (2006). Point: Counterpoint: Cardiovascular variability is/is not an index of autonomic control of circulation. *Journal of Applied Physiology* 101: 688.

Eckberg DL (1997). Sympathovagal Balance : A Critical Appraisal. *Circulation* 96: 3224-3232.

Eckberg DL. (2003). The human respiratory gate. *Journal of Physiology*. 548: 339-52.

Edelberg R (1972). Electrodermal recovery rate, goal-orientation, and aversion. *Psychophysiology* 9: 512-520.

Eisenhofer G, Kopin IJ, & Goldstein DS (2004). Catecholamine metabolism: A contemporary view with implications for physiology and medicine. *Pharmacological Reviews* 56: 331-349.

Elstad M & Toska K (2006). Point: Counterpoint: Cardiovascular variability is/is not an index of autonomic control of circulation. *Journal of Applied Physiology* 101: 687.

Erickson K, Drevets W, & Schulkin J (2003). Glucocorticoid regulation of diverse cognitive functions in normal and pathological emotional states. *Neurosci.Biobehav.Rev* 27: 233-246.

Esler M (2000). The sympathetic system and hypertension. *American Journal of Hypertension* 13: 99S-105S.

Esler M, Jennings G, Korner P, Willett I, Dudley F, Hasking G, Anderson W, & Lambert G (1988). Assessment of Human Sympathetic Nervous-System Activity from Measurements of Norepinephrine Turnover. *Hypertension* 11: 3-20.

Esler M, Jennings G, Lambert G, Meredith I, Horne M, & Eisenhofer G (1990). Overflow of Catecholamine Neurotransmitters to the Circulation - Source, Fate, and Functions. *Physiological Reviews* 70: 963-985.

Evans JM (2006). Point: Counterpoint: Cardiovascular variability is/is not an index of autonomic control of circulation. *Journal of Applied Physiology* 101: 687-688.

Fagard RH & Cornelissen VA (2007). Effect of exercise on blood pressure control in hypertensive patients. *European Journal of Cardiovascular Prevention & Rehabilitation* 14: 12-17.

Fahrenberg J & Myrtek M (1996). *Ambulatory Assessment. Computer assisted Psychological and Psychophysiological methods in monitoring and field studies.* Seattle: Hogrefe & Huber Publishers.

Fahrenberg J & Myrtek M (2001). *Progress in ambulatroy assessment: Computer-Assisted Psychological and Psychophysiological Methods in Monitoring and Field Studies.* Seattle: Hogrefe & Huber Publishers.

Fallo F, Fanelli G, Cipolla A, Betterle C, Boscaro M, & Sonino N (1994). 24-hour blood pressure profile in Addison's disease. *American Journal of Hypertension* 7: 1105-1109.

Federenko I, Wust S, Hellhammer DH, Dechoux R, Kumsta R, & Kirschbaum C (2004). Free cortisol awakening responses are influenced by awakening time. *Psychoneuroendocrinology* 29: 174-184.

Feldman S & Weidenfeld J (2002). Further evidence for the central effect of dexamethasone at the hypothalamic level in the negative feedback mechanism. *Brain research* 958: 291-296.

Foster KG & Weiner JS (1970). Effects of Cholinergic and Adrenergic Blocking Agents on Activity of Eccrine Sweat Glands. *Journal of Physiology-London* 210: 883-895.

Fowles DC (1986). The Eccrine System and Electrodermal Activity. In Coles MGH, Donchin, E, & Porges, SW (Eds.). *Psychophysiology: Systems Processes and Applications.* New York: The Guilford Press.

Fowles DC, Christie MJ, Edelberg R, Grings WW, Lykken DT, & Venables PH (1981). Committee report. Publication recommendations for electrodermal measurements. *Psychophysiology* 18: 232-239.

Fox K, Borer JS, Camm AJ, Danchin N, Ferrari R, Sendon JLL, Steg PG, Tardif JC, Tavazzi L, & Tendera M (2007). Resting heart rate in cardiovascular disease. *Journal of the American College of Cardiology* 50: 823-830.

Franchimont D, Kino T, Galon J, Meduri GU, & Chrousos G (2002). Glucocorticoids and inflammation revisited: The state of the art. *Neuroimmunomodulation* 10: 247-260.

Freixa i Baque E (1982). Reliability of electrodermal measures: a compilation. *Biological Psychology* 14: 219-229.

Freedman RR. (1989). Laboratory and ambulatory monitoring of menopausal hot flashes. *Psychophysiology* 26(5):573-9.

Frey MAB & Kenney RA (1979). Systolic-Time Intervals During Combined Hand Cooling and Head-Up Tilt. *Aviation Space and Environmental Medicine* 50: 218-222.

Fritz I & Levine R (1951). Action of adrenal cortical steroids and nor-epinephrine on vascular responses of stress in adrenalectomized rats. *The American journal of physiology* 165: 456-465.

Furlan R, Ardizzone S, Palazzolo L, Rimoldi A, Perego F, Barbic F, Bevilacqua M, Vago L, Porro GB, & Malliani A (2006). Sympathetic overactivity in active ulcerative colitis: effects of clonidine. *American Journal of Physiology-Regulatory Integrative and Comparative Physiology* 290: R224-R232.

Furlan R, Colombo S, Perego F, Atzeni F, Diana A, Barbic F, Porta A, Pace F, Malliani A, & Sarzi-Puttini P (2005). Abnormalities of cardiovascular neural control and reduced orthostatic tolerance in patients with primary fibromyalgia. *Journal of Rheumatology* 32: 1787-1793.

Furlan R, Porta A, Costa F, Tank J, Baker L, Schiavi R, Robertson D, Malliani A, & Mosqueda-Garcia R (2000). Oscillatory patterns in sympathetic neural discharge

and cardiovascular variables during orthostatic stimulus. *Circulation* 101: 886-892.

Gamelin FX, Berthoin S, Sayah H, Libersa C, & Bosquet L (2007). Effect of Training and Detraining on Heart Rate Variability in Healthy Young Men. *International Journal of Sports Medicine* 564-570.

Gerin W, Pickering TG, Glynn L, Christenfeld N, Schwartz A, Carroll D, & Davidson K (2000). An historical context for behavioral models of hypertension. *Journal of Psychosomatic Research* 48: 369-377.

Gerin W, Rosofsky M, Pieper C, & Pickering TG (1994). A test of generalizability of cardiovascular reactivity using a controlled ambulatory procedure. *Psychosomatic Medicine* 56: 360-368.

Gibson A (1981). The influence of endocrine hormones on the autonomic nervous system. *Journal of autonomic pharmacology* 1: 331-358.

Girdler SS, Pedersen CA, Stern RA, & Light KC (1993). Menstrual cycle and premenstrual syndrome: modifiers of cardiovascular reactivity in women. *Health Psychology* 12: 180-192.

Girdler SS, Turner JR, Sherwood A, Light KC (1990). Gender differences in blood pressure control during a variety of behavioral stressors. *Psychosomatic Medicine* 52, 571-591.

Goedhart AD, Kupper N, Willemsen G, Boomsma DI, & de Geus EJC (2006). Temporal stability of ambulatory stroke volume and cardiac output measured by impedance cardiography. *Biological Psychology* 72: 110-117.

Goedhart AD, van der Sluis S, Houtveen JH, Willemsen G, & de Geus EJC (2007). Comparison of time and frequency domain measures of RSA in ambulatory recordings. *Psychophysiology* 44: 203-215.

Goldsmith RL, Bigger JT, Bloomfield DM, & Steinman RC (1997). Physical fitness as a determinant of vagal modulation. *Medicine and Science in Sports and Exercise* 29: 812-817.

Goldsmith RL, Bigger JT, Steinman RC, & Fleiss JL (1992). Comparison of 24-Hour Parasympathetic Activity in Endurance-Trained and Untrained Young Men. *Journal of the American College of Cardiology* 20: 552-558.

Goldsmith RL, Bloomfield DM, & Rosenwinkel ET (2000). Exercise and autonomic function. *Coronary Artery Disease* 11: 129-135.

Goldstein DS (1995). Clinical assessment of sympathetic responses to stress. *Stress* 771: 570-593.

Goldstein DS, Grossman E, Armando I, Wolfovitz E, Folio CJ, Holmes C, & Keiser HR (1993). Correlates of Urinary-Excretion of Catechols in Humans. *Biogenic Amines* 10: 3-17.

Goldstein DS, Mccarty R, Polinsky RJ, & Kopin IJ (1983). Relationship Between Plasma Norepinephrine and Sympathetic Neural Activity. *Hypertension* 5: 552-559.

Goldstein IB, Shapiro D, & Guthrie D (2006). Ambulatory blood pressure and family history of hypertension in healthy men and women. *Am J Hypertens.* 19: 486-491.

Goodyer IM, Herbert J, Altham PME, Pearson J, Secher SM, & Shiers HM (1996). Adrenal secretion during major depression in 8- to 16-year-olds .1. Altered diurnal rhythms in salivary cortisol and dehydroepiandrosterone (DHEA) at presentation. *Psychological Medicine* 26: 245-256.

Grassi G & Esler M (1999). How to assess sympathetic activity in humans. *Journal of Hypertension* 17: 719-734.

Grossman P (2004). The LifeShirt: a multi-function ambulatory system monitoring health, disease, and medical intervention in the real world. *Stud.Health Technol.Inform.* 108: 133-141.

Grossman P, Karemaker J, & Wieling W (1991). Prediction of tonic parasympathetic cardiac control using respiratory sinus arrhythmia: the need for respiratory control. *Psychophysiology* 28: 201-216.

Grossman P & Kollai M (1993). Respiratory sinus arrhythmia, cardiac vagal tone, and respiration: within- and between-individual relations. *Psychophysiology* 30: 486-495.

Grossman P, van Beek J, & Wientjes C (1990). A comparison of three quantification methods for estimation of respiratory sinus arrhythmia. *Psychophysiology* 27: 702-714.

Grossman P, Wilhelm FH, & Spoerle M (2004). Respiratory sinus arrhythmia, cardiac vagal control, and daily activity. *AJP - Heart and Circulatory Physiology* 287: H728-H734.

Grossman P (1992). Respiratory and cardiac rhythms as windows to central and autonomic biobehavioral regulation: Selection of window frames, keeping the panes clean and viewing the neural topography. *Biological Psychology* 34: 131-161.

Grunfeld JP & Eloy L (1987). Glucocorticoids modulate vascular reactivity in the rat. *Hypertension* 10: 608-618.

Guasti L, Simoni C, Mainardi L, Crespi C, Cimpanelli M, Klersy C, Gaudio G, Grandi AM, Cerutti S, & Venco A (2005). Global link between heart rate and blood pressure oscillations at rest and during mental arousal in normotensive and hypertensive subjects. *Autonomic Neuroscience-Basic & Clinical* 120: 80-87.

Gutin B, Barbeau P, Litaker MS, Ferguson M, & Owens S (2000). Heart rate variability in obese children: Relations to total body and visceral adiposity, and changes with physical training and detraining. *Obesity Research* 8: 12-19.

Gutin B, Howe CA, Johnson MH, Humphries MC, Snieder H, & Barbeau P (2005). Heart rate variability in adolescents: Relations to physical activity, fitness, and adiposity. *Medicine and Science in Sports and Exercise* 37: 1856-1863.

Hadase M, Azuma A, Zen K, Asada S, Kawasaki T, Kamitani T, Kawasaki S, Sugihara H, & Matsubara H (2004). Very low frequency power of heart rate variability is a powerful predictor of clinical prognosis in patients with congestive heart failure. *Circ J* 68: 343-347.

Hagbarth KE, Hallin RG, Wallin BG, Torebjork H, & Hongell A (1972). General Characteristics of Sympathetic Activity in Human Skin Nerves. *Acta Physiologica Scandinavica* 84: 164-&.

Haigh RM & Jones CT (1990). Effect of glucocorticoids on alpha 1-adrenergic receptor binding in rat vascular smooth muscle. *Journal of molecular endocrinology* 5: 41-48.

Haigh RM, Jones CT, & Milligan G (1990). Glucocorticoids regulate the amount of G proteins in rat aorta. *Journal of molecular endocrinology* 5: 185-188.

Handa M, Kondo K, Suzuki H, & Saruta T (1984). Dexamethasone hypertension in rats: role of prostaglandins and pressor sensitivity to norepinephrine. *Hypertension* 6: 236-241.

Harms MP, Wesseling KH, Pott F, Jenstrup M, van Goudoever J, Secher NH, van Lieshout JJ (1999). Continuous stroke volume monitoring by modelling flow from non-invasive measurement of arterial pressure in humans under orthostatic stress. Clinical Science 97, 291-301.

Harris B, Watkins S, Cook N, Walker RF, Read GF, & Riadfahmy D (1990). Comparisons of Plasma and Salivary Cortisol Determinations for the Diagnostic Efficacy of the Dexamethasone Suppression Test. *Biological psychiatry* 27: 897-904.

Harris WS, Schoenfeld CD, & Weissler AM (1967). Effects of adrenergic receptor activation and blockade on the systolic preejection period, heart rate, and arterial pressure in man. *J.Clin Invest* 46: 1704-1714.

Hatfield BD, Spalding TW, Santa Maria DL, Porges SW, Potts JT, Byrne EA, Brody EB, & Mahon AD (1998). Respiratory sinus arrhythmia during exercise in aerobically trained and untrained men. *Medicine and Science in Sports and Exercise* 30: 206-214.

Hautala AJ, Makikallio TH, Kiviniemi A, Laukkanen RT, Nissila S, Huikuri HV, & Tulppo MP (2003). Cardiovascular autonomic function correlates with the response to aerobic training in healthy sedentary subjects. *American Journal of Physiology-Heart and Circulatory Physiology* 285: H1747-H1752.

Hayano J, Yamada M, Sakakibara Y, Fujinami T, Yokoyama K, Watanabe Y, & Takata K (1990). Short-Term and Long-Term Effects of Cigarette-Smoking on Heart-Rate-Variability. *American Journal of Cardiology* 65: 84-88.

Hayano J, Sakakibara Y, Yamada A, Yamada M, Mukai S, Fujinami T, Yokoyama K, Watanabe Y, & Takata K (1991). Accuracy of assessment of cardiac vagal tone by heart rate variability in normal subjects. *The American Journal of Cardiology* 67: 199-204.

Herman JP & Cullinan WE (1997). Neurocircuitry of stress: central control of the hypothalamo-pituitary-adrenocortical axis. *Trends in neurosciences* 20: 78-84.

Hjemdahl P (1990). Physiology of the autonomic nervous system as related to cardio vascular function: implications for stress research. In Byrne DG & Roseman, RH (Eds.). *Anxiety and the heart*. New York: Hemisphere Publishing.

Hjemdahl P (1993). Plasma-Catecholamines - Analytical Challenges and Physiological Limitations. *Baillieres Clinical Endocrinology and Metabolism* 7: 307-353.

Hohnloser SH, Klingenheben T, Zabel M, Schroder F, & Just H (1992). Intraindividual reproducibility of heart rate variability. *Pacing Clin.Electrophysiol.* 15: 2211-2214.

Holsboer F (2000). The corticosteroid receptor hypothesis of depression. *Neuropsychopharmacology* 23: 477-501.

Hopf HB, Skyschally A, Heusch G, & Peters J (1995). Low-Frequency Spectral Power of Heart-Rate-Variability Is Not A Specific Marker of Cardiac Sympathetic Modulation. *Anesthesiology* 82: 609-619.

Hoshikawa Y & Yamamoto Y (1997). Effects of Stroop color-word conflict test on the autonomic nervous system responses. *American Journal of Physiology-Heart and Circulatory Physiology* 41: H1113-H1121.

Hottenga JJ, Boomsma DI, Kupper N, Posthuma D, Snieder H, Willemsen G, & de Geus EJC (2005). Heritability and stability of resting blood pressure. *Twin Research and Human Genetics* 8: 499-508.

Houtveen JH, Groot PFC, & de Geus EJC (2005). Effects of variation in posture and respiration on RSA and pre-ejection period. *Psychophysiology* 42: 713-719.

Houtveen JH, Groot PF, de Geus EJ. (2006). Validation of the thoracic impedance derived respiratory signal using multilevel analysis. *Int J Psychophysiology*. 59(2):97-106.

Houtveen JH & Molenaar PC (2001). Comparison between the Fourier and Wavelet methods of spectral analysis applied to stationary and nonstationary heart period data. *Psychophysiology* 38: 729-735.

Houtveen JH, Rietveld S, & de Geus EJ (2002). Contribution of tonic vagal modulation of heart rate, central respiratory drive, respiratory depth, and respiratory frequency to respiratory sinus arrhythmia during mental stress and physical exercise. *Psychophysiology* 39: 427-436.

Houtveen JH, van Doornen LJ. (2007). Medically unexplained symptoms and between-group differences in 24-h ambulatory recording of stress physiology. *Biological Psychology* 76(3):239-49.

Hull SS, Jr., Evans AR, Vanoli E, Adamson PB, Stramba-Badiale M, Albert DE, Foreman RD, & Schwartz PJ (1990). Heart rate variability before and after myocardial

infarction in conscious dogs at high and low risk of sudden death. *J Am Coll.Cardiol*. 16: 978-985.

Iacono WG, Peloquin LJ, Lykken DT, Haroian KP, Valentine RH, & Tuason VB (1984). Electrodermal Activity in Euthymic Patients with Affective-Disorders - One-Year Retest Stability and the Effects of Stimulus-Intensity and Significance. *Journal of Abnormal Psychology* 93: 304-311.

Ice GH, Katz-Stein A, Himes J, & Kane RL (2004). Diurnal cycles of salivary cortisol in older adults. *Psychoneuroendocrinology* 29: 355-370.

Imaki T, Xiao-Quan W, Shibasaki T, Yamada K, Harada S, Chikada N, Naruse M, & Demura H (1995). Stress-induced activation of neuronal activity and corticotropin-releasing factor gene expression in the paraventricular nucleus is modulated by glucocorticoids in rats. *The Journal of clinical investigation* 96: 231-238.

Iwasaki Ki, Zhang R, Zuckerman JH, & Levine BD (2003). Dose-response relationship of the cardiovascular adaptation to endurance training in healthy adults: how much training for what benefit? *Journal of Applied Physiology* 95: 1575-1583.

Jacobs SC, Friedman R, Parker JD, Tofler GH, Jimenez AH, Muller JE, Benson H, & Stone PH (1994). Use of skin conductance changes during mental stress testing as an index of autonomic arousal in cardiovascular research. *American Heart Journal* 128: 1170-1177.

Jazayeri A & Meyer WJ (1988). Glucocorticoid modulation of beta-adrenergic receptors of cultured rat arterial smooth muscle cells. *Hypertension* 12: 393-398.

Jokkel G, Bonyhay I, & Kollai M (1995). Heart rate variability after complete autonomic blockade in man. *Journal of the Autonomic Nervous System* 51: 85-89.

Julien C (2006). Point: Counterpoint: Cardiovascular variability is/is not an index of autonomic control of circulation. *Journal of Applied Physiology* 101: 684.

Kamarck TW, Jennings JR, Debski TT, Glickman-Weiss E, Johnson PS, Eddy MJ, Manuck SB (1992). Reliable measures of behaviorally-evoked cardiovascular reactivity from a PC-based test battery: results from student and community samples. *Psychophysiology* 29, 17-28.

Kamarck TW, Jennings JR, Stewart CJ, Eddy MJ (1993). Reliable responses to a cardiovascular reactivity protocol: a replication study in a biracial female sample. *Psychophysiology* 30, 627-634.

Kamarck TW & Lovallo WR (2003). Cardiovascular Reactivity to Psychological Challenge: Conceptual and Measurement Considerations. *Psychosomatic Medicine* 65: 9-21.

Kamarck TW, Schwartz JE, Janicki DL, Shiffman S, & Raynor DA (2003). Correspondence between laboratory and ambulatory measures of cardiovascular reactivity: a multilevel modeling approach. *Psychophysiology* 40: 675-683.

Kamiya A, Hayano J, Kawada T, Michikami D, Yamamoto K, Ariumi H, Shimizu S, Uemura K, Miyamoto T, Aiba T, Sunagawa K, & Sugimachi M (2005). Low-frequency oscillation of sympathetic nerve activity decreases during development of tilt-induced syncope preceding sympathetic withdrawal and bradycardia. *American Journal of Physiology-Heart and Circulatory Physiology* 289: H1758-H1769.

Kamphuis A & Frowein HW (1985). Assessment of mental effort by means of heart rate spectral analysis. In Orlebeke JF, van Doornen, LJP, & Mulder, G (Eds.). *The Psychophysiology of Cardiovascular Control*. New York: Plenum Press.

Karssen AM, Meijer OC, Berry A, Pinol RS, & de Kloet ER (2005). Low doses of dexamethasone can produce a hypocorticosteroid state in the brain. *Endocrinology* 146: 5587-5595.

Karssen AM, Meijer OC, van dS, I, de Boer AG, de Lange EC, & de Kloet ER (2002). The role of the efflux transporter P-glycoprotein in brain penetration of prednisolone. *J Endocrinol* 175: 251-260.

