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Kaisu Martinmäki

Transient Changes in Heart Rate Variability in Response to Orthostatic Task, Endurance Exercise and Training

With Special Reference to Autonomic Blockades and Time-Frequency Analysis

JYVÄSKYLÄN YLIOPISTO

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Editors
Harri Suominen
Department of Health Sciences, University of Jyväskylä
Pekka Olsbo, Marja-Leena Tynkkynen
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ABSTRACT

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Heart rate variability (HRV) is generally accepted as an estimate of the autonomic, particularly vagal, control of the heart. The quantitative relationship of HRV to vagal heart rate (HR) control is, however, unclear. In addition, the conventional analysis methods of HRV are not suitable for analyzing rapid adjustments of autonomic control. Time-frequency analysis methods, including short-time Fourier transform (STFT), have been developed for analyzing non-stationary signals. The present study was designed to evaluate the ability of HRV to quantify within-subject changes in autonomic HR control by using autonomic blockades and an active orthostatic task, and to study transient changes in HRV in response to a bout of endurance exercise and to endurance training. The present blockade experiments indicated that the spectral power of HRV at all frequencies, particularly high frequency power [HFP, in $\ln(ms^2)$], was predominantly mediated by the vagal system and that the within-subject relationship between HFP and vagal HR control was essentially linear. HRV provided no specific index of sympathetic HR control. During the active orthostatic task, the STFT method was able to detect and quantify transient changes in vagal HR control. Application of the STFT method to the exercise data showed that HFP decreased during the incremental maximal exercise test up to an intensity of 60-70% of maximal power, reflecting vagal withdrawal. Further, the increase in absolute HFP immediately after exercise was faster after the low than high intensity exercise, suggesting faster recovery of vagal HR control after the low intensity exercise. The effects of aerobic fitness on HRV response to exercise were evaluated by using cross-sectional and longitudinal designs. Two groups differing in age-related aerobic fitness were formed by using age-related aerobic fitness norms as classification criteria. Comparisons between the high and moderate age-related fitness groups showed that high age-related aerobic fitness was related to improved vagal HR control at rest and during recovery from submaximal endurance exercise. In the longitudinal design, previously untrained individuals exercised twice a week for 14 weeks. The results indicated that the low-dose endurance training programme improved vagal HR control at the same absolute submaximal exercise intensities but did not alter vagal HR control at rest in previously untrained individuals. It also increased maximal oxygen uptake. To summarize, the STFT method provided a non-invasive tool for assessing transient changes in vagal HR control without the need to interfere with normal control, e.g. by using blocking drugs. The changes in HRV in response to endurance exercise yielded information on the function of the autonomic nervous system in addition to that obtained by resting measurements of HRV.

Key words: cardiovascular autonomic regulation, parasympathetic activity, pharmacological blockade, aerobic training

Author's address Kaisu Martinmäki
University of Jyväskylä,
P.O. Box 35
FIN-40014 University of Jyväskylä
Finland

Supervisors Professor Emeritus Heikki Rusko, PhD
Department of Biology of Physical Activity
University of Jyväskylä, Finland
KIHU – Research Institute for Olympic Sports
Jyväskylä, Finland

Professor Keijo Häkkinen, PhD
Department of Biology of Physical Activity
Neuromuscular Research Center
University of Jyväskylä, Finland

Reviewers Professor André E. Aubert, PhD
Laboratory of Experimental Cardiology
University Hospital Gasthuisberg
Leuven, Belgium

Docent Mikko Tulppo, PhD
Director of the Department of Exercise and Medical Physiology
Verve, Oulu, Finland

Opponent Professor Benjamin D. Levine, MD
Director of the Institute for Exercise and Environmental Medicine
Professor of Medicine
University of Texas Southwestern Medical Center
Dallas, USA

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Kaisu Martinmäki

ABBREVIATIONS

AOT	active orthostatic task
BLa	blood lactate concentration
DBP	diastolic blood pressure
DBP_{min}	minimum value of diastolic blood pressure during the first 30 s after standing-up during an active orthostatic task
ECG	electrocardiography
FFT	fast Fourier transformation
HFP	high frequency power
HFP_{min}	minimum value of high frequency power preceding minimum value of R-to-R peak interval during the first 30 s after standing-up during an active orthostatic task
HR	heart rate
HR_{AnT}	heart rate at anaerobic threshold
HR_{max}	maximal heart rate
HRV	heart rate variability
LFP	low frequency power
LFP/HFP	ratio of low frequency power to high frequency power
pNN50	percentage of adjacent R-to-R peak interval differences > 50 ms
RMSSD	square root of the mean of the sum of the squares of the differences between adjacent R-to-R peak intervals
RRI	R-to-R peak interval
RRI_{min}	minimum value of R-to-R peak interval during the first 30 s after standing-up during an active orthostatic task
RSA	respiratory sinus arrhythmia
SBP	systolic blood pressure
SBP_{min}	minimum value of systolic blood pressure during the first 30 s after standing-up during an active orthostatic task
SDRRI	standard deviation of R-to-R peak intervals
STFT	short-time Fourier transform
TP	total power
VE_{AnT}	ventilation at anaerobic threshold
VE_{max}	maximal ventilation
VLFP	very low frequency power
VO_2	oxygen uptake
VO_{2AnT}	oxygen uptake at anaerobic threshold
VO_{2max}	maximal oxygen uptake
W_{AnT}	power output at anaerobic threshold
W_{max}	maximal power output

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original articles, which are referred to in the text by their Roman numerals.

I Martinmäki K, Rusko H, Kooistra L, Kettunen J & Saalasti S (2006). Intraindividual validation of heart rate variability indexes to measure vagal effects on hearts. Am J Physiol Heart Circ Physiol 290: H640–H647.

II Martinmäki K, Rusko H, Saalasti S & Kettunen J (2006). Ability of short-time Fourier transform method to detect transient changes in vagal effects on hearts: a pharmacological blocking study. Am J Physiol Heart Circ Physiol 290: H2582–H2589.

III Martinmäki K & Rusko H (2008). Time-frequency analysis of heart rate variability during immediate recovery from low and high intensity exercise. Eur J Appl Physiol 102: 353–360.

IV Martinmäki K & Rusko H (2009). Effects of age-related aerobic fitness on heart rate variability at rest, during exercise and during recovery. Submitted for publication.

V Martinmäki K, Häkkinen K, Mikkola J & Rusko H (2008). Effect of low-dose endurance training on heart rate variability at rest and during an incremental maximal exercise test. Eur J Appl Physiol 104: 541–548.

CONTENTS

1 INTRODUCTION

The cardiovascular system is mostly controlled by the autonomic nervous system through the complex interplay between the vagal and sympathetic divisions (Guyton and Hall 2006). The autonomic nervous system establishes and maintains a dynamic adaptive state, allowing an organism to respond to internal and external demands. It mediates changes in heart rate (HR), blood pressure and peripheral vascular tone in response to daily challenges, including change of posture and physical exercise. A large body of evidence has shown that the functioning of the autonomic nervous system plays a substantial role in cardiovascular health and disease (e.g. Rosenwinkel et al. 2001; Carter et al. 2003b; Harris and Matthews 2004). Recently attention has focused on the HR response to endurance exercise and on the pattern of HR recovery after exercise (Freeman et al. 2006). When compared with resting measurements, the measurement of autonomic HR control during and after endurance exercise may provide additional information on the functioning of the autonomic nervous system. Endurance training improves autonomic HR control at rest and autonomic HR response to endurance exercise (Scheuer and Tipton 1977; Blomqvist and Saltin 1983). It offers a therapeutic modality that can restore the functioning of the autonomic nervous system towards a normal equilibrium in patients with autonomic dysfunction (Rosenwinkel et al. 2001). The improved autonomic HR response to endurance exercise may be one of the most important benefits of endurance training since sudden cardiac death is often associated with an acute bout of endurance exercise (Albert et al. 2000; von Klot et al. 2008).

Vagal control cannot be measured directly in conscious humans. Sympathetic control can be assessed directly by the measurement of plasma noradrenaline and adrenaline, the measurement of the noradrenaline spillover rate, or the recording of muscle sympathetic nerve activity with microneurography (Grassi and Esler 1999). Monitoring changes in HR after the administration of pharmacological blocking agents can be used selectively to evaluate either sympathetic or vagal HR control (Katona et al. 1982). However,

these techniques are invasive, may interfere with normal autonomic control, and are not always feasible during endurance exercise.

The measurement of heart rate variability (HRV) provides a non-invasive tool for assessing autonomic HR control (Akselrod et al. 1981; Akselrod et al. 1985). HRV is a term used to describe the variations in time-intervals between heart beats, i.e. variations in electrocardiographic R-to-R peak interval (RRI) lengths. HRV is primarily due to the changing modulations of vagal and sympathetic control of the heart and may therefore be considered as an estimate of autonomic HR control. From the clinical point of view, the measurement of HRV has received a great deal of attention because abnormalities in HRV after myocardial infarction are strongly associated with an increased risk for death (Kleiger et al. 1987; Bigger et al. 1993; La Rovere et al. 1998). Studies related to exercise physiology have mainly focused on the influence of an acute bout of endurance exercise, endurance training, overtraining, and training status on HRV.

HRV has classically been used to assess autonomic HR control at rest (Task Force 1996). A conventional frequency domain analysis of HRV has been developed essentially for conditions in which the level of HR is unchanged. However, during recent decades, many studies have also reported changes in HRV in response to endurance exercise (see e.g. Aubert et al. 2003; Carter et al. 2003b; Sandercock and Brodie 2006). During exercise, and especially during recovery from exercise, there are technical problems related to non-stationary signals. To avoid these problems, researchers have voluntarily excluded approximately the first five minutes after endurance exercise from HRV analyses (Hayashi et al. 1992; Takahashi et al. 2000; Terziotti et al. 2001; Mourot et al. 2004a; Mourot et al. 2004b). Quite recently, studies have been targeted at developing novel methods of HRV analysis that allow the assessment of HRV also in conditions when HR changes rapidly (e.g. Mainardi et al. 2002). By using time-frequency approaches it is possible to obtain information on autonomic control when HR changes rapidly (Keselbrener and Akselrod 1996; Novak et al. 1996; Akselrod et al. 1997; Jasson et al. 1997; Ramaekers et al. 2002; Yoshiuchi et al. 2004; Verheyden et al. 2005), also immediately after the cessation of endurance exercise (Kaikkonen et al. 2007). Furthermore, the time-frequency approaches may provide more accurate estimates of HRV during endurance exercise and thus improve the detection of possible endurance training-induced changes in autonomic HR response.

The general aim of this thesis was to evaluate the ability of HRV to quantify within-subject changes in autonomic HR control by using autonomic blockades and an active orthostatic task, and to study transient changes in HRV in response to a bout of endurance exercise and to endurance training.

2 REVIEW OF THE LITERATURE

2.1 Autonomic control of heart rate

Although the heart has an inherent ability to initiate contraction, it is also subjected to extrinsic control by nervous reflexes and hormones. In the absence of extrinsic control, intrinsic HR varies between about 90 to 120 bpm whereas the autonomic nervous system together with hormones cause HR to vary from about 28 to 220 bpm. Higher brain centers and cardiovascular control areas in the brain stem control HR through autonomic nerve activity based on sensory information from specific receptors. This section provides the background information on autonomic HR control necessary for understanding HRV, which is discussed in the succeeding sections. First, vagal and sympathetic innervations of the heart are described. The quantitative relationship of autonomic control to HR and RRI are then considered. In addition, the most important reflexes and individual characteristics affecting autonomic control are briefly described.

2.1.1 Vagal control of heart rate

The vagus or tenth cranial nerve is responsible for the parasympathetic control of the heart. Vagal preganglionic neurons originate from the dorsal motor nucleus and nucleus ambiguous in the medulla oblongata (Loewy 1990). Axons of preganglionic myelinated neurons with high conduction velocity synapse to postganglionic unmyelinated neurons with low conduction velocity in the cardiac ganglia. The vagal postganglionic neurons enter the sinoatrial node, atrioventricular conducting pathways and the atria muscle (Loewy 1990) and as recently demonstrated these neurons may also enter the ventricular muscle (Johnson et al. 2004). Both the preganglionic and postganglionic neurons use acetylcholine as a neurotransmitter. Acetylcholine slows the rate of sinoatrial node depolarization and discharge by binding to muscarinic cholinergic receptors and activating transmembrane postassium channels (Parkinson 1990b). The latency between the onset of a vagal stimulus and sinoatrial node

response is between about 0.25 s and 0.6 s (Levy et al. 1970; Spear et al. 1979; Borst and Karemaker 1983; Seidel et al. 1997). The maximum response occurs after about 1.5-2.0 s (Eckberg 1980) and the decay time of the response is about 2 s (Eckberg and Eckberg 1982). This implies that changes in vagal control can influence HR on a beat-to-beat basis. Vagal nerve activity cannot be directly measured in humans, but the effects of vagal control on HR response can be examined by determining the difference between HR response prior to and after blocking the peripheral transduction of vagal outflow with a muscarinic blocking agent (Katona et al. 1982; Berntson et al. 1994).

2.1.2 Sympathetic control of heart rate

Sympathetic preganglionic neurons originate from the intermediolateral cell column of the spinal cord (Loewy 1990). In the sympathetic nervous system, the myelinated preganglionic neurons are much shorter and the unmyelinated postganglionic neurons much longer than those in the parasympathetic nervous system. The preganglionic neurons travel in the ventral roots and the white communicating rami to paravertebral ganglia located close to the spinal cord where they synapse to postganglionic neurons. The postganglionic neurons innervate the entire heart, entering the sinoatrial node, atrioventricular-conducting pathways, the atria and ventricular muscles (Edwards 1990; Loewy 1990). The preganglionic neurons release acetylcholine as a neurotransmitter while the postganglionic neurons release noradrenaline that speeds up the rhythm of the sinoatrial node via a β_1-receptor-mediated second messenger cascade of intracellular signals (Parkinson 1990a). The sympathetically mediated increase in HR is characterized by a time delay of 1 to 2 s, a maximum increase in HR at about 4 s and a return to baseline within 20 s (Spear et al. 1979). In addition to neural control, the sympathetic nervous system can modulate HR through the release of hormones from the adrenal gland (Edwards 1990). Sympathetic activity stimulates to secretion from the adrenal medulla of noradrenaline and adrenaline into systemic circulation. The former mainly affects α-receptors, whereas the latter affects α-receptors as well as both β_1-and β_2-receptors. The activation of β_1-receptors in the heart causes an increase in the firing rate of the sinoatrial node, an increase in the velocity of conduction, and an increase in myocardial contractility. The sympathetic outflow can be evaluated in humans by recording muscle sympathetic nerve activity, measuring plasma adrenaline and noradrenaline concentrations or the noradrenaline spillover rate, or using β-adrenergic blocking agents (e.g. Katona et al. 1982; Christensen and Galbo 1983; Berntson et al. 1994; Grassi and Esler 1999).

2.1.3 Quantitative relationship between autonomic control and heart rhythm

HR is regulated by a complex set of interactions between vagal and sympathetic control. The effects of the two autonomic divisions on the heart can vary reciprocally, independently or coactively (Berntson et al. 1993). Because the two

autonomic divisions exert opposing control over the heart, a given increase in HR may arise from distinct autonomic origins, such as vagal withdrawal, sympathetic activation, or both. Therefore, cardiac chronotropic response per se does not provide specific information on either the vagal or sympathetic control of the heart. Furthermore, the predominance of vagal HR control over sympathetic HR control, termed accentuated antagonism, has been documented (Levy and Zieske 1969). This means that the opposing effects of vagal and sympathetic control are not algebraically additive. Instead, the effects of vagal control on HR are augmented with high levels of sympathetic background control and the effects of sympathetic control on HR are suppressed with high levels of vagal background control (Levy and Zieske 1969; Uijtdehaage and Thayer 2000).

In addition to the true interactions among the autonomic divisions, the use of HR instead of RRI length as a metric of chorotropic response may exaggerate the magnitude of these interactions because of inherent nonlinearities between autonomic control and HR (Berntson et al. 1995). There is a hyperbolic relationship between the vagal firing rate and change in HR, with the largest changes occurring at low firing rates, whereas the relationship between the vagal firing rate and RRI is essentially linear (Parker et al. 1984; Koizumi et al. 1985; Berntson et al. 1995). There may be no fundamental limit to maximal vagal control, prior to the point of sinus arrest and death, but an HR value of 26 bpm (or RRI value of 2332 ms) has been presented as a conservative estimate of the maximal vagal control in humans (Carlson et al. 1992; Berntson et al. 1993). The relationship of the sympathetic firing rate to both measures of heart rhythm (HR and RRI) is approximately linear, up to asymptotic levels (Koizumi et al. 1985; Berger et al. 1989; Berntson et al. 1995). HR may exceed 200 bpm (corresponding to RRI of 300 ms) during maximal exercise. In addition to sympathetic activation, the increase in HR during exercise results from thermal, metabolic, and mechanical variables (McArdle et al. 2006).

Both HR and RRI have similar statistical and distributional characteristics. These two measures are often thought of as reciprocal yet interchangeable, but there is a curvilinear inverse relationship between HR and RRI (O'Leary 1996). There is always a reciprocal change in HR and RRI but the magnitude of the change depends on the baseline level of HR (or RRI). For example, when HR is low, a 10-bpm change in HR corresponds to a large change in RRI whereas when the baseline level of HR is elevated, the same 10-bpm change in HR corresponds to a very small change in RRI. Although no universal consensus has been reached on preference for HR versus RRI as a chronotropic metric, it has been suggested that the use of HR may lead to interpretive difficulties whereas the use of RRI has considerable advantages and minimal disadvantages (Berntson et al. 1995). However, HR may be a more appropriate metric in some circumstances, such as exercise.

2.1.4 Reflexes affecting autonomic control

The increase in HR with inspiration and the decrease in HR with expiration are mediated by reflexive changes in the vagal discharge to the sinoatrial node. The physiological mechanisms that trigger respiratory-related vagal gating are not yet fully understood but it has been suggested that central as well as peripheral mechanisms play a role in generating this phenomenon (Richter and Spyer 1990).

Baroreceptors situated in the walls of some arteries, including carotid sinuses, aortic arch and coronary arteries, are responsible for HR changes secondary to fluctuations in arterial blood pressure (Guyton and Hall 2006). When arterial pressure falls, as occurs during a postural change from supine to standing, fewer impulses are sent to the vasomotor center. In a consequence, there is a decrease in vagal activity and an increase in sympathetic activity, resulting in an increase in HR and vasoconstriction and eventually an increase in arterial pressure towards the appropriate level (Wieling and Shepherd 1992). When arterial pressure increases, the opposite reflex occurs. There is interaction between the baroreceptor reflex and respiratory activity, the baroreceptor-mediated vagal response being greater when a stimulus is administered during expiration than during inspiration (Eckberg et al. 1980).

Also chemo-, metabo- and mechanoreceptors affect autonomic control but to a much lesser extent than the baroreceptors. Stimulation of the carotid chemoreceptors affects vagal control, whereas stimulation of the aortic chemoreceptors affects sympathetic control (Guyton and Hall 2006). Skeletal muscle metabo- and mechanoreceptors become activated due to the accumulation of metabolites and increase in muscle tension, e.g. during exercise, and may induce an increase in sympathetic activity (Rowell and O'Leary 1990; O'Leary 1996).

2.1.5 Individual characteristics affecting autonomic control

Individual characteristics, such as gender, age and aerobic fitness, are related to autonomic control. Resting HR is lower in men than women, which may be explained by several factors including differences in autonomic HR control, intrinsic properties of the sinoatrial node and aerobic fitness (Villareal et al. 2001). The lower risk for cardiovascular disease in women when compared with men has led to a suggestion that pre-menopausal women may have higher vagal control and lower sympathetic control than men. However, studies evaluating gender differences in autonomic control by the use of autonomic blockades, measurements of plasma noradrenaline concentration and muscle sympathetic nerve activity have yielded inconsistent results (Burke et al. 1996; Matsukawa et al. 1998; Evans et al. 2001; Villareal et al. 2001).

In general, autonomic cardiovascular control becomes impaired with aging and there is a shift in autonomic balance towards sympathetic dominance (Matsukawa et al. 1998; Evans et al. 2001; Stratton et al. 2003). However, the effect of aging on autonomic control is greatly modulated by genetic differences

and differences in life-style, including physical activity. The age-related decrease in aerobic fitness may partly explain changes in autonomic control with aging.

Regular endurance training induces a decrease in resting HR, which has been suggested to result from the combination of increased vagal control, decreased sympathetic control and/or decreased intrinsic HR (Ekblom et al. 1973; Scheuer and Tipton 1977; Lewis et al. 1980). Cardiovascular and autonomic adaptations to endurance training are described more detail in chapter 2.4.1.

2.2 Heart rate variability (HRV) as a measure of autonomic control

With electrocardiography (ECG), HRV can be easily observed as variation in the time intervals between RRIs. HRV tends to aggregate at several frequencies, namely at respiratory frequencies (around 0.25 Hz at rest) and at frequencies around 0.1 Hz. As early as 1733, a respiratory pattern in the blood pressure and pulse of the horse was observed by Hales, and approximately a hundred years later, a regular quickening of the pulse rate with inspiration and a slowing with expiration in the dog was observed by Ludwig (Berntson et al. 1997). The studies by Anrep and colleagues (1936a; 1936b) spearheaded modern perspectives on respiratory-related HRV. In the year 1876, Mayer described waves in blood pressure that were slower than the respiratory frequency in animals with normal respiratory movements (Pěnáz 1978). HRV reflects autonomic cardiovascular control and the mechanisms underlying this control, and therefore a variety of methods of HRV analysis has been developed to obtain information non-invasively on autonomic HR control. This section first focuses on the physiological basis of HRV without taking into account what method has been used to assess HRV. Several HRV approaches are then described and the quantitative relationship between autonomic control and HRV is discussed. In addition, the effects of gender and age on HRV are briefly described.

2.2.1 Physiological basis of HRV

HRV occurring at respiratory frequencies, also termed respiratory sinus arrhythmia (RSA), is a well-known cardio-respiratory coupling that results in shortening of RRI during inspiration and lengthening of RRI during expiration. RSA is of vagal origin since it is abolished by muscarinic blockade or functional vagotomy (Katona and Jih 1975; Akselrod et al. 1981; Raczkowska et al. 1983; Fouad et al. 1984; Akselrod et al. 1985; Pomeranz et al. 1985; Pagani et al. 1986; Hayano et al. 1991; Cacioppo et al. 1994; Uusitalo et al. 1996). A strong interindividual correlation between RSA and pharmacologically defined vagal tone has been observed (Fouad et al. 1984; Hayano et al. 1991). RSA has been

proposed to result from the interplay of several factors (Koh et al. 1998). These factors include the respiratory motoneuron gating of the responsiveness of vagal-cardiac motoneurons to stimulation by autonomic sensory input, input from baroreceptors in response to fluctuations of arterial pressure, inputs from pulmonary and thoracic stretch receptors, and phasic stretch of the sinoatrial node (Eckberg et al. 1980; Gilbey et al. 1984; Richter and Spyer 1990; Koh et al. 1998; Badra et al. 2001). Respiratory frequency affects RSA but does not affect the tonic level of vagal HR control (Grossman and Kollai 1993; Hayano et al. 1994). RSA magnitude increases with an increase in tidal volume and decreases with an increase in respiratory frequency (Hirsch and Bishop 1981; Eckberg 1983; Saul et al. 1991; Grossman and Kollai 1993; Taylor et al. 2001; Pöyhönen et al. 2004). The relationship between respiratory frequency and RSA may reflect the kinetics of sinoatrial node responses to fluctuating levels of acetylcholine (Eckberg 2003). Respiratory activity is able to result in small RSA also in the absence of autonomic HR control. RSA has been observed after combined vagal and sympathetic blockade and after cardiac transplantation, which may be explained by intracardiac reflex or mechanical stretch of the sinoatrial node (Bernardi et al. 1989; Bernardi et al. 1990; Saul et al. 1991; Taylor et al. 2001).

