EFFECT OF PHYSICAL EXERCISE ON AUTONOMIC REGULATION OF HEART RATE

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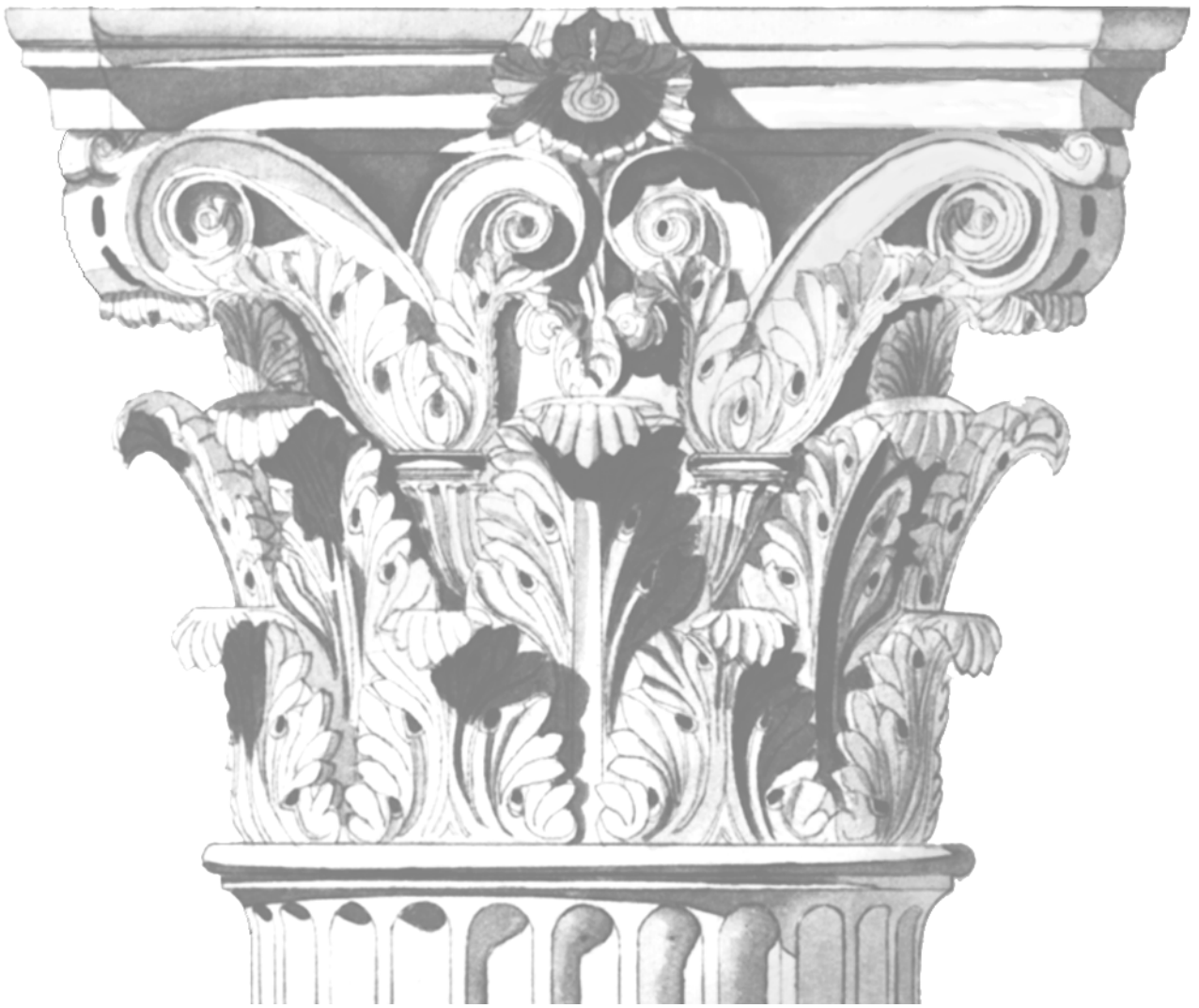

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Abstract

Regular aerobic training has been suggested to protect the heart by increasing cardiac vagal activity. The aims of this study were to evaluate the autonomic regulation of heart rate (HR) during and after exercise, during aerobic training interventions and to study the association between autonomic regulation and the training response in healthy male subjects. HR variability assessment was used to study the effects of exercise on autonomic regulation of HR.

The whole study population consisted of 70 volunteer male subjects (age 36 ± 10 years). The recovery of the autonomic nervous system after prolonged exhaustive exercise was studied in a group of 10 subjects. The training interventions included 51 subjects. The effects of training volume on autonomic regulation were assessed (n = 46) during a controlled eight-week training intervention. The association between training and autonomic regulation was studied (n = 24) during a ten-month period of home-based training based on the American College of Sports Medicine recommendations. Finally, the association between autonomic regulation and the individual training response was analysed (n = 51) after eight weeks of controlled training.

The recovery rate of vagally mediated high-frequency (HF) power of HR variability after prolonged exhaustive exercise was associated with physical fitness ($r = -0.71$, $P < 0.016$). Moderate (3 hours/week) and high-volume (6 hours/week) aerobic training results in a similar increase in HR variability indices. HF power increased from 6.19 ± 1.02 to 6.76 ± 0.96 ln ms^2 ($P < 0.001$) and from 6.61 ± 1.01 to 7.12 ± 0.92 ln ms^2 ($P < 0.001$) after moderate and high-volume training, respectively. During the home-based training program, the changes in HF power were associated with the changes in the fitness ($r = 0.44$, $P < 0.05$), body mass index ($r = -0.44$, $P < 0.05$) and the amount of training ($r = 0.41$, $p < 0.05$). Finally, a significant correlation was observed between the training response and the baseline HF power ($r = 0.52$, $P = 0.001$). HF power accounted for 27 % of the change as an independent predictor of the aerobic training response.

In conclusion, a highly controlled aerobic training intervention of eight weeks, including six 30-min sessions a week at an intensity of 70–80 % of maximum HR, is a sufficient intervention to increase cardiac vagal outflow and the offered home-based training according the current guidelines maintains the high cardiac vagal outflow. Secondly, high vagal activity at baseline is associated with the improvement in aerobic fitness caused by aerobic training, suggesting that the cardiovascular autonomic function is an important determinant of the response to aerobic training.