Kasprowicz AL, Manuck SB, Malkoff SB, & Krantz DS (1990). Individual differences in behaviorally evoked cardiovascular response: temporal stability and hemodynamic patterning. *Psychophysiology* 27: 605-619.

Katona PG & Jih F (1975). Respiratory sinus arrhythmia: noninvasive measure of parasympathetic cardiac control. *J Appl.Physiol* 39: 801-805.

Katona PG, Mclean M, Dighton DH, & Guz A (1982). Sympathetic and Parasympathetic Cardiac Control in Athletes and Non-Athletes at Rest. *Journal of Applied Physiology* 52: 1652-1657.

Kelly JJ, Mangos G, Williamson PM, & Whitworth JA (1998). Cortisol and hypertension. *Clinical and Experimental Pharmacology and Physiology* 25: S51-S56.

Kelsey RM (1991). Electrodermal Lability and Myocardial Reactivity to Stress. *Psychophysiology* 28: 619-631.

Kennedy B & Ziegler MG (1991). Cardiac epinephrine synthesis. Regulation by a glucocorticoid. *Circulation* 84: 891-895.

Kenney WL (1985). Parasympathetic Control of Resting Heart-Rate - Relationship to Aerobic Power. *Medicine and Science in Sports and Exercise* 17: 451-455.

Kingwell BA, Dart AM, Jennings GL, & Korner PI (1992). Exercise Training Reduces the Sympathetic Component of the Blood-Pressure Heart-Rate Baroreflex in Man. *Clinical Science* 82: 357-362.

Kingwell BA, Thompson JM, Kaye DM, Mcpherson GA, Jennings GL, & Esler MD (1994). Heart-Rate Spectral-Analysis, Cardiac Norepinephrine Spillover, and Muscle Sympathetic-Nerve Activity During Human Sympathetic Nervous Activation and Failure. *Circulation* 90: 234-240.

Kirschbaum C & Hellhammer DH (1994). Salivary cortisol in psychoneuroendocrine research: Recent developments and applications. *Psychoneuroendocrinology* 19: 313-333.

Kjaer M, Secher NH, & Galbo H (1987). Physical Stress and Catecholamine Release. *Baillieres Clinical Endocrinology and Metabolism* 1: 279-298.

Kleiger RE, Bigger JT, Bosner MS, Chung MK, Cook JR, Rolnitzky LM, Steinman R, & Fleiss JL (1991). Stability over time of variables measuring heart rate variability in normal subjects. *Am J Cardiol*. 68: 626-630.

Kline KA, Saab PG, Llabre MM, Spitzer SB, Evans JD, McDonald PA, Schneiderman N (2002). Hemodynamic response patterns: responder type differences in reactivity and recovery. *Psychophysiology* 39, 739-746

Knutsson U, Dahlgren J, Marcus C, Rosberg S, Bronnegard M, Stierna P, & AlbertssonWikland K (1997). Circadian cortisol rhythms in healthy boys and girls: Relationship with age, growth, body composition, and pubertal development. *Journal of Clinical Endocrinology and Metabolism* 82: 536-540.

Koh J, Brown TE, Beightol LA, Ha CY, & Eckberg DL (1994). Human Autonomic Rhythms - Vagal Cardiac Mechanisms in Tetraplegic Subjects. *Journal of Physiology-London* 474: 483-495.

Kollai M & Mizsei G (1990). Respiratory Sinus Arrhythmia Is A Limited Measure of Cardiac Parasympathetic Control in Man. *Journal of Physiology-London* 424: 329-342.

Kovacs KJ & Makara GB (1988). Corticosterone and dexamethasone act at different brain sites to inhibit adrenalectomy-induced adrenocorticotropin hypersecretion. *Brain research* 474: 205-210.

Kovacs KJ & Mezey E (1987). Dexamethasone inhibits corticotropin-releasing factor gene expression in the rat paraventricular nucleus. *Neuroendocrinology* 46: 365-368.

Kreft I & de Leeuw J (1998). *Introducing Multilevel Modeling*. London: Sage.

Kronholm E, Hyyppa MT, Jula A, & Toikka T (1996). Electrodermal lability and hypertension. *International Journal of Psychophysiology* 23: 129-136.

Krzeminski K, Kruk B, Nazar K, Ziemba AW, Cybulski G, & Niewiadomski W (2000). Cardiovascular, metabolic and plasma catecholamine responses to passive and active exercises. *J.Physiol Pharmacol*. 51: 267-278.

Kubicek WG, Karnegis JN, Patterson RP, Witsoe DA, Mattson RH (1966). Development and evaluation of an impedance cardiac output system. *Aerospace Medicine* 37, 1208-1212.

Kudielka BM, Broderick JE, & Kirschbaum C (2003). Compliance with saliva sampling protocols: Electronic monitoring reveals invalid cortisol daytime profiles in noncompliant subjects. *Psychosomatic Medicine* 65: 313-319.

Kudielka BM & Kirschbaum C (2003). Awakening cortisol responses are influenced by health status and awakening time but not by menstrual cycle phase. *Psychoneuroendocrinology* 28: 35-47.

Kunz-Ebrecht SR, Kirschbaum C, Marmot M, & Steptoe A (2004). Differences in cortisol awakening response on work days and weekends in women and men from the Whitehall II cohort. *Psychoneuroendocrinology* 29: 516-528.

Kupper N, de Geus EJC, van den Berg M, Kirschbaum C, Boomsma DI, & Willemsen G (2005a). Familial influences on basal salivary cortisol in an adult population. *Psychoneuroendocrinology* 30: 857-868.

Kupper N, Willemsen G, Boomsma DI, & de Geus EJC (2006). Heritability of indices for cardiac contractility in ambulatory recordings. *Journal of Cardiovascular Electrophysiology* 17: 877-883.

Kupper N, Willemsen G, Posthuma D, de Boer P, Boomsma DI, & de Geus EJC (2005b). A genetic analysis of ambulatory cardiorespiratory coupling. *Psychophysiology* 42: 202-212.

Kupper NH, Willemsen G, van den Berg M, de Boer D, Posthuma D, Boomsma DI, & de Geus EJ (2004). Heritability of ambulatory heart rate variability. *Circulation* 110: 2792-2796.

La Rovere MT, Pinna GD, Maestri R, Mortara A, Capomolla S, Febo O, Ferrari R, Franchini M, Gnemmi M, Opasich C, Riccardi PG, Traversi E, & Cobelli F (2003). Short-Term Heart Rate Variability Strongly Predicts Sudden Cardiac Death in Chronic Heart Failure Patients. *Circulation* 107: 565-570.

Lacey JI & Lacey BC (1958). Verification and Extension of the Principle of Autonomic Response-Stereotypy. *American Journal of Psychology* 71: 50-73.

Lander E & Kruglyak L (1995). Genetic Dissection of Complex Traits - Guidelines for Interpreting and Reporting Linkage Results. *Nature Genetics* 11: 241-247.

Langewitz W & Ruddel H (1989). Spectral analysis of heart rate variability under mental stress. *J Hypertens.Suppl* 7: S32-S33.

Laszlo Z, Rossler A, & Hinghofer-Szalkay HG (2001). Cardiovascular and hormonal changes with different angles of head-up tilt in men. *Physiological Research* 50: 71-82.

Laude D (2006). Point: Counterpoint: Cardiovascular variability is/is not an index of autonomic control of circulation. *Journal of Applied Physiology* 101: 687.

Lawler KA, Kline KA, Adlin RF, Wilcox ZC, Craig FW, Krishnamoorthy JS, Piferi RL (2001). Psychophysiological correlates of individual differences in patterns of hemodynamic reactivity. *International Journal of Psychophysiology* 40, 93-107.

Lechin, F, van der, DB, Lechin, AE (2004). Autonomic nervous system assessment throughout the wake-sleep cycle and stress. *Psychosomatic Medicine* 66, 974-976.

Levi GF, Ratti S, Cardone G, & Basagni M (1982). On the Reliability of Systolic-Time Intervals. *Cardiology* 69: 157-165.

Levy MN & Schwartz PJ (1994). *Vagal Control of the Heart: Experimental Basis and Clinical Implications*. Armonk, NY: Futura Publishing Co.

Lewis RP, Rittgers SE, Forester WF, & Boudoulas H (1977). Critical-Review of Systolic-Time Intervals. *Circulation* 56: 146-158.

Lewis SF, Nylander E, Gad P, & Areskog NH (1980). Non-Autonomic Component in Bradycardia of Endurance Trained Men at Rest and During Exercise. *Acta Physiologica Scandinavica* 109: 297-305.

Liggett S (1995). Functional properties of human beta(2)-adrenergic receptor polymorphisms. *News in Physiological Sciences* 10: 265-273.

Light KC, Kothandapani RV, & Allen MT (1998). Enhanced cardiovascular and catecholamine responses in women with depressive symptoms. *International Journal of Psychophysiology* 28: 157-166.

Light KC, Obrist PA, James SA, & Strogatz DS (1987). Cardiovascular-Responses to Stress .2. Relationships to Aerobic Exercise Patterns. *Psychophysiology* 24: 79-86.

Lin YC & Horvath SM (1972). Autonomic Nervous Control of Cardiac Frequency in Exercise-Trained Rat. *Journal of Applied Physiology* 33: 796-&.

Liu J, Haigh RM, & Jones CT (1992). Enhancement of noradrenaline-induced inositol polyphosphate formation by glucocorticoids in rat vascular smooth muscle cells. *The Journal of endocrinology* 133: 405-411.

Llabre MM, Saab PG, Hurwitz BE, Schneiderman N, Frame CA, Spitzer S, & Phillips D (1993). The stability of cardiovascular parameters under different behavioral challenges: one-year follow-up. *International Journal of Psychophysiology* 14: 241-248.

Loimaala A, Huikuri H, Oja P, Pasanen M, & Vuori I (2000). Controlled 5-mo aerobic training improves heart rate but not heart rate variability or baroreflex sensitivity. *Journal of Applied Physiology* 89: 1825-1829.

Lombardi F, Sandrone G, Pernpruner S, Sala R, Garimoldi M, Cerutti S, Baselli G, Pagani M, & Malliani A (1987). Heart rate variability as an index of sympathovagal interaction after acute myocardial infarction. *Am J Cardiol.* 60: 1239-1245.

Lovallo WR (2005). *Stress and Health: Biological and Psychological Interactions (2nd ed)*. Thousand Oaks, CA: Sage.

Lovallo WR & Gerin W (2003). Psychophysiological reactivity: mechanisms and pathways to cardiovascular disease. *Psychosomatic Medicine* 65: 36-45.

Lovallo WR, Pincomb GA, Sung BH, & Wilson MF (1993). Impedance cardiography used to assess patterns of cardiovascular response to behavioral stressors. *Biol.Psychol.* 36: 97-105.

Lovas K & Husebye ES (2003). Replacement therapy in Addison's disease. *Expert opinion on pharmacotherapy* 4: 2145-2149.

Lozano DL, Norman G, Knox D, Wood BL, Miller BD, Emery CF, & Berntson GG (2007). Where to B in dZ/dt. *Psychophysiology* 44: 113-119.

Lucini D, Norbiato G, Clerici M, & Pagani M (2002). Hemodynamic and Autonomic Adjustments to Real Life Stress Conditions in Humans. *Hypertension* 39: 184-188.

Lykken DT, Iacono WG, Haroian K, Mcgue M, & Bouchard TJ (1988). Habituation of the Skin-Conductance Response to Strong Stimuli - A Twin Study. *Psychophysiology* 25: 4-15.

Malik M & Eckberg DL (1998). Sympathovagal Balance: A Critical Appraisal ò Response. *Circulation* 98: 2643-2644.

Malliani A (2006). Point: Counterpoint: Cardiovascular variability is/is not an index of autonomic control of circulation. *Journal of Applied Physiology* 101: 684.

Malliani A, Pagani M, Lombardi F, & Cerutti S (1991). Cardiovascular Neural Regulation Explored in the Frequency-Domain. *Circulation* 84: 482-492.

Malliani A, Pagani M, Montano N, & Mela GS (1998). Sympathovagal Balance: A Reappraisal. *Circulation* 98: 2640a-2643.

Mallion JM, Baguet JP, Siche JP, Tremel F, & de Gaudemaris R (1999). Clinical value of ambulatory blood pressure monitoring. *Journal of Hypertension* 17: 585-595.

Mantero F & Boscaro M (1992). Glucocorticoid-dependent hypertension. *The Journal of steroid biochemistry and molecular biology* 43: 409-413.

Marik PE & Zaloga GP (2002). Adrenal Insufficiency in the Critically Ill: A New Look at an Old Problem. *Chest* 122: 1784-1796.

Martinmaki K, Rusko H, Kooistra L, Kettunen J, & Saalasti S (2006). Intraindividual validation of heart rate variability indexes to measure vagal effects on hearts. *AJP - Heart and Circulatory Physiology* 290: H640-H647.

Martinsson A, Melcher A, Lindvall K, & Hjemdahl P (1991). Comparison Between Isoprenaline Infusions and Bolus Injections to Assess Beta-Adrenoceptor Function in Man, with Special Reference to Cardiac Contractility and the Influence of Autonomic Reflexes. *Acta Physiologica Scandinavica* 141: 167-180.

Matthews KA, Gump BB, & Owens JF (2001). Chronic stress influences cardiovascular and neuroendocrine responses during acute stress and recovery, especially in men. *Health Psychology* 20: 403-410.

Matthews KA, Salomon K, Kenyon K, & Allen MT (2002). Stability of children's and adolescents' hemodynamic responses to psychological challenge: A three-year longitudinal study of a multiethnic cohort of boys and girls. *Psychophysiology* 39: 826-834.

McCaffery JM, Pogue-Geile MF, Ferrell RE, Petro N, & Manuck SB (2002). Variability within alpha- and beta-adrenoreceptor genes as a predictor of cardiovascular function at rest and in response to mental challenge. *Journal of Hypertension* 20: 1105-1114.

McGrath JJ, O'Brien WH (2001). Pediatric impedance cardiography: temporal stability and intertask consistency. *Psychophysiology* 38, 479-484.

Meijer OC, de Lange EC, Breimer DD, de Boer AG, Workel JO, & de Kloet ER (1998). Penetration of dexamethasone into brain glucocorticoid targets is enhanced in mdr1A P-glycoprotein knockout mice. *Endocrinology* 139: 1789-1793.

Meredith IT, Friberg P, Jennings GL, Dewar EM, Fazio VA, Lambert GW, & Esler MD (1991). Exercise Training Lowers Resting Renal But Not Cardiac Sympathetic Activity in Humans. *Hypertension* 18: 575-582.

Mezzacappa ES, Kelsey RM, & Katkin ES (1999). The effects of epinephrine administration on impedance cardiographic measures of cardiovascular function. *International Journal of Psychophysiology* 31: 189-196.

Miller SB, Ditto B (1989). Individual differences in heart rate and peripheral vascular responses to an extended aversive task. *Psychophysiology* 26, 506-513.

Miller SB, Ditto B (1991). Exaggerated sympathetic nervous system response to extended psychological stress in offspring of hypertensives. *Psychophysiology* 28, 103-113.

Miller AH, Spencer RL, Pulera M, Kang S, McEwen BS, & Stein M (1992). Adrenal steroid receptor activation in rat brain and pituitary following dexamethasone: implications for the dexamethasone suppression test. *Biological psychiatry* 32: 850-869.

Miyamoto Y, Higuchi J, Abe Y, Hiura T, Nakazono Y, & Mikami T (1983). Dynamics of cardiac output and systolic time intervals in supine and upright exercise. *J.Appl.Physiol* 55: 1674-1681.

Mohapatra SN (1981). *Non-invasive cardiovascular monitoring by electrical impedance technique*. London: Pittman Medical.

Montano N, Ruscone TG, Porta A, Lombardi F, Pagani M, & Malliani A (1994). Power Spectrum Analysis of Heart-Rate-Variability to Assess the Changes in Sympathovagal Balance During Graded Orthostatic Tilt. *Circulation* 90: 1826-1831.

Mueller PJ (2007). Exercise training and sympathetic nervous system activity: Evidence for physical activity dependent neural plasticity. *Clinical and Experimental Pharmacology and Physiology* 34: 377-384.

Mujika I & Padilla S (2000). Detraining: Loss of training-induced physiological and performance adaptations. Part I Short term insufficient training stimulus. *Sports Medicine* 30: 79-87.

Mujika I & Padilla S (2001). Cardiorespiratory and metabolic characteristics of detraining in humans. *Medicine and Science in Sports and Exercise* 33: 413-421.

Mukai S & Hayano J (1995). Heart-Rate and Blood-Pressure Variabilities During Graded Head-Up Tilt. *Journal of Applied Physiology* 78: 212-216.

Mulder LJ (1992). Measurement and analysis methods of heart rate and respiration for use in applied environments. *Biol.Psychol.* 34: 205-236.

Munck A, Guyre PM, & Holbrook NJ (1984). Physiological functions of glucocorticoids in stress and their relation to pharmacological actions. *Endocrine Reviews* 5: 25-44.

Munck A & Naray-Fejes-Toth A (1994). Glucocorticoids and stress: permissive and suppressive actions. *Annals of the New York Academy of Sciences* 746: 115-130.

Mundy-Castle AC & McKiever BI (1953). The Psychophysiological Significance of the Galvanic Skin Response. *Journal of Experimental Psychology* 46: 15-24.

Muzi M, Ebert TJ, Tristani FE, Jeutter DC, Barney JA, & Smith JJ (1985). Determination of cardiac output using ensemble-averaged impedance cardiograms. *Journal of Applied Physiology* 58: 200-205.

Nagai Y, Critchley HD, Featherstone E, Trimble MR, & Dolan RJ (2004). Activity in ventromedial prefrontal cortex covaries with sympathetic skin conductance level: a physiological account of a "default mode" of brain function. *Neuroimage.* 22: 243-251.

Nakonezny PA, Kowalewski RB, Ernst JM, Hawkley LC, Lozano DL, Litvack DA, Berntson GG, Sollers JJ, III, Kizakevich P, Cacioppo JT, & Lovallo WR (2001). New ambulatory impedance cardiograph validated against the Minnesota Impedance Cardiograph. *Psychophysiology* 38: 465-473.

Negrao CE, Moreira ED, Brum PC, Denadai MLDR, & Krieger EM (1992). Vagal and Sympathetic Control of Heart-Rate During Exercise by Sedentary and Exercise-Trained Rats. *Brazilian Journal of Medical and Biological Research* 25: 1045-1052.

Nelesen RA, Shaw R, Ziegler MG, & Dimsdale JE (1999). Impedance cardiography-derived hemodynamic responses during baroreceptor testing with amyl nitrite and phenylephrine: a validity and reliability study. *Psychophysiology* 36: 105-108.

Neumann SA & Waldstein SR (2001). Similar patterns of cardiovascular response during emotional activation as a function of affective valence and arousal and gender. *Journal of Psychosomatic Research* 50: 245-253.

Newlin DB & Levenson RW (1979). Pre-ejection period: measuring beta-adrenergic influences upon the heart. *Psychophysiology* 16: 546-553.

Nijsen MJ, Croiset G, Diamant M, de Wied D, & Wiegant VM (2001). CRH signalling in the bed nucleus of the stria terminalis is involved in stress-induced cardiac vagal activation in conscious rats. *Neuropsychopharmacology* 24: 1-10.

Nolan J, Batin PD, Andrews R, Lindsay SJ, Brooksby P, Mullen M, Baig W, Flapan AD, Cowley A, Prescott RJ, Neilson JMM, & Fox KAA (1998). Prospective Study of Heart Rate Variability and Mortality in Chronic Heart Failure : Results of the United Kingdom Heart Failure Evaluation and Assessment of Risk Trial (UK-Heart). *Circulation* 98: 1510-1516.

Oelkers W (1996). Adrenal insufficiency. *The New England Journal of Medicine* 335: 1206-1212.

Ovadia M, Gear K, Thoele D, & Marcus FI (1995). Accelerometer Systolic-Time Intervals As Fast-Response Sensors of Upright Posture in the Young. *Circulation* 92: 1849-1859.

Pagani M, Lombardi F, Guzzetti S, Rimoldi O, Furlan R, Pizzinelli P, Sandrone G, Malfatto G, Dellorto S, Piccaluga E, Turiel M, Baselli G, Cerutti S, & Malliani A (1986). Power Spectral-Analysis of Heart-Rate and Arterial-Pressure Variabilities As A Marker of Sympathovagal Interaction in Man and Conscious Dog. *Circulation Research* 59: 178-193.

Pagani M & Malliani A (2000). Interpreting oscillations of muscle sympathetic nerve activity and heart rate variability. *Journal of Hypertension* 18: 1709-1719.

Pagani M, Mazzuero G, Ferrari A, Liberati D, Cerutti S, Vaitl D, Tavazzi L, & Malliani A (1991). Sympathovagal Interaction During Mental Stress - A Study Using Spectral-Analysis of Heart-Rate-Variability in Healthy Control Subjects and Patients with A Prior Myocardial-Infarction. *Circulation* 83: 43-51.

Pagani M, Montano N, Porta A, Malliani A, Abboud FM, Birkett C, & Somers VK (1997). Relationship between spectral components of cardiovascular variabilities and direct measures of muscle sympathetic nerve activity in humans. *Circulation* 95: 1441-1448.