According to the temporal dynamics of vagal and sympathetic HR control, described in the previous section, both autonomic divisions can influence HRV centered at a frequency of about 0.1 Hz. Some investigators have suggested that HRV at low frequencies is of sympathetic origin (Pagani et al. 1986; Malliani et al. 1991) but most investigators have suggested that sympathetic and vagal control interact in the generation of these rhythms (Akselrod et al. 1981; Pomeranz et al. 1985; Saul et al. 1990; Koh et al. 1994). The specific mechanisms responsible for HRV at low frequencies are not known, and thus the significance of these fluctuations has remained controversial. It has been hypothesized that alterations in the amplitude of sympathetic blood pressure rhythms are related to the magnitude of low frequency HRV via vagally mediated baroreflex responses (Berntson et al. 1997). Muscle sympathetic nerve activity is also known to fluctuate at low frequencies but its role in the generation of HRV is controversial (Saul et al. 1990; Kingwell et al. 1994; Koh et al. 1994). In addition, respiratory-related HRV can be detected at low frequencies on occasions when the respiratory rate is lower than 10 breaths per minute.

2.2.2 Methods of analyzing HRV

Time-domain analysis and conventional frequency-domain analysis are the most commonly used and highly validated methods of analyzing HRV. In addition to these methods, this chapter introduces a short-time Fourier transform (STFT) method as an example of a time-frequency analysis. While conventional frequency-domain analysis can be applied only to stationary signals, time-frequency analysis allows HRV also to be assessed from non-stationary signals. This is a major advantage, since autonomic HR control is characterized by transient changes. A wide range of other HRV approaches are

available in the literature as well. Non-linear dynamic methods have quite recently made their appearance in the analysis of HRV. For example Poincaré plot analysis, detrended fluctuation analysis, and approximate entropy have been applied to exercise data with success (see e.g. Tulppo et al. 1996; Tulppo et al. 1998; Hautala et al. 2003; Tulppo et al. 2003; Mourot et al. 2004a). On the one hand non-linear methods are promising tools, especially with regard to the evaluation of function and dysfunction of cardiovascular control but on the other hand their physiological background is not yet completely established (Aubert et al. 2003; Sandercock and Brodie 2006).

Time-domain analysis

Task Force (1996) issues recommendations for the use of most commonly used methods of HRV analysis. The indices in time-domain analysis are easily computed with simple statistical methods. One class of time-domain indices are those derived from direct measurements of RRIs. The simplest index to compute, and the only one applicable to short-term RRI recordings, is the standard deviation of the RRIs over the period recorded or time interval selected (SDRRI). This index represents overall HRV and encompasses RRI fluctuations at all frequencies during the period of recording. The magnitude of the SDRRI depends largely on the duration of the recording and, therefore comparisons should be made between segments of similar length. Another class of time-domain indices consists of indices derived from the differences between RRIs. These indices include the square root of the mean of the sum of the squares of the differences between adjacent RRIs (RMSSD) and the percentage of adjacent RRI differences > 50 ms (pNN50). RMSSD and pNN50 are highly correlated and both indices mainly reflect respiratory-related fluctuations in RRI.

Frequency-domain analysis

Frequency-domain analysis, also termed spectral analysis, is based on a frequency decomposition of steady fluctuating time-dependent signal (i.e. stationary signal). Compared to time-domain analysis, the main advantage of HRV indices derived from spectral analysis is their relation to the mechanisms of autonomic HR control. By definition, power spectral analysis decomposes the RRI signal into its frequency components and quantifies these components in terms of their relative intensity, termed "power" (Task Force 1996). It provides information on how global HRV is distributed as a function of frequency into its different components. The major problem with spectral analysis is its basic theoretical assumptions, which require the processed signal to be stationary throughout the analyzed time period. Thus steady state conditions can be studied and compared, but no information can be obtained during transient changes in autonomic control. This limitation greatly hampers attempts to investigate dynamic changes in autonomic control in response to internal or external stimuli, such as, standing-up, pharmacological manipulations, endurance exercise or recovery from exercise. The most commonly used spectral methods are nonparametric fast Fourier transformation (FFT) and

parametric autoregressive modeling (Task Force 1996). In general, FFT and autoregressive modeling have provided comparable results when applied to steady state exercise data. Alternative data treatments, such as coarse graining spectral analysis[1], have also been introduced (Yamamoto and Hughson 1991) and applied to exercise data (e.g. Yamamoto et al. 1991; Hayashi et al. 1992; Gregoire et al. 1996; Carter et al. 2003a; Mourot et al. 2004a) .

In short-term recordings, ranging generally from about 2 to 10 minutes, power is usually computed within two different frequency bands: low frequency power (LFP, 0.04-0.15 Hz) and high frequency power (HFP, 0.15-0.40 Hz). Power within a very low frequency band (VLFP, < 0.04 Hz) cannot be assessed accurately from short-term recordings and is normally assessed only from the 24-h recordings. However, the above-mentioned frequency ranges are not universally used and nor are they suitable for all physiological conditions, such as endurance exercise, during which respiratory frequency is much higher than the upper boundary recommended for HFP (e.g. Arai et al. 1989). The power of each frequency component can be expressed either as absolute power, i.e. in milliseconds squared (ms^2), or as normalized power, i.e. in normalized units (n.u.). Normalized power expresses LFP or HFP divided by total power (TP) within the high and low frequency bands, and thus represents the relative power of the two frequency components. In addition, the ratio of LFP to HFP (LFP/HFP) is often computed.

Based on evidence from blockade studies, absolute HFP has been accepted as an index of vagal HR control (Akselrod et al. 1981; Pomeranz et al. 1985; Hayano et al. 1991; Cacioppo et al. 1994; Koh et al. 1994; Uusitalo et al. 1996). LFP does not provide a specific index of either vagal or sympathetic HR control since vagal blockade reduces absolute LFP (Akselrod et al. 1981; Pomeranz et al. 1985; Cacioppo et al. 1994; Koh et al. 1994; Uusitalo et al. 1996) and sympathetic blockade carried out after vagal blockade induces in it a further reduction (Akselrod et al. 1981; Uusitalo et al. 1996). The use of normalized spectral components is not unanimously supported (see e.g. Eckberg 2000). In 1986, Pagani, Malliani and co-workers (1986) introduced the mathematical normalization treatment to derive indices of so called sympatho-vagal balance, and suggested that normalized HFP and LFP measure vagal and sympathetic activity, respectively, and that LFP/HFP measures sympatho-vagal balance (Pagani et al. 1986; Malliani et al. 1991). However, experimental evidence supporting the use of the normalization treatment is limited (see 2.2.3).

Short-time Fourier transform – a time-frequency analysis of HRV

It has been recognized that transient changes in HR in response to variety of clinical tasks reveal important information on the functioning of the autonomic nervous system. In addition to clinical tasks, most real-life challenges induce a rapid increase or decrease in HR. However, since conventional spectral analysis

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[1] Coarse graining spectral analysis selectively extracts very low frequency component of HRV, leaving the low and high frequency components intact in HRV spectral analysis. The frequency components are commonly termed LO (<0.15 Hz) and HI (> 0.15 Hz).

is limited to the stationary portions of the RRI signal, the portions with transient changes have deliberately been ignored when assessing HRV. Quite recently, in order to obtain information on autonomic control during strongly time-dependent phases of an intervention, several tools for time-frequency analysis have been applied to RRI data: linear decomposition of the signal (including the STFT method, waveled and waveled packet decomposition), quadratic time-frequency distributions (including Wigner-Ville transform and Cohen's class of distributions) and adaptive or time-variant autoregressive models (Mainardi et al. 2002). The simplest of these methods is the STFT method. It is an extension of the conventional FFT method, and thus it converts time-frequency mapping into HFP and LFP, familiar from the FFT method (see e.g Oppenheim and Schafer 1999; Saalasti 2003). The advantages of the STFT method are computational efficiency, simple implementation and automaticity. It can also be seen as an objective method as after the selection of window length and frequency ranges no further decisions are needed. The STFT method calculates consecutive power spectra of short portions (of constant duration) of the signal and thus informs about changes in the power spectrum as a function of time. The time-frequency distribution can be displayed as a 3D graph, in which the x-axis is the time axis, the y-axis is the frequency axis and the z-axis is the power (or amplitude) axis (see Figure 1).

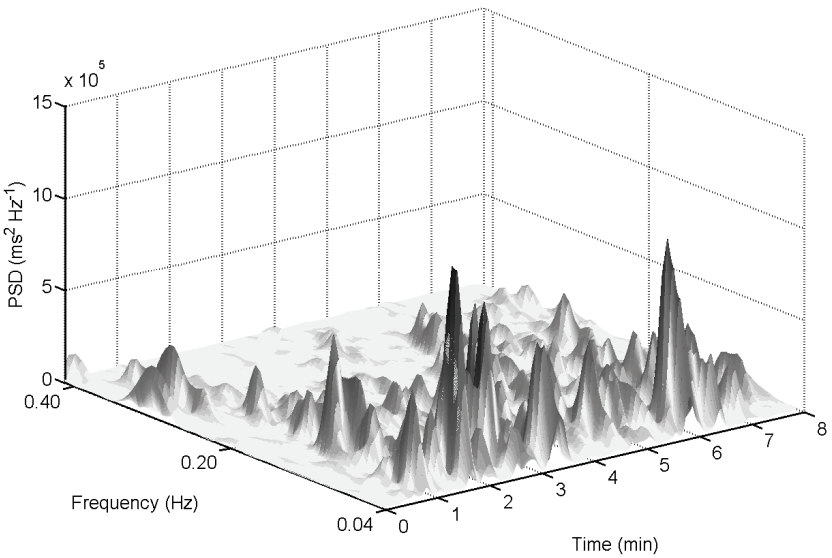

FIGURE 1 Time-frequency distribution of HRV at rest. PSD, power spectral density.

Such a 3D graph allows second-by-second monitoring of any changes in power or frequency. Finally, integrals computed within pre-selected frequency bands as a function of time provide time-dependent information about autonomic HR control. In addition, the instantaneous frequency of the high frequency peak can

be used to obtain information on changes in respiratory frequency during the experiment (Saalasti 2003; Blain et al. 2005; Cottin et al. 2006).

A limitation of the STFT method is that the frequency and time resolutions are inversely related, and therefore a compromise is always required between these two resolutions (Akselrod et al. 1997; Oppenheim and Schafer 1999). A sufficiently high time resolution can be obtained by using short time-windows; however, the use of very short time-windows prevents the assessment of LFP. In general, the duration of the time-window should be at least five times the slowest analyzed wavelength (Challis and Kitney 1991; Keselbrener and Akselrod 1996; Akselrod et al. 1997). The STFT method has successfully been applied to data from endurance exercise and recovery from exercise (e.g. Pichon et al. 2004; Blain et al. 2005; Cottin et al. 2006; Kaikkonen et al. 2007; Kaikkonen et al. 2008)

2.2.3 Quantitative relationship between autonomic control and HRV

In animals, it is has been shown that within-individual variations in cardiac vagal nerve activity are closely related to RRI oscillations associated with respiration and baroreceptor activation (Koizumi et al. 1985). In humans, a dose-response relationship between mean level of vagal effect on the heart and HRV has been studied by graduated administration of atropine. Evidence from studies measuring HRV during graded infusions of atropine have shown that low doses of atropine increase respiratory-related HRV while moderate and large doses decrease it exponentially (Raczkowska et al. 1983; Tulppo et al. 1996; Pichot et al. 1999). One study has also shown that the magnitude of respiratory-related HRV increases progressively during several hours after completion of vagal blockade, (Pichot et al. 1999). Some studies have evaluated the within-subject quantitative relationship between vagal HR control and HRV by using graded infusions of phenylephrine or nitroprusside to achieve baroreflex-mediated increases and decreases in vagal HR control (Bloomfield et al. 1998; Goldberger et al. 2001). One study using this method reported a linear relationship between vagal HR control and absolute HFP (Bloomfield et al. 1998). Another study in turn reported that the relationship is best described by a function in which there is an ascending limb where HFP increases as vagal effect increases until it reaches a plateau level; HFP then decreases as vagal effect increases.

No consistent evidence has been obtained on whether a dose-response relationship exists between sympathetic control and HRV. Some authors have assumed that LFP provides an index of sympathetic control when expressed in normalized units (Pagani et al. 1986; Montano et al. 1994; Pagani et al. 1997). The strongest support for the use of normalized units comes from the measurements by Montano et al. (1994) showing that normalized LFP and LFP/HFP are related to the angle of body tilt in a passive orthostatic task with a high degree of linearity. Since passive orthostatic task increases sympathetic control and decreases vagal control, the authors concluded that normalized LFP is able to quantify the change in sympathetic control. However, this conclusion

has been criticized by Eckberg et al. (2000). His group evaluated the physiological mechanisms involved when healthy humans are tilted passively and found a linear reduction in absolute HFP, but no change in absolute LFP, together with a linear increase in muscle sympathetic nerve activity (Cooke et al. 1999). They suggested that the strong linearity between normalized LFP or LFP/HFP and the angle of upright tilt found by Montano and colleagues most likely reflects a reduction in absolute HFP, not an increase in sympathetic control (Cooke et al. 1999; Eckberg 2000). The validity of normalized spectral powers, in general, has been challenged, because normalization treatment is based on assumptions that absolute LFP is mediated by sympathetic control and that changes in vagal and sympathetic control are reciprocal (see e.g. Eckberg 2000). However, several studies have failed to find a correspondence between LFP and variations in direct measurements of sympathetic outflow to the heart or periphery (Saul et al. 1990; Kingwell et al. 1994; Sloan et al. 1996). Similarly, studies using exercise or induced ischemia to induce increase in sympathetic activity have been unable to produce a gradual increase in LFP (Houle and Billman 1999). Although the quantitative relationship between the magnitude of HRV indices and autonomic control is unclear, HRV indices are employed as measures of within-subject changes in autonomic control in physiological, psychological and clinical examinations.

2.2.4 Effects of gender and age on HRV

There is marked between-subjects variability in HRV, even at rest. Heritable factors may explain a substantial portion of this HRV variance in a population (Singh et al. 1999). Studies have consistently showed that gender affects the time and frequency domain indices of HRV; however such gender differences in HRV depend on the particular indices used and the age range studied (Liao et al. 1995; Ramaekers et al. 1998; Ramaekers et al. 1998; Rossy and Thayer 1998; Fukusaki et al. 2000). The general conclusion is that at ages below 40-50 years, all HRV indices, except those reflecting vagal HR control, are higher in men than women. More specifically, the results of studies reporting absolute power data are conflicting. Some studies have reported that women have significantly higher HFP and similar LFP at rest than men of the same age (Fukusaki et al. 2000). In contrast, other studies have reported that HFP is similar and LFP is significantly lower in women than in men (Liao et al. 1995; Ramaekers et al. 1998; Rossy and Thayer 1998; Sinnreich et al. 1998).

HRV increases in magnitude during early childhood and begins to decrease already after the first decade of life (Finley et al. 1987; van Ravenswaaij-Arts et al. 1991). During adult life, normal aging is associated with a decrease in all the time domain HRV indices and a decrease in HRV spectral power on each frequency band (Liao et al. 1995; Sinnreich et al. 1998; Umetani et al. 1998; Kuo et al. 1999; Pikkujämsä et al. 1999). These changes are related to a decline in the functioning of the autonomic nervous system in general as well as to the deterioration of the cardiovascular system including both structural and functional changes in the heart and in blood vessels. Gender differences

have been observed to decrease with age and they may disappear at advanced age (Ramaekers et al. 1998; Kuo et al. 1999). However, not only aging itself but also a simultaneous decrease in maximal aerobic capacity may result in a decline in HRV at an advanced age (De Meersman and Stein 2007). Findings from studies in senior athletes have shown that regular endurance training that maintains maximal aerobic power at advanced age also maintains resting HRV at a high level (Aubert et al. 2003). It is unclear how these findings in athletes are related to a general population.

2.3 Changes in HRV related to a bout of endurance exercise

Endurance exercise places great demands on the cardiovascular system. The cardiovascular adjustments to a bout of exercise represent an integration of neural, hormonal and mechanical mechanisms. Peripheral sensory information from baroreceptors and receptors originating in the contracting skeletal muscles is integrated in the central nervous system and appropriate output to the peripheral organs is provided. The autonomic nervous system plays a key role in making rapid cardiovascular adjustments at the onset and cessation of exercise and in matching cardiovascular function to the intensity of exercise. This section describes the general cardiovascular adjustments to endurance exercise with a special focus on autonomic HR control during exercise and recovery. In addition, HRV-related findings during and after endurance exercise are reviewed. The validation of HRV indices as measures of autonomic HR control is essentially based on studies conducted at rest and, it is not always clear, how HRV during exercise should be interpreted.

2.3.1 General cardiorespiratory response to a bout of endurance exercise

Maximal oxygen uptake (VO_{2max}) defines the upper functional limit to the capacity of the combined respiratory and cardiovascular system to transport oxygen to muscles and therefore it is used as a laboratory measure of maximum aerobic capacity. By definition, VO_{2max} is achieved during an incremental maximal exercise test when an increase in work load does not elicit any further increase in oxygen uptake (Taylor et al. 1955). According to the Fick equation VO_{2max} equals the the product of maximal values of cardiac output and arteriovenous oxygen difference measured in the left and right atria (McArdle et al. 2006). Thus it can be limited by a central component (cardiac output), which determines oxygen delivered to the tissues, and/or peripheral component (arteriovenous oxygen difference), which refers to oxygen extraction and use by the tissues. VO_{2max} is associated with age, gender, body composition, and genetic factors (Shvartz and Reibold 1990). A reduction in VO_{2max} with aging is associated with reduced maximal HR and left ventricular performance during maximal exercise and consequently reduced maximal cardiac output (Seals et al. 1994).

Cardiac output is defined as the quantity of blood pumped into the aorta each minute by the heart, and thus it equals the product of HR and stroke volume. In a healthy young individual, HR increases in a linear manner from about 50-70 bpm at rest to 195 bpm or more at VO_{2max}, while stroke volume normally reaches its maximum already at moderate exercise intensity (McArdle et al. 2006), although it may also continue to increase, even up to VO_{2max}, with no plateau (Fleg et al. 1994; Gledhill et al. 1994). Thus HR is a major determinant of the increase in cardiac output at high exercise intensities. The increase in HR is primarily mediated by the autonomic nervous system (discussed in detail in chapter 2.3.2.) and the increase in stroke volume is mediated by several extrinsic and intrinsic factors (Rowell et al. 1996). Ventricular contractile force is dependent on ventricular filling pressure (preload) and the velocity of contraction depends on the pressure against which the ventricle exerts its force (afterload). In addition to pre- and afterload, myocardial contractility and distensibility of ventricles contribute to stroke volume. Myocardial contractility increases during endurance exercise due to increased HR per se, increased sympathetic nervous activity and increased concentration of circulating adrenaline.

The performance of the heart during endurance exercise is determined to a great extent by adjustments in the peripheral circulation, including changes in arteriovenous oxygen difference, blood flow and arterial pressure (Rowell et al. 1996). Arteriovenous oxygen difference increases during endurance exercise, the percentage of the available oxygen extracted from the total blood volume increasing from approximately 25% at rest to at least 75% during maximal exercise (McArdle et al. 2006). A redistribution of the blood volume occurs after the onset of endurance exercise due to a vasodilatation in the working skeletal muscles and a vasoconstriction in the inactive tissues (Rowell et al. 1996). Tissue blood flow is regulated by a combination of autonomic nervous activity and locally generated metabolites. In working muscles, local factors override the sympathetically mediated constrictor effect and cause vasodilatation, allowing relatively more of the cardiac output to flow to active areas. The increase in total vascular conductance together with cardiac output maintains aortic mean pressure and pulse pressure within a relatively narrow range during endurance exercise. A considerably increase in SBP, from 120 mmHg up to 200 mmHg or higher, occurs during graded endurance exercise while DBP remains stable or decreases slightly (Åstrand et al. 1965; McArdle et al. 2006).

After the cessation of endurance exercise, cardiac output decreases rapidly. The post-exercise cardiac output in excess of the resting level results from increased HR rather than increased stroke volume (Williams and Horvath 1995). In general, after low intensity exercise, VO_2 recovers monoexponentially and reaches its pre-exercise value within several minutes while, after exhaustive exercise, the recovery of VO_2 consists of several components and can take up to 24 hours (McArdle et al. 2006). Post-exercise VO_2 beyond that required at rest has been termed excess post-exercise oxygen consumption and it reflects the general disturbance to homeostasis due to exercise (Gaesser and Brooks 1984). Excess post-exercise oxygen consumption results from several

metabolic processes including replenishment of oxygen stores in blood and muscle, resynthesis of high-energy phosphates, lactate removal, and elevated body temperature, circulation and ventilation as well as an increase in the rate of the triglyceride/fatty acid cycle and a shift from carbohydrate to fat as a substrate source (Gaesser and Brooks 1984; Børsheim and Bahr 2003).

2.3.2 Autonomic control of heart rate during and immediately after endurance exercise

Findings from studies using autonomic blockades, measurements of plasma catecholamines and mathematical descriptions of HR dynamics have shown that the increase in HR during incremental endurance exercise and the decrease in HR after exercise are primarily mediated by the autonomic nervous system. At the onset of endurance exercise, HR increases rapidly. The increase in HR during exercise is a function of the relative, rather than absolute, intensity of exercise. As the metabolic demands of working muscle increase, there is a further increase in HR. The initial increase in HR up to approximately 100 bpm (or 40% of VO_{2max}) is mainly due to a reduction in vagal activity and the further increase in HR is due to both a decrease in vagal activity and a concomitant increase in sympathetic activity (Robinson et al. 1966; Orizio et al. 1988; Mazzeo 1991). The relative role of sympathetic activation increases with exercise intensity so that at maximal exercise intensity little or no vagal activity can be detected (Robinson et al. 1966; Orizio et al. 1988; Mazzeo 1991). It has been shown that central command and arterial baroreflex resetting result in vagal withdrawal while arterial baroreflex and muscle metabo- and mechano-reflexes are responsible for the increased sympathetic activity during exercise (Rowell and O'Leary 1990; O'Leary 1996). At high exercise intensities the increase in HR is partly mediated by an increased catecholamine concentration in the systemic circulation (Christensen and Brandsborg 1973; Christensen and Galbo 1983; Seals et al. 1988). The increased sympathetic activation also increases the rate of glycolysis and the vasoconstriction effect in inactive tissues (Rowell et al. 1996; McArdle et al. 2006). The acceleration in glycolysis results in pyryvate production in excess of the citric acid cycle and, eventually, accelerated lactate formation in muscle, while the vasoconstriction in inactive tissues causes a decrease in blood flow through the organs that usually remove lactate and produce glucose. Thus, together with other factors, including recruitment of fast twitch skeletal muscle fibers, increased sympathetic activity is associated with an increase in blood lactate concentration during high intensity exercise. The increase in HR in response to exercise becomes smaller with aging, probably due to both reduced vagal withdrawal and diminished β-adrenergic responsiveness (Seals et al. 1994). Also the percentage increase in HR within its operating range decreases with aging.

With the cessation of endurance exercise there is a rapid decrease in HR. Savin et al. (1982), using double blockade, suggested that the exponential decline of HR after peak exercise is an intrinsic property of the intact circulation and that the time-course of the decline is dependent on autonomic activity.