Keywords: aerobic training, cardiovascular autonomic regulation, heart rate variability, individual training response

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Oulu, April, 2004 Arto Hautala

Abbreviations

α_1	short-term fractal scaling exponent
β	slope of the power-law relationship
CCV %	coefficient of component variance
DFA	detrended fluctuation analysis
ECG	electrocardiography
HF	high-frequency
HR	heart rate
LF	low-frequency
SDNN	standard deviation of all R-R intervals
SD1	standard deviation of short-term R-R interval variability
SD2	standard deviation of long-term R-R interval variability
ULF	ultra-low-frequency
VLF	very-low-frequency
$\dot{V}_E$	ventilation
$\dot{V}_{O_2}$	oxygen consumption
$\dot{V}_{O_{2max}}$	maximal oxygen consumption
$\dot{V}_{O_{2peak}}$	peak oxygen consumption

List of original articles

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

I. Hautala A. J., T. H. Mäkikallio, T. Seppänen, H. V. Huikuri, M. P. Tulppo. Short-term correlation properties of R-R interval dynamics at different exercise intensity levels. Clin Physiol 2003; 23: 215-223.

II. Hautala A., M. P. Tulppo, T. H. Mäkikallio, R. Laukkanen, S. Nissilä, H. V. Huikuri. Changes in cardiac autonomic regulation after prolonged maximal exercise. Clin Physiol 2001; 21: 238-245.

III. Tulppo M. P., A. J. Hautala, T. H. Mäkikallio, R. T. Laukkanen, S. Nissilä, R. L. Hughson, H. V. Huikuri. Effects of aerobic training on heart rate dynamics in sedentary subjects. J Appl Physiol 2003; 95: 364-372.

IV. Hautala A. J., T. H. Mäkikallio, A. Kiviniemi, R. T. Laukkanen, S. Nissilä, H. V. Huikuri, M. P. Tulppo. Heart rate dynamics after controlled training followed by a home-based exercise program. Eur J Appl Physiol, in press.

V. Hautala A. J., T. H. Mäkikallio, A. Kiviniemi, R. T. Laukkanen, S. Nissilä, H. V. Huikuri, M. P. Tulppo. Cardiovascular autonomic function correlates with the response to aerobic training in healthy sedentary subjects. Am J Physiol Heart Circ Physiol 2003; 285: H1747-H1752.

Contents

1 Introduction

Marked changes in heart rate (HR) occur during physical exercise and during physical training interventions. The changes in HR are primarily due to alterations in autonomic tone: parasympathetic tone slows down the HR, and sympathetic stimulation increases it. Variability in HR has been extensively studied during the past decade for its value as a predictor of cardiac death (Kleiger *et al.* 1987, Bigger *et al*. 1992, Tsuji *et al.* 1996a, Perkiömäki *et al.* 1997, Huikuri *et al.* 1998, Huikuri *et al.* 1999, Huikuri *et al.* 2000, Mäkikallio *et al.* 2001, Tapanainen *et al.* 2002, Jokinen *et al.* 2003). Measurement of HR variability has become a widely used tool for assessing the cardiovascular autonomic function in various physiological settings (Lipsitz *et al.* 1990, Huikuri *et al.* 1992, Huikuri *et al.* 1993, Davy *et al.* 1996, Tulppo *et al.* 1996, Tulppo *et al.* 1998b, Pikkujämsä *et al.* 2001, Tulppo *et al.* 2001a, Tulppo *et al.* 2001b). However, the number of well controlled studies on changes in HR variability during and after physical exercise and during physical training interventions influencing cardiovascular autonomic regulation is limited.

Regular physical activity and good physical fitness are widely accepted as factors that improve a number of health outcomes and reduce all-cause mortality (Ekelund *et al.* 1988, Blair *et al.* 1989, Kesäniemi *et al.* 2001, Laukkanen *et al.* 2001, Myers *et al.* 2002, Thompson *et al.* 2003). Previous studies have shown that physical fitness is related to cardiac autonomic regulation, providing evidence that aerobic training improves cardiovascular autonomic function (Goldsmith *et al.* 1992, De Meersman 1993, Davy *et al.* 1996, Tulppo *et al.* 1998b). Aerobic exercise has been suggested to protect the heart against harmful cardiac events by increasing parasympathetic tone and also by decreasing cardiac sympathetic activity (Billman 2002). Therefore, physical training has been proposed to reduce harmful cardiac events by improving aerobic capacity.

Substantial heterogeneity in the responsiveness to physical training, assessed by the change in maximal oxygen uptake ($\dot{V}o_{2max}$), has been observed even in highly standardized training programs (Bouchard & Rankinen 2001). The mean improvements of $\dot{V}o_{2max}$ have been about 25% with a range from 0% to 40% (Bouchard 1995, Bouchard & Rankinen 2001). The physiological background for the wide range of responses to physical training remains unclear.

The purpose of this thesis was to study the autonomic regulation of HR during and after dynamic exercise and during aerobic training interventions in healthy subjects by using the HR variability method. We also tested the hypothesis that individual cardiac autonomic function may predict the response to aerobic training.

2 Review of the literature

2.1 Cardiovascular and respiratory response to physical exercise

The onset of dynamic exercise causes a very rapid increase in both respiration and HR. This challenge is met by complex interaction between the local regulation of blood flow to the active skeletal muscles and the neural regulation of the hemodynamic response. The final common denominator of cardiovascular and respiratory function in exercise is delivery of required oxygen and other nutrients to the exercising muscles. During dynamic exercise, a marked increase in oxygen consumption ($\dot{V}o_2$) takes place following an increase in pulmonary ventilation ($\dot{V}_E$), HR, stroke volume and the difference between the oxygen contents of arterial and venous blood (arteriovenous oxygen difference). Moreover, there is an increase in mean arterial pressure and a marked increase in peripheral vascular conductance.

2.1.1 Maximal aerobic power

The region in which $\dot{V}o_2$ becomes constant, with additional increases in exercise intensity, represents the maximal aerobic power ($\dot{V}o_{2max}$). $\dot{V}o_{2max}$ defines the maximal capacity of oxygen delivery. It serves as an objective indicator of overall physiologic functional capacity and aerobic fitness. The dynamic circulatory response is directly related to the intensity of the work load (Taylor *et al.* 1955) and presents a linear increase in oxygen consumption in relation to a progressive increase in the intensity of exercise up to $\dot{V}o_{2max}$ (Mitchell & Blomqvist 1971). $\dot{V}o_{2max}$ can be expressed by the Fick equation (Mitchell & Blomqvist 1971):

$\dot{V}o_{2max}$ = Cardiac output $_{max}$ · Arteriovenous oxygen difference $_{max}$

The limits for aerobic fitness ($\dot{V}o_{2max}$) are therefore defined by the central component (cardiac output), which refers to the amount of blood pumped by the heart during a 1-minute period, and peripheral factors (arteriovenous oxygen difference), which signify the capacity of the lung to oxygenate the blood delivered and the capacity of the muscle to extract this oxygen from blood (Froelicher *et al.* 1993) The average $\dot{V}o_{2max}$ of a 70 kg man is approximately 3 l/min or 43 ml · kg^{-1} · min^{-1}, but it is greatly influenced by genetic factors, fitness and type of training, age, gender and body composition (Shvartz & Reibold 1990).