Palatini P & Julius S (2004). Elevated heart rate: A major risk factor for cardiovascular disease. *Clinical and Experimental Hypertension* 26: 637-644.

Palti H, Gofin R, Adler B, Grafstein O, & Belmaker E (1988). Tracking of blood pressure over an eight year period in Jerusalem school children. *Journal of Clinical Epidemiology* 41: 731-735.

Parati G, Mancia G, Di Rienzo M, Castiglioni P, Taylor JA, & Studinger P (2006). Point: Counterpoint: Cardiovascular variability is/is not an index of autonomic control of circulation. *Journal of Applied Physiology* 101: 676-682.

Penttila J, Helminen A, Jartti T, Kuusela T, Huikuri HV, Tulppo MP, Coffeng R, & Scheinin H (2001). Time domain, geometrical and frequency domain analysis of cardiac vagal outflow: effects of various respiratory patterns. *Clin.Physiol* 21: 365-376.

Perini R, Orizio C, Baselli G, Cerutti S, & Veicsteinas A (1990). The Influence of Exercise Intensity on the Power Spectrum of Heart-Rate-Variability. *European Journal of Applied Physiology and Occupational Physiology* 61: 143-148.

Pfennig A, Kunzel HE, Kern N, Ising M, Majer M, Fuchs B, Ernst G, Holsboer F, & Binder EB (2005). Hypothalamus-pituitary-adrenal system regulation and suicidal behavior in depression. *Biological psychiatry* 57: 336-342.

Pichot V, Busso T, Roche F, Garet M, Costes F, Duverney D, Lacour JR, & Barthelemy JC (2002). Autonomic adaptations to intensive and overload training periods: a laboratory study. *Medicine and Science in Sports and Exercise* 34: 1660-1666.

Pichot V, Gaspoz JM, Molliex S, Antoniadis A, Busso T, Roche F, Costes F, Quintin L, Lacour JR, & Barthelemy JC (1999). Wavelet transform to quantify heart rate variability and to assess its instantaneous changes. *J Appl.Physiol* 86: 1081-1091.

Pickering TG & Devereux RB (1987). Ambulatory monitoring of blood pressure as a predictor of cardiovascular risk. *Am Heart J* 114: 925-928.

Piepoli MF (2006). Point: Counterpoint: Cardiovascular variability is/is not an index of autonomic control of circulation. *Journal of Applied Physiology* 101: 685-686.

Pitzalis MV, Mastropasqua F, Massari F, Forleo C, Di Maggio M, Passantino A, Colombo R, Di Biase M, & Rizzon P (1996). Short- and long-term reproducibility of time and frequency domain heart rate variability measurements in normal subjects. *Cardiovascular Research* 32: 226-233.

Pomeranz B, Macaulay RJB, Caudill MA, Kutz I, Adam D, Gordon D, Kilborn KM, Barger AC, Shannon DC, Cohen RJ, & Benson H (1985). Assessment of Autonomic Function in

Humans by Heart-Rate Spectral-Analysis. *American Journal of Physiology* 248: H151-H153.

Popma A, Jansen LMC, Vermeiren R, Steiner H, Raine A, van Goozen SHM, Engeland Hv, & Doreleijers TAH (2006). Hypothalamus pituitary adrenal axis and autonomic activity during stress in delinquent male adolescents and controls. *Psychoneuroendocrinology* 31: 948-957.

Posener JA, DeBattista C, Williams GH, Chmura KH, Kalehzan BM, & Schatzberg AF (2000). 24-Hour monitoring of cortisol and corticotropin secretion in psychotic and nonpsychotic major depression. *Archives of general psychiatry* 57: 755-760.

Powell KE, Thompson PD, Caspersen CJ, & Kendrick JS (1987). Physical-Activity and the Incidence of Coronary Heart-Disease. *Annual Review of Public Health* 8: 253-287.

Pruessner JC, Kirschbaum C, Meinlschmid G, & Hellhammer DH (2003). Two formulas for computation of the area under the curve represent measures of total hormone concentration versus time-dependent change. *Psychoneuroendocrinology* 28: 916-931.

Quinkler M & Stewart PM (2003). Hypertension and the Cortisol-Cortisone Shuttle. *Journal of Clinical Endocrinology Metabolism* 88: 2384-2392.

Raaijmakers E, Faes TJ, Goovaerts HG, Meijer JH, de Vries PM, Heethaar RM (1998). Thoracic geometry and its relation to electrical current distribution: consequences for electrode placement in electrical impedance cardiography. *Medical & Biological Engineering & Computing* 36, 592-597.

Raaijmakers E, Faes TJ, Scholten RJ, Goovaerts HG, Heethaar RM. (1999). A meta-analysis of three decades of validating thoracic impedance cardiography. *Critical Care Medicine* 27(6): 1203-13.

Raison CL & Miller AH (2003). When Not Enough Is Too Much: The Role of Insufficient Glucocorticoid Signaling in the Pathophysiology of Stress-Related Disorders. *American Journal of Psychiatry* 160: 1554-1565.

Ramey ER, Goldstein MS, & Levine R (1951). Action of nor-epinephrine and adrenal cortical steroids on blood pressure and work performance of adrenalectomized dogs. *The American journal of physiology* 165: 450-455.

Rasmussen JP, Sorensen B, & Kann T (1975). Evaluation of impedance cardiography as a non-invasive means of measuring systolic time intervals and cardiac output. *Acta Anaesthesiologica Scandinavica* 19: 210-218.

Ray CA & Hume KM (1998). Sympathetic neural adaptations to exercise training in humans: insights from microneurography. *Medicine and Science in Sports and Exercise* 30: 387-391.

Reid JD, Intrieri RC, Susman EJ, & Beard JL (1992). The Relationship of Serum and Salivary Cortisol in A Sample of Healthy Elderly. *Journals of Gerontology* 47: 176-179.

Reul JMHM, Gesing A, Droste S, Stec ISM, Weber A, Bachmann C, Bilang-Bleuel A, Holsboer F, & Linthorst ACE (2000). The brain mineralocorticoid receptor: greedy for ligand, mysterious in function. *European Journal of Pharmacology* 405: 235-249.

Rice T, An P, Gagnon J, Leon AS, Skinner JS, Wilmore JH, Bouchard C, & Rao DC (2002). Heritability of HR and BP response to exercise training in the HERITAGE Family Study. *Medicine and Science in Sports and Exercise* 34: 972-979.

Riese H, Groot PF, van den Berg M, Kupper NH, Magnee EH, Rohaan EJ, Vrijkotte TG, Willemsen G, & de Geus EJ (2003). Large-scale ensemble averaging of ambulatory impedance cardiograms. *Behav.Res.Methods Instrum.Comput.* 35: 467-477.

Riese H, Rijsdijk FV, Ormel J, van Roon AM, Neeleman J, & Rosmalen JGM (2006). Genetic influences on baroreflex sensitivity during rest and mental stress. *Journal of Hypertension* 24: 1779-1786.

Riese H, Van Doomen LJP, Houtman ILD, & de Geus EJC (2004). Job strain in relation to ambulatory blood pressure, heart rate, and heart rate variability among female nurses. *Scandinavian Journal of Work Environment & Health* 30: 477-485.

Ring C, Carroll D, Willemsen G, Cooke J, Ferraro A, & Drayson M (1999). Secretory immunoglobulin A and cardiovascular activity during mental arithmetic and paced breathing. *Psychophysiology* 36: 602-609.

Ring C, Burns VE, Carroll D (2002). Shifting hemodynamics of blood pressure control during prolonged mental stress. *Psychophysiology* 39, 585-590.

Ritz T & Dahme B (2006). Implementation and interpretation of respiratory sinus arrhythmia measures in psychosomatic medicine: practice against better evidence? *Psychosomatic Medicine* 68: 617-627.

Roozendaal B (2002). Stress and memory: opposing effects of glucocorticoids on memory consolidation and memory retrieval. *Neurobiology of learning and memory* 78: 578-595.

Roozendaal B & McGaugh JL (1996). The memory-modulatory effects of glucocorticoids depend on an intact stria terminalis. *Brain research* 709: 243-250.

Rosenwinkel ET, Bloomfield DM, Arwardy MA, & Goldsmith RL (2001). Exercise and autonomic function in health and cardiovascular disease. *Cardiology Clinics* 19: 369-387.

Rosmond R & Bjorntorp P (2000). The hypothalamic-pituitary-adrenal axis activity as a predictor of cardiovascular disease, type 2 diabetes and stroke. *Journal of Internal Medicine* 247: 188-197.

Rossy LA & Thayer JF (1998). Fitness and gender-related differences in heart period variability. *Psychosomatic Medicine* 60: 773-781.

Roveda F, Middlekauff HR, Rondon MUPB, Reis SF, Souza M, Nastari L, Barretto ACP, Krieger EM, & Negrao CE (2003). The effects of exercise training on sympathetic neural activation in advanced heart failure - A randomized controlled trial. *Journal of the American College of Cardiology* 42: 854-860.

Roy MP, Kirschbaum C, & Steptoe A (2001). Psychological, cardiovascular, and metabolic correlates of individual differences in cortisol stress recovery in young men. *Psychoneuroendocrinology* 26: 375-391.

Sacknoff DM, Gleim GW, Stachenfeld N, & Coplan NL (1994). Effect of Athletic Training on Heart-Rate-Variability. *American Heart Journal* 127: 1275-1278.

Saito M, Foldager N, Mano T, Iwase S, Sugiyama Y, & Oshima M (1997). Sympathetic control of hemodynamics during moderate head-up tilt in human subjects. *Environmental Medicine* 41: 151-155.

Sakakibara M, Takeuchi S, & Hayano J (1994). Effect of relaxation training on cardiac parasympathetic tone. *Psychophysiology* 31: 223-228.

Sakaue M & Hoffman BB (1991). Glucocorticoids induce transcription and expression of the alpha 1B adrenergic receptor gene in DTT1 MF-2 smooth muscle cells. *The Journal of clinical investigation* 88: 385-389.

Salomon K, Matthews KA, & Allen MT (2000). Patterns of sympathetic and parasympathetic reactivity in a sample of children and adolescents. *Psychophysiology* 37: 842-849.

Sandercock GRH, Bromley PD, & Brodie DA (2005). Effects of exercise on heart rate variability: Inferences from meta-analysis. *Medicine and Science in Sports and Exercise* 37: 433-439.

Sapolsky RM (2003). Stress and plasticity in the limbic system. *Neurochemical research* 28: 1735-1742.

Sapolsky RM, Romero LM, & Munck AU (2000). How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocrine Reviews* 21: 55-89.

Sato K & Sato F (1983). Individual Variations in Structure and Function of Human Eccrine Sweat Gland. *American Journal of Physiology* 245: R203-R208.

Saul JP, Arai Y, Berger RD, Lilly LS, Colucci WS, & Cohen RJ (1988). Assessment of autonomic regulation in chronic congestive heart failure by heart rate spectral analysis. *Am J Cardiol*. 61: 1292-1299.

Saul JP, Rea RF, Eckberg DL, Berger RD, & Cohen RJ (1990). Heart rate and muscle sympathetic nerve variability during reflex changes of autonomic activity. *AJP - Heart and Circulatory Physiology* 258: H713-H721.

Sawchenko PE (1987). Evidence for differential regulation of corticotropin-releasing factor and vasopressin immunoreactivities in parvocellular neurosecretory and autonomic-related projections of the paraventricular nucleus. *Brain research* 437: 253-263.

Scerbo AS, Freedman LW, Raine A, Dawson ME, & Venables PH (1992). A major effect of recording site on measurement of electrodermal activity. *Psychophysiology* 29: 241-246.

Schachinger H, Weinbacher M, Kiss A, Ritz R, & Langewitz W (2001). Cardiovascular indices of peripheral and central sympathetic activation. *Psychosomatic Medicine* 63: 788-796.

Schafer JL & Graham JW (2002). Missing data: our view of the state of the art. *Psychol.Methods* 7: 147-177.

Scheer F. A. J. L. (2003). *Cardiovascular regulation by the biological clock; neural and neuroendocrine mechanisms in human and rat (thesis)*. University of Amsterdam, the Netherlands.

Schell AM, Dawson ME, & Filion DL (1988). Psychophysiological Correlates of Electrodermal Lability. *Psychophysiology* 25: 619-632.

Schell AM, Dawson ME, Nuechterlein KH, Subotnik KL, & Ventura J (2002). The temporal stability of electrodermal variables over a one-year period in patients with recent-onset schizophrenia and in normal subjects. *Psychophysiology* 39: 124-132.

Schermelleh-Engel K, Moosbrugger H, & Müller H (2003). Evaluating the fit of structural equation models: test of significance and descriptive goodness-of-fit measures. *Methods of Psychological Research* 8: 23-74.

Schinkel AH, Wagenaar E, van Deemter L, Mol CA, & Borst P (1995). Absence of the mdr1a P-Glycoprotein in mice affects tissue distribution and pharmacokinetics of dexamethasone, digoxin, and cyclosporin A. *The Journal of clinical investigation* 96: 1698-1705.

Schomig A, Luth B, Dietz R, & Gross F (1976). Changes in vascular smooth muscle sensitivity to vasoconstrictor agents induced by corticosteroids, adrenalectomy and differing salt intake in rats. *Clinical science and molecular medicine.Supplement* 3: 61s-63s.

Schuit AJ, van Amelsvoort LGPM, Verheij TC, Rijneke RD, Maan AC, Sweene CA, & Schouten EG (1999). Exercise training and heart rate variability in older people. *Medicine and Science in Sports and Exercise* 31: 816-821.

Schwartz AR, Gerin W, Davidson KW, Pickering TG, Brosschot JF, Thayer JF, Christenfeld N, & Linden W (2003). Toward a Causal Model of Cardiovascular Responses to Stress and the Development of Cardiovascular Disease. *Psychosomatic Medicine* 65: 22-35.

Schwartz PJ, Larovere MT, & Vanoli E (1992). Autonomic Nervous-System and Sudden Cardiac Death - Experimental Basis and Clinical Observations for Postmyocardial Infarction Risk Stratification. *Circulation* 85: 77-91.

Sherwood A, Allen MT, Obrist PA, & Langer AW (1986). Evaluation of beta-adrenergic influences on cardiovascular and metabolic adjustments to physical and psychological stress. *Psychophysiology* 23: 89-104.

Sherwood A, Light KC, & Blumenthal JA (1989). Effects of Aerobic Exercise Training on Hemodynamic-Responses During Psychosocial Stress in Normotensive and Borderline Hypertensive Type-A Men - A Preliminary-Report. *Psychosomatic Medicine* 51: 123-136.

Sherwood A, Allen MT, Fahrenberg J, Kelsey RM, Lovallo WR, & van Doornen LJP (1990). Methodological Guidelines for Impedance Cardiography. *Psychophysiology* 27: 1-23.

Sherwood A, Carter LS, Jr, Murphy CA (1991). Cardiac output by impedance cardiography: two alternative methodologies compared with thermodilution. *Aviation, Space, and Environmental Medicine* 62, 116-122.

Sherwood A, Royal SA, Light KC (1993). Laboratory reactivity assessment: effects of casual blood pressure status and choice of task difficulty. *International Journal of Psychophysiology* 14, 81-95.

Sherwood A & Turner JR (1993). Postural stability of hemodynamic responses during mental challenge. *Psychophysiology* 30: 237-244.

Sherwood A, Girdler SS, Bragdon EE, West SG, Brownley KA, Hinderliter AL, Light KC (1997). Ten-year stability of cardiovascular responses to laboratory stressors. *Psychophysiology* 34, 185-191.

Sherwood A, McFetridge J, & Hutcheson JS (1998). Ambulatory impedance cardiography: a feasibility study. *Journal of Applied Physiology* 85: 2365-2369.

Shi XR, Stevens GHJ, Foresman BH, Stern SA, & Raven PB (1995). Autonomic Nervous-System Control of the Heart - Endurance Exercise Training. *Medicine and Science in Sports and Exercise* 27: 1406-1413.

Shields SA, MacDowell KA, Fairchild SB, & Campbell ML (1987). Is mediation of sweating cholinergic, adrenergic, or both? A comment on the literature. *Psychophysiology* 24: 312-319.

Shin K, Minamitani H, Onishi S, Yamazaki H, & Lee M (1997). Autonomic differences between athletes and nonathletes: spectral analysis approach. *Medicine and Science in Sports and Exercise* 29: 1482-1490.

Shoemaker JK, Hogeman CS, Khan M, Kimmerly DS, & Sinoway LI (2001). Gender affects sympathetic and hemodynamic response to postural stress. *AJP - Heart and Circulatory Physiology* 281: H2028-H2035.

Singer DH, Martin GJ, Magid N, Weiss JS, Schaad JW, Kehoe R, Zheutlin T, Fintel DJ, Hsieh AM, & Lesch M (1988). Low heart rate variability and sudden cardiac death. *J Electrocardiol.* 21 Suppl: S46-S55.

Singh JP, Larson MG, Tsuji H, Evans JC, O'Donnell CJ, & Levy D (1998). Reduced heart rate variability and new-onset hypertension: insights into pathogenesis of hypertension: the Framingham Heart Study. *Hypertension* 32: 293-297.

Sinnreich R, Kark JD, Friedlander Y, Sapoznikov D, & Luria MH (1998). Five minute recordings of heart rate variability for population studies: repeatability and age-sex characteristics. *Heart* 80: 156-162.

Sleight P & Bernardi L (1998). Sympathovagal Balance. *Circulation* 98: 2640.

Smith JJ, Muzi M, Barney JA, Ceschi J, Hayes J, & Ebert TJ (1989a). Impedance-derived cardiac indices in supine and upright exercise. *Ann.Biomed.Eng* 17: 507-515.

Smith ML, Hudson DL, Graitzer HM, & Raven PB (1989b). Exercise Training Bradycardia - the Role of Autonomic Balance. *Medicine and Science in Sports and Exercise* 21: 40-44.

Snijders TAB & Bosker RJ (1999). *Multilevel Analysis. An introduction to basic and advanced multilevel modeling*. London: SAGE.

Song CG & Kim DW (2003). An improved approach for measurement of stroke volume during treadmill exercise. *Medical Engineering & Physics* 25: 517-522.

Spencer RL, Young EA, Choo PH, & McEwen BS (1990). Adrenal steroid type I and type II receptor binding: estimates of in vivo receptor number, occupancy, and activation with varying level of steroid. *Brain research* 514: 37-48.

Spielberger C.D., Goruch R.L., & Lushene R.E. (1970). *STAI Manual for the State-Trait Anxiety Inventory*. CA: Palto Alto.

Stahle A, Nordlander R, & Bergfeldt L (1999). Aerobic group training improves exercise capacity and heart rate variability in elderly patients with a recent coronary event - A randomized controlled study. *European Heart Journal* 20: 1638-1646.

Stein PK, Rich MW, Rottman JN, & Kleiger RE (1995). Stability of index of heart rate variability in patients with congestive heart failure. *Am Heart J* 129: 975-981.

Svedenhag J, Martinsson A, Ekblom B, & Hjemdahl P (1986). Altered cardiovascular responsiveness to adrenaline in endurance-trained subjects. *Acta Physiol Scand.* 126: 539-550.

Svedenhag J, Martinsson A, Ekblom B, & Hjemdahl P (1991). Altered Cardiovascular Responsiveness to Adrenoceptor Agonists in Endurance-Trained Men. *Journal of Applied Physiology* 70: 531-538.

Svedenhag J, Wallin BG, Sundlof G, & Henriksson J (1984). Skeletal-Muscle Sympathetic Activity at Rest in Trained and Untrained Subjects. *Acta Physiologica Scandinavica* 120: 499-504.

Swain A & Suls J (1996). Reproducibility of blood pressure and heart rate reactivity: a meta-analysis. *Psychophysiology* 33: 162-174.

Tank J, Jordan J, Diedrich A, Stoffels M, Franke G, Faulhaber HD, Luft FC, & Busjahn A (2001). Genetic influences on baroreflex function in normal twins. *Hypertension* 37: 907-910.

Tanz RD (1960). Studies on the action of cortisone acetate on isolated cardiac tissue. *The Journal of pharmacology and experimental therapeutics Links* 128: 168-175.

Task Force of the European Society of Cardiology the North American Society of Pacing (1996). Heart Rate Variability : Standards of Measurement, Physiological Interpretation, and Clinical Use. *Circulation* 93: 1043-1065.

Taylor JA & Studinger P (2006). Cardiovascular variability is/is not an index of autonomic control of circulation. *Journal of Applied Physiology* 101: 690-691.

Ten S, New M, & Maclaren N (2001). Addison's Disease 2001. *Journal of Clinical Endocrinology Metabolism* 86: 2909-2922.

Thurston RC, Blumenthal JA, Babyak MA, Sherwood A. (1994). Emotional antecedents of hot flashes during daily life. *Psychosomatic Medicine* 67(1):137-46.

Tomaka J, Blascovich J, & Swart L (1994). Effects of Vocalization on Cardiovascular and Electrodermal Responses During Mental Arithmetic. *International Journal of Psychophysiology* 18: 23-33.