Since the rapid decrease in HR during first minute after exercise is reduced by vagal blockade and unaffected by sympathetic blockade or exercise intensity, it has generally been thought to reflect vagal reactivation (Perini et al. 1989; Imai et al. 1994; Kannankeril et al. 2004). After the immediate rapid decrease, HR decreases more slowly towards its pre-exercise level due to a further increase in vagal activity and a concomitant decrease in sympathetic activity (Perini et al. 1989; Imai et al. 1994). The relative role of the two autonomic divisions in regulating HR recovery and the mechanism causing changes in autonomic activity are not yet fully known. The immediate post-exercise vagal reactivation has been suggested to result from a loss of central command and activation of the arterial baroreflex (Perini et al. 1989; O'Leary 1996). Fast vagal reactivation may be an important mechanism to avoid excessive cardiac work after endurance exercise (Imai et al. 1994). One factor that affects the time course of HR recovery is the preceding intensity of exercise. Metabolites in skeletal muscle are elevated with increased exercise intensity, and the sustained elevation of such metabolites during post-exercise recovery could result in heightened sympathetic control and/or reduced vagal control (Rowell et al. 1996; McArdle et al. 2006). The myocardium may be susceptible to cardiac arrhythmias and potentially fatal cardiac events throughout the post-exercise period, when resting autonomic balance has not yet been restored (Paterson 1996).

2.3.3 HRV response to endurance exercise

HRV response to endurance exercise has been studied on numerous occasions using constant load exercise sessions (Perini et al. 1990; Casadei et al. 1996; Perini et al. 2000; Cottin et al. 2004; Pichon et al. 2004) or incremental exercise (Arai et al. 1989; Bernardi et al. 1990; Casadei et al. 1995; Tulppo et al. 1996; Warren et al. 1997; Hatfield et al. 1998; Tulppo et al. 1998; Blain et al. 2005; Cottin et al. 2006). A common change pattern is that the transition from rest to exercise induces a decrease in global HRV and that global HRV decreases further with increasing exercise intensity. The results for HFP and LFP are highly inconsistent, which can partly be explained by different exercise protocols. The different behavior of absolute and normalized HRV components during exercise also confounds interpretations and therefore the findings for absolute spectral components and the findings for normalized spectral components are reviewed separately.

Absolute HFP and LFP

As a consequence of the decrease in global HRV absolute HFP and LFP are lower during endurance exercise than at rest and decrease progressively with exercise intensity. HFP tends to decrease gradually from low to moderate exercise intensity and remains unchanged at higher intensities (Arai et al. 1989; Casadei et al. 1995; Tulppo et al. 1996; Warren et al. 1997; Tulppo et al. 1998). The intensity above which no further decrease occurs in HFP has been reported to be 50% of VO_{2max} (Tulppo et al. 1996). The above-mentioned findings,

together with findings that vagal blockade abolishes HFP at low and moderate exercise intensities (Tulppo et al. 1996; Warren et al. 1997), suggest that absolute HFP is a suitable marker of vagal withdrawal during exercise. Since there is complete vagal withdrawal at high exercise intensities, the persistence of small absolute HFP at high exercise intensities has to be explained by other factors than vagal activity. In order to explain the non-autonomic origin of HFP several hypothesis have been presented, the most likely of which is that HFP at high exercise intensities is related to an increase in venous return and a periodic stretching of the sinoatrial node secondary to changes in atrial transmural pressure with ventilation (Casadei et al. 1995; Casadei et al. 1996; Blain et al. 2005; Cottin et al. 2006).

Only a few studies have reported on absolute LFP during incremental exercise. These studies have shown that absolute LFP gradually decreases as a function of exercise intensity (Arai et al. 1989; Casadei et al. 1995; Tulppo et al. 1996) up to an intensity of 80% VO_{2max} (Tulppo et al. 1996) and is no longer detectable at exercise intensities near VO_{2max} (Casadei et al. 1995). Since LFP is mediated by both autonomic divisions, it is likely the decrease in absolute LFP at low exercise intensities reflects a decrease in vagal HR control. However, since sympathetic HR control increases gradually when exercise intensity increases, absolute LFP should increase in response to the increase in sympathetic HR control. In fact, as mentioned above, the opposite of this has been reported and thus absolute LFP cannot be used as an index of sympathetic control during exercise.

Normalized LFP and HFP

TP decreases with increasing exercise intensity, and therefore many researchers have adopted the use of normalized units or LFP/HFP in order to control for this decrease. As exercise induces a decrease in vagal control and an increase in sympathetic control, one would expect normalized HFP to decrease and normalized LFP and LFP/HFP to increase during incremental exercise. However, the use of the normalization procedure has produced conflicting results that have rarely been in line with the knowledge of autonomic control during exercise obtained from pharmacological data and hormonal measures. Normalized HFP has demonstrated an increase or no change with an increase in intensity at low exercise intensities and demonstrated a large increase when intensity approaches VO_{2max} (Bernardi et al. 1990; Casadei et al. 1995). In the same studies, normalized LFP has first increased slightly, although non-significantly, and then decreased gradually in response to an increase in exercise intensity (Bernardi et al. 1990; Casadei et al. 1995). LFP/HFP has been reported to be higher than 1 at exercise intensities below ventilatory threshold and lower than 1 at exercise intensities above ventilatory threshold (Cottin et al. 2004).

Some authors have expressed normalized power for each spectral component as a percentage of TP ranging from 0.00 Hz to the upper boundary of the HFP band, and thus the denominator has also included VLFP (Perini et al. 1990; Casadei et al. 1995; Perini et al. 2000). It has been reported that a greater

proportion of HRV moves into the very low frequency band with increasing exercise intensity and that VLFP represents a major part of the remaining HRV at the highest exercise intensities (Perini et al. 1990; Casadei et al. 1995). Therefore, the magnitude and behavior of normalized HRV indices computed by the different normalization procedures may lead to different interpretations of autonomic HR control during exercise.

Limitations of HRV measurements during exercise

In the studies discussed above, several problems in measuring HRV have become evident at high exercise intensities. First, the progressive increase in sympathetic control during incremental exercise cannot be evaluated precisely with any HRV parameter. Second, there may be problems in the reliable detection of spectral peaks when global HRV is decreased. Third, conventional spectral analysis typically requires RRI stationarity for several minutes, a condition which is rarely encountered during endurance exercise. In order to obtain reliable spectral estimates, the duration of constant load exercise has ranged from 3 to 14 minutes and the duration of each stage during an incremental protocol has been stretched to as long as six minutes. However, one factor that affects HRV response to endurance exercise is the duration of the exercise session. For example, an increase in exercise duration from 3 to 6 or 9 minutes has been shown to induce a decrease in absolute power in both HRV components at an exercise intensity of 60% VO_{2max} (Pichon et al. 2004). Fourth, respiratory frequency increases beyond the upper recommended limit of the bandwidth for HFP. The problems regarding signal stationarity and the detection of respiratory oscillations can be overcome by use of time-frequency analysis and optimal selection of the HFP bandwidth (Lewis et al. 2007). Finally, there is a dramatic increase in ventilation at about 60% of VO_{2max}, and it appears that the non-neural contributions to HFP secondary to the increase in ventilation confound the interpretation of HRV results at high exercise intensities (Bernardi et al. 1990; Casadei et al. 1995; Casadei et al. 1996; Cottin et al. 2004; Cottin et al. 2006). It has been suggested that RSA amplitude detected from RRI data during exercise may be related to ventilation per se and not particularly to either tidal volume or respiratory frequency (Blain et al. 2005). This suggestion is based on the finding that with constant ventilation RSA amplitude remained unchanged during exercise at 70% VO_{2max} irrespective of the values of tidal volume and respiratory frequency. However, it is not meaningful to control ventilation during exercise because, even at rest, the voluntary control of ventilation has been shown to alter autonomic HR control (Stark et al. 2000). Furthermore, not all authors support the view that ventilation must be controlled during exercise. Barterls et al. (2004) concluded that HFP during exercise represents true autonomic HR control rather than the effect of ventilation on HFP. They found no difference in HFP at rest when the respiratory rate was increased from 15 to 32 breaths per minute, whereas HFP was found to decrease in response to a similar increase in respiratory rate along with an increase in exercise intensity from 50 to 100% of the ventilatory threshold.

2.3.4 HRV response to the cessation of endurance exercise

For methodological reasons most studies have deliberately avoided performing spectral analysis during approximately the first five minutes of recovery from endurance exercise during which HR decreases in a markedly non-liner fashion (Hayashi et al. 1992; Takahashi et al. 2000; Terziotti et al. 2001; Mourot et al. 2004b; Mourot et al. 2004a). Very recently, HRV changes during the first minutes of recovery have attracted attention and a variety of methods of HRV analysis have been introduced (Hatfield et al. 1998; Goldberger et al. 2006; Buchheit et al. 2007; Kaikkonen et al. 2007; Kaikkonen et al. 2008). The immediate recovery phase that, has not usually been studied, is termed here "the transient recovery phase" and it includes the first five minutes of recovery during which HR decreases rapidly. The following recovery phase with slower HR decrease or stabilized HR is termed "the slow recovery phase". This division is somewhat artificial and is based on the characteristics of the RRI signal rather than the time course of metabolic adjustments after the cessation of endurance exercise.

The transient recovery phase

Two studies by Kaikkonen et al. (2007; 2008) have presented minute-by-minute values for absolute HFP and LFP immediately after the cessation of endurance exercise by using the STFT method. The results of one study showed no recovery of absolute HFP during the first five minutes after high intensity exercise: the exercise protocols used were two interval interventions at 85 and 93% of the velocity at VO_{2max} and two continuous interventions at 80 and 85% of the velocity at VO_{2max} (Kaikkonen et al. 2008). In contrast to HFP, absolute LFP was found to increase during the first minute of recovery after each intervention, when compared with the exercise value. In another study, five different exercise interventions were used to evaluate the influence of exercise intensity and duration on immediate HRV recovery (Kaikkonen et al. 2007). The main finding was that moderate intensity exercise resulted in slower recovery of absolute HFP and lower levels of HFP than low intensity exercise while doubling the duration of the exercise had no influence on HFP. No other studies have reported spectral powers during the transient recovery phase, and therefore results obtained by using other HRV approaches are presented. Hatfield et al. (1998) evaluated respiratory-related HRV by using a robust measure called RSA and found that this measure increased over the first 2 minutes after the cessation of an incremental maximal test but not from recovery minute 2 to 3. Goldberger et al. (2006) computed RMSSD and a new and simple index, termed the root mean square residual, for consecutive short-scale segments (i.e. 15, 30 and 60 s). RMSSD and the root mean square residual increased rapidly after the cessation of incremental maximal exercise and correlated positively with vagal HR control defined by using blockade. Buchheit et al. (2007) also found an increase in RMSSD immediately after the cessation of constant load exercise at moderate intensity.

The slow recovery phase

Main focus of studies measuring HRV after exercise has been to evaluate whether HRV measured at one or several recovery time points is different from its pre-exercise baseline value. HRV has been assessed during time periods when HR has already settled at a relatively stable level. In general, exercise-reduced HRV has been reported to return gradually to the pre-exercise level, the time course of recovery being highly dependent on the exercise protocol. Mourot et al. (2004a) reported that absolute HFP was below its pre-exercise value 10 minutes after cessation of a 10-min exercise at 50% of VO_{2max}. There was no difference between the pre-exercise and recovery value for absolute LFP. Also a 5-min exercise at 80% VO_{2max} has been reported to induce lower absolute HFP 10 minutes after exercise when compared with the pre-exercise value (Takahashi et al. 2000). In addition, it has been shown that after a prolonged exercise session at high intensity (> 45 minutes at power corresponding to the ventilatory threshold, or 6 x 800 m at 1 km h^{-1} below the velocity corresponding the VO_{2max}) more than 1 hour is needed to restore pre-exercise levels of absolute HFP and LFP (James et al. 2002; Mourot et al. 2004b).

Some studies have evaluated the effects of two or more exercise intensities on HRV recovery. Terziotti et al. (2001) compared the effects of 20-min exercise at 50% and at 80% of the anaerobic threshold on HRV recovery. After a 15-min recovery time, absolute HFP was below the pre-exercise value after both intensities but the decrease was greater after exercise at 80% than at 50% of the anaerobic threshold. Absolute LFP during recovery showed no difference from the pre-exercise value after either exercise intensity. Hayashi et al. (1992), using coarse graining spectral analysis, found that 10 minutes after exercise at 100% of the ventilatory threshold, the high frequency component, termed HI, was lower than the pre-exercise value. No such difference was observed after exercise at 20% of the ventilatory threshold. Parekh and Lee (2005) evaluated HRV recovery in 5-min segments after two exercise bouts of similar caloric costs but performed at intensities of 50% and 80% of VO_2 reserve. They found a significant intensity by recovery time interaction for absolute HFP and LFP, the restoration of both spectral components being slower after the higher exercise intensity.

Taken together, HRV recovery is affected by exercise intensity and duration. Based on the above findings, the changes observed in absolute HFP during recovery reflect the restoration of resting vagal control, which is highly affected by exercise intensity. Compared to the changes in HFP, the behavior of absolute LFP after exercise is more difficult to explain. Many of the studies cited above have also reported on normalized HFP and LFP, but the results regarding these values are controversial and no common pattern of change can be found (James et al. 2002; Mourot et al. 2004a; Mourot et al. 2004b; Parekh and Lee 2005). Since several studies have reported no difference between pre-exercise and recovery values for absolute LFP, changes in normalized HFP and LFP result from changes in absolute HFP.

2.4 Changes in HRV related to endurance training

The cardiovascular response to endurance training depends on the intensity, frequency and duration of the training. Regular endurance training induces cardiovascular adaptations that improve the transport of oxygen to working muscles. A common observation related to endurance training is a decrease in resting HR. Intra-cardiac adaptations, such as left ventricular hypertrophy and increased myocardial contractility together with a variety of extra-cardiac adaptations, including changes in autonomic outflow, contribute to training-induced bradycardia. This section focuses, first, on general cardiovascular adaptations to endurance training, with an emphasis on training-induced lower HR, and, second, on the chronic and acute effects of endurance training on HRV.

2.4.1 Cardiovascular and autonomic adaptations to endurance training

General cardiovascular adaptations to endurance training are well-documented (e.g. Blomqvist and Saltin 1983; Rowell et al. 1996). In a young healthy individual, about a half of the increase in VO_{2max} caused by endurance training is attributable to increased cardiac output and a half is attributable to increased arteriovenous oxygen difference (Rowell et al. 1996). Endurance training induces a small decrease or no change in HR_{max} and the greater maximal cardiac output after endurance training results from an increase in stroke volume. Due to the increased stroke volume the same cardiac output at rest and at any given absolute submaximal exercise intensity can be attained by lower HR. These adaptations decrease metabolic load on the heart at any submaximal exercise intensity. Integrated cardiovascular adaptation to endurance training includes an increase in blood volume and an increase in total vascular conductance. Due to the close relationship between VO_{2max} and total vascular conductance, SBP at any given fraction of VO_{2max} is not altered by endurance training. SBP is reduced by endurance training at the same absolute submaximal exercise intensity but not during maximal exercise.

Studies using blockades or other invasive measures have suggested that several mechanisms may contribute to training-induced bradycardia. Evidence from several studies supports the vagal origin of training-induced bradycardia (Smith et al. 1989; Shi et al. 1995). Increased vagal control may be associated with a greater activation of the cardiac baroreceptors due to increased blood volume after training (Mack et al. 1991). There are also data supporting the sympathetic or intrinsic origin of training-induced bradycardia (Ekblom et al. 1973; Lewis et al. 1980; Katona et al. 1982; Smith et al. 1989; Stein et al. 2002). Since sympathetic control is low in the supine posture, it is unlikely that a reduction in resting sympathetic control could completely explain training-induced resting bradycardia. However, decreased sympathetic neural activity and a decrease in the release of catecholamines could contribute to relative

bradycardia during exercise (Hartley et al. 1972; Peronnét et al. 1981; Lehmann et al. 1984).

Another well-documented effect of endurance training is that in general, trained individuals need a shorter time than untrained individuals for HR to recover from an acute bout of exercise (Hagberg et al. 1979; Hagberg et al. 1980; Imai et al. 1994). HR has been shown to decrease more rapidly during the transient recovery phase after exercise in trained than untrained individuals (Imai et al. 1994). The mechanisms responsible for these changes have not yet been conclusively identified. However, it has been proposed that the more rapid recovery of HR associated with endurance training is mediated by the vagal system (Imai et al. 1994) and may be independent of the sympathetic system (Hagberg et al. 1979). It has also been suggested that a slower decrease in HR after exercise in older individuals is due to lower maximal aerobic capacity (Darr et al. 1988).

Exercise-induced changes in the cardiovascular system, accompanied by improvements in aerobic capacity, depend highly on the duration, intensity and frequency of training. It has been suggested that there is a fundamental difference betwen short- and long-term adaptations to endurance training (e.g. Stein et al. 2002; Aubert et al. 2003). Short-term training may result in autonomic adaptations, with a decrease in sympathetic control and an increase in vagal control while long-term training, increasing the diameter of the left ventricle, may result in intrinsic electrophysiological adaptations and enhance vagal control. In general, adaptations to endurance training also depend on individual characteristics, such as age, gender, training background and genetically determined responsiveness (McArdle et al. 2006). In older individuals, an appropriate endurance training programme induces a similar decrease in resting and exercise HR to that observed in younger individuals (Seals et al. 1994).

2.4.2 Endurance training-induced changes in HRV at rest, during exercise and during recovery

In a number of studies, HRV has been compared between groups with differing aerobic fitness. As a rule, these groups have differed in terms of resting HR and thus researchers have assumed to find differences in HRV indices, especially in those reflecting specifically vagal HR control. In longitudinal studies, it has generally been hypothesized that the training-induced decrease in HR is accompanied by an increase in HRV.

Cross-sectional studies

The majority of cross-sectional studies have compared HRV measured at rest between a sedentary group and a group of endurance athletes. In general, absolute or normalized HFP have been shown to be higher in trained than untrained subjects (Macor et al. 1996; Shin et al. 1997; Mourot et al. 2004a), although conflicting data also exist (Furlan et al. 1993; Middleton and De Vito 2005). Absolute LFP at rest has consistently shown no difference between

trained and untrained subjects while there is less consistency in the results for normalized LFP (Furlan et al. 1993; Macor et al. 1996; Mourot et al. 2004a; Middleton and De Vito 2005).

A few studies have compared the HRV response to exercise between groups with different aerobic fitness. Using incremental exercise Tulppo et al. (1998) found that subjects with good aerobic fitness had lower HR accompanied by higher HFP (normalized in mean RRI) at submaximal exercise intensities than subjects with poor aerobic fitness. Studies using exercise intensities determined in relation to the maximal aerobic power have reported no difference in either absolute HFP or LFP during exercise between trained and untrained subjects (Macor et al. 1996; Mourot et al. 2004a). Interestingly, Mourot et al. (2004a) found that compared to untrained subjects trained subjects had higher absolute LFP and similar HFP during recovery from exercise at 50% of VO_{2max}. On the basis of these studies, it seems that HRV indices that reflect vagal HR control are higher at rest and during absolute submaximal exercise in trained than untrained subjects, supporting the view that the lower HR observed in trained subjects may partly be due to increased vagal activity. HRV data collected during recovery are scarce and no information is available on fitness-related differences in the time course of HRV recovery.

Longitudinal studies

Various longitudinal studies have investigated the effects of endurance training on HRV indices at rest. Although the findings have been conflicting, most studies have reported an increase in absolute HFP with a decrease in HR (Carter et al. 2003a; Leicht et al. 2003b; Tulppo et al. 2003; Hautala et al. 2004; Pichot et al. 2005). Others have reported that despite increased VO_{2max} there has been no alteration in absolute HFP (Stein et al. 1999; Loimaala et al. 2000) or no alteration in HFP and HR measured at rest or during 24 hours (Perini et al. 2002; Verheyden et al. 2006). While the majority of the above-cited studies have reported no change in absolute LFP at rest, several studies using 24 hour recordings have reported an increase in absolute LFP due to endurance training (Tulppo et al. 2003; Hautala et al. 2004; Pichot et al. 2005). The studies reporting absolute as well as normalized powers have not always observed any change in normalized spectral components despite changes in absolute HFP or LFP (Leicht et al. 2003b; Mourot et al. 2004a).

Studies on the effects of endurance training on HRV during exercise have yielded conflicting results. Leicht et al. (2003b) reported that an intensive 8-week endurance training programme induced a decrease in HR accompanied by an increase in absolute HFP and LFP at the same absolute exercise intensities. They further reported no difference between pre- and post-training values for HR or absolute spectral powers measured at the same relative work loads in relation to maximal HR. No systematic changes were observed in normalized spectral components. In contrast, Leicht et al. (2003a) found a decrease in HR but no systematic significant change in either absolute or normalized spectral components at the same absolute exercise intensities after a 16-week moderate intensity endurance training programme. Carter et al.

(2003a) reported no change in either absolute HFP or LFP at the same absolute exercise intensity level despite an increase in TP and a decrease in HR after a 12-week intense running programme. Perini et al. (2002) reported that an intense 8-week training programme induced no significant change in the submaximal HR versus VO_2 relationship during incremental or constant load tasks. Changes in normalized HFP and LFP as a function of metabolic demand also were unaltered due to training. In addition, they reported that after maximal exercise, restoration of HRV was similar prior to and after training. Mourot et al. (2004a) found that a 6-week intense training programme induced a decrease in HR and an increase in absolute LFP during exercise at 50% of VO_{2max}, but there was no change in absolute HFP. Neither absolute HFP nor LFP during recovery from exercise was altered due to training.

These inconsistent results are probably attributable to the differences in the duration, intensity and frequency of training between studies. In their review, Achten and Jeukendrup (2003) suggested that vigorous training programmes are necessary to induce changes in HRV. They also pointed out that in addition to exercise duration, exercise intensity and training volume play an important role. The role of exercise frequency remains unclear. Age also may confound the effects of endurance training on HRV, especially on HRV during exercise, since age is related to decline in cardiovascular function and this age-related decline is more apparent during exercise than at rest (Stratton et al. 1994). The inconsistency in the results may also be due to the considerable variation between studies in the exercise intensities at which HRV has been measured.

3 AIMS OF THE STUDY

The general aim of this thesis was to evaluate the ability of HRV to quantify within-subject changes in autonomic HR control by using autonomic blockades and an active orthostatic task, and to study transient changes in HRV in response to a bout of endurance exercise and to endurance training.

The specific aims were:

1) To evaluate vagal and sympathetic contributions to HRV at rest. (I)
2) To define the within-subject quantitative relationship between the magnitude of HRV and the vagal effects on the heart in different body postures in persons with different endurance training backgrounds. (I)
3) To evaluate the ability of the short-time Fourier transform (STFT), a time-frequency analysis, to track the transient changes in vagal effects on the heart during an active orthostatic task. (II)
4) To examine the effects of exercise intensity on HRV during exercise and immediately after the cessation of exercise. (III-V)
5) To examine the effects of age-related aerobic fitness on HRV at rest, and during exercise at different levels of intensity and during subsequent recovery. (IV)
6) To examine the effects of low-dose endurance training on HRV at rest, during incremental exercise, and during subsequent recovery. (V)

4 METHODS

4.1 Subjects

All the subjects were healthy volunteers free of hypertension or other systemic diseases. They were non-smokers, and were not taking any medication or drugs that would alter cardiovascular control. The procedures were conducted according to the declaration of Helsinki and were approved by the Ethics Committee of the Central Hospital of Central Finland (I-IV) or by the Ethics Committee of the University of Jyväskylä, Finland (V). The subjects gave their written informed consent prior to participation and they had a right to withdraw from the experiments at any time. The general health status of the subjects was assessed with a questionnaire (I-V), a resting ECG (for all subjects in studies I-II, for subjects > 40 years in studies III-IV), and a standard medical examination (for subjects > 40 years in studies III-IV, for all subjects in study V). The questionnaire prescreened for inherited propensity to cardiovascular diseases, autonomic nervous system abnormalities, and contraindications to pharmacological blocking agents or maximal exercise test. The subjects were asked to refrain from any physical exertion and consumption of alcohol for two days before the testing day. The consumption of caffeinated beverages was prohibited on test days. All the testing sessions were carried out in a quiet laboratory (22-24°C) between 8:00 a.m. and 18:00 p.m. When the same subject was tested on two or more separate days, the repeated testing sessions were performed at the same time on each day. Table 1 shows characteristics of the subjects.