2.1.2 Cardiac output

The cardiac output reflects the functional capacity of the cardiovascular system. Cardiac output is computed as HR multiplied by stroke volume. For an average person at rest, an average HR of 70 beats/min sustains a 5 l · min^{-1} cardiac output. During exercise, systemic blood flow and HR increase in direct proportion to exercise intensity. Cardiac output increases from a resting value of 5 l · min^{-1} to 30 l · min^{-1} during maximal exercise. The cardiac output increases linearly from the resting HR level (~70 beats/min) to the individual maximal HR (~170-200 beats/min). Stroke volume increases when the half capasity of $\dot{V}o_{2max}$ is reached. The increased stroke volume response during exercise is caused by enhanced cardiac filling in diastole followed by more forceful systolic contraction, a neurohormonal activation improving ejection accompanied by acceleration of systole leading to an expansion of blood volume and a reduction of circulatory resistance. (Gledhill *et al.* 1994, Stevenson *et al.* 1994, Krip *et al.* 1997, Hagberg *et al.* 1998, Sun *et al.* 2000). Acceleration of HR during exercise is due to both increased sympathetic nervous system activity and withdrawal of parasympathetic nervous tone (Rosenblueth & Simeone 1934, Robinson *et al.* 1966). The HR response to exercise is caused by several factors, including age, type of exercise, body position, aerobic fitness, blood volume and environment. A decline of HR response occurs with aging, which appears to be influenced by intrinsic cardiac and humoral alterations (Jose & Collison 1970, Craft & Schwartz 1995)

2.1.3 Peripheral factors

Arterial blood distributes about 25% of its total oxygen to tissues at rest. Arteriovenous oxygen difference at rest indicates that there is a reserve of oxygen available for sudden demands. During exercise, this difference increases, as the tissues extract more oxygen, exceeding 85 % extraction of oxygen from blood at $\dot{V}o_{2max}$. The arteriovenous oxygen difference may not explain the individual differences in $\dot{V}o_{2max}$ in a homogeneous group of subjects, because the exercise is generally considered to influence the difference in a fixed amount (Froelicher *et al.* 1993).

Any increase in energy expenditure requires rapid adjustments in blood flow that affect the entire cardiovascular system. Exercise causes dilatation of the vasculature of active muscles in contrast to constriction of blood vessels in inactive tissues (Buckwalter *et al.* 1997). Blood flow in muscle from a resting level of <10 ml · min^{-1} · 100 g^{-1} may increase up to 250-400 ml · min^{-1} · 100 g^{-1} during exercise, accounting for the increase in peripheral vascular conductance (Andersen & Saltin 1985, Rowell 1988). Additionally, temperature, carbon dioxide, acidity, adenosine, magnesium and potassium ions and nitric oxide production by the endothelial cells lining the blood vessels trigger the discharge of relaxing factors to maintain enhanced regional blood flow (Shen *et al.* 1995, Dietz *et al.* 1997, Joyner & Dietz 1997, Balon 1999, Delp 1999, Goto *et al.* 2003, Joyner & Tschakovsky 2003).

Arterial blood pressure reflects the combined effects of arterial blood flow per minute (i.e. cardiac output) and the resistance to that flow offered by the peripheral vasculature. The resting systolic and diastolic blood pressures are approximately 120 mmHg and 75 mmHg in healthy subjects, respectively, indicating a calculated mean arterial pressure of 90 mmHg. During dynamic exercise, systolic blood pressure increases markedly to around 240 mmHg at $\dot{V}o_{2max}$, while diastolic blood pressure decreases to approximately 60 mmHg at $\dot{V}o_{2max}$, resulting in a moderate increase in mean arterial pressure.

2.1.4 Pulmonary ventilation

Respiratory rate and inspired or expired volume, tidal volume (TV), determine pulmonary minute ventilation ($\dot{V}E$). $\dot{V}E$ averages 6 l · min^{-1} at rest, but may increase to 200 l · min^{-1} during maximal exercise. The maximal value depends on the aerobic fitness and body characteristics. During light-to-moderate exercise, $\dot{V}E$ increases linearly according to oxygen consumption and carbon dioxide production, mainly through an increase in TV. At higher exercise intensities, acceleration of respiration takes a more important role. The term 'ventilatory threshold' refers to the point at which $\dot{V}E$ increases disproportionately relative to $\dot{V}o_2$ or the work load during graded exercise (Wasserman *et al.* 1973).

2.2 Cardiovascular and respiratory adaptation to aerobic training

Regular aerobic training induces significant adaptations both at rest and during exercise in a variety of dimensional and functional capacities related to the cardiovascular and respiratory regulation system, enhancing the delivery of oxygen into active muscles. These changes include decreases in resting and submaximal exercise HR, enhanced stroke volume and cardiac output, an increasing arteriovenous oxygen difference and reduction in $\dot{V}E$ during submaximal exercise. With an adequate training stimulus, most of these responses are independent of race, gender and age (O'Toole 1989, Skinner *et al.* 2001). The mean improvement in maximal aerobic power has generally been about 15 to 25% of the baseline values over the first 2 to 3 months of regular aerobic training (ACSM

1998). However, substantial heterogeneity in the responsiveness to aerobic training, assessed by the change in $\dot{V}o_{2max}$, has been observed even in highly standardized training programs (Bouchard & Rankinen 2001). The mean improvements of $\dot{V}o_{2max}$ have been about 25% with a range 0% to a 40% increase in $\dot{V}o_{2max}$ (Bouchard *et al.* 1995, Bouchard & Rankinen 2001).

2.2.1 Cardiac structure and function

The increase in maximal cardiac output following aerobic training results from improved stroke volume with unchanged or slightly reduced maximal HR. While the size of the heart is correlated to the total body and to genetic factors the higher stroke volume caused by aerobic training is attributed to enlargement of the cardiac chambers and to expansion of the total blood volume during rest and exercise (Pelliccia *et al.* 1991). However, cardiac hypertrophy is dependent on the type of exercise. Aerobically trained subjects have an increased heart volume and cavity diameter with a proportional increase in wall muscle thickness (Huston *et al.* 1985). Moderate cardiac hypertrophy reflects a fundamental and normal training-induced aerobic adaptation of muscle to an increased workload, regardless of age (Moore & Palmer 1999). This enlargement, characterized by dilatation of the left ventricular cavity and a modest ventricular hypertrophy restores upon detraining (Hickson *et al.* 1985).