Toska K & Walloe L (2002). Dynamic time course of hemodynamic responses after passive head-up tilt and tilt back to supine position. *Journal of Applied Physiology* 92: 1671-1676.

Treiber FA, Kamarck T, Schneiderman N, Sheffield D, Kapuku G, & Taylor T (2003). Cardiovascular Reactivity and Development of Preclinical and Clinical Disease States. *Psychosomatic Medicine* 65: 46-62.

Trinder J, Kleiman J, Carrington M, Smith S, Breen S, Tan N, Kim Y (2001). Autonomic activity during human sleep as a function of time and sleep stage. *Journal of Sleep Research* 10, 253-264.

Tsigos C & Chrousos GP (2002). Hypothalamic-pituitary-adrenal axis, neuroendocrine factors and stress. *Journal of Psychosomatic Research* 53: 865-871.

Tsuji H, Larson MG, Venditti FJ, Jr., Manders ES, Evans JC, Feldman CL, & Levy D (1996). Impact of reduced heart rate variability on risk for cardiac events. The Framingham Heart Study. *Circulation* 94: 2850-2855.

Tulen JHM, Boomsma F, & Veld AJMI (1999). Cardiovascular control and plasma catecholamines during rest and mental stress: effects of posture. *Clinical Science* 96: 567-576.

Tulppo MP, Makikallio TH, Takala TE, Seppanen T, & Huikuri HV (1996). Quantitative beat-to-beat analysis of heart rate dynamics during exercise. *The American journal of physiology* 271: H244-H252.

Ukkola O, Gagnon J, Rankinen T, Thompson PA, Hong Y, Leon AS, Rao DC, Skinner JS, Wilmore JH, & Bouchard C (2001). Age, body mass index, race and other determinants of steroid hormone variability: the HERITAGE Family Study. *European Journal of Endocrinology* 145: 1-9.

Uusitalo ALT, Laitinen T, Vaisanen SB, Lansimies E, & Rauramaa R (2004). Physical training and heart rate and blood pressure variability: a 5-yr randomized trial. *American Journal of Physiology-Heart and Circulatory Physiology* 286: H1821-H1826.

Uusitalo ALT, Tahvanainen KUO, Uusitalo AJ, & Rusko HK (1996). Non-invasive evaluation of sympathovagal balance in athletes by time and frequency domain analyses of heart rate and blood pressure variability. *Clinical Physiology* 16: 575-588.

Van de Borne P, Neubauer J, Rahnama M, Jansens JL, Montano N, Porta A, Somers VK, & Degaute JP (2001). Differential characteristics of neural circulatory control - Early versus late after cardiac transplantation. *Circulation* 104: 1809-1813.

Van de Kar LD & Blair ML (1999). Forebrain Pathways Mediating Stress-Induced Hormone Secretion. *Frontiers in Neuroendocrinology* 20: 1-48.

Van Doornen LJP, Knol D, Willemsen AHM, & de Geus EJC (1994). The relationship between stress reactivity in the lab and in real-life: is reliability the limiting factor? *Journal of Psychophysiology* 8: 297-304.

Van Doornen LJP & de Geus EJC (1989). Aerobic Fitness and the Cardiovascular Response to Stress. *Psychophysiology* 26: 17-28.

Van Eekelen AP, Houtveen JH, Kerkhof GA (2004). Circadian variation in base rate measures of cardiac autonomic activity. *European Journal of Applied Physiology* 93, 39-46.

Vanoli E, de Ferrari GM, Stramba-Badiale M, Hull SS, Jr., Foreman RD, & Schwartz PJ (1991). Vagal stimulation and prevention of sudden death in conscious dogs with a healed myocardial infarction. *Circ Res.* 68: 1471-1481.

Venables PH & Christie MJ (1980). Electrodermal activity. In Martin I & Venables, PH (Eds.). *Techniques in Psychophysiology*. U.K.: Wiley.

Verdecchia P (2000). Prognostic value of ambulatory blood pressure - Current evidence and clinical implications. *Hypertension* 35: 844-851.

Verdecchia P, Porcellati C, Schillaci G, Borgioni C, Ciucci A, Battistelli M, Guerrieri M, Gatteschi C, Zampi I, & Santucci A (1994). Ambulatory blood pressure. An independent predictor of prognosis in essential hypertension. *Hypertension* 24: 793-801.

Verdecchia P, Schillaci G, Borgioni C, Ciucci A, Pede S, & Porcellati C (1998). Ambulatory pulse pressure: a potent predictor of total cardiovascular risk in hypertension. *Hypertension* 32: 983-988.

Verdecchia P, Schillaci G, Reboldi G, Franklin SS, & Porcellati C (2001). Ambulatory monitoring for prediction of cardiac and cerebral events. *Blood Press Monit.* 6: 211-215.

Vissing SF, Scherrer U, & Victor RG (1994). Increase of Sympathetic Discharge to Skeletal-Muscle But Not to Skin During Mild Lower-Body Negative-Pressure in Humans. *Journal of Physiology-London* 481: 233-241.

Vossel G & Zimmer H (1990). Psychometric Properties of Nonspecific Electrodermal Response-Frequency for A Sample of Male-Students. *International Journal of Psychophysiology* 10: 69-73.

Vrijkotte TG, van Doornen LJ, & de Geus EJ (2000). Effects of work stress on ambulatory blood pressure, heart rate, and heart rate variability. *Hypertension* 35: 880-886.

Vrijkotte TG, van Doornen LJ, & de Geus EJ (2004). Overcommitment to work is associated with changes in cardiac sympathetic regulation. *Psychosomatic Medicine* 66: 656-663.

Vrijkotte TGM, Riese H, & de Geus EJC (2001). Cardiovascular reactivity to work stress assessed by ambulatory blood pressue, heart rate, and heart rate variability. In Fahrenberg J & Myrtek, M (Eds.). *Progress in ambulatory assessment. Computer assisted psychological and psychophysiological methods in monitoring and field studies.* Seattle: Hogrefe & Huber.

Waldstein SR, Neumann SA, & Merrill JA (1998). Postural effects on hemodynamic response to interpersonal interaction. *Biol.Psychol.* 48: 57-67.

Wallin BG (1981). Sympathetic-Nerve Activity Underlying Electrodermal and Cardiovascular Reactions in Man. *Psychophysiology* 18: 470-476.

Wallin BG, Sundlof G, & Delius W (1975). Effect of Carotid-Sinus Nerve-Stimulation on Muscle and Skin Nerve Sympathetic Activity in Man. *Pflugers Archiv-European Journal of Physiology* 358: 101-110.

Wang JS, Jen CJ, & Chen HI (1997). Effects of chronic exercise and deconditioning on platelet function in women. *Journal of Applied Physiology* 83: 2080-2085.

Weber EJ, Molenaar PC, & van der Molen MW (1992). A nonstationarity test for the spectral analysis of physiological time series with an application to respiratory sinus arrhythmia. *Psychophysiology* 29: 55-65.

Weinstein AA, Deuster PA, & Kop WJ (2007). Heart rate variability as a predictor of negative mood symptoms induced by exercise withdrawal. *Medicine and Science in Sports and Exercise* 39: 735-741.

Weissler AM, Harris WS, & Schoenfeld CD (1968). Systolic time intervals in heart failure in man. *Circulation* 37: 149-159.

Wellcome Trust Case Control Consortium. (2007). Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. *Nature*. 447(7145):661-78.

Wiklund U, Akay M, & Niklasson U (1997). Short-term analysis of heart-rate variability by adapted wavelet transforms. *IEEE Eng Med Biol.Mag.* 16: 113-8, 138.

Wilhelm FH, Roth WT, & Sackner MA (2003). The lifeShirt. An advanced system for ambulatory measurement of respiratory and cardiac function. *Behav.Modif.* 27: 671-691.

Willemsen G, Ring C, Carroll D, Evans P, Clow A, & Hucklebridge F (1998). Secretory immunoglobulin A and cardiovascular reactions to mental arithmetic and cold pressor. *Psychophysiology* 35: 252-259.

Willemsen GH, de Geus EJ, Klaver CH, van Doornen LJ, & Carroll D (1996). Ambulatory monitoring of the impedance cardiogram. *Psychophysiology* 33: 184-193.

Williams PT (2001). Physical fitness and activity as separate heart disease risk factors: a meta-analysis. *Medicine and Science in Sports and Exercise* 33: 754-761.

Wilson TE, Cui J, & Crandall CG (2001). Absence of arterial baroreflex modulation of skin sympathetic activity and sweat rate during whole-body heating in humans. *Journal of Physiology-London* 536: 615-623.

Winzer A, Ring C, Carroll D, Willemsen G, Drayson M, & Kendall M (1999). Secretory immunoglobulin A and cardiovascular reactions to mental arithmetic, cold pressor, and exercise: Effects of beta-adrenergic blockade. *Psychophysiology* 36: 591-601.

Woelk G (1994). Blood pressure tracking from child to adulthood: a review. *Central African Journal of Medicine* 40: 163-169.

Woodside DB, Winter K, & Fisman S (1991). Salivary Cortisol in Children - Correlations with Serum Values and Effect of Psychotropic-Drug Administration. *Canadian Journal of Psychiatry-Revue Canadienne de Psychiatrie* 36: 746-748.

Wurtman RJ & Axelrod J (1966). Control of enzymatic synthesis of adrenaline in the adrenal medulla by adrenal cortical steroids. *Journal of Biological Chemistry* 241: 2301-2305.

Wurtman RJ (2002). Stress and the adrenocortical control of epinephrine synthesis*. *Metabolism* 51: 11-14.

Wust S, Wolf J, Hellhammer DH, Federenko I, Schommer N, & Kirschbaum C (2000). The cortisol awakening response - normal values and confounds. *Noise and Health* 2: 79-88.

Yusuf S, Hawken S, Ounpuu S, Dans T, Avezum A, Lanas F, McQueen M, Budaj A, Pais P, Varigos J, & Liu LS (2004). Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. *Lancet* 364: 937-952.

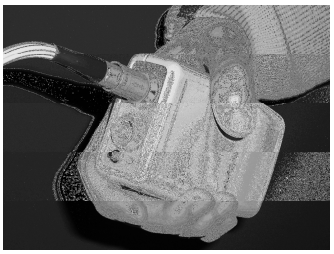
Zaloga GP & Marik P (2001). Hypothalamic-pituitary-adrenal insufficiency. *Critical care clinics* 17: 25-41.

Appendices

Appendix I: VU-AMS.5fs

The Vrije Universiteit Ambulatory Monitoring System, or VU-AMS for short, is a system for measuring bio-signals during normal daily activities, in real-life settings. The complete VU-AMS consists of:

- AMS device, a small lightweight device for the actual ambulatory recording
- AMS*i* interface cable to connect the VU-AMS device to your PC via a USB or RS232 connection
- AMS software



The VU-AMS.5fs is the most recent version of the VU-AMS. The VU-AMS.5fs measures the electrocardiogram (ECG), the thorax impedance (Z0), the changes in thorax impedance (dZ), the impedance cardiogram (ICG), the skin conductance level (SCL), phonocardiogram (PCG) and the vertical and horizontal acceleration of the subject (body movement).

The main features of the VU-AMS.5fs device are:

- Full ECG signal recording at a maximum sampling rate of 1000Hz with a 16-bit resolution. The ECG signal is used to extract the interbeat interval time series.
- Full recording of thorax impedance (dZ) at 1000Hz (16 bit). From the dZ signal the ICG (dZ/dt) is calculated offline and used to extract systolic time intervals.
- Full recording of skin conductance in DC or AC (10Hz) mode (16 bit) from which skin conductance level or skin conductance responses can be computed.
- Recording of vertical and horizontal acceleration to index gross body movement of the subject.
- Optional: Full recording of heart sound (PCG) at a maximum sampling rate of 1000Hz (16 bits).
- All signals (ECG, ICG, skin conductance, motility, heart sounds) are recorded simultaneously from a single device.
- Data is stored on Compact Flash memory cards. Storage capacities of 1 gigabyte (and higher) make it possible to record all raw signals at their highest sampling rates for at least 24 hours, and up to 72 hours at typical sampling rates.
- The VU-AMS.5fs uses two standard AA-type batteries. This makes it possible to use high capacity rechargeable NiMh batteries. Recordings of up-to 48 hours are possible when using 2600mAh types, without changing batteries. Longer recordings are possible with additional batteries. The memory cards allow repeated replacement of the batteries without any data loss.
- An infrared interface cable connects the VU-AMS device to the PC for online monitoring (serial RS232 or USB ports).

Changes from the older 4.6 version

The new 5fs version stores the complete ECG/dZ signals allowing a higher sampling frequency (form 250 to 1000 Hz) and increased sampling resolution (16-bit). In combination with improved filtering techniques this increases the signal-to-noise ratio and allows greatly improved offline analysis and artifact correction strategies. Batteries can now be changed without data loss.

For **backward compatibility**, a special data conversion program is available to convert the new data files back to the old 4.6 file format. This makes it possible to use the original VU-AMS analysis software package (AMSGRA, AMSRES, AMSIMP) to label data and (interactively) score parameters like heart rate, pre-ejection period and respiratory sinus arrhythmia.

Appendix II: User Manual

Ambulatory monitoring with the VU-AMS version 5fs

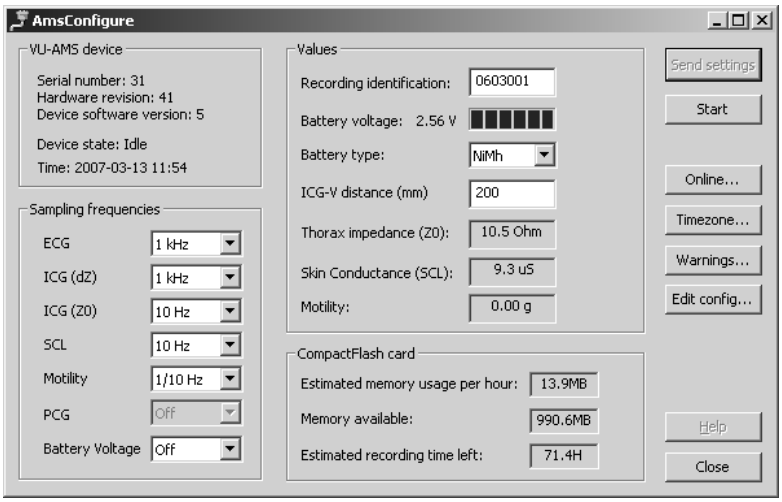
Requirements
Two AA batteries: Use 1.2V rechargeable NiMh batteries or non-rechargeable 1.5V alkaline batteries.
Compact flash card: External memory card. The VU-AMS.5fs has been extensively tested with the 1GB 80x Compact Flash card from Transcend (TS1GCF80). Other flash cards may work too.
Compact flash card reader: Card reader unit to extract the AMS data from the flash card after recording and to erase the card for a next recording. Any brand or built-in flash card reader will do.
Electrodes: Seven electrodes are needed for a single recording. We use the 'UltraTrace®' single use clear tape ECG electrode with Wet Gel.
Lead wire connector: A blue lead wire connector with 7 lead wires is used for the recording of the ECG and thorax impedance. Optionally a second yellow connector with two lead wires for skin conductance recording is needed.
VU-AMS.5fs: The ambulatory recording device.
VU-AMS*i* (with optional RS232-to-USB converter): An infrared interface cable that either connects to the RS232 serial port of a PC directly or through an RS232-to-USB converter.

Signal recording
Use an empty Flash Card for each new measurement. First, put the flash card bottom up in the VU-AMS and then place two completely charged AA batteries in the battery holder. Successful placement is signaled by a beep tone and a green light. When the VU-AMS has started, you will hear a triple beep tone. After you close the battery lid, the VU-AMS is ready for use. The green light will flash twice every ten seconds. This indicates the VU-AMS is ready, but *not* recording.

Connect the VU-AMS to the PC with the interface cable (marked: AMS*i*). Connect the infrared end of the interface cable to the VU-AMS; the electronic end of the interface cable goes to the serial port of the PC (or to the serial-to-USB converter).

Now start the AmsConfigure program. It tries to automatically detect the VU-AMS device on all available COM ports. If successful, the opening screen (see figure below) will be displayed. Check the battery type, battery voltage indication (should be about 3 Volt for alkaline and about 2.6 Volt for rechargeable NiMh batteries), and re-check time and date. Fill out the identification field. *NB:* If you intend to use our preprogrammed SPSS scripts, make sure the identifier is exactly 7 characters long. Try to use a numerical name only (e.g. 0603001).

NOTE: Before you start AmsConfigure, make sure to set date and time of the PC correctly. All dates and times in the AMS data files will be based on the time and date read from the PC at start-up, so it is important to make sure your PC has the correct time and date. The VU-AMS will verify time and date of the PC against its internal Real Time clock; deviations > 5 minutes (configurable in 'Warnings') will be flagged by AmsConfigure.



The typical sampling frequencies are as shown in the figure. AmsConfigure allows you to set sampling frequencies for the various signals. You can also disable signals here by setting them to 'Off'. When you change the settings, **make sure to send the settings to the device before closing the screen!**

Attach the electrodes as explained in the instruction leaflet "How to attach the VU-AMS.5fs" (Appendix III). This instruction leaflet is used by our subjects to (re)attach the device themselves (for instance after taking a shower).

After connecting the ECG/ICG lead wire plug the 'Online' option of the AmsConfigure program can be used to display the ECG, Z0, dZ and ICG. The dZ should be within -0.5 and +0.5 Ohm most of the time. Z0 should always stay within an 8 to 20 Ohm range. The dZ signal should reflect deep breathing clearly. In the ICG the typical waveform of the cardiac ejection phase should be clearly detectable. Light movement of the subject should not overly distort it. If these criteria are not met, re-attach the electrodes in the order 7,6,1,3,4,5,2 until satisfactory signals are obtained.

NOTE: In ambulatory paradigms, this is your only opportunity to re-attach faulty electrodes.

When satisfied, start data recording by pressing the 'start' button. A beep will be heard to acknowledge the start of the recording and the green light will start flashing once every three seconds. You may now disconnect the VU-AMS device from the interface.

Synchronize the watch of the subject to the exact time of your VU-AMS for optimally time-locked self-report diary and physiological data.

At the end of the recording the measurement is stopped by pressing the event button for three seconds. You may now disconnect the lead wire plug(s) from the connector(s) and the lead wires from the electrodes. The subjects can also do this themselves at home at a designated time.

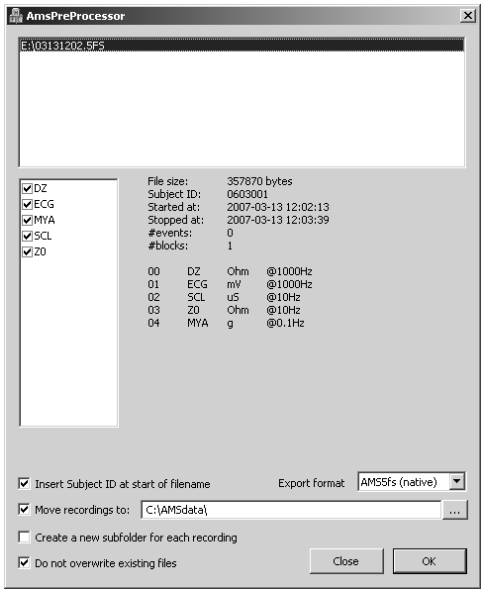
Once the device is returned to you, check if the measurement is stopped (the light flashes twice every ten seconds or not at all in case the batteries are discharged), remove the

batteries and place the Compact Flash Card in the reader unit. Copy the AMS files to a designated directory. Use the same name for the directory as was used as a subject identifier. It is best to backup the original '.5fs' AMS-data files as soon as possible (extension '.5fs' discriminates the version 5fs AMS-data files from the '.ams' files generated by previous versions).

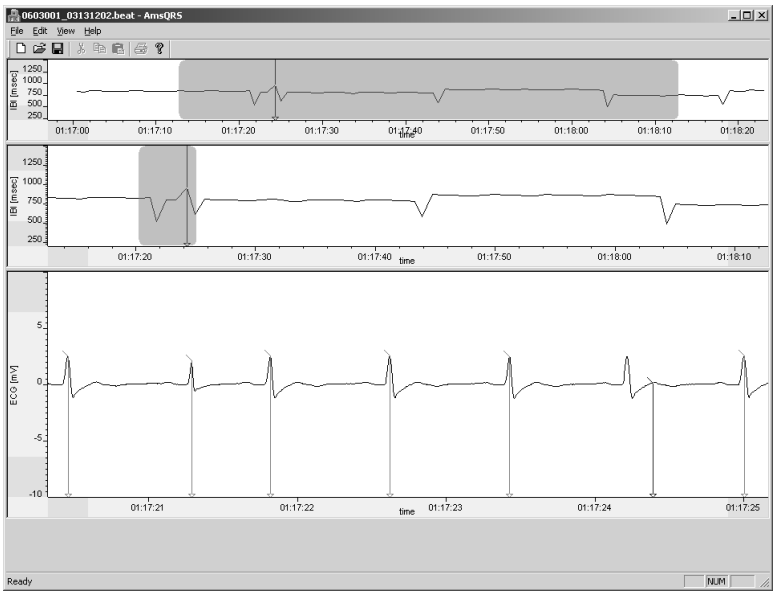
If the recording has been interrupted by the experimenter or by the subject, multiple .5fs files with different start times will be generated Use the AmsMerge tool to concatenate the .5fs files into a single .5fs file that spans the entire recording. Interruptions will be marked in this file as hold-continue periods.

