Chapter 1

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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; Kasprówicz 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.
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(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 Xth 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.

![Diagram of pre- and post-ganglionic neurons]

**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; Penttilä 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; Penttilä 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 $\alpha$-adrenergic (e.g. in arterioles) or $\beta$-adrenergic receptors (e.g. on the heart). $\alpha$-adrenergic stimulation causes vasoconstriction by acting on the smooth muscles in the medial layer of the blood vessels. Stimulation of the cardiac $\beta$-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 $\beta_2$-receptors in the vessels and on the heart, causing vasodilatation (mostly in muscle tissue) and increases in heart rate and contractility.
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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 \( \beta_1 \) (e.g. metoprolol) or \( \beta_2 \)-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/mm², 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 \( \beta \)-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 β-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 β-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 $B_1$- and $B_2$-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 $\beta$-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 authoritative 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 & Björntorp, 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 adrenocorticotropic hormone (ACTH) from the anterior
pituitary. ACTH in turn stimulates the adrenal gland to release cortisol in the bloodstream. 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.,
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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.