Aerobic training also improves cardiac performance during exercise. The most notable improvement is the more rapid early and peak ventricular filling during exercise. An enlarged blood volume, together with greater ventricular compliance and distensibility, and more complete ventricular relaxation are important factors increasing stroke volume during exercise (Gledhill *et al.* 1994). The enhanced diastolic filling and the reduced afterload ensure that stroke volume is maintained or even slightly increased from submaximal to maximal exercise (Gledhill *et al.* 1994). As a result of the enlarged end-diastolic volume, left ventricular systolic performance is improved, mainly by the Frank-Starling mechanism (Levine 1993).

2.2.2 Blood pressure

Regular aerobic training at moderate intensity levels elicits significant reductions in systolic and diastolic blood pressure (Fagard 2001). The average training-induced reductions in systolic and diastolic blood pressure have varied from 2 to 11 mmHg and from 1 to 8 mmHg, respectively (Kelley & Tran 1995, Rankinen *et al.* 2000, Rice *et al.* 2000). The magnitude of the training responses increases as a function of initial blood pressure levels, being more pronounced in patients with mild to moderate hypertension than in normotensive subjects (Fagard 2001). The reductions in blood pressure are seen at rest and during exercise at a given submaximal load. At high exercise intensities and at maximal exercise, blood pressure is generally similar before and after training.

2.2.3 Blood volume

Aerobic training increases blood volume. Plasma volume increases after a few days of training, while the expansion of erythrocyte volume takes longer (Harrison 1985). A 12 to 20% increase in plasma volume occurs after three to six aerobic training sessions (Sawka *et al.* 2000). The plasma volume increase enhances the circulatory reserve and contributes to increased end-diastolic volume, stroke volume, oxygen transport and temperature regulation during exercise (Hagberg *et al.* 1998).

2.2.4 Peripheral vascular adaptations

Peripheral vascular adaptation, which includes enhanced perfusion and flow capacity, has been observed after regular aerobic training. Total leg blood flow during strenuous exercise increases in parallel with a rise in maximal aerobic power. In addition, the arteriovenous oxygen difference in muscle increases after aerobic training. These adaptations may arise from structural modifications of the vasculature and alterations in the control of vascular tone (Ingjer 1979). An increase in the capillary density of muscle has also been shown after training. Both capillary density and blood flow increase in proportion to the rise in maximal aerobic power during long-term aerobic training interventions (Ingjer 1979, Hepple 2000).

The rise in peak muscle blood flow appears to be achieved by enhanced endothelium-dependent dilatation in the muscle, which increases its vasodilator capacity. Enhanced peak hyperemic blood flow appers to be an early adaptation to regular aerobic training (Sinoway *et al.* 1987, Laughlin 1995). The increase in blood flow induced by training may exert its effect on endothelial function by modulating the expression of endothelial cell nitric oxide synthase (Niebauer & Cooke 1996). Nitric oxide seems to be an important and potent endothelium-derived relaxing factor that facilitates blood vessel dilatation and decreases vascular resistance (Joyner & Tschakovsky 2003).

2.2.5 Pulmonary adaptation

Aerobic training stimulates adaptations in pulmonary ventilation during submaximal and maximal exercise. During maximal exercise, V_E increases as Vo_{2max} increases. The increase in Vo_{2max} increases both the oxygen requirement and the corresponding need to eliminate carbon dioxide via alveolar ventilation. In general, during submaximal exercise, TV increases and respiratory rate decreases. Aerobic training reduces the ventilatory equivalent for oxygen during submaximal exercise and lowers the percentage of the total energy demand attributable to respiration (Casaburi *et al.* 1987). The reduced oxygen consumption by the ventilatory musculature enhances endurance by reducing the fatigu-

ing effects of exercise on the ventilatory musculature adding oxygen supply for the active muscles (Johnson *et al.* 1993, Harms *et al.* 1997).

2.2.6 Individual differences in adaptation to aerobic training

Despite the fact that the mean improvements of $\dot{V}o_{2max}$ have generally been of the order of 15 to 25 % of the baseline values over the first 2 to 3 months of regular aerobic training (ACSM 1998), there is considerable heterogeneity in the responsiveness to aerobic training. Large individual differences in the response to regular aerobic training have been observed after highly standardized exercise programs in healthy subjects, ranging from 0% to a 40 % increase in $\dot{V}o_{2max}$ (Lortie *et al.* 1984, Kohrt *et al.* 1991, Bouchard 1995, Bouchard & Rankinen 2001). The HERITAGE Family Study based on 720 healthy subjects summarized the contributions of age, sex, race and baseline fitness level to the response to aerobic training. All these variables together accounted for only 11 % of the variance in the response to 20 weeks of standardized training. The gender was the most powerful predictor of the training response with a contribution of 5.4 %, followed by age with a 4 % contribution (Bouchard & Rankinen 2001). Genetic background causes considerable variation in both the baseline aerobic capacity and the changes in aerobic fitness after aerobic training interventions (Bouchard *et al.* 1999, Rankinen *et al.* 2001, Rankinen *et al.* 2002). However, the mechanisms responsible for the heterogeneity in the response to regular training is largely unknown.

2.3 Autonomic nervous system

The human nervous system is composed of three major parts: the sensory input portion, the central nervous system, and the motor output portion. The portion of the nervous system that controls the visceral functions of the body at a subconcious level is called the autonomic nervous system. The autonomic nervous system provides the innervation for the heart. Although cardiac muscle has an intrinsic mechanism for HR, neural influences superimpose on the inherent rhythm of the myocardium. These influences originate from the cardiovascular centre in the medulla oblongata and flow through the sympathetic and parasympathetic (vagal) components of the autonomic nervous system. These divisions operate in parallel, but differ from each other due to differences in anatomy, neurotransmitters, receptors and physiological actions (Bannister 1990). The vagal nerves innervate the sinoatrial node, the atrioventricular (AV) conducting pathways and the atrial muscle. Vagal innervation has also proposed to influence on the cardiac ventricular function (Standish *et al.* 1994). The parasympathetic influence on HR is mediated via release of acetylcholine by the vagus nerve. Muscarinic acetylcholine receptors respond to this release, mostly by an increase in cell membrane potassium conductance (Osterrieder *et al.* 1980, Sakmann *et al.* 1983). Sympathetic postganglionic fibres innervate the entire heart, including the sinoatrial node, the AV conducting pathways and the atrial and ventricular

myocardium. The sympathetic influence on HR is mediated by release of epinephrine and norepinephrine (Opie 1998).