Processing VU-AMS.5fs data
To further process the data you need to convert the raw '.5fs' AMS-data files to a format of choice, using the AmsPreProcessor program. Different formats are supported including Biopac, ASCII, EDF and EDF+.

The default is to convert the files to the native VU-AMS format. Native format creates a different file for each of the signals which is visible in the file name e.g.
0603001_03131202$DZ.bch;
0603001_03131202$DZDT.bch;
0603001_03131202$ECG.bch;
0603001_03131202$MYA.bch;
0603001_03131202$SCL.bch;
0603001_03131202$Z0.bch;
0603001_03131202.amsinv.
The binary amsinv (AMS inventory) file indicates the exact times of event button pushes and restarts).



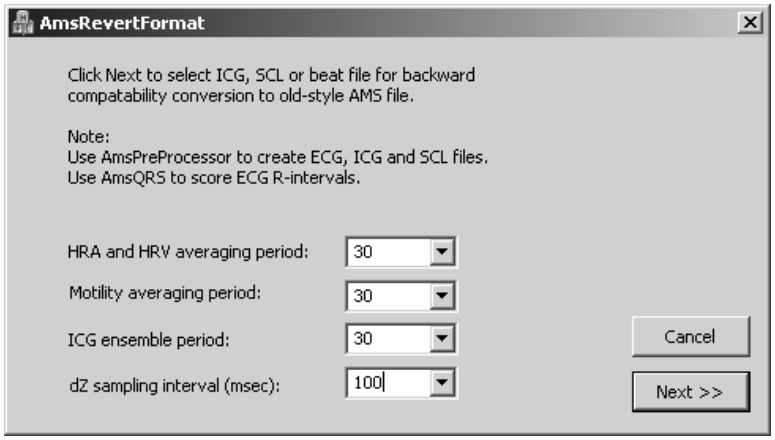
After conversion to native AMS format **FIRST** extract the IBI time series from the ECG signal. This is done with the AmsQRS program. The AmsQRS program displays the cardiotachogram of the complete recording in the top panel and the raw ECG signal in the lower panel. The middle panel also displays the cardiotachogram, but only for the interval selected in the top panel.



Clicking in the top panel will automatically scroll the middle and lower panel to the corresponding point in time. Visual inspection of the top panel will rapidly identify 'spikes' which can occur because an R-wave was missed and/or a T-wave was used instead. The spikes can be corrected in the lower window by dragging the cursor to the correct R-wave peak. A single left-click in the ECG panel will automatically insert a new R-peak cursor at the selected location. A single right-click on an existing cursor will remove that cursor. The left and right arrow keys of the keyboard allow you to step through all scored R-peaks. The active (blue) cursor can also be deleted by pressing the delete key. The corrected time series is saved in the '.beat' file. Detailed information on how to tailor automatic scoring is found under the 'Help' option of the AmsQRS program.

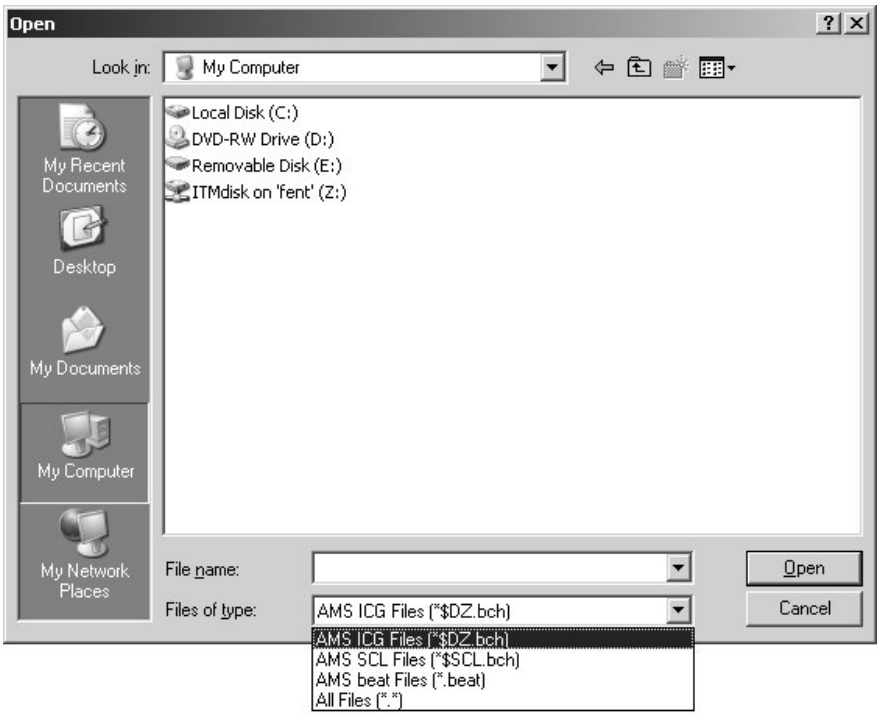
NOTE: All time spent correcting the IBI time series is well spent, since it is the basis of many other variables extracted from the VU-AMS.

How to score IBI, PEP and RSA in VU-AMS.5fs data?
After corrections with the AmsQRS program, the AMS.5fs signals can be converted to the old VU-AMS file format of the VU-AMS 4.6 series (.ams) with the AmsRevertFormat program. The immediate advantage of this is that all existing software for labeling (AMSGRA) and impedance scoring (AMSIMP) and respiration scoring (AMSRES) is now available. Manual for these programs are on the website (www.psy.vu.nl/vu-ams).

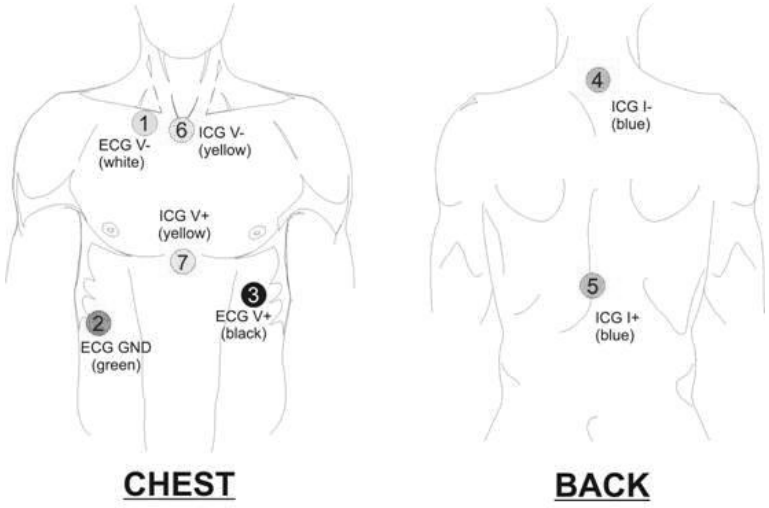


The VU-AMS 4.6 series did not yet combine ECG/SCL and ECG/ICG in a single device. You will have to choose the appropriate signals at the AmsRevertFormat option screen. For instance to create an .ams file with ECG/ICG signals use the following settings in AmsRevertFormat:

Then save the file as an impedance recording ($DZ.bch).



Appendix III: How to attach the VU-AMS.5fs?



Attachment of the ECG/ICG electrodes
Clean the skin at the 7 positions indicated in the figure. Rub the skin firmly with an alcohol soaked tissue or, if alcohol is not available, use a clean dry tissue. Attach an electrode by pressing the sticky plastic brim of the electrode on the skin and subsequently pushing the metal stud at the center of the electrode firmly, to properly spread the contact gel.

ECG:

1. (V-) Slightly below the right collar bone 4 cm to the right of the sternum
2. (GND) On the right side, between the lower two ribs
3. (V+) On the left side, on the ribcage, about 4 cm (1.3") below the nipple

ICG:

4. (I-) At the back, on the spine, at least 3 cm (1") above electrode 6
5. (I+) At the back, on the spine, at least 3 cm (1") below electrode 7
6. (V-) At the top end of the sternum, between the tips of the collarbones
7. (V+) At the low end of the sternum, where the ribs meet

Attachment of the lead wires and lead wire connector
Attach the lead wires to the electrodes according to the color -coding scheme in the figure above. Next, the blue ECG/ICG lead wire connector has to be plugged in the blue socket.

Starting the measurement by plugging in
The VU-AMS device is always on standby. Measurement will (re-)start after you plug in the lead wire connector and press the event button for about three seconds. A beep will be heard to acknowledge the start of the recording and the green light will start flashing about once every three seconds.

Wearing the device
Put the VU-AMS device in its carrier bag with the lead wire connector facing up. Fasten the device with the Velcro strap in the bag and gird it on with the VU-AMS belt (if it is more convenient, you can also use your own belt). Make sure the device remains in a vertical position as much as possible.

Marking special events
A small black button is placed on top of the VU-AMS device next to the two lead wire plug connectors. To mark a special event, push this button for about one second. Pushing it will be confirmed by a short beep.

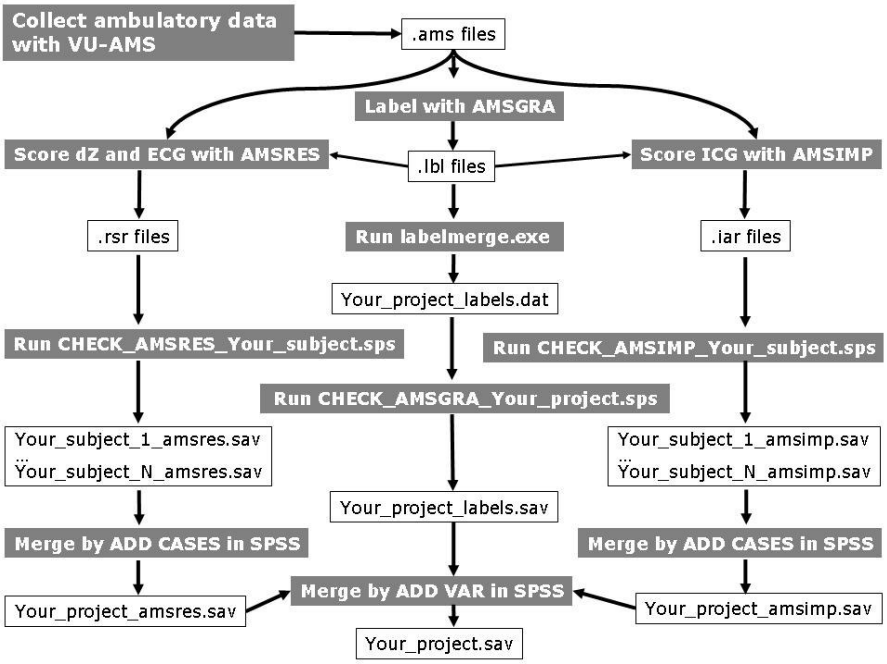
Stopping the measurement
If you want to stop the measurement temporarily (e.g. for taking a shower) press the event button for at least 3 seconds until the green light ceases flashing. Next, unplug the lead wire connector from its socket and disconnect the lead wires from the electrodes. The electrodes themselves are waterproof and need not be removed from the skin. To restart the measurement, simply follow the instructions above starting at 'Attachment of the lead wires'.

Still working?
A small indicator light on top of the device will be flashing about once every three seconds as long as the VU-AMS is recording.

Something is going wrong.
• The green light is flashing very rapidly
Diagnosis: The Compact Flash card is not (properly) installed.
Solution: Install the Compact Flash card in the proper way.
• The green light is flashing rapidly
Diagnosis: The battery lid is not (properly) fastened.
Solution: Fasten the battery lid in the proper way.
• You hear a double beep (the ‘alert beep’).
Diagnosis: The battery voltage is becoming low.
Solution: Replace the batteries with fresh ones.
• An electrode comes off, a lead wire gets disattched, or the lead wire connector is pulled out by accident.
Solution: No worries. Just attach the electrode again (use a spare one if necessary), reattach the lead wire, or plug the connector back into the socket.

Otherwise, for online help dial the number printed on your diary.

Appendix IV: Data reduction strategy (based on AMGRA labeling)
A graphical depiction of a strategy to handle VU-AMS data proven fruitful in our own research is given below. We combine AMS software with SPSS scripts:



How?
When you performed all labeling (AMSGRA) and all scoring (AMSIMP, AMSRES) of the VU-AMS data you can download the four SPSS scripts provided here to optimally organize your data under SPSS. Make a copy of each script where you change "generic" in the file names to "Your_project" or "Your_subject" (see below) and where you change the extension of the script files from .txt to .sps.

Then work with these copies only, i.e. with
- LABEL_VARIABLE&VALUES_Your_project.sps
- CHECK_AMSGRA_allsubjects_Your_project.sps
- CHECK_AMSRES_Your_subject_1.sps
- CHECK_AMSIMP_Your_subject_1.sps

In these SPSS scripts, a number of critical assumptions are made regarding the structure of your AMS data, specifically the file names of the AMS-files. If you do not meet these assumptions it may need substantial effort to get these scripts to run!! So much so that you should consider renaming your file names to meet these assumption.

ASSUMPTIONS:
1. The AMS file names are always of the same length (7 digits).
2. The AMS file names are numerical only (e.g. 0603001).
3. The AMS file names identify the subject (and the session), i.e. 0045601.ams for subject 456 at the first test session or 0003603.ams for subject 36 at the third test session.
4. You do not use AMSRES or AMSIMP before labeling the data with AMSGRA.
5. You did not have the labels in AMSGRA overlap in time.

FIRST
Provide appropriate variable and value labels for your database in the SPSS script LABEL_VARIABLE&VALUES_Your_project.sps. The examples given in this script should be able to guide you in doing this correctly.

SECOND
Concatenate the AMSGRA .lbl-files to yield a single large ASCII input text file for SPSS, which we refer to further as Your_project_labels.dat. To concatenate the AMGRA .lbl-files use the tool 'labelmerge.exe' under the MS-DOS prompt (see other downloadable files on the VU-AMS website). It removes the five lines of header information at the start of the .lbl-files. It then inserts the name of the .lbl-file (which according to assumption 3 is a subject identifier) at the beginning of each line. Check that the string 'LBL' does not occur in Your_project_labels.dat. In the CHECK_AMSGRA_allsubjects_Your_project.sps script, change the file names and directory structure used in the GET DATA and SAVE OUTFILE commands to the appropriate file names and directory structure of your own project. This is done by changing the strings "Your_full_directory_tree" and "Your_file_with_all_subjects" in the DEFINE statements at the top of the script. Then run the SPSS script to create the target SPSS file Your_file_with_all_subjects.sav.

THIRD
Now run the two remaining jobs SEPARATELY ON EACH SUBJECT. For AMSRES data, you generate a new script for each subject that in turn yields a separate target SPSS file for each subject 1 to N: Your_subject_1_AMSRES.sav, Your_subject_2_AMSRES.sav until Your_subject_N_AMSRES.sav. For AMSIMP data, you generate a new script for each subject that in turn yields the SPSS target file Your_subject_1_AMSIMP.sav. Note that in the CHECK_AMSRES_Your_subject.sps and CHECK_AMSIMP_Your_subject.sps scripts you need to change the directory structure used in the GET DATA and SAVE OUTFILE commands to the appropriate directory structure of your own project. For each of the subjects in the project you need to change the file name (i.e. subject_1, subject_2, etc) to the appropriate file name. Finally, in the CHECK_AMSIMP_Your_subject.sps scripts you may want to set the electrode distance separately for each subject by a DEFINE. When all subjects are done, use SPSS to MERGE (ADD CASES) Your_subject_1_AMSRES.sav to Your_subject_N_AMSRES.sav files into a single SPSS file that now contains AMSRES data of all subjects. We refer to this file as Your_project_AMSRES.sav. Do similar for the AMSIMP files of all subjects to obtain Your_project_AMSIMP.sav.

FOURTH
You can now MERGE the data from all three domains (Your_project_labels.sav, Your_project_AMSRES.sav and Your_project_AMSIMP.sav) using the interactive menu of SPSS. It is critical that you use the subject identifier variable and the category identifiers as the BREAK variables in the MERGE (ADD VAR) command.

Appendix V: SPSS scripts

LABEL_VARIABLE&VALUES_Your_project.sps

```
** This SPSS include file is used by other VU-AMS SPSS jobs to label the time
** periods over which the data have been aggregated. Aggregation uses the categories
** that were used in the label configuration file (label.cfg) during labeling under
** AMSGRA. These categories and there levels will be completely study-specific
*################################################
** This means that you need to change EVERYTHING below to make this job
** properly reflect your own study protocol and labeling. The good thing, however, is
** that you need to do this only ONCE
*################################################
** An example of a valid job for a specific study is given below. The study had five
** categories, each with multiple levels.
*********************************************************************************
*** ADD STUDY SPECIFIC VARIABLE LABELS.
*********************************************************************************.
VARIABLE LABEL posture 'main posture during labeled period'.
VARIABLE LABEL physical 'physical load during labeled period'.
VARIABLE LABEL activity 'main activity during labeled period'.
VARIABLE LABEL location 'location of subject during labeled period'.
VARIABLE LABEL social 'social situation during labeled period'.
EXECUTE.
*********************************************************************************
*** ADD STUDY SPECIFIC VALUE LABELS.
*********************************************************************************.
VALUE LABELS posture
            10          'lying'
            11          'sitting'
            12          'standing'
            13          'walking'
            14          'lie/sit'
            15          'sit/stand'
            16          'sit/stand/walk'
            17          'stand/walk'
            18          'bicycling'
            19          'unknown'.
EXECUTE.

VALUE LABELS physical
            20          'light physical activity'
            21          'medium physical activity'
            22          'heavy physical activity'
            23          'very heavy physical activity'
            24          'sleep'
            25          'unknown'.
EXECUTE.

VALUE LABELS activity
            30          'deskwork (PC)'
            31          'administrative work'
            32          'general activities at work'
            33          'household activities'
            34          'active transport (driving yourself)'
            35          'passive transport (passenger)'
            36          'telephone /talking business'
            37          'telephone/talking private'
```

```
            38          'reading / PC recreative'
            39          'eating / drinking'
            40          'watching television'
            41          'recreative activity'
            42          'sleep'
            43          'unknown'
            81          'sleep1'
            82          'sleep2'
            83          'sleep3'
            84          'sleep4'
            85          'sleep5'
            86          'sleep6'
            87          'sleep7'
            88          'sleep8'
            89          'sleep9'
            90          'sleep10'
            91          'sleep11em'.
EXECUTE.

VALUE LABELS location
            40          'work'
            41          'home'
            42          'outside'
            43          'friend'
            44          'on the road'
            45          'public'
            46          'hospital/medical doctor'
            48          'family elsewhere'
            49          'unknown'.
EXECUTE.

VALUE LABELS social
            50          'alone'
            51          'with SO'
            52          'with own kids'
            53          'with friends'
            54          'with colleagues'
            55          'with others'
            56          'with family'
            57          'unknown'.
EXECUTE.
```