Eighteen men participated in a blockade experiment. In study I, the subjects were divided into an endurance sports group (n = 8) and a non-endurance sports group (n = 10). The endurance sports group consisted of cross-country skiers and distance runners with a mean training history of 9±4 years. Average training volume during the previous year was 436±111 h, and weekly volume during the 4 weeks preceding the study was 6.5±1.6 h. The non-endurance sports group consisted of participants in such sports as judo, shot put, and basketball. Study II included the data of 11 of the original 18 subjects

regardless of training background. A respiratory frequency > 0.20 Hz during the experiment was used as an inclusion criterion (see 4.7).

TABLE 1 Characteristics of the subjects.

	I		II	III	IV		V
	ES	NES			HAF	MAF	
	n = 8	n = 10	n = 11	n = 26	n = 15	n = 13	n = 11
Gender	M	M	M	M	M/F	M/F	M
Age, years	23±4	24±3	24±2	37±9	37±10	37±9	37±7
Height, cm	176±4	181±7	181±5	172±8	173±8	171±9	181±8
Weight, kg	69±6	77±10	77±9	70±10	68±9	74±12	80±13
Body fat, %	11±2	17±4[a]	16±5	21±7	18±6	23±6[b]	18±4
HR_{max}, bpm				180±9	180±9	181±10	189±11
W_{max}, W				228±56	259±43	206±47[c]	254±29
VO_{2max}, L min^{-1}				3.1±0.8	3.4±0.7	2.9±0.7[b]	2.9±0.4
VO_{2max}, mL kg^{-1} min^{-1}				44±10	50±7	39±7[d]	37±4
$W_{AnT,}$ W				157±46	188±36	135±34[d]	179±23
$W_{AnT,}$ % of W_{max}				68±6	73±5	65±5[c]	70±5
$VO_{2AnT,}$ L min^{-1}				2.2±0.6	2.5±0.5	1.9±0.4[c]	2.1±0.3
$VO_{2AnT,}$ mL kg^{-1} min^{-1}				31±7	36±5	26±4[d]	27±4

The values are means±sd. HR_{max}, maximal heart rate; W_{max}, maximal power output; VO_{2max}, maximal oxygen uptake; W_{AnT}, power output at anaerobic threshold; VO_{2AnT}, oxygen uptake at anaerobic threshold; ES, the endurance sports group; NES, the non-endurance sports group; HAF, the high age-related fitness group; MAF, the moderate age-related fitness group; M, male; F, female. Significant difference at [a] $p < 0.01$ compared to ES, at [b] $p < 0.05$, [c] $p < 0.01$, and [d] $p < 0.001$ compared to HAF.

Thirty-two subjects from a large group of volunteers were selected to participate in study III and IV by using gender, age, physical activity and predicted aerobic fitness (Jackson et al. 1990) as selection criteria. The age distribution of the subjects was as follows: 8 subjects were 25-30 years, 8 subjects 31-35 years, 4 subjects 36-40 years, 4 subjects 41-45 years, 4 subjects 46-50 years and 4 subjects 51-55 years. In each age category, gender and age-related aerobic fitness were counterbalanced. In study III, the subjects constituted a single group regardless of the selection criteria and the number of subjects was 26 as the data on 6 subjects were excluded from the results. Two subjects did not complete all the exercise conditions and 4 discontinued respiratory gas collection. In study IV, the subjects were classified into a high fitness group and a moderate fitness group according to the age-related aerobic fitness norms of Shvartz and Reibold (1990): high age-related fitness consisted of fitness categories 1 (= excellent) and 2 (= very good) and moderate age-related fitness consisted of fitness categories 3 (= good), 4 (= average) and 5 (= fair). The number of subjects in study IV was 28. The data of 4 subjects were excluded from results because of technical problems with the RRI data (2 subjects) or testing procedure (2 subjects).

Twelve previously untrained men volunteered for study V. Some subjects had previously been involved in various recreational physical activities, but none of the subjects had any background in regular endurance or competitive

sports. During the 2 years preceding the study, the subjects had not been involved in regular endurance training of any kind. One subject did not complete all training sessions and his data was excluded.

4.2 Pharmacological blockades (I, II)

Subjects underwent a vagal and sympathetic blocking procedure on separate days starting at 8:15 a.m. The order of blockades was counterbalanced across days and groups (i.e. the endurance and non-endurance sports groups). After catheter insertion into the antecubital vein, instrumentation and instructions, the subjects were allowed to rest quietly for 10 min. During this rest period ECG and blood pressure were checked in order to confirm health status. Before administration of the blocking agent, recordings were obtained at supine rest for 5 min and during an active orthostatic task (AOT). During the AOT the subjects sat quietly for 5 min, stood up unaided, and remained standing for 3 min. Then, either selective vagal or sympathetic blocking was performed according to the procedure describe by Katona et al. (1982). Recordings at supine rest and during the AOT were repeated after the selective blockade. On the day with the vagal blockade, the same recordings were repeated an additional four times at regular 15-min intervals over a 150-min recovery period, during which the effects of the vagal blockade diminished.

Atropine sulfate, a muscarinic antagonist, was used to block the vagal effects on the heart, and metoprolol, a β_1-antagonist, was used to block the sympathetic effects on the heart. The blockade was performed by administrating four equal intravenous doses of either atropine sulfate (4×0.01 mg kg^{-1}; Atropin, Leiras Oy, Helsinki, Finland) or metoprolol (4×0.05 mg kg^{-1}; Spesicor, Leiras Oy, Helsinki, Finland). The doses were given at 3-min intervals, and after each dose, 5 ml of saline (Natrosteril, 0.9%, Medipolar, Oulu, Finland) was flushed. The last dose of the blocking drug produced no further change in HR obtained from online ECG. The dosages were selected on the basis of the literature to achieve a relatively complete selective blockade while minimizing the possible side effects (Berntson et al. 1994; Cacioppo et al. 1994).

4.3 Incremental maximal exercise test (III-V)

A standardized incremental maximal exercise test was performed in a seated position on an electronically braked cycle ergometer (Ergoline Ergometrics 800S, Bitz, Germany) with standardized pedaling frequency (60 rev min^{-1}) and seat and handlebar heights. After a 5-min pre-exercise sitting baseline, exercise workload was increased in a ramp fashion every second minute up to a voluntary maximum. In study III and IV the initial workload was 50 W and each increment was 20 W and in study V the corresponding workloads were 75 W and 25 W. The maximal exercise test was followed by a controlled recovery

period during which the subject was either in the sitting posture for 10 min (III-IV) or supine posture for 15 min (V). All subjects older than 40 years performed the maximal exercise test under the control of a physician. On the same day as the maximal exercise test, resting measurements were performed for 5 min in the supine, sitting and standing posture.

A plateau of oxygen consumption, respiratory exchange ratio exceeding 1.10, and HR and blood lactate concentration (BLa) approximating the age-predicted maximum were employed as criteria for the attainment of maximal power output (W_{max}) and oxygen uptake (VO_{2max}) (Taylor et al. 1955). All subjects met at least two of these criteria. W_{max} was assessed as the mean power during the last 2 min of the exercise test. VO_{2max}, maximal HR (HR_{max}), and ventilation (VE_{max}) were determined at W_{max}. Anaerobic and aerobic thresholds were determined as described in detail previously (Aunola and Rusko 1984), and power output, oxygen uptake, HR and ventilation at the anaerobic threshold (W_{AnT}, VO_{2AnT}, HR_{AnT}, and VE_{AnT} respectively) and at the aerobic threshold were computed.

4.4 Constant load exercise sessions (III, IV)

The subjects performed two constant load exercise sessions: a low intensity [LI, 29±6% of W_{max}] session and a high intensity [HI, 90±1% of W_{AnT} equal to 61±6% of W_{max}] session. The exercise sessions were carried out on different testing days, separated by at least 2 days, at the same time of the day. The day with the LI exercise session always preceded the day with the HI exercise session. Each exercise session consisted of a 5-min pre-exercise sitting baseline, a 10-min exercise on a cycle ergometer (Ergoline Ergometrics 800S, Bitz, Germany) and 10-min recovery in the sitting posture. The exercise intensities were chosen on the one hand to induce two clearly different demands on autonomic HR control (Orizio et al. 1988; Rowell and O'Leary 1990; Mazzeo 1991) and on the other hand to avoid such the high intensity at which HRV is mainly of non-neural origin (Casadei et al. 1995; Casadei et al. 1996). HR adjustments at the intensity chosen for the LI exercise are mediated predominately by the vagal system. Both autonomic divisions are responsible for HR adjustments at the intensity chosen for the HI exercise. Due to large between-subject variation in the aerobic capacity of the present subjects, the higher intensity was determined in relation to W_{AnT} instead of W_{max}.

4.5 Endurance training programme (V)

The endurance training experiment consisted of a 7-week preparatory period followed by a 14-week endurance training period. The incremental maximal exercise test was carried out before the preparatory period and before (pre-training, Pre-T) and after (post-training, Post-T) the endurance training period.

During the 7-week preparatory period, the subjects participated in supervised low intensity training twice a week. They became familiarized with the regular training and laboratory testing procedures. The preparatory period included 30-min exercise sessions below the aerobic threshold (43±4% of W_{max}) twice a week. One exercise session was performed on a cycle ergometer under the supervision of the researcher. The other exercise session was unsupervised and to increase the subject retention rate, it consisted of cycling, running and Nordic walking.

The 14-week endurance training period was designed to improve aerobic power and consisted of low and high intensity training twice a week. Again, one exercise session was performed on a cycle ergometer under the supervision of the researcher while the other exercise session was unsupervised and consisted of the above-mentioned training modes. During the first seven weeks of the endurance training period, the supervised exercise session lasted for 45 min and comprised four stages: a 15-min warm-up below the aerobic threshold (45±5% of W_{max}), 10 min between the aerobic and anaerobic threshold (70±5% of W_{max}), 5 min above the anaerobic threshold and a 15-min cool-down below the aerobic threshold. The unsupervised exercise session lasted for 60 min and was below the aerobic threshold. During the remaining seven weeks of the endurance training period, the supervised exercise session lasted for 75 min and comprised the following stages: a warm-up below the aerobic threshold, 2 x 10 min between the aerobic and anaerobic thresholds, 2 x 5 min above the anaerobic threshold and a cool-down below the aerobic threshold. The unsupervised exercise session lasted for 60-90 min and was below the aerobic threshold. The subjects used a HR monitor during the training sessions in order to maintain exercise intensity at the required level. The subjects were not involved in any regular physical activities other than those in the present training programme.

4.6 Data collection

In studies I and II, ECG and blood pressure were continuously recorded with a sampling frequency of 1000 Hz by a computer-based data acquisition system (model MP1000, Biopac Systems, Inc., Goleta, CA, USA). Standard disposable electrodes were placed on the chest and connected to the amplifier. The ECG data was digitized with a 16-bit analog-to-digital converter. To measure blood pressure a finger cuff from a plethysmograph was installed around the second phalanx of the middle finger of the left hand (Finapres, Ohmeda, Inc. Englewood, CO, USA). During the experiment, the cuff finger was carefully kept at heart level to avoid changes in hydrostatic pressure. An automatic peak trigger was used to detect the R-waves and convert the ECG signal to RRIs. A similar technique was used to convert the continuous blood pressure signal to systolic and diastolic blood pressure (SBP and DBP) values. In studies III-V, RRIs were continuously recorded using a RR-Recorder (Polar Electro Ltd.,

Kempele, Finland), with a sampling frequency of 1000 Hz from the ECG signal, providing an accuracy of 1 ms for each RRI. Resting blood pressure was measured to ensure that all subjects were normotensive.

In studies I and II, respiratory frequency was measured on the basis of thoracic movements registered with a strain-gauge transducer (TSD101C, Biopac Systems, Inc., Goleta, CA, USA). In studies III-V, fractional gas concentrations of expired O_2 and CO_2, respiratory frequency, and tidal volume were recorded breath-by-breath V_{max} 229, Sensormedics, Palo Alto, CA, USA). Prior to each separate measurement condition (e.g. rest, exercise), the gas analyzer was calibrated using ambient air (20.9% O_2 and 0.04% CO_2) and calibration gas (15.87% and 4.17%). The calibration of the flow-meter of the analyzer was performed with a 3-L syringe.

Mean values for all the continuously collected variables (i.e. RRI, HR, SBP, DBP and the respiratory parameters) were calculated from the same time periods which were used for HRV analysis. These time periods are described in detail at the end of chapter 4.7.

Blood samples from the antecubital vein were taken to determine plasma noradrenaline and adrenaline concentrations, providing blunt estimates of sympathetic nervous system activity (Christensen and Galbo 1983). In study I, a blood sample of 10 ml was taken before selective blockade, under full blockade and at the end of the recovery period after blockade. In study V, a blood sample of 10 ml was taken at rest, at the end of maximal exercise test and after the 15-min recovery period. Within 15 min after sampling, the blood sample was centrifuged (3600 r min^{-1}) at + 4°C for 7 min. A plasma sample was aliquoted for noradrenaline and adrenaline measurements and stored at – 80°C until assayed. Determinations were done by using high-pressure liquid chromatography with an electrochemical detector (™ESA Coulochem II detector, Model 5011 Analytical Cell, ESA, Inc., Chelmsford, MA, USA) at Kuopio University Hospital.

BLa (Eppendorf EBIO 6666, Eppendorf, Hamburg, Germany) was determined from fingertip venous blood samples. During the incremental maximal exercise sessions (III-V), a blood sample was taken after the pre-exercise sitting baseline, and after each workload. During the constant load exercise sessions (III-IV), a blood sample was taken after the sitting baseline, at the end of the 10-min exercise period and at the recovery minutes 2, 5 and 10.

4.7 HRV analysis

RRI-signal processing and HRV computations were performed with MATLAB software (The MathWorks, Inc., Natick, MA, USA). RRI series were checked and edited for artifacts using a detecting algorithm and subsequently verified by visual inspection. The original RRI series were resampled at a rate of 5 Hz by using linear interpolation to obtain equidistantly sampled time series. In order to remove low frequency trends and variances below and above the frequency

band of interest, a polynomial filter and a digital FIR band-pass filter were used (see e.g. Oppenheim and Schafer 1999).

The FFT method with a Hanning window (512 samples) was used to obtain power spectrum estimates of HRV during the stationary periods of the RRI time series (I and III) according to the recommendations of Task Force (1996). Since the RRI time series were not stationary during the AOT (II), the incremental maximal exercise test (IV-V) or the recovery period after exercise (III-V), conventional spectral analysis could not be used for analysing HRV. Therefore, the STFT method was used to compute a time-frequency decomposition of the RRI time series (see e.g. Oppenheim and Schafer 1999; Cottin et al. 2006). The method calculated consecutive power spectra of short sections of the signal: a section of a predetermined number of samples was multiplied by the Hanning window function and the fast Fourier transform of their product was taken. The window was then shifted one sample ahead and the same calculations were performed again. This process was repeated until the whole RRI time series of interest was covered. Finally, integrals of the power spectral density curve within each frequency band were computed as a function of time. The frequency and time resolutions of the STFT method are inversely related, and therefore, a compromise is always required between these resolutions. It is recommended that the duration of the time window should be at least five times the slowest analyzed wavelength (Challis and Kitney 1991; Keselbrener and Akselrod 1996; Akselrod et al. 1997). The selected window lengths were 125 (II), 256 (IV-V) and 512 samples (III). In study II, a relatively short window (125 samples equal to 25 s) was selected in order to obtain a sufficiently high time resolution. The length of the window was five times the duration of the lowest variations in the selected high-frequency band, ranging from 0.20 to 0.40 Hz, in study II. Only the data on subjects with a respiratory frequency > 0.20 Hz during the AOT were included in the results.

Low frequency (LFP, 0.04–0.15 Hz), high frequency [HFP, 0.15–0.40 Hz (I); 0.20-0.40 (II); 0.15-1.0 (III); 0.15-1.2 (IV-V)], and total power (TP) corresponding to LFP+HFP were calculated as integrals under the power spectral density curve within the pre-determined frequency bands and expressed in terms of ms^2. In addition, spectral power was expressed in normalized units (n.u.). Normalized LFP (in n.u.) was calculated by dividing LFP (in ms^2) by TP (in ms^2) and normalized HFP (in n.u.) was calculated by dividing HFP (in ms^2) by TP (in ms^2). In addition, LFP/HFP was calculated by dividing HFP (in ms^2) by LFP (in ms^2).

HRV (together with RRI, HR, SBP, DBP and the respiratory parameters) was calculated from the following time periods. In the pharmacological blockade experiment, HRV was assessed by using either FFT (I) or STFT (II). HRV was calculated by using FFT with a Hanning window of 512 samples during each posture starting from 150 s, 150 s, and 80 s after assuming the supine, sitting and standing postures, respectively (I). During the AOT, only HFP at three different steps of the AOT was calculated by STFT as follows: 1) mean of the instantaneous values from 150 to 270 s in the sitting posture, 2) the minimum value of HFP (HFP_{min}) preceding the minimum value of RRI (RRI_{min})

during the first 30 s after standing up[2], and 3) mean from 60 to 160 s in the standing posture (II). In addition, reactivity scores describing a fast and slow response to the AOT were computed as follows: the fast response to the AOT (Δ_{fast}) = sitting value – minimum value and the slow response to the AOT (Δ_{slow}) = sitting value – standing value. During the constant load exercise session, HRV was calculated by either FFT (III) or STFT (IV) as mean for 3 min at the end of the sitting posture, at the end of constant load exercise, and at the end of the recovery period. In addition, after the cessation of the exercise, instantaneous HRV derived from STFT was averaged for each successive recovery minute. During the maximal exercise test, instantaneous HRV derived from STFT was averaged for 1 min at the end of each workload (IV-V). After the maximal exercise test, instantaneous HRV was averaged for 3 min starting from recovery minute 4 and from recovery minute 12 (V). In addition, regarding the resting measurements performed preceding the maximal exercise test instantaneous HRV was averaged for 3 min at the end of the supine, sitting and standing posture (IV-V).

4.8 Statistical analysis

Statistical analyses were performed with SPSS for Windows statistical software (SPSS, Inc., Chicago, ILL, USA) and MATLAB software (The MathWorks, Inc., Natick, MA, USA). Values are means ± sd. To meet the assumptions of parametric statistical analysis, a natural logarithm transformation of the absolute LFP, HFP and TP values was used. A standard of 1 was added to the HRV values before calculating the natural logarithm so that the values would not be negative, e.g. $y=\ln(1 + x)$. In general, an appropriate parametric or nonparametric test was chosen, depending on the distributional characteristics of the variable. In all statistical tests, differences were considered significant when $p < 0.05$, and alpha-level adjustments were made to account for numerous pairwise comparisons as needed.

In the pharmacological blockade experiment, the paired sample t-test or Wilcoxon signed rank test was used to compare variables before and after blockade. One-way repeated measures ANOVA or the Friedman's test was used to compare variables across three body postures or across several time points. The independent samples t-test or Mann-Whitney test was used to compare variables between the endurance and non-endurance sports groups. The effects due to blockade and due to the different steps of the AOT were evaluated by 2 (drug condition: no drug and drug) × 3 (AOT step: sitting, minimum, and standing) repeated measures ANOVAs.

The relationship between the magnitude of HRV and vagal effects on the heart was evaluated by calculating a within-subject linear regression analysis of recovery time (expressed in min) for each HRV index. Values obtained during

[2] The minimum value during the first 30 s after standing-up was also calculated for SBP and DBP (SBP_{min} and DBP_{min}).

the recovery from vagal blockade were fitted to a linear model, separately for the supine, sitting, and standing postures. The slopes and intercepts of the linear regression model, as defined for each individual, were interpreted as describing within-subject dynamics between each HRV index and vagal effects on the heart. The coefficient of determination (R^2) was interpreted as an estimate of the signal-to-noise ratio when using a particular HRV index as a measure of vagal effects on the heart.

The data from the constant load exercise sessions were used to evaluate, first, the effects of exercise intensity and second, the effects of age-related aerobic fitness on HRV during and after exercise. The effects of exercise intensity on the minute-by-minute recovery dynamics of the dependent variables were evaluated by 2 (exercise intensity: LI and HI) × 11 (recovery minute: recovery minutes 0-10) repeated measures ANOVAs. Differences between successive recovery minutes were determined separately for LI and HI by repeated measures ANOVAs with a 'repeated' contrast. A paired t-test or the Wilcoxon signed rank test was used to compare the effects of exercise intensity on the variables measured at the pre-exercise sitting baseline, at the end of the constant load exercise or at the end of the recovery period. Differences in dependent variables between the high and moderate age-related fitness groups at rest and at the end of exercise were evaluated by Student's t-test for independent samples. During the recovery periods, the effects of fitness on the dependent variables were evaluated by 2 (fitness: the high and moderate fitness group) × 5 (recovery minute: recovery minutes 1-5) repeated measures ANOVAs. In addition, the effects of fitness group on the dependent variables during the incremental maximal exercise test were evaluated by 2 (fitness: the high and moderate fitness group) × 7 (exercise intensity: 40, 50, … , 100 % of W_{max}) repeated measures ANOVAs.

In the endurance training experiment, a paired sample t-test or Wilcoxon signed rank test was used to compare the dependent variables before and after the preparatory training period and before and after the endurance training period. The effects of endurance training and exercise intensity were assessed by 2 (endurance training: Pre-T and Post-T) × 5 (absolute exercise intensity: 75, 100, 125, 150, and 175 W) repeated measures ANOVAs and 2 (endurance training: Pre-T and Post-T) × 7 (exercise intensity: 40, 50, ... , 100 % of W_{max}) repeated measures ANOVAs.

5 RESULTS

5.1 HRV before and after autonomic blockades (I)

RRI and HRV

Table 2 shows RRI and HRV before blockade and after the selective vagal and sympathetic blockades. The vagal blockade significantly decreased mean RRI, SDRRI, TP, absolute LFP and HFP, and normalized HFP. Vagal blockade significantly increased normalized LFP and LFP/HFP. Sympathetic blockade significantly increased mean RRI, SDRRI and absolute HFP. The effects of body posture in each of the drug conditions are shown in Table 2.

When RRI was compared between the endurance sports group and the non-endurance sports group in the drug-free condition, no significant difference was found in any posture. None of HRV indices differed significantly between the groups before the sympathetic blockade.

In contrast, before the vagal blockade, SDRRI, TP and absolute HFP in the supine posture were higher in the endurance than non-endurance sports group [SDRRI, 82.11±38.23 ms vs. 43.74±16.02 ms, $p < 0.05$; TP, 8.92±1.32 ln(ms^2) vs. 7.49±0.94 ln(ms^2), $p < 0.05$; absolute HFP, 8.18±1.22 ln(ms^2) vs. 6.96±0.86 ln(ms^2), $p < 0.05$; respectively]. The decrease in MRRI caused by the vagal blockade was not significantly different between the groups.