2.3.1 Vagal and sympathetic regulation of heart

The intrinsic HR, in the absence of any neurohumoral influence, is about 90 to 120 beats/min (Craft & Schwartz 1995). The two branches of the autonomic nervous system work in a synchronized way on HR. In a healthy unblocked individual, the HR at any time represents the net effect of parasympathetic (vagal) nervous activity and sympathetic nervous activity. Under resting conditions, as during the night, vagal tone prevails (Levy 1971) and the variations in HR period are largely dependent on vagal modulation (Chess *et al.* 1975). The effect of vagal stimulation is to slow down, or even stop, the heart. The latency of the sinus node response is very short. After a single stimulus, the maximum response has been reported to occur within 400 milliseconds (Levy et al. 1970). Thus, vagal stimulation results in a peak response in either the first or the second beat after its onset. After the end of vagal stimulation, HR rapidly returns to its previous level. The rate of recovery is somewhat slower than that of onset, but HR is usually restored in less than 5 to 10 seconds (Revington & McCloskey 1990). Increased activity in the sympathetic nerve pool results in increases in both HR and the force of contraction. Following the onset of sympathetic stimulation, there is a latency period of up to 5 seconds followed by a progressive increase in HR, which reaches a steady state in 20 to 30 seconds (Hainsworth 1995).

2.3.2 Physiological modulators of autonomic nervous function

Baroreflex has been proposed to be mainly a vagally mediated control system between HR and blood pressure, where the R-R interval is changed in response to changes in arterial pressure (Eckberg *et al.* 1971, Eckberg 1980). Any rise in blood pressure is sensed by the baroreceptors, which are pressure-sensitive nerve endings mainly found in the wall of aortic arch and in the carotid sinuses. The baroreceptor reflex is stimulated, which results in a reduction of HR and cardiac contractility and, thus, a drop in blood pressure. An initial decrease in blood pressure has opposite effects.

When the oxygen supply to active muscle is inadequate for the ongoing rate of metabolism, metabolites accumulate and stimulate the sensory nerves within the muscle. Activation of these nerves elicits a muscle chemoreflex or metaboreflex that increases sympathetic nerve activity and mean arterial pressure (O'Leary 1993, Sheriff *et al.* 1998, Ansorge *et al.* 2002). It has also been assumed that afferents within the active skeletal muscle, termed muscle mechanoreceptors, mediate reflex changes in HR during dynamic exercise (O'Leary 1993).

The renin-angiotensin system has been proposed to modulate autonomic nervous function on a time scale of seconds to minutes, indicating that the renin-angiotensin system effects short-term cardiovascular control (Akselrod *et al.* 1981, Taylor *et al.* 1998).

Stimulation of sympathetic nerves to adrenal medullae causes release of the epinephrine and norepinephrine hormones (circulating catecholamines), which have almost the same effects througout the body as direct sympathetic stimulation, except that these effects are prolonged, lasting for 1 to 3 minutes after the stimulation is over (Herd 1991, Rowell 1997).

2.3.3 Autonomic nervous function during exercise

Marked changes in HR modulation occur at different exercise intensity levels mediated primarily by the sympathetic and parasympathetic nervous systems. At the beginning of dynamic exercise, HR increases rapidly, mainly due to the withdrawal of vagal activity (Rosenblueth & Simeone 1934, Robinson *et al.* 1966). If the exercise is light (HR < 100 beats/min), the sympathetic activity applied to the heart does not increase, and tachycardia occurs solely due to the reduction in parasympathetic tone (Victor *et al.* 1987). As the workload increases, HR increases due to further vagal withdrawal and concomitant sympathetic activation (Rosenblueth & Simeone 1934, Robinson *et al.* 1966). The increase in sympathetic activation may be due to arterial baroreflex resetting, the muscle metaboreflex or muscle mechanoreceptor activation (O'Leary & Seamans 1993). During heavy exercise, parasympathetic activity wanes and sympathetic activity increases in such a way that little or no parasympathetic tone remains (Rosenblueth & Simeone 1934, Robinson *et al.* 1966, Tulppo *et al.* 1996, Tulppo *et al.* 1998b).

2.3.4 Adaptation of autonomic nervous system to aerobic training

An obvious indicator of cardiovascular adaptation to regular aerobic training is a lowering of HR at rest and during submaximal exercise. Maximal HR remains unchanged or may even be slightly reduced. The lowering of resting and submaximal HR is mediated by alterations in the autonomic nervous system and by changes in the intrinsic mechanism of the sinus node and right atrial myocytes (Ekblom *et al.* 1973, Lewis *et al.* 1980). During exercise, in a trained subject, a given increase in cardiac output requires less increase in HR due to the maintenance of a larger stroke volume. Studies focusing on autonomic responses to training indicate that HR is reduced during submaximal exercise due to a lower intrinsic HR, a reduction in sympathetic activity and circulating catecholamines and a greater parasympathetic influence (Ekblom *et al.* 1973, Lewis *et al.* 1980, Tulppo *et al.* 1998b). The lower sympathetic activation of the heart at submaximal working levels derives in part from the diminished reflex signals originating from skeletal muscle due to less abundant metabolite accumulation and attenuated discharge of metaboreceptors (Mostoufi-Moab *et al.* 1998). The mechanisms underlying the training-

induced increase in vagal activity are thought to consist of greater activation of the cardiac baroreceptors in response to the enlargement of blood volume and ventricular filling (Convertino *et al.* 1991, Levine 1993, Spinelli *et al.* 1999) as well as changes in the opioid (Angelopoulos *et al.* 1995) and dopaminergic modulation of parasympathetic tone (Slavik & LaPointe 1993). Lower intrinsic HR may be one adaptation mechanism after aerobic training (Hughson *et al.* 1977, Bonaduce *et al.* 1998). Subjects with enlarged heart have lower intrinsic HR. Therefore it has been hypothesized that cardiac enlargement caused by training accounts for the lower intrinsic HR. Another possible mechanism for reduced intrinsic HR is that atrial enlargement reduces the stretch-depolarization stimulus altering the resting regulation of heart muscle.

2.4 Heart rate variability

Heart rate (HR) variability is a term applied to variations in beat-to-beat fluctuations around the mean HR. It reflects the effects of sympathetic and parasympathetic tone and other physiological control mechanisms on cardiac function. Continuous changes in sympathetic and parasympathetic neural impulses result in changes in HR and cause oscillation around the mean HR. HR variability has been extensively studied during the past decade for its value as a predictor of cardiac death (Kleiger *et al.* 1987, Bigger *et al.* 1992, Tsuji *et al.* 1996a, Perkiömäki *et al.* 1997, Huikuri *et al.* 1998, Huikuri *et al.* 1999, Huikuri *et al.* 2000, Mäkikallio *et al.* 2001, Tapanainen *et al.* 2002, Jokinen *et al.* 2003). Measurement of HR variability has become a widely used tool for assessing cardiovascular autonomic function in various physiological settings (Lipsitz *et al.* 1990, Huikuri *et al.* 1992, Huikuri *et al.* 1993, Davy *et al.* 1996, Tulppo *et al.* 1996, Tulppo *et al.* 1998b, Pikkujämsä *et al.* 2001, Tulppo *et al.* 2001a, Tulppo *et al.* 2001b).