CHECK_AMSGRA_allsubjects_Your_project.sps

```
SET PRINTBACK = OFF.

** File name = CHECK&_AMSGRA_generic.sps.
** Version July 2007.
** label Data File version 101.
** Some easy to locate DEFINEs that set the WORKING DIRECTORY, the input file with the
concatenated LBL-files of all subjects, and
** a file that defines the variable and value labels for the categories of your labeled periods.

DEFINE   !DIRY()     'C:\Your_full_directory_tree\' !ENDDEFINE.
DEFINE   !FILE()     'Your_file_with_all_subjects'  !ENDDEFINE.
DEFINE   !LABELF()  'label_VAR&VALUES_your_project'        !ENDDEFINE.
SET PRINTBACK = OFF.

***ATTENTION*****************************************************************.
** Although this job is fairly generic, it may need some adjustments to  fit your data.
** Search for the sections labeled with '@@@@@@' to bring this job in accordance
** with the protocol of your specific study and your own wishes regarding data
** handling (what to save and what not).
*****************************************************************************.
**SCRIPT HISTORY*************************************************************.
** Eco de Geus. Version 13-02-1996
** Modified for validation study AMS-Portapres by Harriette Riese
** Modified for applying under SPSSWIN 6.1 Tanja Vrijkotte Harriette Riese
** Modified for Slotervaartziekenhuis data 25 maart 1997 Harriette Riese
** Modified for OLVG data 1-7-98 Harriette Riese under SPSS7.5 under windows95.
** Modified for 'la nurses data complete' 7-4-99 Harriette Riese
** Modified for FFA-insuline 18-07-2002 Harriette Riese SPSS 11.0
** Modified for NETSAD 27-11-2002 Harriette Riese SPSS 11.0
** Modified for NETSAD 28-03-2003 Nina Kupper SPSS 11.5
** Modified for website vu-ams 03-02-2004 Nina Kupper SPSS 11.5
** Modified for Psychophysiology course 2006 Eco de Geus & Annebet Goedhart
** Modified for Rosa project Eco de Geus 01-04-2007
** Lay-out & Logic update Annebet Goedhart July 2007
*****************************************************************************.
**GENERAL DESCRIPTION********************************************************.
** This is a syntax file for SPSS for Windows version 13.
** INPUT: It will read a label.dat file with the concatenated LBL-files of all subjects.
** label.dat files are created by preprocessing individual LBL-files with
** 'labelmerge.exe' in DOS (see other downloadable files on the VU-AMS website).
** labelmerge.exe removes the 5 lines of header information at the start of the
** lbl-files. It then inserts the name of  the lbl-file at the beginning of each line.
** We assume that this file name acts as a 7-digit subject identifier.
** All processed ".lbl" files are then concatenated and the resulting file
** (with extension .dat) is read in SPSS to make a database of the labeled periods
** under AMSGRA, together with the average motility, heart rate and heart rate
** variability parameters for each of these labeled periods as found in the ".lbl" file.

** ACTION: The AMSGRA data are read into SPSS.
** LIST OF VARIABLES TO BE READ:
** ppname          = subject identification
** strtdate        = start date of the labeled period in dd:mm:yy
** enddate         = end date of the labeled period in dd:mm:yy
** strttime        = start time of the labeled period in hh:mm:ss
** endtime         = end time of the labeled period in hh:mm:ss
**                  (Don't make too much of 'seconds' here, since labeling accuracy is
```

```
**                  usually not more than 30 s)
** label 1 .. n = Here a number of labeling categories may be read; the exact number
**                  depends on the configuration of your label.cfg file
** hramean         = the mean of the average HRs found in this labeled period
**                  Yes, this is an average of averages! Each 30 seconds (by default) an
**                  average heart rate is computed from all interbeat intervals (IBIs)
**                  found in that period. The variable 'hrmean' gives the mean of these
**                  averages over the entire labeled period. Please note that we have no
**                  information on the number or the integrity of IBIs constituting the
**                  30-s average HR!  Note that 30 seconds is the default, you may
**                  have changed it, but the same principle will apply. To get the
**                  complete IBI time series you can convert the AMS file to an ascii
**                  file containing all ibi's by AMSASC.
** hra#            = the number of average HRs found in this labeled period
** hramin          = the lowest average HR found in this period
** hramax          = the highest average HR found in this period
** hravar          = the variance in the average HRs found in this period
** hrasd           = the standard deviation of the average HRs found in this period
** msdmean         = the mean of all 30-second msd scores found in this labeled period
**                  The same 30-second fragment (by default) that is used to compute
**                  the average heart rate is used to compute the Mean Square of
**                  Successive Differences in interbeat interval (in ms). The latter index
**                  of heart rate variability may be used to get a quick impression of vagal
**                  tone. Again, make sure to note that we have no information on the
**                  number or the integrity of IBIs constituting the mssd.
** msd#            = the number of average HRs (and thus msds) found in this labeled
**                  period
** msdmin          = the lowest msd found in this period
**                  Yuck! A bug in AMSGRA (up to version 4.4) sometimes produces
**                  negative values here. See below on how to deal with this.
** msdmax          = the highest msd found in this period
** msdvar          = the variance in the msds found in this period
** msdsd           = the standard deviation of the msds found in this period
** motmean         = the average of all motility scores found in this labeled  period.
**                  Each motility score represents the sum of vertical acceleration over a
**                  30-s period (30 seconds is thedefault, you may have changed it,
**                  even independently of the period for heart rate averaging, but the
**                  same principle will apply).
** mot#            = the number of 30-s motility scores found in this labeled period
** motmin          = motility during the 30-second fragment with lowest motility
** motmax          = motility during the 30-second fragment with highest motility
** motvar          = the variance in the motility scores found in this period
** motsd           = the standard deviation of motility scores found in this period
** ibimean         = the mean of the average IBIs found in this labeled period
**                  Yes, this is an average of averages! Each 30 seconds (by default) an
**                  average IBI is computed from all  interbeat intervals found in that
**                  period. The variable 'ibimean' gives the mean of these averages over
**                  the entire labeled period. Please note that we have no information on
**                  the number or the integrity of IBIs constituting the 30-sec average
**                  IBI!  Note that 30 seconds is the default, you may have changed it,
**                  but the same principle will apply. To get the complete IBI time series
**                  you can convert the AMS file to an ASCII file containing all IBIs by
**                  using AMSASC.
** ibi#            = the number of average IBIs found in this labeled period
** ibimin          = the lowest average IBIs found in this period
** ibimax          = the highest average IBIs found in this period
** ibivar          = the variance in the average IBIs found in this period
** ibisd           = the standard deviation of the average IBIs found in this period
```

```
** Outliers are detected, variables and values are labeled and indices of total recording
**    time and data loss are added.
** OUTPUT: The resulting .SAV file will consider each labeled period to be a "case".
** Therefore, per subject there will be a PEP, SV etc for each labeled period.
*************************************************************************.
** @@@@@@ FILE AND DIRECTORY STRUCTURE**********************.
** WE ASSUME THAT FILE NAMES ARE EXACTLY 7 CHARACTERS LONG
** & REFLECT THE ID OF THE SUBJECT NOTE THAT THE FILENAMES
** USED IN DATA LIST AND SAVE OUTFILE COMMANDS MUST BE
** CHANGED TO CORRESPOND TO YOUR OWN FILENAMES AND
** DIRECTORY STRUCTURE THIS CAN BE DONE BY CHANGING THE
** DEFINE STATEMENTS AT THE START OF THIS JOB
*************************************************************************.
** @@@@@@ PROJECT SPECIFIC LABELING*************************.
** The job text below assume that you used the following categories to label the data:
** posture= a code stating the dominant posture during that period (10 levels)
** physical load      = a code indicating the level of physical load (6 levels)
** activity           = type of activity the subject is engaged in (24 levels)
** location           = a code for the location of the subject (9 levels)
** social situation   = indicating the social situation the subject is in (8 levels)
**
** This is unlikely to correspond to your own categories. Change the command syntax
** accordingly, i.e. change
** posture 47-51 F5.0 physical 52-55 F4.0 activity 56-59 F4.0 location 60-63 F4.0
** social 64-67 F4.0
**
** to the appropriate description of the categories you used to label the data
*************************************************************************.
***WARNING FOR POTENTIAL PROBLEMS
*************************************************************************.
** The largest problem that arises during the GET DATA statement is that the input
** has a comma-notation for floating point notation (e.g. 8,03) whereas SPSS expects
** a dot-notation (8.03) or vice versa. When the need arises: In the control panel of
** Windows select 'regional and language options' / 'customize' and set the decimal
** symbol for numbers to 'dot'.
*************************************************************************.
***** READING MULTIPLE LBL-FILES ***********************************************************************.
TITLE 'PROCESSING VU-AMS LBL-FILES CREATED WITH AMSGRA AND CONCATENATED WITH labelMERGE'.

GET DATA  /TYPE = TXT /FILE = !DIRY+!FILE+'.dat'
 /FIXCASE = 5  /ARRANGEMENT = FIXED  /FIRSTCASE =1  /IMPORTCASE = ALL
 /VARIABLES =
 /1 subject 0-6 F7.0 strtdate 11-18 EDATE8  strttime 20-27 TIME11.2 enddate 30-37 EDATE8 endtime
39-46 TIME11.2 posture 47-51 F5.0 physical 52-55 F4.0 activity 56-59 F4.0 location 60-63 F4.0 social
64-67 F4.0
/2 hra# 16-24 F9.2 hramin 25-33 F9.2 hramax 34-42 F9.2 hramean 43-51 F9.2 hravar 52-60 F9.2 hrasd
61-69 F9.2
/3 msd# 16-24 F9.2 msdmin 25-33 F9.2 msdmax 34-42 F9.2 msdmean 43-51 F9.2 msdvar 52-60 F9.2
msdsd 61-69 F9.2
/4 mot# 16-24 F9.2 motmin 25-33 F9.2 motmax 34-42 F9.2 motmean 43-51 F9.2 motvar 52-60 F9.2
motsd 61-69 F9.2
/5 ibi# 16-24 F9.2 ibimin 25-33 F9.2 ibimax 34-42 F9.2 ibimean 43-51 F9.2 ibivar 52-60 F9.2 ibisd 61-
69 F9.2 .
CACHE.
EXECUTE.
*************************************************************************.
*****ADD VARIABLE LABELS*********************************************.
VARIABLE LABEL strtdate 'start date of the labeled period in dd:mm:yy'.
```

```
VARIABLE LABEL enddate 'end date of the labeled period in dd:mm:yy'.
VARIABLE LABEL strttime 'start time of the labeled period in hh:mm:ss'.
VARIABLE LABEL endtime 'end time of the labeled period in hh:mm:ss'.
VARIABLE LABEL hra# 'the number of 30-second average HRs found during this period'.
VARIABLE LABEL hramin 'the lowest 30-second average HR found during this period'.
VARIABLE LABEL hramax 'the highest 30-second average HR found during this period'.
VARIABLE LABEL hramean 'the mean HR across this period'.
VARIABLE LABEL hrasd 'the standard deviation of the 30-second average HRs found during this period'.
VARIABLE LABEL msdmin 'the lowest 30-second average RMSSD found during this period'.
VARIABLE LABEL msdmax 'the highest 30-second average RMSSD found during this period'.
VARIABLE LABEL msdmean 'the mean RMSSD across this period'.
VARIABLE LABEL msdsd 'the standard deviation of all 30-second RMSSD averages found in this period'.
VARIABLE LABEL motmin 'motility during the 30-second fragment in this period with lowest motility'.
VARIABLE LABEL motmax 'motility during the 30-second fragment in this period with highest motility'.
VARIABLE LABEL motmean 'the average motility across this period'.
VARIABLE LABEL motsd 'the standard deviation of all 30-second motility averages found during this
period'.
VARIABLE LABEL IBImin 'IBI in the 30-second fragment with fastest heart rate in this period '.
VARIABLE LABEL IBImax 'IBI in the 30-second fragment with slowest heart rate in this period '.
VARIABLE LABEL IBImean 'the average IBI across this period'.
VARIABLE LABEL IBIsd 'the standard deviation of all 30-second IBI averages found during this period'.
EXECUTE.
*************************************************************************.
** VALUE AND VARIABLE LABELING ***********************************************************************.
** Use an INCLUDE FILE to supply the correct variable and value labels for (all of)
** the numerical codes used when labeling the AMS-file with AMSGRA. Note that
** the file name needs to be changed to the name of your study specific include file
** that you prepared before running this job. In the example below the include file
** supplies VARIABLE names and VALUES for posture, physical load, type of the
** activity, location and social situation. This may, of course, be different in
** your include file.
*************************************************************************.
INCLUDE !DIRY+!LABELF+'.sps'.
**@@@@ CATEGORY DEPENDENT CHECKS OF LABELING IN AMSGRA
*************************************************************************.
** The checks below assumes that you used the following five CATEGORIES for
** labeling with AMSGRA;
** posture             = a code stating the dominant posture during that period (10 levels)
** physical load = a code indicating the level of physical load (6 levels)
** activity            = type of activity the subject is engaged in (24 levels)
** location            = a code for the location of the subject (9 levels)
** social situation    = indicating the social situation the subject is in (8 levels)
** This is unlikely to correspond to your own categories. Change the command syntax
** accordingly, i.e. change
**           posture physical activity location social
** to the appropriate description of the categories you used to label the data
*************************************************************************.
**CHECKS OF LABELING IN AMSGRA *********************************************************************.
** Carefully check the frequency distribution of the labels used.
** Identify the problematic frequencies and go back to the original diary data where
** needed to resolve this.
FREQUENCIES
  VARIABLES=posture physical activity location social
  /ORDER=  ANALYSIS .

** Test for events that are unlike to happen (e.g. watching television while using
** public transportation; being asleep while driving a car etc. If they occur check the
** original diaries whether a false entry was made during labeling and resolve this.
```

```
CROSSTABS
  /TABLES=activity BY posture
  /FORMAT= AVALUE TABLES
  /CELLS= COUNT .
EXECUTE.
CROSSTABS
  /TABLES=physical  BY posture
  /FORMAT= AVALUE TABLES
  /CELLS= COUNT .
EXECUTE.
CROSSTABS
  /TABLES=physical  BY location
  /FORMAT= AVALUE TABLES
  /CELLS= COUNT .
EXECUTE.
CROSSTABS
  /TABLES=social  BY location
  /FORMAT= AVALUE TABLES
  /CELLS= COUNT .
EXECUTE.
*************************************************************************.
**REMOVING EMPTY LABELS*************************************************.
** Remove labeled periods that have no physiological data. This occurs in between
** hold and continues.
SELECT IF hramin ~= 0.
SELECT IF hra#  > 1.
EXECUTE .
*************************************************************************.
**OUTLIER DETECTION*****************************************************.
** Check whether your physiological variables remain within their plausible
** physiological range:
**  HRAMEAN                    = the average HR  35-200 bpm
**  HRAMIN                     = the lowest HR   30-180 bpm
**  HRAMAX                     = the highest HR  50-240 bpm
**  HRASD            = HR standard deviation  1-25 bpm
**  MOTMEAN          = average motility of subject *device dependent*
**  MOTMIN                     = lowest motility *device dependent*
**  MOTMAX                     = highest motility *device dependent*
**  MOTSD                      = standard deviation *device dependent*
**  MSDMEAN                    = the average MSSD 3-195
**  MSDMIN                     = lowest MSSD      0-200
**  MSDMAX                     = highest MSSD     5-550
**  MSDSD            = MSSD standard deviation 0-90
**  This is a topic that is subject to personal opinion, you must ultimately decide how to deal ** with
outliers yourself.

FREQUENCIES
  VARIABLES=HRAMEAN HRASD HRAMAX HRAMIN MSDMEAN MSDSD
MSDMAX MSDMIN
  /FORMAT=LIMIT (1)
  /PERCENTILES= 5 95
  /STATISTICS=STDDEV VARIANCE RANGE MINIMUM MAXIMUM MEAN
  /HISTOGRAM  NORMAL.
EXECUTE.
```

```
** Generic exclusion criteria.
SELECT IF (35 < HRAMEAN < 200).
SELECT IF (MSDMAX < 550).
SELECT IF (MSDMIN < 200).
SELECT IF (1 < HRASD < 25).
EXECUTE.
** Project specific exclusion criteria.
** @@@@@ (FILL IN AS REQUIRED)
EXECUTE.
*************************************************************************.
**SAVING DATA************************************************************.
** We happily throw away redundant or unimportant information at this point.
** DROP  mot# msd# (number of 30-s averages in each of the labeled periods is
** already indicated by hra#, which we keep)
** DROP hravar  motvar msdvar (variance, we already have SD)
** Compute the duration of each of the labeled periods and adds this variable
*************************************************************************.
FORMATS strttime endtime (TIME11.2).
IF (strtdate eq enddate) duration =  CTIME.MINUTES(endtime - strttime).
IF (strtdate ne enddate) duration =  CTIME.MINUTES(endtime + (TIME.HMS(24,0,0) - strttime)).
EXECUTE.
VARIABLE label duration 'Duration of this labeled period in minutes (NB: floating point notation)'.
EXECUTE.

SAVE OUTFILE=!DIRY+!FILE+' + '_labels.sav'/ DROP mot# msd# ibi# hravar motvar msdvar ibivar.
EXECUTE.
```