Plasma catecholamine concentrations

The vagal blockade induced no significant change in either plasma noradrenaline (no drug, 1.51±0.30 nmol L^{-1}; atropine, 1.57±0.53 nmol L^{-1}) or adrenaline concentration (no drug, 0.11±0.05 nmol L^{-1}; atropine, 0.09±0.03 nmol L^{-1}). Plasma noradrenaline concentration increased from 1.70±0.55 nmol L^{-1} before the sympathetic blockade to 2.22±0.73 nmol L^{-1} after the sympathetic blockade ($p < 0.05$), while there was no significant change in adrenaline concentration (no drug, 0.12±0.05 nmol L^{-1}; metoprolol, 0.16±0.10 nmol L^{-1}).

TABLE 2 HRV indices before and after the vagal and sympathetic blockades.

	Vagal blockade			Sympathetic blockade		
	No drug	Atropine	Drug effect	No drug	Metoprolol	Drug effect
MRRI, ms						
Supine	1028±168[c]	613±51[c]	p<0.001	1031±160[c]	1263±165[c]	p<0.001
Sitting	915±152[e]	574±57[e]	p<0.001	922±121[e]	1130±170[e]	p<0.001
Standing	762±117[ef]	506±50[ef]	p<0.001	790±114[ef]	1013±136[ef]	p<0.001
SDRRI, ms						
Supine	60.79±33.46[a]	8.77±2.47	p<0.001	56.10±20.86	80.61±39.27	p<0.05
Sitting	65.95±31.00	8.92±3.33	p<0.001	59.71±22.49	70.67±26.66	p<0.05
Standing	46.67±25.89[f]	8.32±4.52	p<0.001	48.48±26.33	57.86±29.77	ns.
LFP, ln(ms^2)						
Supine	6.89±1.77[a]	1.76±0.91[b]	p<0.001	7.17±1.07	7.54±1.31	ns.
Sitting	8.00±1.11[e]	3.11±1.11[e]	p<0.001	7.67±1.21	7.94±1.20	ns.
Standing	7.39±1.39	2.72±1.09	p<0.001	7.44±1.46	7.09±1.41	ns.
HFP, ln(ms^2)						
Supine	7.50±1.18[b]	1.29±0.69[c]	p<0.001	7.46±0.96[c]	8.23±1.19[c]	p<0.05
Sitting	7.15±1.17	1.28±0.91	p<0.001	7.12±1.12	7.31±1.40	ns.
Standing	5.64±1.37[ef]	0.71±0.35[ef]	p<0.001	5.44±1.44[ef]	6.00±1.41[ef]	p<0.05
TP, ln(ms^2)						
Supine	8.12±1.31	2.19±0.82[b]	p<0.001	8.14±0.92[a]	8.77±1.04[b]	p<0.05
Sitting	8.44±1.02	3.30±1.05[e]	p<0.001	8.35±0.92	8.51±1.10	ns.
Standing	7.62±1.33	2.82±1.03	p<0.001	7.63±1.42[e]	7.52±1.26[ef]	ns.
LFP, n.u.						
Supine	0.38±0.27[c]	0.63±0.24[b]	p< 0.01	0.43±0.20[c]	0.36±0.24[b]	ns.
Sitting	0.66±0.15[e]	0.84±0.20[e]	p<0.05	0.61±0.29	0.61±0.23	ns.
Standing	0.81±0.15[e]	0.90±0.11[e]	P<0.05	0.84±0.15[ef]	0.69±0.22[e]	p<0.05
HFP, n.u.						
Supine	0.62±0.27[c]	0.37±0.24[b]	p< 0.01	0.57±0.20[c]	0.64±0.24[b]	ns.
Sitting	0.34±0.15[e]	0.16±0.20[e]	p<0.05	0.39±0.29	0.39±0.23	ns.
Standing	0.19±0.15[e]	0.10±0.11[e]	P<0.05	0.16±0.15[ef]	0.31±0.22[e]	p<0.05
LFP/HFP						
Supine	1.30±1.85[c]	3.37±3.45[b]	p<0.01	1.59±3.42[c]	1.06±1.66[b]	ns.
Sitting	5.70±13.13[e]	17.18±15.21[e]	P<0.01	3.75±3.86	3.80±5.32	ns.
Standing	8.96±9.02[e]	27.20±26.12[e]	p<0.05	12.62±12.47[ef]	7.36±12.59[e]	ns.

Values are means±sd. MRRI, mean R-R interval; SDRRI, standard deviation of R-R intervals; LFP, low frequency power; HFP, high frequency power; n.u., normalized units; LFP/HFP, LFP-to-HFP ratio; ns., non-significant. Significant difference at [a] $p < 0.05$, [b] $p < 0.01$ and [c] $p < 0.001$ across all postures within the drug condition. Significant difference in paired contrast at [e,f] $p < 0.05$ when compared with supine and sitting postures, respectively, within the drug condition.

5.2 Changes in HRV during recovery from vagal blockade (I)

RRI and HRV

The RRI and HRV indices before the vagal blockade and during recovery from the blockade are shown in Figure 2. At the end of the recovery period, mean RRI and absolute LFP in the supine posture had returned to their pre-blockade values, whereas absolute HFP and TP in the supine posture were 25±21% ($p < 0.001$) and 15±14% ($p < 0.001$), respectively, below their pre-blockade values. In the sitting and standing postures, mean RRI, TP and absolute LFP and HFP obtained at the end of the recovery period were significantly below their pre-blockade values ($p < 0.05$ - 0.001). Normalized LFP and HFP and LFP/HFP showed minor changes during the recovery period.

The within-subject slopes, intercepts, and R^2s of the linear fits are shown in Table 3. Linear fits explained a high percentage of the variance between recovery time and the absolute spectral components but a low percentage of the variance between recovery time and the normalized spectral components. In the supine posture, the individual R^2s for absolute HFP ranged from 49% to 98%, being lower than 85% for only 4 of the 18 subjects, and the individual R^2s for absolute LFP ranged from 60% to 97%, being lower than 85% for 7 of the 18 subjects.

Significant differences in the slopes, intercepts, and R^2 values of the within-subject linear fits across all postures are shown in Table 3. There were no significant differences between the endurance and non-endurance sports groups in the slopes, intercepts, or R^2 values of the linear fits in any posture.

Plasma catecholamine concentrations

Plasma noradrenaline concentration increased from 1.57±0.53 nmol L^{-1} to 1.98±0.60 nmol L^{-1} ($p < 0.05$) during the recovery period after the vagal blockade. Plasma adrenaline concentrations measured under the full blockade (0.09±0.03 nmol L^{-1}) and after the recovery period (0.07±0.04 nmol L^{-1}) were not significantly different.

Respiratory frequency

Respiratory frequency increased slightly in each posture during recovery from the vagal blockade ($p < 0.05$). However, respiratory frequency remained within a narrow range in the supine (from 0.23±0.04 Hz to 0.25±0.04 Hz), sitting (from 0.20±0.06 Hz to 0.23±0.06 Hz) and standing postures (from 0.21±0.07 to 0.24±0.06 Hz) during the recovery period.

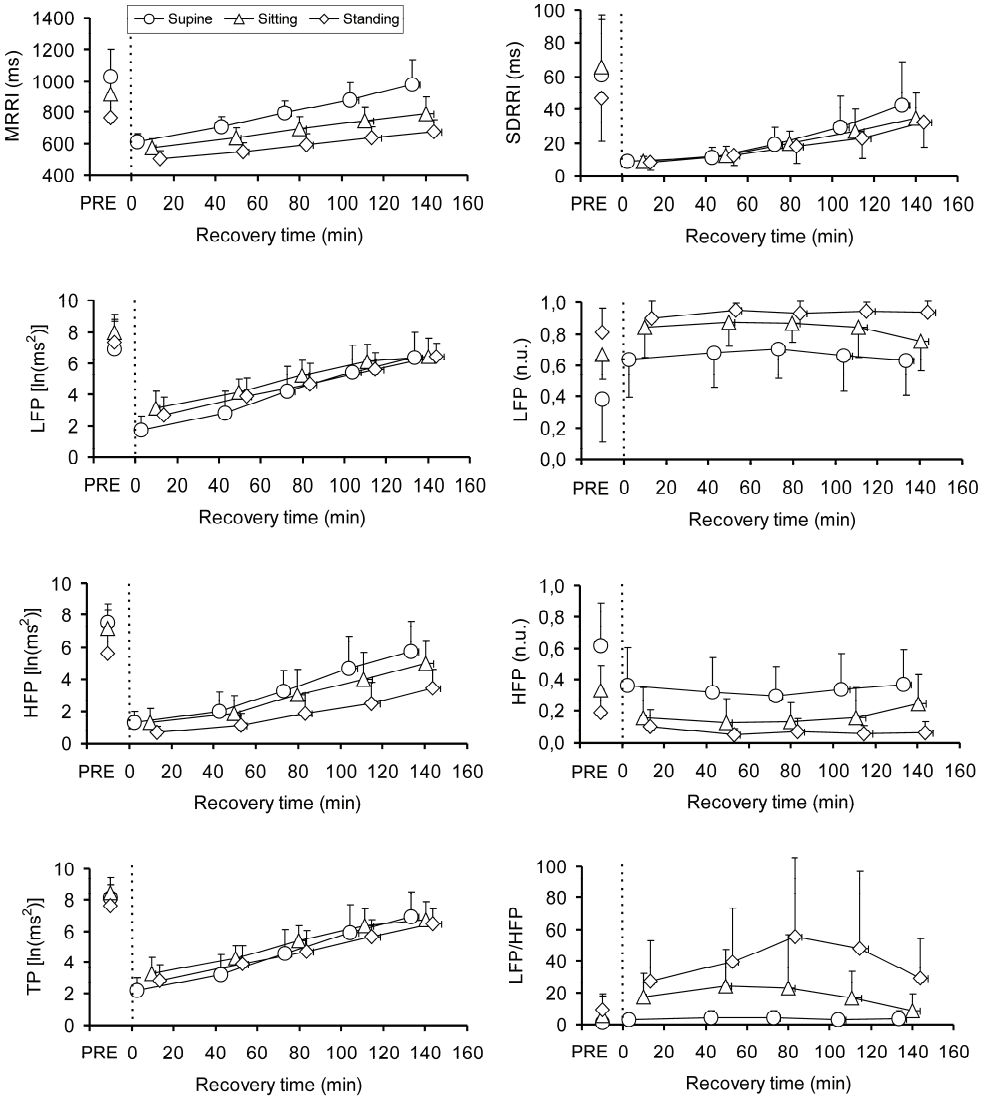

FIGURE 2 RRI and HRV indices before the vagal blockade (PRE) and during recovery from the blockade. MRRI, mean R-R interval; LFP, low frequency power; HFP, high frequency power; TP, total power; SDRRI, standard deviation of R-R intervals; n.u., normalized units; LFP/HFP, LFP-to-HFP ratio. The vertical hairline shows the time when four intravenous boluses of atropine have been administered.

TABLE 3 Individual slopes, intercepts and coefficients of determination of linear fits between recovery time from the vagal blockade and HRV indices.

	Slope	Intercept	R^2 (%)
MRRI, ms			
Supine	2.76±1.00[c]	596±50[c]	98±2
Sitting	1.69±0.50[d]	557±52[d]	97±1
Standing	1.30±0.35[d]	486±50[de]	97±2
SDRRI, ms			
Supine	0.26±0.18	3.72±5.05	77±17
Sitting	0.20±0.11	4.87±4.29	83±9
Standing	0.18±0.11	4.08±5.26	81±17
LFP, ln(ms^2)			
Supine	0.036±0.012[b]	1.53±1.13[b]	87±10
Sitting	0.027±0.007[d]	2.93±0.92[d]	83±14
Standing	0.028±0.008[d]	2.34±1.29	85±16
HFP, ln(ms^2)			
Supine	0.035±0.015[b]	0.86±0.87[a]	87±13
Sitting	0.029±0.011	0.74±0.97	85±12
Standing	0.021±0.009[d]	0.20±0.55[de]	77±16
TP, ln(ms^2)			
Supine	0.037±0.012[a]	1.91±1.03[b]	91±10
Sitting	0.028±0.007[d]	3.05±0.92[d]	88±11
Standing	0.028±0.008[d]	2.42±1.23	86±15
LFP, n.u.			
Supine	0.00040±0.00078	0.37±0.28[c]	55±20
Sitting	0.00019±0.00036	0.66±0.15[d]	68±16
Standing	0.00044±0.00092	0.80±0.17[d]	64±23
HFP, n.u.			
Supine	-0.00040±0.00078	0.63±0.28[c]	55±20
Sitting	-0.00019±0.00036	0.34±0.15[d]	68±16
Standing	-0.00044±0.00092	0.20±0.17[d]	64±23
LFP/HFP			
Supine	-0.00021±0.00327	1.30±1.91[c]	54±17
Sitting	-0.00275±0.00874	5.81±13.40[d]	62±21
Standing	0.00322±0.00604	8.80±8.90	60±20

Values are means±sd. MRRI, mean R-R interval; SDRRI, standard deviation of R-R intervals; LFP, low frequency power; HFP, high frequency power; n.u., normalized units; LFP/HFP, LFP-to-HFP ratio; R^2, coefficient of determination of a linear fit; ns., non-significant. Significant difference at [a] $p < 0.05$, at [b] $p < 0.01$ and [c] $p < 0.001$ across all postures within the drug condition. Significant difference in paired contrast at [d,e] $p < 0.05$ when compared with supine and sitting postures, respectively.

5.3 Transient changes in HRV during an active orthostatic task (II)

RRI and HRV

No significant difference between the two testing days was observed in RRI or absolute HFP values in the drug-free condition. Figure 3 shows an example of the time-frequency distribution of HRV calculated with the STFT method during the AOT before blockade and after the selective autonomic blockades.

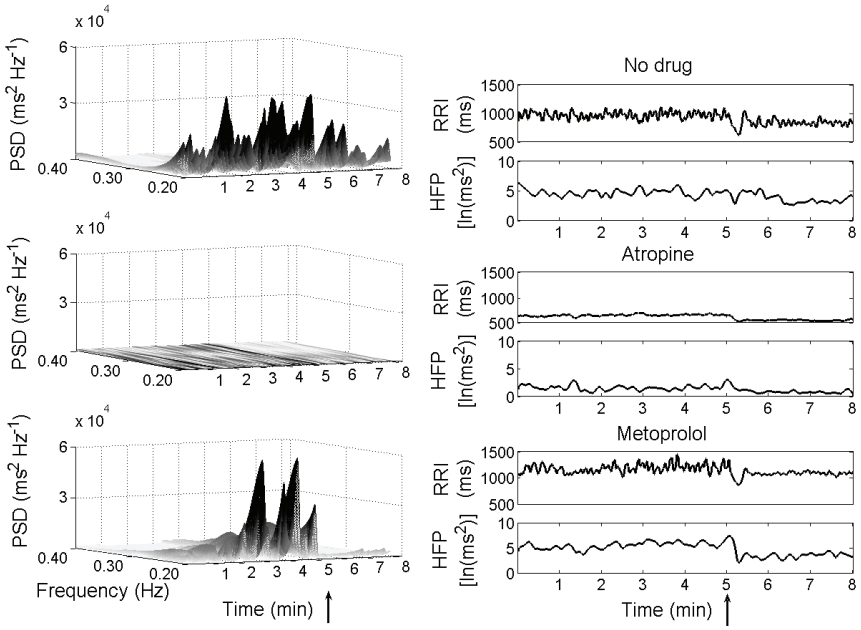

FIGURE 3 Time-frequency distribution of HRV (on the left), R-R-peak interval (RRI), and instantaneous high frequency power (HFP) during the active orthostatic task in the drug-free condition (no drug), after the vagal blockade (atropine), and after the sympathetic blockade (metoprolol, on the right). Example from one subject. The subject sat for 5 min, stood up (see arrow), and remained standing for 3 min. PSD, power spectral density.

Before the vagal blockade, RRI_{min} (i.e. the minimum value during the first 30 s after standing-up; see 4.7) occurred 17±7 s and HFP_{min} occurred 13±6 s after standing-up. Before the sympathetic blockade, the corresponding times were 15±3 s and 10±4 s. Neither the vagal nor the sympathetic blockade had significant effects on the time course of the transient changes in RRI and absolute HFP after standing up.

Figure 4 shows RRI and absolute HFP at the different steps of the AOT before and after the blockades. The vagal blockade decreased RRI and absolute HFP at all steps of the AOT ($p < 0.001$). The sympathetic blockade increased RRI at all steps of the AOT ($p < 0.001$) and HFP_{min} and HFP in the standing posture ($p < 0.01$), but not HFP in the sitting posture. Figure 4 shows the results of

pairwise comparisons between the different steps of the AOT within the drug condition. In the drug-free conditions and after the sympathetic blockade, HFP_{min} and HFP in the standing posture were significantly lower than HFP in the sitting posture and HFP in the standing posture was significantly higher than HFP_{min}. After the vagal blockade, there was no significant difference in HFP values across the different steps of the AOT.

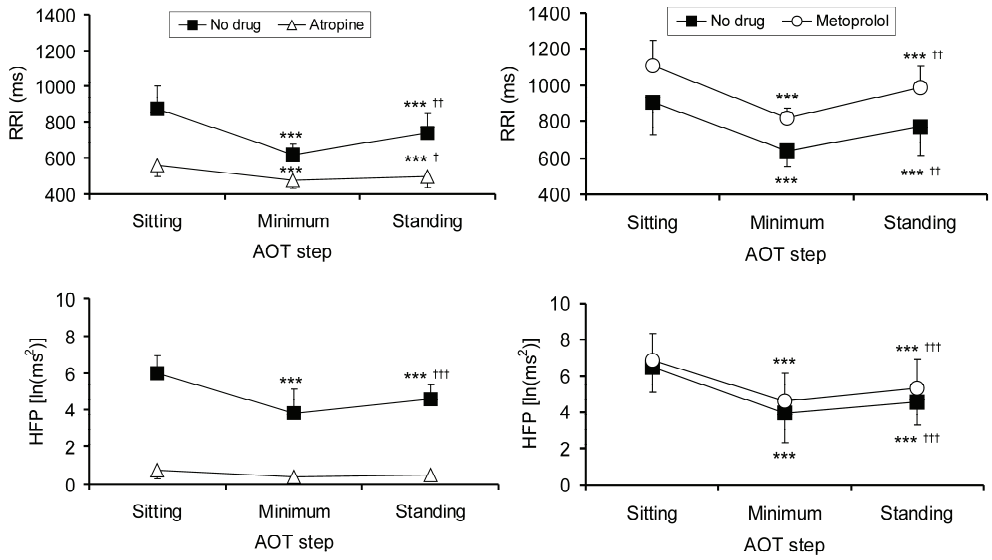

FIGURE 4 R-R interval (RRI) and high frequency power (HFP) at different steps of the active orthostatic task (AOT step) before blockade (no drug), after the vagal blockade (atropine) and after the sympathetic blockade (metoprolol). Minimum, minimum during the first 30 s after active standing up. Significant difference at ***$p < 0.001$ when compared with sitting and at †$p < 0.05$, ††$p < 0.01$, and †††$p < 0.001$ when compared with minimum within the drug condition.

Effects of the selective blockades on the RRI and HFP reactivity scores describing the fast and slow autonomic cardiac response to the AOT are shown in Table 4. The vagal blockade significantly decreased all reactivity scores while the sympathetic blockade had no effect on the reactivity scores.

Blood pressure

In the drug-free condition, there was no difference between the testing days in blood pressure values. Before the vagal blockade, the time delay between standing-up and the occurrence of SBP_{min} and DBP_{min} was 12±2 s and 12±2 s, respectively. Before the sympathetic blockade, the time delay was 12±3 s for SBP_{min} and 11±2 s for DBP_{min}. Neither the vagal nor sympathetic blockade significantly affected the time delays. Before the vagal blockade, SBP decreased due to standing-up from 122±20 mm Hg in the sitting posture to its minimum value of 97±22 mm Hg ($p < 0.001$) and then increased to 117±22 mm Hg in the standing posture ($p < 0.001$). Correspondingly, DBP decreased due to standing-

up from 76±15 mm Hg in the sitting posture to the minimum of 55±14 mm Hg ($p < 0.001$) and then increased to 77±17 mm Hg in the standing posture ($p < 0.001$). A similar response pattern of a significant rapid decrease followed by a significant increase in SBP and DBP after standing-up was found after the vagal blockade as well as before and after the sympathetic blockade. The vagal or sympathetic blockade induced no significant systematic change in the level of SBP or DBP.

Respiratory frequency

Respiratory frequency was lower before (0.24±0.04 Hz, range 0.20–0.32 Hz) than after the vagal blockade (0.27±0.05 Hz, range 0.21–0.35 Hz, $p < 0.05$). There was no significant difference in respiratory frequency before (0.27±0.05 Hz, range 0.21–0.35 Hz) and after the sympathetic blockade (0.27±0.05 Hz, range 0.20–0.34 Hz).

TABLE 4 Reactivity scores before and after the selective autonomic blockades.

	Vagal blockade			Sympathetic blockade		
	No drug	Atropine	Drug effect	No drug	Metoprolol	Drug effect
ΔRRI_{fast}, ms	255±77	82±18	p<0.001	270±82	293±110	ns.
ΔRRI_{slow}, ms	137±78	63±23	p<0.001	137±68	123±77	ns.
ΔHFP_{fast}, ln(ms^2)	2.17±1.16	0.34±0.50	p<0.001	2.52±0.99	2.24±1.04	ns.
ΔHFP_{slow}, ln(ms^2)	1.45±0.97	0.26±0.51	p<0.001	1.91±0.98	1.51±1.04	ns.

Values are means±sd. RRI, R-R interval; HFP, high frequency power; Δ_{fast}, fast response to the active orthostatic task (i.e. sitting– minimum during the first 30 s after active standing-up; see 4.7); Δ_{slow}, slow response to the active orthostatic task (i.e. sitting– standing); ns., non-significant.

5.4 HRV during and immediately after low and high intensity exercise (III)

HR and HRV

The HR and HRV values at the pre-exercise sitting baseline, at the end of exercise, and at the end of the recovery period are shown in Table 5. The LI exercise induced a significant increase in HR and a significant decrease in absolute LFP and HFP with no change in the normalized spectral components. Similarly, the HI exercise induced a significant increase in HR and a significant decrease in absolute LFP and HFP. In addition, the HI exercise significantly decreased normalized LFP and significantly increased normalized HFP.

TABLE 5 HR and HRV at the end of the 5-min pre-exercise sitting baseline, the 10-min exercise and the 10-min recovery period.

	Baseline	Exercise	Recovery
LI, 30% of W_{max}			
HR, bpm	72±10	107±10[a]	72±10[b]
LFP, ln(ms^2)	7.25±1.13	4.96±0.99 [a]	6.91±1.13[ab]
HFP, ln(ms^2)	6.62±1.08	4.12±1.19[a]	6.35±1.11[ab]
LFP, n.u.	0.63±0.20	0.69±0.14	0.61±0.21
HFP, n.u.	0.37±0.20	0.31±0.14	0.39±0.21
HI, 90% of W_{AnT}			
HR, bpm	72±10	145±11 [a]	84±10[ab]
LFP, ln(ms^2)	7.29±0.960	1.71±0.70 [a]	6.34±1.03[ab]
HFP, ln(ms^2)	6.58±1.20	1.98±0.60 [a]	5.12±1.01[ab]
LFP, n.u.	0.64±0.20	0.42±0.22 [a]	0.74±0.17[ab]
HFP, n.u.	0.36±0.20	0.58±0.22 [a]	0.26±0.17[ab]

Values are means±sd. LI, the low intensity exercise; HR, heart rate; LFP, low frequency power; HFP, high frequency power; n.u., normalized units; HI, the high intensity exercise. Significant difference at [a] $p < 0.01$ when compared with baseline and at [b] $p < 0.01$ when compared with exercise.