2.4.1 Physiological background of heart rate variability

Breathing exerts profound influences on autonomic neural outflow. Beat-to-beat fluctuation in HR is related to respiration due to the inspiratory inhibition of vagal tone. Inspiratory inhibition is evoked primarily by central impulses from the medullary and cardiovascular centre (Davidson *et al.* 1976). In addition, peripheral reflexes due to hemodynamic changes and thoracic stretch receptors contribute to respiratory sinus arrhythmia (Hirsch & Bishop 1981, Akselrod *et al.* 1985). This parasympathetically mediated fluctuation can be minimized by atropine or vagotomy (Akselrod *et al.* 1985, Hayano *et al.* 1991). The amount of HR variability in relation to respiration is used as a non-invasive index of vagal nerve activity in humans (Eckberg 1983, Montano *et al.* 1994). Total HR variability has been shown to decrease by 80-90 % after complete vagal blockade in laboratory conditions. Therefore, vagal activity has been proposed to be the major determinant of HR variability in healthy humans (Hayano *et al.* 1991, Taylor *et al.* 1998).

Phasic autonomic motoneurone firing depends significantly on the intensity of the stimulatory inputs (Taylor *et al.* 2001, Penttilä *et al.* 2003). Respiratory sinus arrythmia is minimal at rapid and extensive at slow breathing rates (Eckberg 2003). The magnitude of respiratory sinus arrhythmia is maximal at around six breaths per minute (Hirsch & Bishop 1981, Hayano *et al.* 1994). It is also influenced by TV, but the effect is relatively weak, as an increase of 50 % increases the respiration-related variation in HR by only 15 % (Eckberg 1983). Furthermore, the depth of respiration does not influence respiratory sinus arrhythmia significantly if the TV is greater than 40 % of forced vital capacity, which is the case during maximal respiration (Bennett *et al.* 1978).

Sympathetic activity has been proposed to correspond to R-R interval fluctuation at around 0.1 Hz frequency (Malliani *et al.* 1991, Pagani *et al.* 1997). However, some evidence fails to support the notion that 0.1 Hz oscillation of HR reflects changes in the sympathetic inputs to the sinoatrial node (Saul *et al.* 1990, Koh *et al.* 1994, Taylor & Eckberg 1996, Eckberg 1997). One physiological mechanism affecting the fluctuation of HR variability at the frequency of 0.1 Hz is the vasomotor part of the baroreflex control, which is responsible for arterial pressure oscillations (Madwed *et al.* 1989, Furlan *et al.* 2001, Malpas 2002). Peripheral vascular resistance also exhibits intrinsic oscillation with that frequency (Rosenbaum & Race 1968, Kitney 1975). These fluctuations can be influenced by thermal skin stimulation and may arise from thermoregulatory peripheral blood flow adjustments. The fluctuations in peripheral vascular resistance are accompanied by fluctuations of similar frequency in blood pressure and HR and are mediated by the sympathetic nervous system (Lindqvist *et al.* 1989). The renin-angiotensin system may also play a minor role in the genesis of HR variability (Akselrod *et al.* 1981), as may also presso- and chemoreceptors, which affect HR through rapid control mechanisms (van Ravenswaaij-Arts *et al.* 1993).

2.4.2 Technical requirements for heart rate variability measurements

In long-term recordings, i.e. during 24 hours, good short-term and long-term reproducibility of HR variability has been reported when measured from surface ECGs (Huikuri *et al.* 1990, Kleiger *et al.* 1991, Nolan *et al.* 1996). Long-term recordings have appeared to be a reliable and practical method that is not dependent on the co-operation of the subject and does not involve any placebo effect on the results (Stein *et al.* 1994, Task Force 1996). The reproducibility of 24-hour HR variability measurements may be even better compared to the assessment of HR dynamics from short-term recordings (usually 5-minute) in controlled situations (Breuer *et al.* 1992, Tulppo *et al.* 1998a). However, good reproducibility of HR variability parameters has also been observed in short-term recordings (Melanson 2000, Kowalewski & Urban 2003) and especially during standardized exercise (Tulppo *et al.* 1998b).

The extent and type of editing R-R interval data have remarkably different effects on various HR variability indices (Huikuri *et al.* 1999, Salo *et al.* 2001). Even though there is no universal method for editing R-R interval data, there is general consensus that arti-

facts, premature beats and non-sinus tachycardia episodes should be deleted before running the analyses (Task Force 1996).

The sampling frequency is an important factor for accurate detection of beat-to-beat fluctuations in R-R intervals (Tapanainen *et al.* 1999). A sampling frequency of 250-500 Hz has been recommended (Task Force 1996), but most of the older studies on HR variability have gathered data by using 24-hour Holter ECG systems with a sampling frequency of 128 Hz. A low sampling frequency may cause jitter in the recognition of the QRS complex, creating an error in the R-R interval measurement (Merri *et al.* 1990). In a previous study, a sampling frequency of 128 Hz vs. 1000 Hz resulted in a significant difference in the values of the HR variability parameters (Tapanainen *et al.* 1999). Therefore, it is preferable, especially during dynamic HR variability measurements, to collect the data with a high sampling frequency.

2.4.3 Convential methods of analysing heart rate variability

The conventional HR variability measures can be assessed by calculating indices based on statistical methods, derived from either the R-R intervals or the differences between them (time domain analysis), by spectral (frequency domain) analysis or by geometrical methods of R-R interval analysis. The analysis can be performed on short ECG segments (lasting for 0.5 to 5 minutes) or on 24-hour ECG recordings (Akselrod *et al.* 1985, Task Force 1996).

The most widely used time domain index is the average HR. It is easy to calculate over a suitable length of time. Another commonly used parameter is the standard deviation of all normal-to-normal R-R intervals over an entire recording (usually 24-hour recording) (SDNN). These variables are considered to reflect both parasympathetic and sympathetic influences on HR variability (Bigger *et al.* 1989, Kleiger *et al.* 1992). In the landmark study of Kleiger *et al*., SDNN < 50 ms was associated with 5.3-fold mortality when compared to patients with preserved HR variability (SDNN > 100 ms) (Kleiger *et al.* 1987). There are also other time domain parameters, which are closely intercorrelated, but they have not gained much popularity in the recent years.

Power spectrum analysis reflects the amplitude of HR fluctuations present at different oscillation frequencies. The R-R interval signal is disintegrated into numerous sinusoidal functions of different frequencies, and a power spectrum is created, in which the amplitude is plotted as a function of each frequency. Spectral analysis methods are based on either nonparametric (fast Fourier transformation, FFT) or parametric techniques (autoregressive model estimation). In most cases, the results are comparable regardless of the technique applied. The following limits of the frequency bands have been recommended: ultra-low-frequency < 0.0033 Hz (ULF), very-low-frequency from 0.0033 to 0.04 Hz (VLF), low-frequency from 0.04 to 0.15 Hz (LF) and high-frequency from 0.15 to 0.4 Hz (HF). The measurement of different power components is usually made in absolute values of power (milliseconds squared). LF and HF may also be measured in normalised units, which represent the relative value of each power component in proportion to the total power minus the VLF component (Pagani *et al.* 1986, Malliani *et al.* 1991).