CHECK_AMSRES_Your_subject_1.sps

```
** File name = CHECK_AMSRES_singlesubject_generic.sps
** Version July 2007
** AMS version 4.6 / 5fs (back conversion)
** Some easy to locate DEFINEs that set the WORKING DIRECTORY, the
** RSR-file to be checked, and a file that defines the variable and value labels for the
** categories of your labeled periods.

DEFINE   !DIRY()     'C:\Your_full_directory_tree\'          !ENDDEFINE.
DEFINE   !FILE()     'Your_file_for_subject_1'              !ENDDEFINE.
DEFINE   !LABELF()  'label_VAR&VALUES_your_project'      !ENDDEFINE.

***ATTENTION*****************************************************************.
** Although this job is fairly generic, it may need some adjustments to  fit your data.
** Search for the sections labeled with '@@@@@' to bring this job in accordance
** with the protocol of your specific study and your own wishes regarding
** data handling (what to save and what not).
******************************************************************************.
**SCRIPT HISTORY*************************************************************.
** Original version 1996 Eco de Geus
** [many hacks by Riese & Vrijkotte]
** Revised extensively by Dolf de Boer 2004
** Modified for Psychophysiology course 2006 Eco de Geus & Annebet Goedhart
** Updated for thesis Annebet Goedhart, summer 2007
******************************************************************************.
**GENERAL DESCRIPTION********************************************************.
** This is a syntax file for SPSS for Windows version 13.
** INPUT: It will read individual ".rsr" files into SPSS.
** ACTION: This syntax file is an example of how one could use SPSS for the
** detection of outliers and final aggregation of the data obtained from AMSRES.
** This syntax has been developed in our department through years of experience and
** final improvements have been made and tested in a dataset of the ambulatory
** monitoring of >1000 participants. The selection criteria in this syntax have
** provided satisfactory results for this dataset. However, for other populations,
** circumstances or research protocols, different criteria might be required.
** First the raw data is imported into SPSS. This DATA LIST statement will differ
** according to the settings used when creating  the .rsr file (see "Edit", "Options"
** in AmsRes).
** @@@@@ WE ASSUME THAT ALL OUTPUT FIELDS HAVE BEEN
** GENERATED, i.e. ALL BOXED TICKED, WITH EXCEPTION OF
** 'write file header'. ALSO WE ASSUME THAT THE BREATH IS
** TIMESTAMPED WITH A LONG DATE/TIME FORMAT. FINALLY WE
** ASSUME THAT 5 DIFFERENT CATEGORIES WERE USED IN LABELING
** THE DATA UNDER AMSGRA
** In short the file header of the AMSRES report file would have looked like
** column  1 (width= 8): Subject ID
** column  2 (width =7): Breath Number
** column  3 (width= 7): Beat-to-beat sequence number (starting at 0)
** (historic/no longer used)
** column  4 (width= 9): Date (dd-mm-yy)
** column  5 (width= 9): Time (hh:mm:ss)
** column  6 (width= 6): Inspiration time [ms]
** column  7 (width= 6): Expiration time  [ms]
** column  8 (width= 6): Shortest accelerating inspiration [ms]
** column  9 (width= 6): Longest decelerating expiration [ms]
** column 10 (width= 6): Respiration Rate [ms]
** column 11 (width= 6): Respiratory Sinus Arrhythmia (RSA) [ms]
```

```
** column 12 (width= 6): Mean IBI (across the duration of the breath) [ms]
** column 13 (width= 8): correlation between mean IBI and RSA
** (running average on 30 s)
** column 14 (width= 8): dZ Amplitude - raw signal (min) [Ohm]
** column 15 (width= 8): dZ Amplitude - raw signal (max) [Ohm]
** column 16 (width= 8): dZ Amplitude - filtered signal (min) [Ohm]
** column 17 (width= 8): dZ Amplitude - filtered signal (max) [Ohm]
** column 18 (width= 2): Rejected ('R') or accepted ('A')
** column 19 (width= 4): label number (0 if N/A)
** column 20+ (width=99): Values in all categories used for labeling (integers)
**
** LIST OF VARIABLES TO BE READ:
** subjectSubject ID
** breath Breath Number
** bbb              Beat-to-beat sequence number (starting at 0)
** date             Date       Date (dd-mm-yy)
** time             Time (hh:mm:ss)
** insp             Inspiration time [ms]
** exp              Expiration time  [ms]
** shortibi         Shortest accelerating inspiration [ms]
** longibi Longest decelerating expiration [ms]
** rr               Respiration Rate [ms]
** rsa              Respiratory Sinus Arrhythmia (RSA) [ms]
**                          -1 undetectable 'shortest IBI'
**                          -2 undetectable 'longest IBI'
**                          -3 both 'longest IBI' and 'shortest IBI' were undetectable
**                          -4 'longest IBI' is shorter than the 'shortest IBI'
** ibi              Mean IBI (across the duration of the breath) [ms]
** ibirsa_corr      correlation between mean IBI and RSA (running average on 30 s)
** dzMinR dZ Amplitude - raw signal (min) [Ohm]
** dzMaxRdZ Amplitude - raw signal (max) [Ohm]
** dzMinf  dZ Amplitude - filtered signal (min) [Ohm]
** dZMaxf dZ Amplitude - filtered signal (max) [Ohm]
** reject  Rejected ('R') or accepted ('A')
** labelnr label number (0 if N/A)
** (labels) Values in all categories used for labeling (integers)
**

** VARIABLES ADDED:
** RSAZERO is calculated out of shortibi and longibi. In contrast to the original RSA
** value, values for RSAZERO are set to zero when neg=1 (absence of a valid
** shortibi and/or longibi) or reverse=1 (shortibi>longibi). In our experience, a lot
** of cases of neg=1 or reverse=1 are due to a true low RSA and RSA should be set
** to zero. We also keep the original RSA value (RSA). For breaths with neg =1 or
** reverse=1, however, RSA is interpreted to be missing and these breaths are
** excluded.
**
** DATA LOSS PARAMETERS Outliers are detected in various procedures are
** removed; an indicator of the total data loss caused by each procedure is temporarily
** added.
**
** OUTPUT: The resulting .SAV file will aggregate across labeled periods. Each
** labeled period will considered to be a "case".
** Therefore, per subject there will be a RSA, RR etc for each labeled period. These
** data will be "clean".
*************************************************************************.
** @@@@@ FILE AND DIRECTORY STRUCTURE**********************.
** WE ASSUME THAT FILE NAMES ARE EXACTLY 7 CHARACTERS LONG
```

```
** & REFLECT THE ID OF THE SUBJECT  NOTE THAT THE FILENAMES
** USED IN DATA LIST AND SAVE OUTFILE COMMANDS MUST BE
** CHANGED TO CORRESPOND TO YOUR OWN FILENAMES AND
** DIRECTORY STRUCTURE THIS CAN BE DONE BY CHANGING THE
** DEFINE STATEMENTS AT THE START OF THIS JOB
*****************************************************************************.
** @@@@@ PROJECT SPECIFIC LABELING*****************************.
** The job text below assume that you used the following categories to label the data:
** posture= a code stating the dominant posture during that period (10 levels)
** physical load      = a code indicating the level of physical load (6 levels)
** activity           = type of activity the subject is engaged in (24 levels)
** location           = a code for the location of the subject (9 levels)
** social situation = indicating the social situation the subject is in (8 levels)
**
** This is unlikely to correspond to your own categories. Change the command syntax
** accordingly, i.e. change
**          posture 129-133 physical 134-137 activity 138-141 location 142-145
**          social 146-149
** to the appropriate description of the categories you used to label the data
*****************************************************************************.
***WARNING FOR POTENTIAL PROBLEMS
*****************************************************************************.
** The largest problem that arises during the GET DATA statement is that the input
** has a comma-notation for floating point notation (e.g. 8,03) whereas SPSS expects
** a dot-notation (8.03) or vice versa. When the need arises: In the control panel of
** Windows select 'regional and language options' / 'customize' and set the decimal
** symbol for numbers to 'dot'.
** Another nicety is that SPSS in some version stopped counting empty lines as 'true'
** lines. This may mean that you need to adjust the FIRSTCASE parameter in the
** GET DATA statement.
*****************************************************************************.
TITLE 'PROCESSING VU-AMS RSR-FILES CREATED WITH AMSRES'.
SUBTITLE 'READING THE AMBULATORY BREATH-TO-BREATH RR, RSA AND IBI DATA'.

DATA LIST FILE= !DIRY+!FILE+'.rsr' FIXED RECORDS=1
/ subject 1-7 breath 8-15 bbb 16-22 date 23-31 (DATE) time 33-40 (TIME)
insp 41-46 exp 47-52 shortibi 53-58 longibi 59-64 rr 65-70 (2) rsa 71-76 ibi 77-82 rrrsa_corr 83-90 (4)
DzMinr 91-98 (4) DzMaxr 99-106 (4) dzMinf 107-114 (4) dzMaxf 115-122 (4) reject 124 (a) labelnr 125-
128 posture 129-133 physical 134-137 activity 138-141 location 142-145 social 146-149.
EXECUTE.

**OUTLIER DETECTION*************************************************.
** Now we apply various selection criteria to remove nonreliable data for this subject.
** Exact documentation of the data-loss after each selection is done with the
** cases_1 .. cases_n variables
*****************************************************************************.
COMPUTE cases_1 = $CASENUM.
VARIABLE LABLE cases_1 'Total number of breaths'.
AGGREGATE  /OUTFILE=*  MODE=ADDVARIABLES  OVERWRITE=YES /BREAK=subject /cases_1 =
MAX(cases_1).

** The first two breaths at the beginning of the signal have to rejected because the
** FIR-filter needs some samples to start-up.

SELECT IF breath > 2.
SELECT IF labelnr > 0.
EXECUTE.
```

```
COMPUTE cases_2 = $CASENUM.
VARIABLE LABLE cases_2 'Excluding the unlabeled parts of the recording'.
AGGREGATE  /OUTFILE=*  MODE=ADDVARIABLES  OVERWRITE=YES /BREAK=subject /cases_2 =
MAX(cases_2).

**With this statement, we exclude all breaths that have been rejected in AmsRes.
** For rejection criteria, see the AMSRES manual.

SELECT IF (reject = 'A').
EXECUTE.

COMPUTE cases_3 = $CASENUM.
VARIABLE LABLE cases_3 'Excluding breaths that were interactively rejected in AMSRES'.
AGGREGATE  /OUTFILE=*  MODE=ADDVARIABLES  OVERWRITE=YES /BREAK=subject /cases_3 =
MAX(cases_3).

** Here we exclude off hand expirations and inspirations that we consider too long or
** too short to be physiologically plausible.

SELECT IF (exp < 10000).
SELECT IF (exp > 300).
SELECT IF (insp <9000).
SELECT IF (insp > 300).
EXECUTE.

COMPUTE cases_4 = $CASENUM.
VARIABLE LABLE cases_4 'Excluding non-plausible long or short breaths'.
AGGREGATE  /OUTFILE=*  MODE=ADDVARIABLES  OVERWRITE=YES /BREAK=subject /cases_4 =
MAX(cases_4).
*************************************************************************.
**With these former selection criteria the loss of data in our population was generally
** about 7%. More than 20% is considered a lot. One might want to check whether a
** lot of unreliable data is correctly rejected or whether the automatic scoring of
** AmsRes has incorrectly rejected a lot of reliable data.
*************************************************************************.
**In the remaining data, the distributions of inspiration and expiration are inspected.
** @@@@@ this has been commented out, to speed things up. A more careful, but
** less "automated" and slower, strategy would leaves this intact

** FREQUENCIES VARIABLES=insp exp/FORMAT=LIMIT (1) /PERCENTILES= 3 97 /STATISTICS=NONE
/HISTOGRAM  NORMAL.

** REMOVE OUTLIERS!! *************************************************************************.
**Usually the distribution is skewed to the right so removing with a 3 SD criterion
** eliminates about 1 to 2%
*************************************************************************.
** Add the Z-scores of inspiration and expiration time.

DESCRIPTIVES VARIABLES=insp exp /SAVE /STATISTICS=MEAN STDDEV MIN MAX .

SELECT IF Zinsp > -3 .
SELECT IF Zexp > -3 .
SELECT IF Zinsp < 3 .
SELECT IF Zexp < 3 .
EXECUTE .

COMPUTE cases_5 = $CASENUM.
VARIABLE LABLE cases_5 'Excluding breaths 3SD deviated from the mean'.
```

```
AGGREGATE  /OUTFILE=*  MODE=ADDVARIABLES  OVERWRITE=YES /BREAK=subject /cases_5 =
MAX(cases_5).
*****************************************************************************.

**With the next statements we will compute the tidal volume as the difference
** between the dZ amplitude at the peak and the troughs. The filtered signal will be
** used; note that any calibration to spirometric volumes must be done using the
** filtered volumes
*****************************************************************************.
COMPUTE Vt = dzMaxf - dzMinf.
EXECUTE.

** CHECK!! ******************************************************************************.
** Check for tidal volumes that are extremely deep or shallow.  Breaths with zero
** amplitude must always be removed There is usually a tail with very large breaths
** due to sighs. We have not removed these in previous analyses , but we
** grant this is a matter for debate.
*****************************************************************************.
** @@@@@ this has been commented out, to speed things up. A more careful, but
** less "automated" and slower, strategy would leaves this intact
**
** FREQUENCIES  VARIABLES=Vt /FORMAT=LIMIT (10) /STATISTICS=NONE /HISTOGRAM  NORMAL.

SELECT IF (Vt > 0).
*SELECT IF (Vt < 3).
EXECUTE.

COMPUTE cases_6 = $CASENUM.
VARIABLE LABLE cases_6 'Excluding zero-amplitude breaths'.
AGGREGATE  /OUTFILE=*  MODE=ADDVARIABLES  OVERWRITE=YES /BREAK=subject /cases_6 =
MAX(cases_6).
*****************************************************************************.
**With the next statements we will check for inter beat intervals that are extremely
** short or extremely long. First we temporary select longibi>0 and shortibi>0
** so values flagging missing data (-1) (i.e. breaths where no valid shoritibi
**and/or longibi could be found) are not inadvertedly included.
*****************************************************************************.
** @@@@@ this has been commented out, to speed things up. A more careful, but
** less "automated" and slower, strategy would leaves this intact
**
** TEMPORARY.
** SELECT IF longibi > 0.
** SELECT IF shortibi > 0.
** FREQUENCIES VARIABLES=shortibi longibi ibi /FORMAT=LIMIT (1)  /PERCENTILES= 5 95
/STATISTICS=NONE /HISTOGRAM NORMAL.

** CHECK!! ******************************************************************************.
**A HR lower than 35 (shortibi > 1700) is very uncommon and unlikely, although not
** impossible. The next statement excludes these beats. If these ibi's>1700 cannot be
** considered outliers (see histogram) it is recommended to check the AmsRes
** interface to see whether such beats derive from spikes or whether they are true and
** reliable. If so, disable or change the select statements below.
** In a 24 hour recordings do not be alarmed by a mixture distribution; this is what
** you expect (NIGHT vs. DAYTIME)
*****************************************************************************.
** @@@@@ THIS SET OF STATEMENTS MAY REQUIRE DIFFERENT
** PARAMETERS IN YOUR SAMPLE!!!
```

```
SELECT IF (shortibi < 1700).
SELECT IF (longibi < 1800).
EXECUTE.

COMPUTE cases_7 = $CASENUM.
VARIABLE LABLE cases_7 'Excluding very slow heart beats'.
AGGREGATE  /OUTFILE=*  MODE=ADDVARIABLES  OVERWRITE=YES /BREAK=subject /cases_7 =
MAX(cases_7).
*****************************************************************************.
** IBI's of 250 ms (HR = 240) and shorter are extremely uncommon and may very
** well be unrejected spikes. With the next statements they will be rejected. Note that
** we keep shortibi<0 and longibi<0 since these values flag the cases where no valid
** shortibi or longibi could be found within the inspirational and expirational
** intervals.
*****************************************************************************.

SELECT IF  ((shortibi > 250) OR (shortibi < 0)).
EXECUTE.
SELECT IF  ((longibi > 270) OR (longibi < 0)).
EXECUTE.
*both shortibi and longibi may not have been found.

SELECT IF  ((ibi > 260) OR (ibi < 0)).
EXECUTE.

COMPUTE cases_8 = $CASENUM.
VARIABLE LABLE cases_8 'Excluding very fast heart beats'.
AGGREGATE  /OUTFILE=*  MODE=ADDVARIABLES  OVERWRITE=YES /BREAK=subject /cases_8 =
MAX(cases_8).
*****************************************************************************.
**Here, we create a dummy variable that allows us to compute the frequency of
** breaths where RSA cannot be computed due to the absence of a valid shortibi
** and/or longibi within the respiratory cycle.
*****************************************************************************.

COMPUTE neg=0.
IF ((shortibi<0) or (longibi <0)) neg=1.
EXECUTE.
*****************************************************************************.
**The next dummy variable that is computed allows us to calculate the percentage of
** breaths where shortibi is longer than longibi (reverse=1).
*****************************************************************************.

COMPUTE reverse=0.
IF ((NEG EQ 0) AND (shortibi>longibi)) reverse=1.
EXECUTE.

FREQUENCIES VARIABLES= neg reverse /FORMAT=LIMIT(10) /STATISTICS=NONE.

** CHECK!! ******************************************************************************.
**On average, (neg=1) was about 20% in our dataset. If (neg=1) is a lot higher than
** usual in your population it might be worth to inspect the data in AmsRes to see
** whether this is due to breaths with a truly low RSA (i.e. where the
**coupling of shortibi and longibi to the respiratory cycle is lost) or whether the
** AmsRes automatic scoring program hopelessly failed for this participant
*****************************************************************************.
**RSAZERO is calculated out of shortibi and longibi. In contrast to the original RSA
** value, values for RSAZERO are set to zero when neg=1 (absence of a valid
```

```
** shortibi and/or longibi) or reverse=1 (shortibi>longibi). In our experience, a lot
**of cases of neg=1 or reverse=1 are due to a true low RSA and RSA should be set to
** zero. We also keep the original RSA value (RSA). For breaths with neg =1 or
** reverse=1, however, RSA is interpreted to be missing and these breaths are
** excluded.
*****************************************************************************.

COMPUTE rsazero = longibi - shortibi.
IF ((reverse=1) or (neg=1)) rsazero=0.
IF ((reverse=1) or (neg=1)) rsa=-1.
EXECUTE.

** This statement makes sure that missing values are excluded from the calculation of
** the means of these variables. Note that we could do this earlier than at this point
** because -1 values of longibi and shortibi were used as meaningful signals.

MISSING VALUES LONGIBI SHORTIBI RSA (-1).
EXECUTE.

** CHECK!! *****************************************************************************.
**OUTLIERS OF RR,  RSA and IBI:
**The RR, RSA and IBI histograms are visually inspected to see whether there are
** outliers. If there are outliers, it is advised to return to the AmsRes interface to
** check a couple of high RSA's to see whether these are based on reliable signal.
** In our view, the main reason for distrust of outliers would be the presence of
** unrejected spikes. In other words, in case of a good manual scoring in AmsRes,
** outliers of RSA usually are true high RSA's. If outliers of RSA are
** invalid, they can be rejected manually in AmsRes. A more time-efficient but less
** elegant method would be to exclude these outliers with a selection statement in
** SPSS instead of a manual rejection in AmsGra. In this case the selection
** statement has to be adjusted to a specific cut-off value for  each participant. This
** cut-off value can be based on a visual check of the RSA histogram
** (this is what we did 800 times) or one could exclude a set percentage for all
** subjects, for instance the highest 1%.
*****************************************************************************.
** adjust value based on the visual check of the histograms below or to the
** 99-percentile.
** @@@@@ THIS SET OF STATEMENTS MAY REQUIRE DIFFERENT
** PARAMETERS IN YOUR SAMPLE!!

*FREQUENCIES VARIABLES=rr rsa rsazero ibi /FORMAT=LIMIT (10) /PERCENTILES= 99
/STATISTICS=NONE /HISTOGRAM  NORMAL.

SELECT IF rr < 30.
SELECT IF MISSING(rsa) OR (rsa < 250).
SELECT IF rsazero < 300.
EXECUTE.

COMPUTE cases_9 = $CASENUM.
VARIABLE LABLE cases_9 'Excluding the extremes in the tail of the RR and RSA distributions'.
AGGREGATE  /OUTFILE=*  MODE=ADDVARIABLES  OVERWRITE=YES /BREAK=subject /cases_9 =
MAX(cases_9).

** CHECK!! *****************************************************************************.
** Next we correlate the RSA values with the respiration rate  C(RR, RSA) should be
** between -0.7 and -0.1, although exceptions may occur. C(RSA0, RSA) should be
** higher than 0.7
*****************************************************************************.
```

```
CORR rr rsa WITH rsazero.
EXECUTE.

**This concludes outlier detection and the selected remaining breaths can be saved**.
**SAVING RAW DATA**********************************************************.
** What we are SAVING here is a set of variables that have a value for each
** BREATH, i.e. each BREATH is treated as a single case by SPSS.
** All _amsres.sav files can be added in an additional job to make one final RSA
** datafile including all your subjects.
*****************************************************************************.

SAVE OUTFILE=!DIRY+!FILE+'_amsres'+'.sav'.
EXECUTE.

** CHECK!! ******************************************************************.
** Be aware of how much signal you throw away and why. In our dataset, about 11%
** of the data was thrown away on average. About ten percent was rejected through
** the AmsRes automatic scoring program and manual rejection. The SPSS outlier
** detection job was responsible for an additional 1%. However the percentage of
** breaths that need to be rejected varies between subjects. In participants with a lot
** of bad signal for example (spikes, clipping, abdominal breathing) this could go up
** to 40%. But: better safe than sorry: Only parts of the signal that show no evidence
** of artifacts should be saved. Do not worry about removing a couple of breaths too
** many when dealing with  a 24hr ambulatory monitoring signal. Clearly, this
** nonchalance is only  justified when rejected data is spread randomly
** throughout the signal. When bad signals always occur at certain emotions or events,
** it would be a changing in the data to systematically remove these breaths.
*****************************************************************************.

COMPUTE dataloss= ((cases_1 - cases_9)/cases_1)*100.
EXECUTE.
VARIABLE LABEL dataloss 'Percent of breaths that were lost due to artifact and outlier rejection'.

DESC /VAR cases_1 cases_2 cases_3 cases_4 cases_5 cases_6 cases_7 cases_8 cases_9 dataloss /STAT
=MEAN.

** AGGREGATING DATA ACROSS CATEGORIES
*****************************************************************************.
** Usually, the analyses will need a data set that is aggregated across longer time
** periods with fixed values for the labeling categories. This is done by the SPSS
** AGGREGATE COMMAND
** @@@@@ The AGGREGATE COMMAND below assumes that you used the
** following five CATEGORIES for labeling with AMSGRA;
** posture= a code stating the dominant posture during that period (10 levels)
** physical load      = a code indicating the level of physical load (6 levels)
** activity= type of activity the subject is engaged in (24 levels)
** location           = a code for the location of the subject (9 levels)
** social situation = indicating the social situation the subject is in (8 levels)
** This is unlikely to correspond to your own categories. Change the command syntax
** accordingly, i.e. change
**
**     /BREAK= subject posture physical activity location social
**
** to the appropriate description of the categories you used to label the data
*****************************************************************************.
GET FILE=!DIRY+!FILE+'_res'+'.sav'.
```

```
AGGREGATE
  /OUTFILE=*
  /BREAK= subject posture physical activity location social
  /strtdate = MIN(date)
  /enddate = MAX(date)
  /strttime = MIN(time)
  /endtime = MAX(time)
  /mrsa = MEAN(rsa)
  /mrsa0 = MEAN(rsazero)
  /mibi = MEAN(ibi)
  /mrr = MEAN(rr)
  /mvt = MEAN(Vt)
  /sdrsa = SD(rsa)
  /sdrsa0 = SD(rsazero)
  /sdibi = SD(ibi)
  /sdrr = SD(rr)
  /sdvt = SD(Vt).

*****ADD VARIABLE LABELS*********************************************.
VARIABLE LABEL strtdate 'start date of the labeled period in dd:mm:yy'.
VARIABLE LABEL enddate      'end date of the labeled period in dd:mm:yy'.
VARIABLE LABEL strttime     'start time of the labeled period in hh:mm:ss'.
VARIABLE LABEL endtime      'end time of the labeled period in hh:mm:ss'.
VARIABLE LABEL mrsa         'aggregated RSA (ms) across all breaths in the label (not including zero)'.
VARIABLE LABEL mrsa0        ' aggregated RSA (ms) across all breaths in the label (including zero)'.
VARIABLE LABEL mibi         'aggregated IBI across all breaths in the  label (ms)'.
VARIABLE LABEL mrr          'aggregated RR across all breaths in the label (breaths per minute)'.
VARIABLE LABEL mvt          'aggregated Vt across all breaths in  the label (arbitrary units)'.
VARIABLE LABEL sdrsa        'SD of RSA across all breaths in the label (RSA not including zeros)'.
VARIABLE LABEL sdrsa0       'SD of RSA across all breaths in the label (RSA including zeros)'.
VARIABLE LABEL sdibi        'SD of IBI across all breaths in the label'.
VARIABLE LABEL sdrr         'SD of RR across all breaths in the label'.
VARIABLE LABEL sdvt         'SD of Vt across all breaths in the label'.
EXECUTE.

** VALUE AND VARIABLE LABELING ***************************************************************************.
** Use an INCLUDE FILE to supply the correct variable and value labels for (all of)
** the numerical codes used when labeling the AMS-file with AMSGRA. Note that
** the file name needs to be changed to the name of your study specific include file
** that you prepared before running this job.
** In the example below the include file supplies VARIABLE names and VALUES
** for posture, physical load, type of the activity, location and social situation.
** This may, of course, be different in  your include file.
*********************************************************************************.
INCLUDE !DIRY+!labelF+'.sps'.

**SAVING DATA*************************************************************.
** What we are finally SAVING is a set of variables that have a value for each labeled
** period. Each labeled period is treated as a single case by SPSS. Although the code
** of the subject is available for each labeled period, an SPSS "case"
** now does NOT equal a "case" (subject) in your study!
*********************************************************************************.

SAVE OUTFILE=!DIRY+!FILE+'_amsres.sav'.
EXECUTE.
```