There was no significant difference in HR or any of HRV indices at the pre-exercise sitting baseline between the LI and HI exercise sessions. HR was lower during the LI than HI exercise ($p < 0.001$). Both the absolute LFP and HFP were higher during the LI than HI exercise ($p < 0.001$). Normalized LFP was higher and normalized HFP was lower during the LI than HI exercise ($p < 0.001$). At the end of the recovery period, HR ($p < 0.001$) and all the HRV indices ($p < 0.05$-0.001) differed between the LI and HI exercise sessions.

Figure 5 shows the results for the effects of exercise intensity and recovery time on HR and HRV during the recovery period after the LI and HI exercise. The successive minute-by-minute values for absolute LFP increased during the first recovery minute after the LI exercise and during the first three recovery minutes after the HI exercise. After the LI exercise, absolute HFP increased significantly during the first recovery minute, decreased slightly after the second and third recovery minutes and stabilized thereafter. After the HI exercise, absolute HFP increased significantly during the first two recovery minutes.

Blood lactate concentration

Baseline BLa was not significantly different between the LI (1.44±10.45 mmol L^{-1}) and HI exercise sessions (1.34±0.44 mmol L^{-1}). BLa at the end of the exercise (1.60±0.69 mmol L^{-1} vs. 4.64±1.41 mmol L^{-1}, $p < 0.001$) and at the end of the recovery period (1.06±0.40 mmol L^{-1} vs. 2.73±1.06 mmol L^{-1}, $p < 0.001$) were lower for the LI than HI exercise session.

Respiratory parameters

None of the baseline respiratory parameters differed between the LI and HI exercise sessions. All the respiratory parameters were lower during the LI than HI exercise (VO_2, 16.26±3.47 mL kg^{-1} min^{-1} vs. 29.49±6.37 mL kg^{-1} min^{-1}; respiratory frequency, 0.34±0.05 Hz vs. 0.43±0.08 Hz; tidal volume, 1.41±0.37 L vs. 2.07±0.42 L, $p < 0.001$, respectively). The minute-by-minute recovery dynamics of all the respiratory parameters were significantly affected by exercise intensity, recovery minute, and their interaction ($p < 0.001$). After the LI exercise, respiratory frequency and VO_2 decreased through recovery minute four ($p < 0.05$–0.001) and tidal volume through recovery minute five ($p < 0.001$). After the HI exercise, respiratory frequency and VO_2 decreased through recovery minute seven ($p < 0.05$–0.001) and tidal volume through recovery minute six ($p < 0.001$). All the respiratory parameters reached the pre-exercise baseline values during the 10-min recovery period, except the respiratory frequency after the HI exercise.

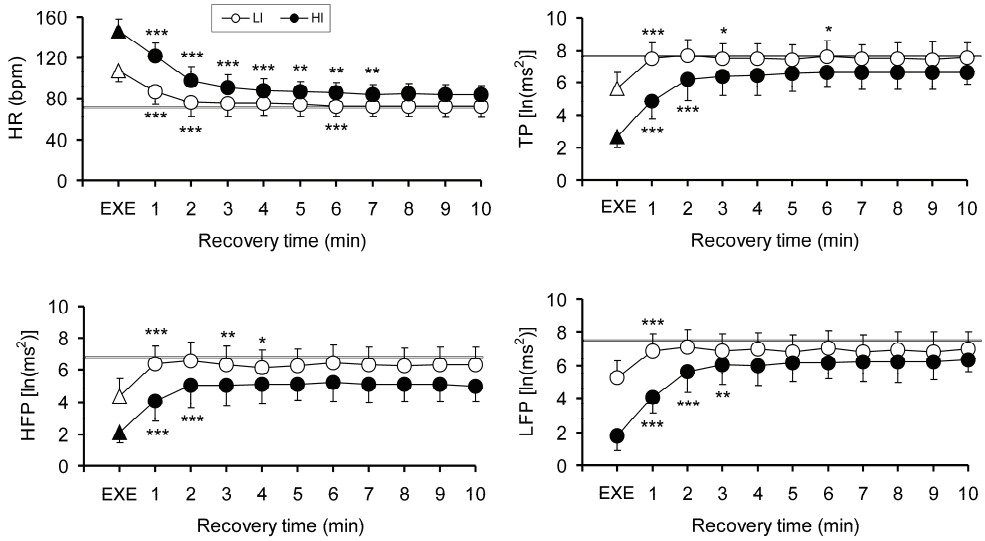

FIGURE 5 HR and HRV during the recovery period after the low (LI, 30% of W_{max}) and high intensity exercise (HI, 90% of W_{AnT}). HR, heart rate; HFP, high frequency power; TP, total power; LFP, low frequency power. Significant difference between successive recovery minutes, separately for LI and HI, at *p < 0.05, **p < 0.01 and ***p < 0.001. The reference line represents the pre-exercise value.

5.5 Effects of age-related aerobic fitness on HRV (IV)

Aerobic capacity

Table 1 (see 4.1) shows that the high age-related fitness group had significantly greater aerobic power than the moderate fitness group as expressed by a significantly greater group mean of VO_{2max}, W_{max}, VO_{2AnT}, and W_{AnT}. The ranges (minimum-maximum) of these parameters widely overlapped since

aerobic fitness norms in relation to age and gender were used when the subjects were classified into the two groups: VO_{2max} (high fitness, 40-66 mL kg^{-1} min^{-1}; moderate fitness, 30-53 mL kg^{-1} min^{-1}), W_{max} (high fitness, 190-326 W; moderate fitness, 150-290 W) , VO_{2AnT} (high fitness, 31-47 mL kg^{-1} min^{-1}; moderate fitness, 20-35 mL kg^{-1} min^{-1}), and W_{AnT} (high fitness, 138-245 W or 64-82% of W_{max}; moderate fitness, 91-205 W or 52-73% of W_{max}).

HR and HRV

HR in the supine and sitting postures was lower in the high than in the moderate age-related fitness group (supine, 62±8 bpm vs. 72±8 bpm, $p < 0.01$; sitting, 63±7 bpm vs. 75±9 bpm, $p < 0.01$). Absolute HFP was higher in the supine posture and tended to be higher in the sitting posture in the high than in the moderate fitness group [supine, 7.73±1.39 ln(ms^2) vs. 6.56±1.13 ln(ms^2), $p < 0.05$; sitting, 7.35±1.28 ln(ms^2) vs. 6.44±1.08 ln(ms^2), $p = 0.054$]. There was no difference in absolute LFP in either posture between the high and moderate fitness group [supine, 7.66±1.26 ln(ms^2) vs. 6.99±0.99 ln(ms^2); sitting, 7.50±1.15 ln(ms^2) vs. 6.80±0.78 ln(ms^2), respectively]. TP was higher in the high than in the moderate fitness group in both postures [supine, 8.57±1.21 ln(ms^2) vs. 7.63±1.04 ln(ms^2), $p < 0.05$; sitting, 8.35±1.08 vs. 7.49±0.88 , $p < 0.05$].

Figure 6 shows HR and HRV during the LI and HI exercise sessions. During the LI exercise, neither HR nor any of HRV indices were significantly different between the age-related fitness groups. During the recovery period after the LI exercise, HR was lower ($p < 0.05$) and absolute HFP tended to be higher ($p = 0.054$) in the high than in the moderate age-related fitness group. During the HI exercise, there was no difference in HR between the age-related fitness groups whereas all HRV indices were significantly lower in the high than in the moderate age-related fitness group. During the recovery period after the HI exercise, there was a significant fitness × recovery minute interaction for HR ($p < 0.01$), HR decreasing more rapidly in the high age-related fitness group. A further analysis showed that HR was not significantly different between the age-related fitness groups during the first three recovery minutes but was significantly lower thereafter in the high than in the moderate age-related fitness group. Absolute HFP ($p < 0.01$) and TP ($p < 0.05$) during the recovery period after the HI exercise were higher in the high than in the moderate age-related fitness group. When the pre-exercise sitting baseline value was subtracted from the recovery values, there was no significant difference between the age-related fitness groups in HR or any of HRV indices after either the LI or HI exercise; however a significant fitness group × recovery minute interaction for HR continued to remain after the HI exercise ($p < 0.01$).

Figure 7 shows the effects of age-related fitness and exercise intensity on HR and HRV during the maximal exercise test. There was no difference in HR between the fitness groups, when HR was expressed as a function of relative exercise intensity ranging from 40 to 100% of W_{max}. TP at the same relative exercise intensity levels was, however, lower ($p < 0.05$) and absolute HFP ($p = 0.062$) and LFP ($p = 0.064$) tended to be lower in the high than in the moderate age-related fitness group.

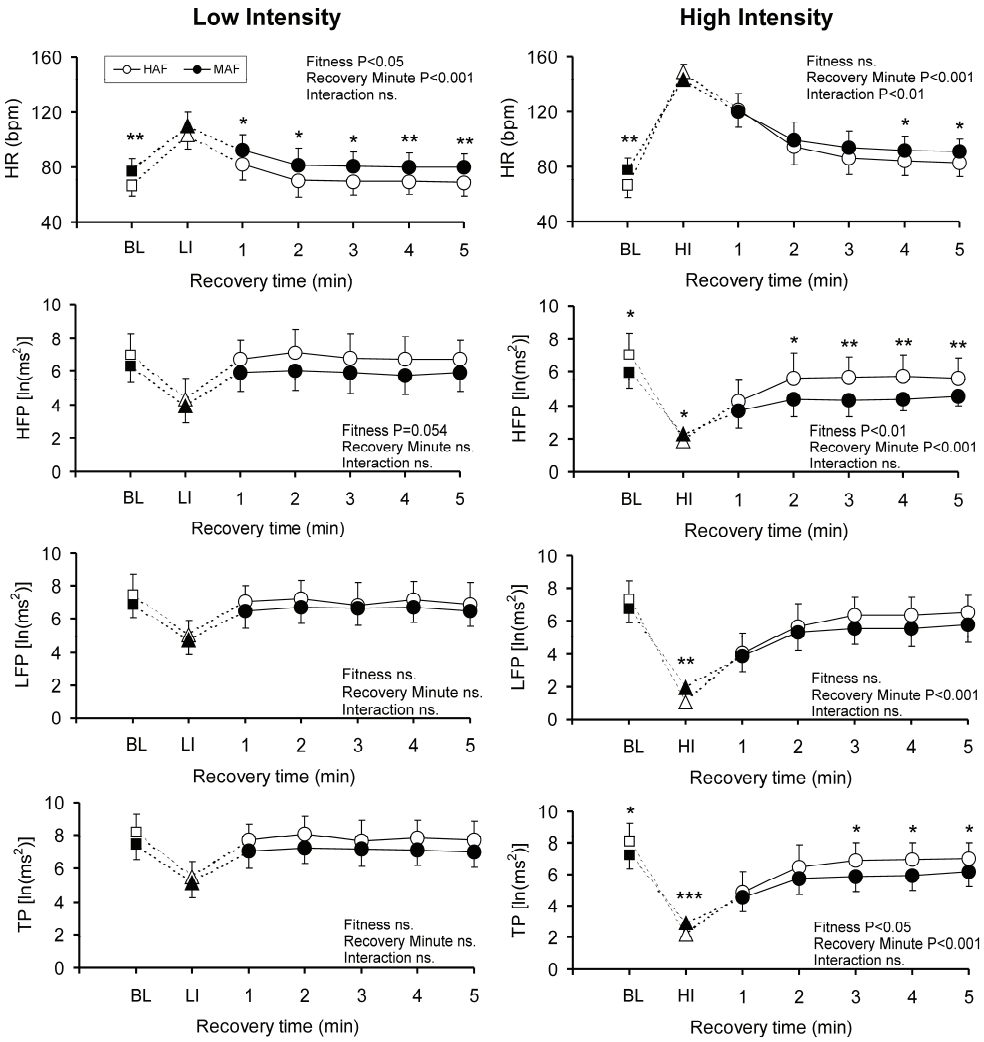

FIGURE 6 HR and HRV at the pre-exercise sitting baseline (BL), during the low (LI, 30% of W_{max}, on the left) and high intensity exercise (HI, 90% of W_{AnT}, on the right) and during recovery from exercise. HAF, a high age-related fitness group; MAF, a moderate age-related fitness group; HR, heart rate; HFP, high frequency power; LFP, low frequency power; TP, total power; ns., non-significant. Results from the repeated measures ANOVAs with 'fitness' as a between-subjects factor and 'recovery minute' as a within-subject factor are shown in the figure. Significant difference in paired comparisons between the age-related fitness groups * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

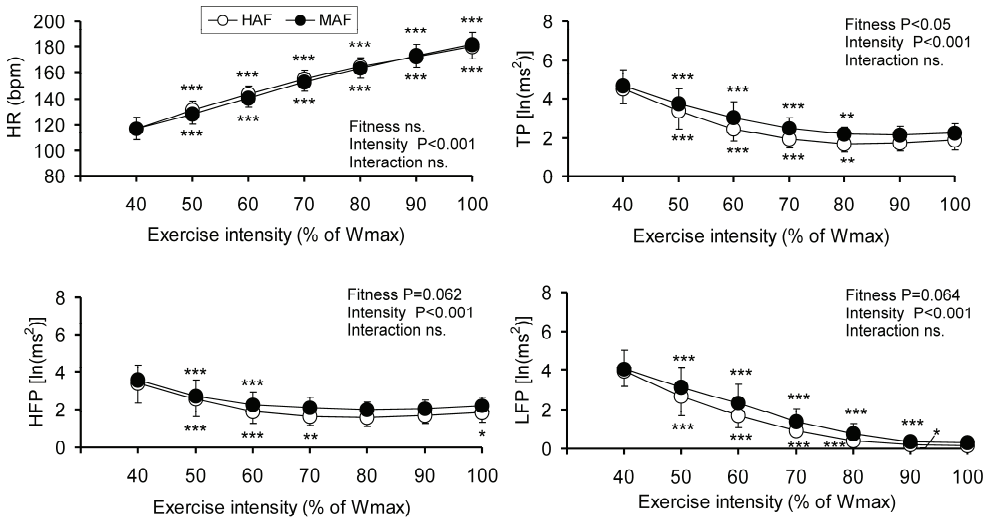

FIGURE 7 HR and HRV as a function of relative exercise intensity. HAF, a high age-related fitness group; MAF, a moderate age-related fitness group; HR, heart rate; HFP, high frequency power; TP, total power; LFP, low frequency power; ns., non-significant. Results from the repeated measures ANOVAs with 'fitness' as a between-subjects factor and 'relative exercise intensity' as a within-subject factor are shown in the figure. Significant difference between successive exercise intensity levels, separately for HAF and MAF, at *p < 0.05, **p < 0.01 and ***p < 0.001.

Blood lactate concentration

During the LI and HI exercise sessions, there was no significant difference in BLa between the high and moderate age-related fitness groups. There was no significant group difference in BLa expressed as a function of relative exercise intensity during the maximal exercise test or in the maximal value of BLa.

Respiratory parameters

Tidal volume did not differ significantly between the age-related fitness groups in any condition during the experiment. There was no significant difference between the fitness groups in respiratory frequency at rest, during the LI or HI exercise or during the recovery period after the HI exercise. However, during the recovery period after the LI exercise, respiratory frequency decreased more rapidly in the high than in the moderate age-related fitness group (fitness × recovery minute interaction, $p < 0.05$). During the maximal exercise test, the high age-related fitness group showed a more pronounced increase in respiratory frequency with relative exercise intensity when compared with the moderate age-related fitness group (fitness × relative exercise intensity, $p < 0.05$).

5.6 Effects of low-dose endurance training on HRV (V)

Aerobic capacity

The 7-week preparatory period induced no change in any of the parameters of aerobic power. HR, HRV, plasma catecholamine concentrations, resting blood pressure, weight or body fat measured prior to and after the preparatory period were not significantly different.

The 14-week endurance training period induced a significant increase in aerobic power as expressed by an increase from Pre-T to Post-T in VO_{2max} (37±4 mL kg^{-1} min^{-1} vs. 39±4 mL kg^{-1} min^{-1}, $p < 0.001$), VO_{2AnT} (27±4 mL kg^{-1} min^{-1} vs. 30±4 mL kg^{-1} min^{-1}, $p < 0.01$), W_{max} (254±29 W vs. 285±30 W, $p < 0.001$) and W_{AnT} (179±23 W vs. 203±24 W, $p < 0.001$). Also, ventilation increased from Pre-T to Post-T (VE_{max}, 142.0±22.2 L min^{-1} vs. 151.7±19.3 L min^{-1}, $p < 0.05$; VE_{AnT}, 54.6±10.4 L min^{-1} vs. 60.1±9.4 L min^{-1}, $p < 0.05$). However, respiratory frequency and tidal volume at the same absolute submaximal exercise intensity levels were not significantly altered after the endurance training period. No significant change from Pre-T to Post-T was observed in HR_{max} (189±11 bpm vs. 187±12 bpm) or HR_{AnT} (160±3 bpm vs. 159±13 bpm). There was no significant change in body weight, body fat or resting blood pressure.

HR and HRV

Table 6 shows the Pre-T and Post-T values of HR and HRV observed in the supine, sitting and standing postures. Neither HR nor the HRV indices were significantly different between Pre-T and Post-T.

TABLE 6 HR and HRV at rest and during recovery from the maximal exercise test.

	Rest			Recovery	
	Supine	Sitting	Standing	5-7 min	12-14 min
Pre-T					
HR, bpm	61±5	68±5	80±7	105±7	102±6
LFP, ln(ms^2)	6.77±0.61	6.96±0.68	7.09±0.65	3.46±0.78	3.38±0.84
HFP, ln(ms^2)	6.80±1.02	6.25±0.83	5.57±0.78	2.81±0.54	2.41±0.67
TP, ln(ms^2)	7.62±0.73	7.47±0.66	7.38±0.63	4.00±0.59	3.77±0.75
Post-T					
HR, bpm	61±8	67±9	78±10	105±10	102±9
LFP, ln(ms^2)	6.77±0.79	7.15±0.66	7.13±0.71	3.39±0.87	3.38±1.09
HFP, ln(ms^2)	6.90±0.94	6.53±1.00	5.75±0.75	2.88±0.67	2.67±0.91
TP, ln(ms^2)	7.69±0.77	7.75±0.62	7.44±0.64	3.91±0.78	3.85±0.99

Values are means±sd. Pre-T, before the endurance training period; HR, heart rate; LFP, low frequency power; HFP, high frequency power; TP, total power; Post-T, after the endurance training period. N = 10 for the supine, sitting and standing postures and n = 9 for recovery.

Figure 8 shows the effects of training and exercise intensity on HR and HRV. At the same absolute submaximal exercise intensities, HR was significantly lower and all HRV indices were significantly higher at Post-T than Pre-T.

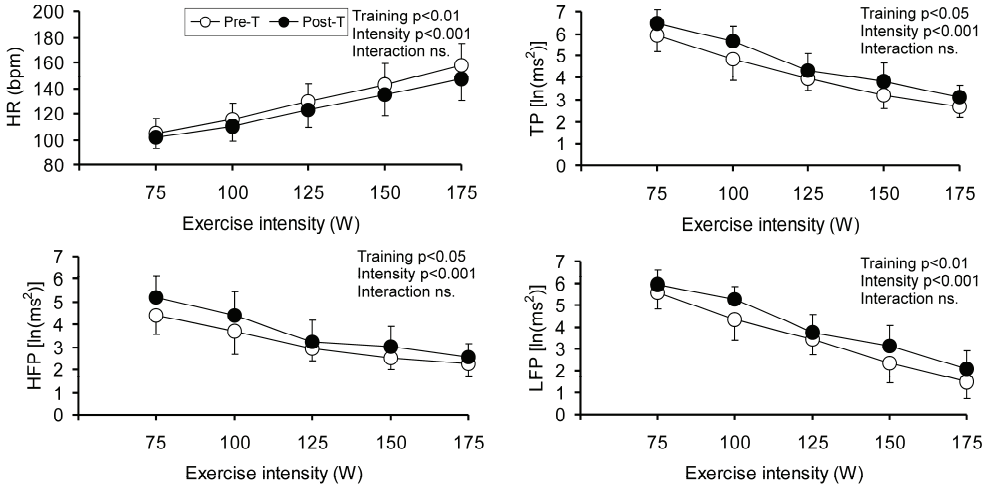

FIGURE 8 HR and HRV as a function of submaximal exercise intensity prior to (Pre-T) and after the 14-week endurance training period (Post-T). HR, heart rate; HFP, high frequency power; TP, total power; LFP, low frequency power; ns., non-significant. Results from the 'endurance training' × 'absolute exercise intensity' repeated measures ANOVAs are shown in the figure.

When exercise intensity was expressed in relation to maximal power, the Post-T values for HR and all HRV indices were similar to their Pre-T values at the same relative exercise intensity levels. Comparisons of the successive exercise intensity levels showed that absolute HFP and LFP significantly decreased in response to the increment in exercise intensity up to the intensity of 70 and 90% of W_{max}, respectively. A significant decrease in TP occurred up to the intensity of 80% of W_{max}. At higher exercise intensities, the HRV indices remained unchanged despite the increments in exercise intensity.

Plasma catecholamine concentration

Plasma noradrenaline concentration at rest was higher at Pre-T than Post-T (2.01±0.63 nmol L^{-1} vs. 1.55±0.36 nmol L^{-1}, $p < 0.05$). Plasma adrenaline concentration at rest was not different between Pre-T (0.18±0.05 nmol L^{-1}) and Post-T (0.19±0.07 nmol L^{-1}). At the end of the maximal exercise, there was no significant difference between the Pre-T and Post-T in noradrenaline (27.35±6.66 nmol L^{-1} vs. 24.95±5.18 nmol L^{-1}, respectively) or adrenaline (2.52±1.62 nmol L^{-1} vs. 2.68±1.46 nmol L^{-1}, respectively). Similarly, at the end of the recovery period no significant difference was observed between the Pre-T and Post-T in noradrenaline (5.45±1.76 nmol L^{-1} vs. 5.55±1.36 nmol L^{-1}, respectively) or adrenaline (0.42±0.31 nmol L^{-1} vs. 0.53±0.48 nmol L^{-1}, respectively).

Blood lactate concentration

BLa at rest did not differ between Pre-T and Post-T. BLa at the same absolute exercise intensity levels was significantly lower at Post-T than Pre-T, whereas BLa at the similar relative exercise intensity levels was not significantly

different between Pre-T and Post-T. The maximal value for BLa increased from 14.0±2.1 mmol L^{-1} at Pre-T to 15.2±2.2 mmol L^{-1} at Post-T ($p < 0.01$).

6 DISCUSSION

The methodological part of this thesis evaluated HRV as a measure of autonomic HR control. In order to examine the effects of vagal and sympathetic contributions to HRV, comparisons were made between HRV values obtained before and after the selective autonomic blockades. In addition to this standard blocking approach, HRV was monitored during recovery from the vagal blockade, when the effects of vagal control on the heart increased gradually, and the within-subject quantitative relationship between HRV and vagal HR control was computed. Third, the blockade data was used to evaluate the ability of the STFT method to detect autonomic cardiac response to the AOT. In the second part of this thesis, the STFT method was applied to monitor changes in HRV in response to a bout of endurance exercise. The effects of exercise intensity on HRV were evaluated not only during exercise but also during immediate recovery after exercise. The effects of aerobic fitness on HRV response to exercise were evaluated by using cross-sectional and longitudinal designs.