Efferent cardiac vagal outflow has been generally proposed to contribute to the HF spectral power of HR variability (Hayano *et al.* 1991, Task Force 1996, Hayano & Yasuma 2003). Both clinical and experimental studies, including muscarinic receptor blockade, vagotomy and electrical vagal nerve stimulation, have shown vagal activity to be the major contributor to the HF component (Akselrod *et al.* 1981, Parker *et al.* 1984, Akselrod *et al.* 1985, Hayano *et al.* 1991, Pyetan & Akselrod 2003). More controversial is the interpretation of the LF component, which is considered by some authors a marker of sympathetic modulation (Malliani *et al.* 1991, Montano *et al.* 1994, Montano *et al.* 1998, Furlan *et al.* 2000) and by some others a parameter that includes both sympathetic and vagal influences (Akselrod *et al.* 1981, Akselrod *et al.* 1985). The ratio of absolute LF to absolute HF power (LF/HF ratio) has been used as an index of sympathovagal balance (Montano *et al.* 1994) or as an index of sympathetic activity (Pagani *et al.* 1986, Yamamoto *et al.* 1991, Pagani *et al.* 1997), but the value of this variable has been questioned (Eckberg 1997). In long-term recordings (24-hour), the HF and LF components account for only approximately 5-10 % of total power. Although the ULF and VLF components account for the remaining 90-95 % of total power, the physiological backround is not well known. Atropin abolishes almost completely all variation of HR, suggesting that vagal activity is also the main denominator of these components (Akselrod *et al.* 1981, Hayano *et al.* 1991, Taylor *et al.* 1998). However, the ULF and VLF components may also reflect changes in thermoregulation due to pheripheral blood flow adjustments (Lindqvist *et al.* 1989) or changes in everyday physical activities (Bernardi *et al.* 1996).

Geometrical methods are techniques in which R-R intervals are converted into various geometrical forms. In Poincarè scatterograms, each R-R interval is plotted as a function of the previous one. The plots can be either visually or quantitatively interpreted (Huikuri *et al.* 1996b, Tulppo *et al.* 1996). The standard deviation of the longitudinal axis of the plot (SD2) is a marker of long-term HR variability, while the standard deviation of the vertical axis is a marker of short-term beat-to-beat variability (SD1) (Huikuri *et al.* 1996b). The analysis of Poincarè plots has proven especially useful during exercise, as it reveals the changes in the vagally mediated beat-to-beat R-R interval fluctuation that are not easily detectable by linear summary measures of HR variability (Tulppo *et al.* 1996, Tulppo *et al.* 1998b).

2.4.4 Nonlinear dynamic methods of analysing heart rate variability

HR is not generated by simple periodic oscillations, but nonlinear phenomena are involved in the genesis of this process (Peng *et al.* 1995a, Task Force 1996, Mäkikallio *et al.* 1996, Mäkikallio *et al.* 1998, Ivanov *et al.* 1999). Therefore, new analysis techniques have also been developed to probe features in HR behaviour that are not detectable by the traditional HR variability methods based on moment statistics. The Detrended Fluctuation Analysis (DFA) technique is a nonlinear method to detect qualitative rather than quantitative changes in HR dynamics (Peng *et al.* 1995a, Iyengar *et al.* 1996). DFA quantifies the fractal correlation properties of the R-R interval data (Peng *et al.* 1995b). If the analysed scaling exponent α is near 1, it indicates fractal-like behaviour of the signal. The DFA

method has been used as a new approach to evaluate the mortality risk in various patient groups, after the short-term scaling exponent (α_1) was shown to be decreased in patients with congestive heart failure (Peng *et al.* 1995b, Ho *et al.* 1997). The decreased α_1 has been shown to be a strong independent predictor of cardiac and total mortality in different patient populations (Mäkikallio *et al.* 1999, Huikuri *et al.* 2000, Mäkikallio *et al.* 2001, Perkiömäki *et al.* 2001, Tapanainen *et al.* 2002, Jokinen *et al.* 2003).

The physiologic background of altered fractal correlation properties has not been fully established. A change in the HR dynamics from fractal-like behaviour towards a stronger correlation in HR behaviour (from 1.0 to 1.5) has been observed after parasympathetic blockade by atropin (Tulppo *et al.* 2001b) and during maneuvers designed to alter sympathetic tone, e.g. low-intensity exercise and passive head-up tilting (Tulppo *et al.* 2001a). On the contrary, a reduction in the short-term correlation properties of HR dynamics from 1.0 to 0.5 has been observed after the administration of physiological doses of norepinephrine (Tulppo *et al.* 2001b). This suggest that altered behaviour of the fractal correlation properties of HR can be a specific marker of neurohumoral and sympathetic activation.

The distribution of power spectral density can be described by the linear inverse power-law relationship of power (on a logarithmic scale) to frequency (on a logarithmic scale (Bigger *et al.* 1996). The power-law relationship of R-R interval variability can be quantified by calculating the slope (β), which is usually done over the VLF and ULF frequency bands ranging from 10^{-4} to 10^{-2}. It is also a marker of the fractal-like correlation properties of R-R interval data in this power range. A low power-law slope has predicted mortality in a general elderly population (Huikuri *et al.* 1998) as well as in different patient groups (Mäkikallio *et al.* 1999, Huikuri *et al.* 2000).

2.4.5 Effects of age, gender and circadian profile on heart rate variability

The effect of age on HR variability has been observed in many studies. The magnitude and course of this effect depend on the age range studied, the experimental conditions and the methods used to assess HR variability. Increased HR variability occurs during childhood (Korkushko *et al.* 1991, Finley & Nugent 1995). On the other hand, increasing age during adult life is associated with a reduction of total variance and a smaller power at all frequencies of R-R intervals (Hayano *et al.* 1990, Lipsitz *et al.* 1990, Bigger *et al.* 1995, Jensen-Urstad *et al.* 1997, Umetani *et al.* 1998, Stolarz *et al.* 2003). Furthermore, altered fractal scaling properties have been observed at advancing age (Pikkujämsä *et al.* 1999, Jokinen *et al.* 2001). Age has also remained an independent determinant of HR variability in multivariate analysis (Tsuji *et al.* 1996b). The data on age-related changes in HR variability have been explained on the basis of changes in autonomic innervation or responsiveness (Lakatta 1993, Taylor *et al.* 1995). Age-related differences in HR variability are abolished after the administration of a pharmacological autonomic blockade, while intrinsic HR and paced atrioventricular dissociation have been observed to be lower in old than young subjects (Craft & Schwartz 1995). Age differences in HR variability can be ex-

plained by autonomic influences, but HR and atrioventricular conduction differences exist independently of β-adrenergic and/or parasympathetic influences (Craft & Schwartz 1995).