CHECK_AMSIMP_Your_subject_1.sps

```
SET PRINTBACK = OFF.

** File name = CHECK&_AMSIMP_singlesubject_generic.sps.
** Version July 2007.
** label Data File version 101.
** Some easy to locate DEFINEs that set the WORKING DIRECTORY, the
** input file with the large scale ensemble averaged impedance data (.iar) for a single
** subject and a file that defines the variable and value labels for the categories of your
** labeled periods. We also define the electrode distance between the measurement
** electrodes for this specific individual (default = 20).

DEFINE   !WD()       'C:\Your_full_directory_tree\'          !ENDDEFINE.
DEFINE   !FILE()     'Your_file_for_subject_1'               !ENDDEFINE.
DEFINE   !Electrode_distance 20                                      !ENDDEFINE
DEFINE   !LABELF()  'label_VAR&VALUES_your_project'      !ENDDEFINE.

***ATTENTION*****************************************************************.
** Although this job is fairly generic, it may need some adjustments to  fit your data.
** Search for the sections labeled with '@@@@@@' to bring this job in accordance
** with the protocol of your specific study and your own wishes regarding
** data handling (what to save and what not).
*******************************************************************************.
**SCRIPT HISTORY**************************************************************.
** Eco de Geus Version 13-02-1996
** Adjusted for A'dam Work Stress studies: Tanja Vrijkotte & Harriette Riese 1997
** Adjusted for NETAMB: Nina Kupper 2004
** SV parameter computation added Annebet Goedhart 2005
** Lay-out & Logic update Eco de Geus & Annebet Goedhart July 2007
*******************************************************************************.
**GENERAL DESCRIPTION*********************************************************.
** This is an SPSS syntax script for SPSS for windows version 13 or higher.
** INPUT: The script will read an individual .iar file delivered by AMSIMP using the
** DATA LIST command below. It is assumed that each line of the .iar file represents
** ICG data from the large scale ensemble average across an entire labeled period.
** When saving the .iar file make sure to create an average report file (iar) with option
** "average over labels" checked also make sure that UNLABELED PERIODS are
** NOT WRITTEN to the outputfile by AMSIMP (uncheck option "write unlabeled
** periods")
** Stroke volume based parameters are meaningful only if Z0 and L have been
** appropriately filled in for the subject.
** ACTION: The AMSIMP data are read into SPSS. Integrity of the impedance data
** is checked in various ways and a number of additional parameters are computed.
** LIST OF VARIABLES TO BE READ:
** Subject = Subject identification (1-8 string)
** ensemble_no      = Number of the ensemble average since start of recording,
** starts at 0 (9-15 integer)
** block = Beat to beat block number, starts at 0... can still be > 1 in
** continuous recordings because of hold continues (16-22 integer)
** date = Start date of the ensemble average (24-31 date dd-mm-yy)
** time     = Exact start time of the ensemble average (33-40 time hh:mm:ss)
** PEP      = Time of the upstroke (B-point), this time is in reference to Q-ONSET
** in ms (41-46 integer)
** dzdt_min          = Time of the dz/dt-min point (Z-point), this time is in reference
** to Q-ONSET in ms (47-52 integer)
** incis     = Time of the incisura (X-point), this time is in reference to Q-ONSET
** in ms (53-58 integer)
```

```
** Zo = Average thorax impedance (Ohm) during the entire ensembling
** period (59-65 float)
** ibi = Average R-wave to R-wave interval in ms (66 - 71 integer)
** amp_upstroke = Amplitude of dZ/dt signal at the B-point from x-axis in
** Ohm/s (72 - 77 float)
** amp_dzdtmin = Amplitude of dZ/dt-min point from x-axis in Ohm/s
** (78 - 83 float)
** amp_inci = Amplitude of dZ/dt signal at the incisura in Ohm/s (84 - 89 float)
** LVET = Left ventricular ejection time in ms (90 - 95 integer)
** Rw_dz = R-peak to dz/dt min interval in ms (96 - 101 integer)
** SV = Stroke volume in cc (102 - 109 float)
** Whether this is in relation to signal amplitude at dZ/dt=0 or at the B-point
** depends on what option was checked in AMSIMP!
** heather = Heather Index in ohm/s^2 (110 - 117 float)
** accep = During visual scoring you interactively set this variable to A=accepted,
** R=REJECTED or C=CORRECTED (119 char)
** Optional descriptions of the period (N/A if not available) columns 120+
**   label# = labeled period number (starting at 1, 0 if N/A)
**   postu = a code describing the predominant posture during that period
**   physic = a code describing the predominant physical load during that period
**   activ = an activity code categorizing possible daily activities of the study group
**   locat = code describing the main location during that period
**   socia = code describing the main social situation during that period
**   stress = code describing subjective stress during that period
**   These final 6 categories are labels specification, which you can change at will when
** you have used other labels to label the AMSgra file.
**
** NOTA BENE: Time of Q-ONSET is imputed by AMSIMP by subtracting 48
** ms from the time of the R-wave onset
**
** ADDITIONAL PARAMETERS COMPUTED:
** We compute Stroke Volume by the Kubicek formula
** Kubicek (1966) SVkubi = rho * ( (L*L)/(Z0*Z0) ) * (dZ/dt)max * LVET
** rho = 135 ohm/s.
** L defined above
** Z0 = average thorax impedance in the ensemble average
** Cardiac output is computed as SV * Heart rate
** SVb follows the official stroke volume computation. It should correspond to what
** AMSIMP has computed if the electrode distance had been filled out correctly.
** If L had not been filled out properly it can in principle be computed from the
** difference between the computed SVb and the original AMSIMP SV.
** As an alternative stroke volume measure SV can also be computed as SV0.
** In this computation the B point is measured in relation to the dZ/dt=0 baseline.
** We have not implemented this formula in the syntax.
** For further information see Goedhart et al., 2006 (Biol Psych 72:110-17).
** We also compute the Heather index by the Kelsey formula.
** Kelsey proposed to scale the dZ/dt min amplitude to total Z before computing the
** HI.
** Heather_kelsey=(SVb_ampl/Z0)/dzdt_min.
** SVb_ampl = dZ/dt amplitude from B-point to dz/dt-min peak
** Z0 = average thorax impedance in the ensemble average
** dZ/dt_min = time of the dz/dt-min point in reference to Q-ONSET

** OUTPUT: The resulting .SAV file will consider each labeled period to be a "case".
** Therefore, per subject there will be a PEP, SV etc for each labeled period.
*********************************************************************.
** @@@@@@ FILE AND DIRECTORY STRUCTURE**********************.
** WE ASSUME THAT FILE NAMES ARE EXACTLY 7 CHARACTERS LONG
```

```
** & REFLECT THE ID OF THE SUBJECT NOTE THAT THE FILENAMES
** USED IN DATA LIST AND SAVE OUTFILE COMMANDS MUST BE
** CHANGED TO CORRESPOND TO YOUR OWN FILENAMES AND
** DIRECTORY STRUCTURE. THIS CAN BE DONE BY CHANGING THE
** DEFINE STATEMENTS AT THE START OF THIS JOB
*****************************************************************************.
** @@@@@@ PROJECT SPECIFIC LABELING**************************.
** The job text below assume that you used the following categories to label the data:
** posture= a code stating the dominant posture during that period (10 levels)
** physical load      = a code indicating the level of physical load (6 levels)
** activity           = type of activity the subject is engaged in (24 levels)
** location           = a code for the location of the subject (9 levels)
** social situation   = indicating the social situation the subject is in (8 levels)
** This is unlikely to correspond to your own categories. Change the command syntax
** accordingly, i.e. change
**          posture 124-128(F) physical 129-132(F) activity 133-136(F)
**          location 137-140(F) social 141-144(F)
** to the appropriate description of the categories you used to label the data.
*****************************************************************************.
***WARNING FOR POTENTIAL PROBLEMS
*****************************************************************************.
** The largest problem that arises during the GET DATA statement is that the input
** has a comma-notation for floating point notation (e.g. 8,03) whereas SPSS expects
** a dot-notation (8.03) or vice versa. When the need arises: In the control panel of
** Windows select 'regional and language options' / 'customize' and set the decimal
** symbol for numbers to 'dot'.
*****************************************************************************.
***** READING AN INDIVIDUAL IAR-FILE ******************************.
TITLE 'PROCESSING VU-AMS IAR-FILES CREATED WITH AMSIMP'.

DATA LIST FILE= !WD + !FILE + '.iar'
  fixed records=1 /1  subject 1-8(F) ensemble_no 9-15(F) block#  16-22(F) strtdate 24-31(edate)
strttime 33-40(time) PEP 41-46(F) dZdt_min 47-52(F) incisura 53-58(F) Z0 59-65(2) IBI 66-71(F)
amp_upstroke 72-77(F) amp_dzdtmin 78-83(F) amp_inci 84-89(F) LVET 90-95(F) Rw_dZdT 96-101(F) SV
102-109(F) heather 110-117(F) Accepted  119(A) label# 120-123(F) posture 124-128(F) physical 129-
132(F) activity 133-136(F) location 137-140(F) social 141-144(F).
EXECUTE.

FORMATS pep TO heather (F8.2).

** An error in your output file indicating that the string 'N/A ' cannot be read in the
** 'labelno' field, indicates that you have included the non labeled ICG complexes in
** your *.iar file. Fix this (one of the options in the AMSIMP menu).
*****************************************************************************.
*****ADD VARIABLE LABELS**********************************************.
VARIABLE LABEL strtdate 'Start date of the ensemble average (dd-mm-yy)'.
VARIABLE LABEL strttime 'Start time of the ensemble average (hh:mm:ss)'.
VARIABLE LABEL ensemble_no 'Number of the ensemble average since start of recording, starts at 0'.
VARIABLE LABEL block#  'Beat to beat block number'.
VARIABLE LABEL label#  'Sequential label number'.
VARIABLE LABEL PEP 'PEP:Time of the upstroke (B-point) in reference to Q-ONSET (ms)'.
VARIABLE LABEL dzdt_min 'time of the dz/dt-min point in reference to Q-ONSET (ms)'.
VARIABLE LABEL incisura 'time of the incisura (X-point) in reference to Q-ONSET (ms)'.
VARIABLE LABEL Z0 'Average thorax impedance (Ohm)'.
VARIABLE LABEL IBI 'average R-wave to R-wave interval (ms)'.
VARIABLE LABEL amp_upstroke ' Amplitude of dZ/dt signal at the B-point from x-axis in Ohm/s'.
VARIABLE LABEL amp_dzdtmin 'Amplitude of dZ/dt-min point from x-axis in Ohm/s'.
VARIABLE LABEL amp_inci 'Amplitude of dZ/dt signal at the incisura in Ohm/s'.
```

```
VARIABLE LABEL LVET 'LVET: Left ventricular ejection time in ms'.
VARIABLE LABEL Rw_dzdt 'R-peak to dz/dt min interval in ms'.
VARIABLE LABEL SV 'Stroke volume in cc'.
VARIABLE LABEL heather 'Heather Index in ohm/s^2'.
VARIABLE LABEL Accepted 'During visual scoring you interactively set this variable to A=accepted,
R=REJECTED or C=CORRECTED'.
EXECUTE.
*****************************************************************************.
 ** VALUE AND VARIABLE LABELING ***********************************************************************************.
** Use an INCLUDE FILE to supply the correct variable and value labels for (all of)
** the numerical codes used when labeling the AMS-file with AMSGRA. Note that
** the file name needs to be changed to the name of your study specific include file
** that you prepared before running this job.
** In the example below the include file supplies VARIABLE names and VALUES
** for posture, physical load, type of the activity, location and social situation. This
** may, of course, be different in your include file.
*****************************************************************************.
INCLUDE !WD + !LABELF+'.sps'.

**REMOVING REJECTED DATA ***************************************************************************.
** Remove data that was rejected during interactive visual scoring

SELECT IF accepted NE 'R'.
EXECUTE.
*****************************************************************************.
**CALCULATIONS SECTION*****************************************.
** We compute Stroke Volume by the Kubicek formula
** Kubicek (1966) SVkubi = rho * ( (L*L)/(Z0*Z0) ) * (dZ/dt)max * LVET
** rho = 135 ohm/s.
** L defined above
** Z0 = average thorax impedance in the ensemble average
** Cardiac output is computed as SV * Heart rate
** SVb follows the official stroke volume computation. It will differ from the SV
** computed by AMSIMP for one or two of the reasons below:
** - AMSIMP uses the amplitude of dZ/dt_min in relation to the zero-amplitude at
** the dZ/dt baseline instead of the amplitude at the B-point.
** - The electrode distance L may not yet have been entered properly during
** AMSIMP scoring
** As an alternative stroke volume measure SV can also be computed as SV0.
** In this computation the B point is scored in relation to the dZ/dt=0 baseline.
** We have not implemented this formula in the syntax.
** For further information see Goedhart et al., 2006 (Biol Psych 72: 110-117).
** We also compute the Heather index by the Kelsey formula.
** Kelsey proposed to scale the dZ/dt_min amplitude to total Z before computing
** the HI.
** Heather_kelsey=(SVb_amp/Z0)/dzdt_min.
** SVb_amp = dZ/dt amplitude from B-point to dz/dt_min peak
** Z0 = average thorax impedance in the ensemble average
** dZ/dt_min = time of the dz/dt_min point in reference to Q-ONSET.
*****************************************************************************.
COMPUTE L = !electrode_distance.
COMPUTE SVb_ampl = amp_dzdtmin - amp_upstroke.
COMPUTE SVbamp_x_lvet = SVb_ampl * (lvet/1000).
COMPUTE L02Z02 = ( (L*L)  / ( Z0 * Z0 ) ).
EXECUTE.
COMPUTE SVb =  -135 * L02Z02 * SVbamp_x_lvet.
EXECUTE.
COMPUTE HR = 60000/IBI.
```

```
COMPUTE CO  =   (SV   * HR) /1000.
COMPUTE COb =  (SVb * HR) /1000.
EXECUTE.

** Kelsey proposed to scale the dZ/dt min amplitude to total Z before computing the
** HI.
COMPUTE Heather_kelsey=(SVb_ampl/Z0)/dzdt_min.
EXECUTE.

VARIABLE LABEL SV 'Stroke volume (from B-point) in cc (correct if L was filled out during AMSIMP
scoring)'.
VARIABLE LABEL SVb 'Stroke volume (from B-point) in cc (correct if L was defined in the SPSS job)' .
VARIABLE LABEL HR  'Heart rate in bpm'.
VARIABLE LABEL CO 'Cardiac Output (from B-point) in liters'.
VARIABLE LABEL COb 'Cardiac Output (from B-point) in liters'.
VARIABLE LABEL L02Z02 'Electrode distance squared divided by resting impedance squared'.
VARIABLE LABEL SVb_ampl 'dZ/dt amplitude from B-point to dz/dt-min peak'.
VARIABLE LABEL SVbamp_x_lvet 'product dZ/dt amplitude from B-point en LVET'.
VARIABLE LABEL L 'distance between the front electrodes (cm)'.
VARIABLE LABEL Heather_kelsey 'Alternative heather index according to Kelsey (arb units)'.
EXECUTE.

**OUTLIER DETECTION**************************************************.
** Check whether your physiological variables remain within their plausible
** physiological range, for example by looking at histograms.
** This is a topic that is subject to personal opinion, you must ultimately decide how
** to deal with outliers yourself. A first rough selection of possible outliers can be
** based on physiologically implausible values that are outside these ranges:
** Plausible physiological range:
** DZDTMIN          dZdt-min amplitude          -2.5 -   -0.15 Ohm/s
** PEP              Pre-ejection period         50   -  170    ms
** LVET             Left ventricular ejection   150   -  500    ms
** HR               Heart rate                  30   -  180    bpm
** IBI              Inter beat interval         333   - 2000    ms
** ZO               Average thorax impedance    6   -   17    Ohm
** STROKE           Stroke volume               40 -  200      cc
** CO               Cardiac Output              3 -   35      l
** HI               Heather index              -40 -    0      Ohm
******************************************************************************.
EXAMINE VARIABLES=ibi pep lvet SV SVb /PLOT  HISTOGRAM  /STATISTICS NONE /MISSING LISTWISE
/NOTOTAL.

** Generic exclusion criteria
COMPUTE exclude = 0.
IF (HR< 30) OR (HR > 200) exclude = 1.
IF (Z0 < 6) OR (Z0 > 17) exclude = exclude + 10.
IF (PEP < 50) OR (PEP > 170) exclude =exclude + 20.
IF (LVET < 150 ) OR (LVET > 450) exclude = exclude + 30.
EXECUTE.

SELECT IF (exclude eq 0).
EXECUTE.
** Project specific exclusion criteria.
** @@@@@ (FILL IN AS REQUIRED)
EXECUTE.

** How many labels were excluded and for what reason?.
FREQ exclude.
```

```
**DETECTION OF POTENTIAL INCONSISTENCIES IN INTERACTIVE
** SCORING ************************************************************.
** Please check the correlations and the graphs carefully. If an outlier can be
** identified, go back to AMSIMP, to decide whether you are convinced the B
** or X point should be where you put it.
** CHANGE THINGS ONLY IF YOU REALLY MADE AN OBVIOUS
** MISTAKE!
** The correlation between PEP and SV will mostly be positive (0.2-0.6)
** The correlations of PEP with IBI (0.2-0.5) and LVET with IBI (0.3-0.7) will also be
** generally positive.
** For PEP these rules do not always work, a failure to find lvet-ibi correlations is
** more alarming.
** If the graph shows a clear outlier in either PEP or LVET it is almost always your
** scoring that caused it, and not physiology. Go back to the AMSIMP file for closer
** inspection.
** If you have doubts about this scoring, don't hesitate to ask for another (our?)
** opinion. If you're sure about your scoring, ALWAYS let ICG morphology prevail
** over 'desirable correlations between PEP and IBI and PEP and LVET'.
*************************************************************************.
CORR /VAR PEP LVET with IBI SV Heather.

GRAPH /SCATTERPLOT(BIVAR)=SV WITH pep /MISSING=LISTWISE .

GRAPH /SCATTERPLOT(BIVAR)=ibi WITH pep  /MISSING=LISTWISE .
GRAPH /SCATTERPLOT(BIVAR)=ibi WITH lvet  /MISSING=LISTWISE .

*************************************************************************.
**SAVING DATA*********************************************************.
** We happily throw away redundant or unimportant information at this point.
** Save your icg outfile now. All _amsimp.sav files can be added in an additional job
** to make one final ICG datafile including all your subjects.
*************************************************************************.

SAVE OUTFILE= !WD + !FILE + '_amsimp.sav'
/KEEP subject ensemble_no strtdate strttime Z0 L PEP LVET IBI Heather Heather_kelsey SV SVb HR CO
COb label# posture physical activity location social.
```

List of publications

Papers

Goedhart AD, Kupper N, Willemsen G, Boomsma DI, de Geus EJC (2006). Temporal stability of ambulatory stroke volume and cardiac output measured by impedance cardiography. *Biological Psychology* 72, 110-117.

Goedhart AD, van der Sluis S, Houtveen JH, Willemsen G, de Geus EJC (2007). Comparison of time and frequency domain measures of RSA in ambulatory recordings. *Psychophysiology* 44(2), 203-215.

Goedhart AD, Bakker F, de Vries M, Kreft J, de Geus EJC (revision submitted). No effects of two weeks of detraining on ambulatory measures of cardiac autonomic control. *Journal of Psychophysiology*.

Goedhart AD, Willemsen G, Hoogendijk WJG, van Weissenbruch MM, de Geus EJC (submitted). No evidence for permissive effects of the early morning cortisol rise on daytime sympathetic and cardiovascular reactivity to stress. *Biological Psychology*.

Goedhart AD, Houtveen JH, de Geus EJC (accepted). Comparing low frequency heart rate variability and pre-ejection period: two sides of a different coin. *Psychophysiology*.

Book chapter

Goedhart AD, Willemsen G, de Geus EJC (2008). Sympathetic nervous system activity in the heart and the skin: are they comparable? In Kaneko M (Ed): *Sympathetic Nervous System Research Developments*. New York: Nova Science publishers.

Dankwoord

Nu 4 jaar na het begin van mijn promotie traject is het tijd geworden om de laatste bladzijde van mijn proefschrift te schrijven! Dit is de gelegenheid om de mensen die, allen op hun eigen manier, een bijdrage hebben geleverd aan dit proefschrift te bedanken.

Dit proefschrift zou niet tot stand zijn gekomen zonder de hulp van mijn promotor en copromotor: prof. de Geus en dr. Willemsen. Beste Eco, ik voel me bevoorrecht met jou als begeleider. Zonder jouw wetenschappelijke inzicht, inspiratie en enthousiasme was dit proefschrift nooit zo geworden als het nu is. De openheid en humor heb ik erg gewaardeerd als ook de manier waarop je mij begeleid hebt. De deur stond altijd open om vragen te stellen en commentaar op mijn werk was altijd binnen no-time geleverd. De manier waarop jij onsamenhangende stukken tekst weet om te schrijven tot een pakkend verhaal is verbluffend. Heel veel dank voor je dagelijkse begeleiding en alle energie die je in dit proefschrift hebt gestopt. Beste Gonneke, ook jouw bijdrage was van essentieel belang voor het tot stand komen van dit proefschrift. Bedankt voor jouw kritische blik, precisie en hulp bij de laatste loodjes.

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Het gebruiken van apparaten tijdens onderzoek of practica gaat natuurlijk altijd gepaard met allerlei onvoorspelbare problemen. Draadbreuken, verloren data, slechte signalen en nog duizend andere probleempjes hebben de revue gepasseerd. Zonder Paul Groot en Jarik den Hartog, de VU-AMS experts, was er al heel wat in de soep gelopen. Heel erg bedankt voor jullie altijd snelle hulp en deskundigheid.

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Annebet, 14 april 2008.