6.1 Vagal and sympathetic contributions to HRV

The main findings regarding the pre-blockade versus post-blockade comparisons were that the vagal blockade shortened mean RRI and decreased HRV at all frequencies, while the sympathetic blockade lengthened mean RRI and slightly increased SDRRI and absolute HFP. This was what was expected on the basis of the literature. The literature is consistent in indicating that absolute HFP reflects respiratory-related fluctuations in vagal control and that absolute HFP is largely abolished by vagal blockade and not reduced by sympathetic blockade (Akselrod et al. 1981; Pomeranz et al. 1985; Cacioppo et al. 1994; Uusitalo et al. 1996; Taylor et al. 1998). In accordance with the present study, a study by Taylor et al. (1998) showed an increase in absolute HFP after sympathetic blockade. Selective autonomic blocking of one division of the autonomic nervous system may induce indirect or reflexive alterations in the

unblocked division (Berntson et al. 1994) and thus, the observed increase in absolute HFP may be due to a reflexive augmentation of vagal control. The specific mechanisms responsible for the generation of LFP are not yet fully understood. LFP has been suggested to be associated with low frequency blood pressure fluctuations and baroreflex function (Berntson et al. 1997; Eckberg 2000). Also, muscle sympathetic nerve activity is known to fluctuate at frequencies around 0.1 Hz and these fluctuations may contribute to some way to low frequency fluctuations in arterial pressure (Eckberg 2000). The present observations that absolute LFP was reduced by the vagal blockade and unaffected by the sympathetic blockade are consistent with the findings from previous blockade studies (Cacioppo et al. 1994; Taylor et al. 1998). However, some studies have also reported that LFP is reduced when sympathetic blockade is carried out after vagal blockade in order to complete a state of dual blocking (Akselrod et al. 1981; Pomeranz et al. 1985; Uusitalo et al. 1996). This suggests the vagal as well as the sympathetic system can contribute to absolute LFP.

At rest, sympathetic HR control is minimal in healthy young subjects with a physically active background. Therefore, the effects of blockades on HRV were studied also in the sitting and standing postures. Vagal control is highest and sympathetic control lowest in the supine posture, the reverse occurs in the standing posture, and autonomic control in the sitting posture tends to fall between these extremes (Cacioppo et al. 1994). The present data demonstrated that mean RRI was longest in the supine posture, shortest in the standing posture and intermediate in the sitting posture. This pattern of differences across the three postures was observed in each drug condition, which simply indicates that mean RRI is directly influenced by both autonomic divisions. Consistently with other studies (Pomeranz et al. 1985; Cacioppo et al. 1994; Taylor et al. 1998), the present study further demonstrated that body posture influenced the magnitude of HRV, the influence being most pronounced for absolute HFP. In the standing posture, absolute HFP was lower than in the other postures. Unexpectedly, absolute HFP was slightly, but significantly, altered by postural manipulation also when HFP was attenuated by the vagal blockade. This suggests that vagal control may not be the only determinant of absolute HFP. Absolute HFP in all three postures was, however, largely mediated by the vagal system. It has been reported that LFP in the standing posture is jointly mediated by the vagal and sympathetic systems (Pomeranz et al. 1985). However, the present study showed no evidence for sympathetic contributions to absolute LFP in any posture. Taken together, the decreases after the vagal blockade in absolute LFP and HFP in each posture together with the lack of reduction in HRV after the sympathetic blockade suggest that HRV at all frequencies was primarily mediated by the vagal system.

Normalized HFP and LFP have been proposed as markers of vagal and sympathetic HR control, respectively, and the LFP/HFP ratio has been proposed as a marker of sympatho-vagal balance (Pagani et al. 1986). The present changes in the normalized spectral components after the vagal blockade appear to lend support to this proposition. However, the sympathetic blockade

failed to show systematic changes in the normalized spectral components, and thus the proposition was not supported. When the effects of the blockades on the normalized spectral components together with the effects of blockades on the absolute spectral components are considered, the present data do not support the use of normalized HFP as a vagal index or normalized LFP as a sympathetic index. In concert with other authors (Cacioppo et al. 1994) this conclusion is based on the demonstrated substantial vagal contributions to absolute LFP rather than a failure to demonstrate sympathetic contributions to absolute LFP and normalized LFP.

Regular endurance training designed to increase aerobic fitness is associated with alterations in autonomic HR control. Several HRV studies have evidenced that the low resting HR often observed in endurance athletes is associated with higher vagal HR control (Macor et al. 1996; Shin et al. 1997; Mourot et al. 2004a). No significant difference in mean RRI at supine rest between the endurance sports group and non-endurance sports group was found. Moreover, the "gold standard" for estimating resting vagal control, a decrease in mean RRI due to the vagal blockade, was similar in both groups. The large variability in mean RRI at rest (range from 798 ms to 1,390 ms) in the non-endurance sports group probably explains the insignificant difference in mean RRI at rest. Absolute HFP, TP and SDRRI were higher in the endurance than non-endurance sports group in the drug-free condition before the vagal blockade. These group differences were not, however, replicated in the drug-free condition on the day with the sympathetic blockade. Thus, no systematic group difference in either mean RRI or HRV was found, suggesting that there was no difference in vagal HR control between the present subjects with different training backgrounds. Also the subjects in the non-endurance sports group trained and thus, they are not comparable with the sedentary control subjects used in other studies.

6.2 Within-subject quantitative relationship between vagal HR control and HRV

The effects of gradually decreasing vagal blockade on HRV were monitored to evaluate from the quantitative point of view whether HRV could be used to estimate within-subject changes in vagal HR control. Mean RRI, SDRRI and natural log-transformed values for HFP, LFP and TP were found to increase linearly with the decreasing effects of the vagal blockade. Correspondingly, when expressed without log transformation, the spectral components increased exponentially as a function of the decreasing effects of the vagal blockade.

A linear fit explained 98% of the within-subject variance between recovery time and mean RRI in the supine posture. This is consistent with the previous studies reporting that RRI is closely linearly related to the vagal-nerve firing rate (Parker et al. 1984; Koizumi et al. 1985; Berntson et al. 1995). Absolute HFP in the supine posture was found to increase with increasing recovery time in an

essentially linear manner, a linear fit explaining 87% of the within-subject variance. Plasma noradrenaline concentration increased slightly during the recovery period after the vagal blockade. Thus, the change in sympathetic HR control during the recovery period may have altered the relationship between mean RRI and recovery time, although the relationship was essentially linear. It is unlikely that such a small change in sympathetic HR control had any effect on the relationship between absolute HFP and recovery time. The present study evaluated the relationship between vagal HR control and HRV within the range of vagal control that is typical for most real-life situations during waking hours. The half-life of atropine elimination is 3.7±2.4 hours (Ali-Melkkila et al. 1993), and thus it is likely that the effects of atropine on the heart were not completely eliminated during the 150-min recovery period. In fact, absolute HFP was found to be 25% below its pre-blockade value after the recovery period, supporting the likelihood that the effects of atropine on the heart were most probably still present at the end of the recovery period.

Previous studies evaluating the within-subject quantitative relationship between HRV and vagal HR control have used a different methodology to that used in this study. When HRV has been monitored during the incremental administration of atropine, it has been found that respiratory-related HRV increases after the low doses of atropine and decreases rapidly after the moderate and high doses of atropine (Raczkowska et al. 1983; Tulppo et al. 1996; Pichot et al. 1999). Atropine not only blocks vagal outflow to the heart but also has a central stimulating effect on vagal outflow (Katona et al. 1977), which partly explains the reported augmentation of HRV after low doses of atropine (Raczkowska et al. 1983). Studies using baroreflex-mediated vagal stimulation and/or withdrawal have shown that, within subjects, with increasing vagal effects on the heart absolute HFP increases within a wide range of vagal HR control (Bloomfield et al. 1998; Goldberger et al. 2001). At extremely high levels of vagal HR control, absolute HFP has been reported to decrease with a further increase in vagal HR control (Goldberger et al. 2001). Several explanations have been presented for the "ceiling effect" of absolute HFP at very high levels of vagal HR control. It has been suggested that acetylcholine is released to such an extent during expiration that its concentration in the sinoatrial node remains high also during inspiration or, alternatively, that there is a loss of phasic respiratory changes in vagal nerve discharges at high blood pressure levels (Goldberger et al. 2001). On the basis of the present data the ceiling effect of absolute HFP cannot be evaluated because no such high levels of vagal HR control were studied. Moreover, the present study focused on a different dimension of vagal HR control than baroreflex studies.

Large between-subjects variability in the R^2s, slopes and intercepts of the linear model between absolute HFP and recovery time was found in this study. An equivalent increase in recovery time produced a non-equivalent increase in absolute HFP between individuals, as revealed by the substantial between-subjects variability in the slopes of the linear fits. This may reflect real between-subject differences in the quantitative relationship between HFP and vagal HR control, or alternatively, it may simply reflect between-subject differences in

atropine metabolism (Ali-Melkkila et al. 1993). No group difference was found in the relationship of absolute HFP to vagal HR control between the subjects with an endurance and those with a non-endurance training background. However, as discussed in chapter 6.1 no significant systematic difference in resting vagal HR control emerged between the groups either.

Body posture was found to alter the quantitative relationship of vagal control to mean RRI and to absolute HFP. A similar increase in recovery time induced a significantly greater increase in mean RRI and absolute HFP in the supine than in the standing posture. An explanation for this difference may arise from the various combinations of vagal and sympathetic control in different body postures. Mean RRI and absolute HFP may be less sensitive to the increase in vagal control in the standing than supine posture, because of greater sympathetic activation. In addition, phasic respiratory gating of vagal motoneurons occurs only when vagal activity level is high enough (Cooke et al. 1999), and such a level of vagal activity is likely to be achieved more rapidly in the supine than standing posture after vagal blockade. Moreover, in the standing posture, vagal activity is always below the level that is necessary for maximal respiratory gating (Cooke et al. 1999). Although body posture altered the quantitative relationship between HFP and vagal HR control, it had no effect on the goodness-of-fit of the linear model. Thus, HFP increased in a highly linear manner with increasing vagal HR control in all three body postures.

Not only absolute HFP but also absolute LFP demonstrated a pronounced linear increase as a function of increasing vagal HR control. Given its sympatho-vagal origin, absolute LFP does not, however, provide a selective index of vagal HR control. It was found that at the end of the recovery period absolute LFP in the supine posture, together with mean RRI, had recovered to the pre-blockade values while absolute HFP was still below the pre-blockade value. This supports the suggestion that some other mechanisms in addition to the vagal system contribute to absolute LFP.

On the basis of the measurements made at different angles of head-up tilt, it has been proposed that the normalized spectral components and LFP/HFP detect changes in sympatho-vagal balance with a high degree of linearity (Montano et al. 1994). However, the present study showed that the normalized spectral components and LFP/HFP showed minor changes during the recovery period after the vagal blockade. Furthermore, the variance between the recovery time and normalized LFP, normalized HFP or LFP/HFP explained by linear fit was only 54-68%, depending on the body posture. Thus, neither the normalized spectral components nor LFP/HFP were able accurately to detect vagally-induced within-subject change in the sympatho-vagal balance.

6.3 Ability of STFT to detect transient changes in vagal HR control

The blockade data was used to evaluate the capability of the STFT method to detect transient change in autonomic HR control induced by the AOT. In addition to the passive head-up tilt, the AOT is a simple non-invasive test that provokes well-documented abrupt cardiovascular changes, eliciting a fast response in the different divisions of the autonomic nervous system (Ewing et al. 1978; Ewing et al. 1980; Wieling and Shepherd 1992). It results in a shift of blood away from the chest to the venous system below the diaphragm, and thus arterial blood pressure decreases rapidly. In normal subjects, compensatory mechanisms are activated immediately after standing-up in order to maintain arterial blood pressure at an appropriate level of perfusion for all the vital organs, especially the brain. The initial adjustments to standing-up are primarily mediated by the autonomic nervous system, and the humoral regulatory system only becomes involved during prolonged standing.

The present data from the drug-free condition showed that blood pressure decreased rapidly due to standing-up, reached its minimum value in 12 s, and then increased gradually. The initial decrease in blood pressure was immediately followed, within several seconds by a shortening in RRI length to its local minimum, and then an increase to the level characteristic of the standing posture. The immediate shortening of RRI length results primarily from vagal withdrawal and is related to arterial baroreflex compensation for the transient fall in arterial blood pressure (Ewing et al. 1978; Ewing et al. 1980). However, the sympathetic system has also been reported to influence this part of the response (Wieling and Shepherd 1992). On the basis of study I absolute HFP was selected from among all of the tested HRV indices as the most selective index of vagal HR control. The present data showed a fast decrease in absolute HFP at the onset of standing and then an increase in HFP to an intermediate level, lower than that characteristic of the sitting baseline posture. This suggests that the STFT method successfully detected vagal response to the AOT. The immediate decrease observed in HFP most likely reflects vagal withdrawal in response to the fall in blood pressure and the later increase in HFP reflects vagal activation associated with the recovery of arterial blood pressure. The observed drug-free changes in HFP due to an upright posture are in line with findings by studies using other time-frequency approaches, such as the selective discrete Fourier transform algorithm (Keselbrener and Akselrod 1996; Akselrod et al. 1997) and the smoothed Wigner-Ville transformation (Novak and Novak 1993; Novak et al. 1996; Jasson et al. 1997; Yoshiuchi et al. 2004).

Vagal and sympathetic blockades were used to confirm the presumed vagal origin of the fast autonomic response to standing-up. A similar response pattern of a significant rapid decrease followed by a significant increase in RRI was observed after the vagal blockade as well as after the sympathetic blockade, indicating that both autonomic divisions are able to elicit compensatory

changes in RRI in response to the fall in arterial blood pressure due to standing. However, the fast and slow RRI reactivity scores, defined as a decrease from the sitting value to the minimum value immediately after standing-up and a decrease from the sitting to standing value, respectively, were significantly decreased by the vagal blockade but unaffected by the sympathetic blockade. Thus, the fast and slow cardiac response to the AOT seems to be mediated by the vagal system alone. This is consistent with findings by Ewing et al. (1978; 1980) who reported that the sympathetic system is responsible for RRI response to standing-up only if the vagal system is blocked or damaged. Due to non-linear transformation of RRI to HR this interpretation may contrast to interpretations of studies using HR as a chronotropic metric (Cacioppo et al. 1994; Berntson et al. 1995, see also original study II). A given millisecond change in RRI represents an equivalent change in autonomic outflow independent of the baseline state and therefore RRI provides a reliable estimate of chronotropic response when the baseline state is altered e.g. due to pharmacological blockades (see e.g. Berntson et al. 1995). The vagal blockade eliminated the fast and slow HFP responses to standing-up while the sympathetic blockade induced no significant change in the magnitude of HFP reactivity scores. Although there was still some variation in the instantaneous HFP values after the vagal blockade, allowing the detection of HFP minimum, no significant difference was observed across the HFP values calculated for the different steps of the AOT. These findings suggest that the magnitude of HFP response was mediated by the vagal system. To conclude, HFP derived from the STFT method successfully detected and quantified a decrease in vagal activity.

Since the purpose of the present study was to monitor HFP only, it was possible to use a short time-window in the HRV analysis. The mean time delay between standing-up and the local HFP minimum illustrated that absolute HFP seemed to reach its local minimum approximately when information from the sitting posture was no longer included in the 25-s time-window. A major advantage of such a short time-window is the high time resolution when analyzing a short-term change in vagal HR control. If the window lengths typical for a conventional spectral analysis, such as the FFT, are used, a transient change lasting only 20-30 s would hardly appear in the power spectrum or even not be detected at all (Keselbrener and Akselrod 1996). The use of the 25-s time-window caused, however, some limitations. It was impossible to assess LFP, because the duration of the time-window should be at least five times the slowest analyzed wavelength (e.g. Challis and Kitney 1991; Keselbrener and Akselrod 1996). Due to the short time-window a frequency of 0.20 Hz was selected as the lower boundary of the high frequency band. In order to confirm that all the HRV related to RSA was included in the HFP value, only the data on subjects whose respiratory frequency ranged from 0.20 Hz to 0.40 Hz were included to the results.

6.4 Effects of exercise intensity on HRV

Technically, several problems arise in performing HRV analysis during exercise. The majority of studies using the standard spectral analysis have reported the technical problem of not being able to deal with stationary time series (Aubert et al. 2003). In addition, the decrease in TP may affect the accuracy of the estimation of the spectral components (Sandercock and Brodie 2006). The present study adopted the STFT method to overcome the stationarity limitation of the conventional spectral analysis. The STFT method was able to detect HFP and LFP also at high exercise intensities despite a decrease in TP. Although the decrease in TP during exercise probably impaired the accuracy of the analysis, the limit of resolution was not reached.

The effects of exercise intensity on HRV were studied by using two constant load exercise sessions and an incremental exercise up to the voluntary maximum. The exercise intensities were chosen so that during the LI exercise, the HR adjustments to exercise were mainly mediated by the vagal system and during the HI exercise vagal HR control was highly reduced and sympathetic HR control was substantially elevated (Orizio et al. 1988; Rowell and O'Leary 1990; Mazzeo 1991). The present results showed that when compared with the resting values, HR increased and absolute HFP and LFP decreased during both exercise intensities. In addition, HR was lower and absolute HFP and LFP were higher during the LI than HI exercise. The results of the maximal incremental exercise test showed further that the increase in HR with an increment in exercise intensity was accompanied by a decrease in TP and absolute HFP and LFP at the moderate intensity levels. These findings are in agreement with previous findings that absolute HFP as well as LFP decrease with increasing metabolic demand (Arai et al. 1989; Tulppo et al. 1996; Warren et al. 1997; Tulppo et al. 1998). As demonstrated by study I and study II, the vagal system contributes to absolute HFP and LFP, and both spectral components are linearly related to changes in vagal HR control. Therefore, decreases in absolute HFP and LFP at low and moderate exercise intensities probably reflect a reduction in vagal activity due to increased metabolic demand, as previously proposed (Arai et al. 1989).

Due to the decrease in TP with exercise many researchers have adopted the use of normalized HFP and LFP in order to gain insight into the changes in autonomic HR control associated with exercise. Normalized HFP would be expected to decrease and normalized LFP to increase with the increase in HR during exercise. In the present study, the normalized spectral components failed to detect the assumed reciprocal changes in vagal and sympathetic HR control during the constant load exercise sessions. The LI exercise induced no significant change in the normalized spectral components, while the HI exercise induced an increase in normalized HFP and a decrease in normalized LFP. Consequently, normalized HFP was lower and normalized LFP was higher during the LI than HI exercise. These findings, together with the observation that absolute LFP decreased up to the intensity of 90% of $W_{max,}$ support the

suggestion by Casadei et al. (1995) that neither absolute nor normalized LFP reflects the increase in sympathetic HR control during exercise.

The present data from the maximal exercise test showed that absolute HFP decreased up to the intensity of 60-70% of W_{max}, after which it was still detectable up to W_{max}. In addition, the intensity level above which no further decrease in absolute HFP was observed despite the increase in exercise intensity seemed to be associated with the anaerobic threshold. In study IV, absolute HFP decreased up to 60% of W_{max} in the moderate fitness group, with W_{AnT} corresponding to 65% of W_{max}, and up to 70% of W_{max} in the high fitness group, with W_{AnT} corresponding to 73% of W_{max}. Similarly in study V, absolute HFP decreased up to W_{AnT}. It is evident that the persistence of absolute HFP at intensities near W_{max} cannot reflect vagal HR control, because vagal HR control is known to be abolished during exhaustive exercise. Moreover, it has been shown that HFP at high exercise intensities is not attenuated by the vagal blockade, suggesting non-neural genesis of the remaining HFP (Warren et al. 1997). Non-neural mechanisms probably become evident when vagal HR control is low and ventilation is increased. A likely mechanism for the generation of HFP during high intensity exercise is periodic stretching of the sinoatrial node secondary to the change in atrial transmural pressure with ventilation (Bernardi et al. 1990; Casadei et al. 1995; Casadei et al. 1996; Blain et al. 2005). A recent study using the STFT method has even demonstrated that ventilatory thresholds can be detected on the basis of changes in amplitude and frequency of the high frequency component of HRV (Cottin et al. 2006).

6.5 Effects of exercise intensity on transient changes in HRV after exercise

It is generally agreed that spectral analysis of HRV estimates autonomic HR control in resting conditions as well as during post-exercise recovery due to the return of the cardiovascular control mechanisms to the pre-exercise condition (Perini and Veicsteinas 2003). Although the importance of immediate autonomic adjustments to the cessation of exercise has been recognized from the clinical point of view, the literature on post-exercise HRV dynamics is scarce. This is mainly due to the fact that the conventional spectral analysis cannot be applied to time periods during which HR changes rapidly. In this study the STFT method was applied in order to obtain HRV data and to be able to evaluate the time evolution of autonomic HR control immediately after the cessation of exercise at different intensities.

It has been recommended that submaximal exercise should be used in the evaluation of post-exercise vagal reactivity as high sympathetic control and the accumulation of metabolites during intense exercise could attenuate post-exercise vagal reactivation (Imai et al. 1994). Therefore, HRV recovery was studied after exercise at two different intensities below W_{AnT}. The main finding was a rapid increase in absolute HFP, along with the decrease in HR, during the

first recovery minute after the LI exercise and through the second recovery minute after the HI exercise. The increase in HFP supports the conclusion drawn in previous blocking studies (Imai et al. 1994; Kannankeril et al. 2004) that the immediate decrease in HR after exercise is associated with vagal reactivation. Possible mechanisms that may lead to vagal reactivation are loss of central command and the activation of arterial baroreflex (O'Leary 1996). Comparisons of the present results with those of other studies that have reported HRV immediately after exercise is difficult owing to the use of different exercise intensities and HRV analysis methods (Hatfield et al. 1998; Goldberger et al. 2006; Buchheit et al. 2007). In general, regardless of the method of analysis HRV has been reported to increase immediately after the cessation of exercise. The blockade approach has shown that after a large increase in vagal HR control during the first minute after maximal exercise, vagal HR control further increases until the fourth recovery minute, and then remains rather stable (Kannankeril et al. 2004). On the basis of the present HFP dynamics vagal HR control stabilized within one or two minutes after exercise depending on exercise intensity. Exercise intensity also influenced HFP values at the end of the 10-min recovery period. The present findings are consistent with those by Kaikkonen et al. (2007) who used the same HRV methodology as in this study. They concluded that the increased intensity of the exercise resulted in slower recovery of absolute HFP and in lower levels of HFP immediately after exercise when compared to the low intensity exercise. However, in another study HFP dynamics showed no significant recovery during the first five minutes after different exercise interventions at 80-93% of the velocity at VO_{2max} (Kaikkonen et al. 2008). This suggests that vagal HR recovery may be delayed after highly demanding exercise. Studies measuring HRV after the transient recovery phase, when HR is stabilized, have also shown that restoration of resting vagal control is affected by exercise intensity (Hayashi et al. 1992; Terziotti et al. 2001; Parekh and Lee 2005).

The present results demonstrated that absolute LFP showed highly parallel increases with absolute HFP after the cessation of exercise. Absolute LFP increased during the first recovery minute after the LI exercise, as did also absolute HFP. After the HI exercise, absolute LFP increased for one minute longer than absolute HFP. LFP may be associated with the function of sympathetic limb of the baroreflex response to changes in blood pressure. Based on this, Casadei et al. (1995) have suggested that LFP depends on the presence of both the baroreflex and the activity of the sympathetic system, and consequently, restoration of LFP after exercise reflects restoration of baroreflex sensitivity. However, although different mechanisms are responsible for the generation of HRV at different frequency bands, absolute LFP appeared to add no information to that provided by absolute HFP during the present experiment. When interpreting the changes in HRV, changes in HR should also take into account. It was observed that despite stabilization of HRV, HR continued to decrease through the second recovery minute after the LI exercise and the seventh recovery minute after the HI exercise. A likely explanation is that HI exercise induces changes in several metabolic processes and the

disturbance of the homeostasis is sustained for some time during recovery. Furthermore, plasma noradrenaline concentration remains constant or peaks during the first minutes after exercise and then decreases gradually to the pre-exercise level (Perini et al. 1989). Thus, the observed continued recovery of HR after the HI exercise may reflect a slow gradual decrease in sympathetic HR control. While HRV alone does not provide information on sympathetic HR control, consideration of the changes in HR together with the changes in HRV may provide some insight into sympathetic HR control.

6.6 Effects of age-related aerobic fitness on HRV at rest, during exercise and during recovery

Although VO_{2max} decreases with aging, the decrease can be delayed by a physically active lifestyle and endurance training. On the basis of comparisons between senior endurance athletes and sedentary age-matched subjects it has been suggested that the age-related decrease in HRV may also be overcome to some extent by sustained endurance training at older age (Aubert et al. 2003). In contrast to absolute aerobic fitness (i.e. VO_{2max}) used in previous studies, the age-related VO_{2max} norms by Shvartz and Reibold (1990) were used as the criteria when classifying the present subjects into the high and moderate age-related fitness groups. Within the groups, which included men and women with age range from 25 to 55 years, the VO_{2max} values ranged from 40 to 66 mL kg^{-1} min^{-1} in the high age-related fitness group and from 30 to 53 mL kg^{-1} min^{-1} in the moderate age-related fitness group. The group mean for VO_{2max} was greater in the high than in the moderate age-related fitness group despite the considerable overlap of the VO_{2max} distributions of these two groups. A novel finding was that HR was lower and absolute HFP was higher at supine rest in the high than in the moderate age-related fitness group. In addition, also in the sitting posture, HR was lower and absolute HFP tended to be higher in the high than in the moderate age-related fitness group. These results suggest that not only high VO_{2max}, as previously shown by several cross-sectional studies (Macor et al. 1996; Shin et al. 1997; Mourot et al. 2004a), but also high VO_{2max} in relation to age and gender is associated with enhanced vagal HR control at rest. The present findings based on a cross-sectional design do not permit conclusions to be drawn on whether high age-related aerobic fitness results in high vagal HR control at rest or whether both factors are already genetically determined.

HRV was assessed at similar relative exercise intensities because any given absolute work load would have demanded a different metabolic effort in accordance with each individual's aerobic capacity. During the LI exercise, no significant difference in HR or HRV between the age-related fitness groups was found. Likewise Macor et al. (1996) reported no difference in absolute LFP or HFP at either 20% or 40% of maximal workload between cyclists and controls. During the present HI exercise, no significant difference was found in HR

between the age-related fitness groups but TP, absolute HFP and LFP were unexpectedly lower in the high than in the moderate age-related fitness group. Neither tidal volume nor respiratory frequency differed between the age-related fitness groups, indicating that the difference in HRV during the HI exercise was not due to ventilation. Mourot et al. (2004a) compared HRV during exercise at 50% of peak power output between cyclists and controls and found no difference in absolute LFP or HFP. However, they found measures of vagal HR control derived from time-domain and Poincaré plot analysis during exercise to be lower in the cyclists than the controls and speculated that this could be explained by the greater absolute exercise intensity in the cyclists. In the present study, absolute power output in the high age-related fitness group was 1.4 times as great as that in the moderate age-related fitness group. The reason for the difference was that the HI exercise was performed at the same exercise intensity in relation to the anaerobic threshold. It has been proposed that input from active muscles, which is mainly related to absolute exercise intensity, may play a role in the changes in HRV during exercise (Perini et al. 2002). This is consistent with the present results regarding the HI exercise as well as the maximal exercise test. When HRV during the maximal exercise test was expressed as a function of relative exercise intensity, not only was TP significantly lower but absolute HFP and LFP also tended to be lower in the high than in the moderate age-related fitness group. However, there was a more pronounced increase in respiratory frequency with relative exercise intensity in the high age-related fitness group, which may have affected the HRV results with respect to the maximal exercise test.

The effects of aerobic fitness on HRV dynamics immediately after exercise are not known. The present data demonstrated that HRV during the transient recovery phase differed between the age-related fitness groups after the HI exercise but not after the LI exercise. During the 5-min recovery period after the HI exercise, the high age-related fitness group showed a greater decrease in HR and greater overall levels of TP and absolute HFP when compared with the moderate age-related fitness group. However, when the pre-exercise sitting baseline values were subtracted from the recovery values, HFP and TP after the HI exercise were not significantly different between the age-related fitness groups. This indicates that after the HI exercise, vagal HR control was equally reduced from its pre-exercise level in the both age-related fitness groups. The present data failed to find any significant group difference in HRV after the LI exercise. One would have expected the lower HR in the high than in the moderate age-related fitness group after the LI exercise to be associated with higher absolute HFP. However, only a tendency towards higher absolute HFP was found. A large between-subjects variation in absolute HFP, in general, and a wide variation in absolute HFP within both the high and moderate age-related fitness groups, in particular, may explain the lack of a significant difference between the fitness groups in absolute HFP during recovery. It is also possible, although unlikely, that the lower post-exercise HR observed in the high age-related fitness group is explained by less sympathetic activation during exercise and consequently a lower post-exercise level of sympathetic

activity in that group than in the moderate age-related fitness group. However, sympathetic activation plays a minor role in HR control during exercise at such a low intensity level as in the present study, where HR was 104 bpm in the high age-related fitness group and 110 bpm in the moderate age-related fitness group (Rowell and O'Leary 1990).

6.7 Effects of low-dose endurance training on HRV at rest, during exercise and during recovery

The 7-week preparatory period was designed to familiarize the subjects with low intensity training and, as expected, it induced no significant change in aerobic power, HR or HRV. The measurements performed after the preparatory period constituted a stable reference level against which the effects of the 14-week endurance training period could be evaluated.

The endurance training programme, consisting of low to high intensity training only twice a week, was effective in inducing a mean increase of 12% in W_{max} and 8% in VO_{2max}, and an increase in the anaerobic threshold. No significant changes in HR and HRV at rest were observed due to the training programme. This does not support the general assumption that endurance training improves resting vagal control. Many studies have reported a decrease in HR accompanied by an increase in absolute HFP after endurance training (Carter et al. 2003a; Leicht et al. 2003b; Tulppo et al. 2003; Hautala et al. 2004; Pichot et al. 2005). However, not all studies have reported an increase in absolute HFP at rest (Stein et al. 1999; Loimaala et al. 2000; Perini et al. 2002; Verheyden et al. 2006; Karavirta et al. 2009). After the present training programme, the resting plasma noradrenaline concentration was slightly but significantly lower than its pre-training value. This suggests a training-induced decrease in resting sympathetic HR control. However, no decrease was observed in resting HR in response to the training programme.

The endurance training period induced a decrease in HR accompanied with an increase in all the HRV indices, including absolute HFP, at exercise intensity levels ranging from 75 W to 175 W. This suggests that vagal HR control during submaximal exercise was higher after the training period as suggested previously by Ekblom and colleagues (1973) on the basis of their blocking experiment. Respiratory frequency and tidal volume during submaximal exercise were not altered due to the low-dose of endurance training used. This supports that the conclusion that the observed increase in HRV at the same absolute submaximal exercise intensity levels resulted from the changes in autonomic HR control and was not mediated by training-induced alterations in ventilatory pattern. The observed training-induced increase in absolute HFP during submaximal exercise in the present study is in line with findings with a similar endurance training programme in older men (Karavirta et al. 2009) and, with a shorter but more intense training programme in younger subjects (Leicht et al. 2003b). Some studies have reported no

significant change in HRV measured during submaximal exercise after endurance training (Carter et al. 2003a; Leicht et al. 2003a). The present results showed that when the data from the present maximal exercise test was expressed as a function of relative exercise intensity, the training period induced no change in HR or HRV at exercise intensities ranging from 40% to 100% of $W_{max.}$ Also this finding is in line with the results reported by Leicht et al. (2003b). They found no difference between pre- and post-training values for HR or absolute spectral powers measured at three work loads determined in relation to HR_{max}. These findings support the suggestion that the relationship between autonomic HR control and relative exercise intensity is not altered by endurance training as was observed earlier by Ekblom and colleagues (1973).

The observed training-induced increase in vagal HR control may not be the only determinant of the decrease in HR during submaximal exercise after training. A decrease in sympathetic HR control was reported to partially explain the decrease in HR during submaximal exercise after endurance training (Ekblom et al. 1973). In the present study, plasma catecholamine concentration was not, however, measured at submaximal exercise intensities. Thus, the question whether the low-dose endurance training programme decreased sympathetic HR control during submaximal exercise remains unanswered. The endurance training period had no significant effect on either plasma noradrenaline or adrenaline concentration measured immediately after the maximal exercise test, indicating that the training period induced no change in the maximal sympathetic response to the incremental maximal exercise test. Findings from cross-sectional studies have suggested that training-induced lower HR at rest and during endurance exercise may also be explained in part by reduced intrinsic HR (Lewis et al. 1980; Katona et al. 1982; Smith et al. 1989). It is unlikely that the present training-induced reduction in HR during submaximal exercise would have been due to a decrease in intrinsic HR as resting HR as well as HR_{max} remained unchanged.

Since the venous blood samples were taken immediately after the cessation of exercise, it was impossible to obtain reliable estimates of the transient changes in HRV during the first minutes of recovery. Therefore, HR and HRV were not measured until after the fourth recovery minute. No significant changes were observed in HR and HRV during the 15-min recovery period after the maximal exercise test.

Taken together, the present findings suggest that although the training stimulus was sufficient to improve vagal HR control during exercise, it was insufficient to alter vagal HR control at rest. Training-induced changes in HRV depend on the duration, intensity and frequency of training, which makes comparisons between studies difficult (Achten and Jeukendrup 2003). The American College of Sports Medicine recommends that in order to promote and maintain health, adults should do at least 30 min of moderate intensity endurance exercise on five days each week (i.e. 150 min per week), or 20 min of vigorous intensity endurance exercise on three days each week (i.e. 60 min per week) (Haskell et al. 2007). The recommendation can also be met by an equivalent combination of moderate and vigorous intensity exercise. The

present study together with a recent study with a similar training programme (Karavirta et al. 2009) showed that as few as two well-designed and well-executed exercise sessions per week were able to increase maximal aerobic power as well as vagal HR control during submaximal exercise. After the first half of the the present 14-week endurance training period, the intensity and duration of the exercise sessions were increased. The total duration of exercise ranged from 100 to 160 min during the endurance training period, meeting the minimum of the recommended weekly duration. In addition to differences in training protocols individual differences in the responsiveness of resting HRV to endurance training may account for the differences between studies (al-Ani et al. 1996; Leicht et al. 2003a). Al-Ani et al. (1996) found that vagal tone (determined by using the change in RRI in response to isometric muscle contraction) was increased due to endurance training in all their subjects, and that this was accompanied by an increase in absolute HFP at rest in most subjects but a decrease in absolute HFP in subjects with a resting HR lower than 50 bpm. In the subjects with low resting HR, the endurance training programme probably increased vagal HR control to a level high enough to saturate the vagal effects on the sinoatrial node. In the present study, the resting HR of the previously untrained male subjects ranged from 55 to 71 bpm prior to training. The low-dose training programme induced no significant change in resting HR. Thus it is likely that the training stimulus provided by the low-dose training programme may simply have been insufficient to produce improved vagal HR control at rest. In contrast to most cross-sectional studies, which show a positive relationship between aerobic fitness and resting HRV, longitudinal training studies have not yielded a general agreement that with endurance training, an increase in HRV can be achieved (Achten and Jeukendrup 2003). There is a fundamental difference between cross-sectional and longitudinal designs. While differences in HRV between endurance-trained individuals and age-matched controls may be due to a combination of genotype and vigorous long-term endurance training, a longitudinal design enables determination of the effects of an actual short-term training programme on HRV.

6.8 Limitations

There are some limitations in the present study. Although autonomic pharmacological blockade is considered as the "gold standard" for assessing autonomic contributions to the heart, systematic biases may arise, for example, from interaction between the sympathetic and vagal systems, nonselective actions of the blocking drugs, and incomplete blockades (Berntson et al. 1994). During graded infusion of atropine, respiratory-related HRV decreases rapidly after moderate doses of atropine have been administered (Raczkowska et al. 1983; Tulppo et al. 1996; Pichot et al. 1999). Therefore, in order to define the quantitative relationship between HRV and vagal HR control, the measurements were performed during recovery from the vagal blockade, when

the vagal effects on the heart increased, and not during the infusion of the incremental doses of atropine. Atropine has an elimination half-life of 3.7±2.3 h (Ali-Melkkila et al. 1993), allowing the assumption that the cardiac effects of atropine decrease in an almost linear manner over a recovery period of 2.5 h. The effects of atropine on the heart were, on the other hand, probably still present at the end of the recovery period and thus vagal control was not completely restored at that point. Due to the substantial between-subjects variance in the pharmacokinetics of atropine, between-subjects differences in atropine metabolism could partly explain the large variance in the within-subject relationship between recovery time and HRV.

Without measuring sympathetic HR control a profound evaluation of autonomic HR control is not possible. Plasma noradrenaline and adrenaline concentrations were measured in the resting conditions (i.e. at rest and during recovery) in the blockade experiment and in the endurance training experiment. Since the plasma catecholamine concentration provides only a momentary surrogate of sympathetic control, evaluation of the dynamics of sympathetic control would have required a great number of blood samples. Such invasive measures could have interfered with the reliable assessment of HRV dynamics. Muscle sympathetic nerve activity, which provides a continuous estimate of sympathetic activity, cannot be recorded from the peroneal nerve during leg exercise. It is clear that the observed changes in HR, especially in response to exercise, resulted partly from changes in sympathetic control. The possible indirect effects of sympathetic control on HRV cannot be excluded.

Third, the present data were collected in the setting of spontaneous ventilation. On the one hand changes in ventilation confound HFP as an index of vagal control at rest and, especially, during endurance exercise (Bernardi et al. 1990; Casadei et al. 1995) while on the other hand, even at rest, the voluntary control of ventilation may alter autonomic HR control (Stark et al. 2000). In addition, it is difficult to select the criteria for controlling respiratory frequency and tidal volume during endurance exercise and recovery. Furthermore, not all authors support the view that ventilation must be controlled during endurance exercise. Barterls et al. (Bartels et al. 2004) concluded that HFP during endurance exercise represents true cardiovascular autonomic modulation rather than the effect on ventilation on HFP. In the present experiments, respiratory frequency and tidal volume were measured. No differences in these parameters were found that would have systematically confounded the present HRV results obtained in the controlled laboratory environment. Nevertheless, the accuracy of absolute HFP in measuring vagal HR control during recovery from the vagal blockade was high even in the setting of spontaneous ventilation. However, it remains speculative to what extent changes in ventilation increase the signal-to-noise ratio when estimating vagal HR control during exercise and recovery with HFP.

7 MAIN FINDINGS AND CONCLUSIONS

To summarize, the vagal blockade decreased HRV at all frequencies in the supine, sitting and standing postures, the decreases being most pronounced in absolute HFP. The sympathetic blockade had no systematic effect on HRV in any posture. Within subjects, TP, absolute HFP and LFP after natural log transformation increased in an essentially linear manner as the effects of the vagal blockade decreased in each body posture and in persons with different endurance-training backgrounds. Normalized HFP and LFP as well as LFP/HFP were significantly affected by the vagal blockade but showed no systematic pattern of changes during recovery from the vagal blockade or due to the sympathetic blockade. Absolute HFP derived from the STFT method successfully detected the rapid decrease and the following increase in vagal activity induced by the active orthostatic task. Absolute HFP and LFP decreased with the increase in HR during the incremental maximal test up to the exercise intensity of 60-70% and 90% of maximal power output, respectively, and were detectable during maximal exercise. HRV increased rapidly during the first minutes after the cessation of exercise, and the restoration of absolute HFP and LFP was faster after the low than high intensity exercise. The effects of age-related aerobic fitness on HRV were most pronounced at rest and during post-exercise recovery, as illustrated by the lower HR and higher TP and absolute HFP at rest and during recovery from the high intensity exercise. During the high intensity exercise there was no significant difference in HR between the age-related fitness groups but TP and absolute HFP and LFP were lower in the high than in the moderate age-related fitness group. The low-dose endurance training programme induced lower HR and higher TP and absolute HFP and LFP at the same absolute submaximal exercise intensities while it did not alter either HR or HRV at rest.

The conclusions of the present thesis are as follows:

1) Absolute spectral power at all frequencies, particularly HFP, was predominantly mediated by the vagal system in the supine, sitting and standing postures.
2) The within-subject relationship of vagal HR control to absolute HFP and LFP was essentially linear in each body posture and in persons with different endurance-training backgrounds. Normalized HFP and LFP did not accurately measure the changes in sympatho-vagal balance induced by the decreasing effects of the vagal blockade.
3) The STFT method was able to detect the magnitude and time course of transient changes in vagal HR control during the active orthostatic task without the need to interfere with normal control, e.g. by using blocking drugs.
4) The time course of the increase in absolute HFP after submaximal exercise was dependent on exercise intensity and suggested that the recovery of vagal HR control was faster after the low than high intensity exercise.
5) High age-related aerobic fitness was related to improved vagal HR control at rest and during recovery from submaximal endurance exercise.
6) The low-dose endurance training improved vagal HR control during submaximal endurance exercise but did not alter resting vagal HR control. Thus, vagal HR control may be more responsive to training stimulus during endurance exercise than at rest.

YHTEENVETO

Sykevaihtelun muutokset ortostaattisessa testissä, kestävyysliikunnassa ja kestävyysharjoittelussa käyttäen hyväksi autonomisen säätelyn salpauskokeita ja aika-taajuusanalyysiä

Autonominen hermosto, joka koostuu sympaattisesta ja parasympaattisesta hermostosta, säätelee sydämen ja verenkiertoelimistön toimintaa. Päivittäiset ärsykkeet, kuten istumasta seisomaannousu ja liikunta aiheuttavat autonomisen hermoston säätelemiä muutoksia syketaajuudessa, ääreisverenkierron vastuksessa ja verenpaineessa. Eräs keskeinen tekijä verenkiertoelimistön sairauksissa on heikentynyt autonomisen hermoston toiminta, joka ilmenee joko lisääntyneenä sympaattisena aktiivisuutena, alentuneena parasympaattisena aktiivisuutena tai molempina. Aerobisen kunnon ja kestävyysharjoittelun on todettu olevan positiivisesti yhteydessä autonomisen hermoston toimintaan.

Sydämen autonomista säätelyä, pääasiassa parasympaattista säätelyä, voidaan arvioida noninvasiivisesti mittaamalla sydämen sykevaihtelua. Sydämen parasympaattisen säätelyn ja sykevaihtelun välinen kvantitatiivinen yhteys on kuitenkin epäselvä. Lisäksi yleisesti käytössä olevat konventionaaliset sykevaihtelun analyysimenetelmät on tarkoitettu sykevaihtelun määrittämiseen tilanteissa, joissa autonominen säätely pysyy muuttumattomana. Uusilla sykevaihtelun aikataajuusmenetelmillä, kuten short-time Fourier transform (STFT) menetelmällä, on mahdollista määrittää sykevaihtelu myös sydämen autonomisen säätelyn muutosten aikana.

Tämän tutkimuksen tarkoituksena oli ensinnäkin arvioida sykevaihtelumuuttujien kykyä mitata sydämen autonomisen säätelyn nopeita muutoksia yksilön sisällä ja toiseksi selvittää akuutin kestävyysliikuntakuormituksen ja kestävyysharjoittelun vaikutuksia sykevaihteluun.

Sympaattisen ja parasympaattisen säätelyn vaikutusta sykevaihteluun arvioitiin salpauskokeilla, joissa estettiin lääkeaineella joko sympaattisen tai parasympaattisen hermoaktiivisuuden vaikutus sydämeen. Sydämen parasympaattisen säätelyn ja sykevaihtelun välinen kvantitatiivinen yhteys määritettiin parasympaattisen salpausvaikutuksen poistumisen aikana mitattujen sykevaihtelumuutosten perusteella. Salpauskokeet osoittivat, että sykevaihtelu kokonaisuudessaan ja erityisesti korkeataajuuksinen sykevaihtelu [ilmaistuna yksikköä ln(ms^2) käyttäen] kuvasi sydämen parasympaattista säätelyä. Sydämen parasympaattisen säätelyn ja korkeataajuuksisen sykevaihtelun välinen yhteys oli lineaarinen yksilön sisällä. Mikään tutkituista sykevaihtelumuuttujista ei kuvannut spesifisti sydämen sympaattista säätelyä. Lisäksi salpauskokeet osoittivat, että STFT-menetelmällä lasketun korkeataajuuksisen sykevaihtelun avulla voidaan arvioida sydämen parasympaattisen säätelyn nopeita muutoksia aktiivisen ortostaattisen kokeen aikana.

Sykevaihtelu määritettiin maksimaalisen portaittaisen kuormituskokeen sekä kevyen ja raskaan vakiotehoisen pyöräilykuormituksen aikana sekä kuor-

mituksesta palautumisen aikana. Akuutit liikuntakuormitukset osoittivat, että kuormitustehon noustessa korkeataajuuksinen sykevaihtelu pieneni, kunnes kuormitusteho oli 60–70 % maksimaalisesta tehosta, heijastaen parasympaattisen aktiivisuuden pienenemistä. Kevyen vakiotehoisen kuormituksen jälkeen korkeataajuuksinen sykevaihtelu suureni nopeammin kuin raskaan vakiotehoisen kuormituksen jälkeen viitaten siihen, että sydämen parasympaattinen säätely palautui nopeammin kevyen kuin raskaan kuormituksen jälkeen.

Aerobisen kunnon vaikutusta sykevaihteluun levossa, akuutin liikuntakuormituksen aikana ja palauduttaessa liikuntakuormituksesta tutkittiin käyttäen poikittais- ja pitkittäisasetelmaa. Poikittaisasetelmassa koehenkilöt jaettiin ikävakioidun aerobisen suorituskyvyn mukaan kohtuukuntoiseen ryhmään ja hyväkuntoiseen ryhmään. Tulokset osoittivat, että sydämen parasympaattisen säätelyn oli hyväkuntoisilla suurempaa kuin kohtuukuntoisilla sekä levossa että palauduttaessa submaksimaalisesta liikuntakuormituksesta. Pitkittäisasetelmassa aikaisemmin harjoittelemattomat koehenkilöt noudattivat 14-viikon ajan harjoitusohjelmaa, joka sisälsi kaksi viikoittaista kestävyysharjoitusta. Tulokset osoittivat, että aerobisen suorituskyvyn paranemisen lisäksi määrältään vähäinen kestävyysharjoittelu lisäsi sydämen parasympaattista säätelyä submaksimaalisilla kuormitustehoilla, mutta ei vaikuttanut sydämen parasympaattiseen säätelyyn levossa.

Tutkimuksen perusteella voidaan todeta, että tutkittua sykevaihtelun aikataajuus laskentamenetelmää voidaan käyttää sydämen parasympaattisen säätelyn mittaamiseen tilanteissa, joissa autonomisessa säätelyssä tapahtuu nopeita muutoksia. Sykevaihtelun mittaaminen asennonmuutoksen aikana, kestävyysliikuntakuormituksen aikana tai palauduttaessa kuormituksesta tuottaa non-invasiivisesti tietoa sydämen syketaajuudessa tapahtuvien nopeiden muutosten taustalla olevasta autonomisesta säätelystä. Tutkimustuloksia voidaan hyödyntää tutkittaessa autonomisen säätelyn yhteyksiä aerobiseen suorituskykyyn, urheilijoiden ylirasitustilaan, stressiin, ikääntymiseen sekä sydän- ja verisuonitauteihin.

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