Gender has been reported to influence autonomic nervous functions. In cross-sectional studies, women have been reported to show higher vagal modulation of HR as compared with men (Ryan *et al.* 1994, Gregoire *et al.* 1996, Huikuri *et al.* 1996a, Kuo *et al.* 1999). Autonomic activity also shows a circadian profile with an increase in sympathetic tone during the day and in parasympathetic tone at night (Molgaard *et al.* 1991, Huikuri *et al.* 1994, Peckova *et al.* 1998).

2.4.6 Effects of physical fitness on heart rate variability

Good physical fitness and regular exercise training induce adaptations of the autonomic nervous system, most commonly seen in the form of a decrease in the basal HR. It is evident that cardiac vagal tone is higher in well-trained individuals as compared that of controls (Goldsmith *et al.* 1992, De Meersman 1993, Davy *et al.* 1996, Davy *et al.* 1998, Tulppo *et al.* 1998b, Aubert *et al.* 2001, Ueno *et al.* 2002, Rennie *et al.* 2003). Middle-aged healthy subjects with a better exercise capacity had significantly higher vagal activity, especially during exercise than those with poor physical fitness (Tulppo *et al.* 1998b). Tulppo *et al.* (1998b) also showed that the exercise intensity level at which the vagally mediated beat-to-beat R-R interval fluctuation disappeared was significantly higher in subjects with good fitness compared to those with poor physical fitness.

2.4.7 Heart rate variability during exercise

Changes in the autonomic regulation of HR during exercise have been traditionally assessed by spectral measures of HR variability (Arai *et al.* 1989, Yamamoto *et al.* 1991, Nakamura *et al.* 1993). However, the use of these indices in exercise has shown contradictory findings across studies, especially at high intensity levels of exercise (Arai *et al.* 1989, Yamamoto *et al.* 1991, Nakamura *et al.* 1993, Casadei *et al.* 1995, Casadei *et al.* 1996, Gregoire *et al.* 1996, Tulppo *et al.* 1996, Warren *et al.* 1997, Tulppo *et al.* 1998b, Perini & Veicsteinas 2003). For example, some studies have shown the LF/HF ratio to increase (Yamamoto *et al.* 1991, Nakamura *et al.* 1993, Casadei *et al.* 1996, Gregoire *et al.* 1996) during exercise, whereas in some others it is decreased (Breuer *et al.* 1993). The controversial results may be due to differences in the units or methods of spectral analysis (coarse-gained spectral analysis vs. fast Fourier transformation or autoregressive analysis) or in the length of data collection (from 1 to 10 minutes). The sampling frequency during exercise is another important factor for accurate detection of beat-to-beat fluctuations in R-R intervals, which may contribute to the controversial findings. Because the total variance of the R-R interval drops progressively at increasing work rates, the presence of a nonneural component of respiratory sinus arrhythmia would lead to an overes-

timation of vagal activity, and this could partly explain the behaviour of the spectral parameters during heavy exercise (Casadei *et al.* 1996).

Poincarè plots have revealed patterns of HR dynamics during exercise that are not easily detected by time domain and spectral measures of HR variability (Tulppo *et al.* 1996). Tulppo *et al.* (1996) showed that short-term vagally mediated beat-to-beat variability (SD1) disappeared at 50-60 % of maximal O_2 consumption, whereafter the increase in HR was mainly mediated by sympathetic activation. The nonlinear methods of HR variability during exercise have not been extensively studied. However, a change in the fractal-like behaviour towards a stronger correlation in HR behaviour (α_1) has been observed during low-intensity exercise and passive head-up tilting (Tulppo *et al.* 2001a).

In summary, several previous studies have shown the poor ability of the conventional HR variability measures to reveal changes in the autonomic regulation of HR at exercise intensity levels > 70 % of $\dot{V}o_{2max}$. (Yamamoto & Hughson 1991, Casadei *et al.* 1995, Tulppo *et al.* 1996, Warren *et al.* 1997, Tulppo *et al.* 1998b). The ability of the fractal correlation properties of HR to reveal changes in HR behaviour during high-intensity exercise is not known.

2.4.8 Heart rate variability during recovery

Delayed recovery of HR after maximal or submaximal exercise is a powerful predictor of overall mortality among population-based data (Cole *et al.* 1999, Cole *et al.* 2000, Nissinen *et al.* 2003). Recovery of autonomic regulation of HR has been proposed to occur within a few minutes after short-term (10-20 min) maximal or submaximal exercise (Arai *et al.* 1989, Perini *et al.* 1989, Kannankeril & Goldberger 2002). Parasympathetic effects in early postexercise recovery have been proposed to be the underlying mechanism (Arai *et al.* 1989, Perini *et al.* 1989, Sugawara *et al.* 2001, Kannankeril & Goldberger 2002). However, there are no HR variability data on the changes and recovery times of cardiovascular autonomic function after prolonged exercise.

2.4.9 Effects of aerobic training on heart rate variability

Effects of long-term aerobic training have been suggested to be associated with increased HR variability, especially with vagally mediated respiratory sinus arrythmia during short-term (2-10 min) rest recordings (Seals & Chase 1989, De Meersman 1992, Shi *et al.* 1995, al-Ani *et al.* 1996, Gregoire *et al.* 1996, Levy *et al.* 1998, Melanson & Freedson 2001, Yamamoto *et al.* 2001, Myslivecek *et al.* 2002, Carter *et al.* 2003) and during long-term (24 hours) dynamic recordings (Stein *et al.* 1999, Pigozzi *et al.* 2001). In contrary, some controlled studies have failed to show any association between aerobic training and HR variability during short-term (Maciel *et al.* 1985, Boutcher & Stein 1995) or long-term recordings (Loimaala *et al.* 2000).

Several mechanisms or reasons may explain the controversial results of HR variability studies after aerobic training. Long-term adaptation to regular aerobic training results from a complex combination of biochemical, structural, metabolic, humoral and neural factors (Furlan *et al.* 1993). Additional modulatory influences originate from genetic components, the age, the intensity of the exercise training routine and the simultaneous presence of short-time after-effects from the previous activity (Pagani *et al.* 1995). Furthermore, the previous contradictory findings of several aerobic training studies are partly limited by either the small sample size (Maciel *et al.* 1985, Lazoglu *et al.* 1996) or the characteristics of the subjects, e.g. a narrow range of aerobic fitness (Byrne *et al.* 1996). Also, the duration of interventions (from 6 to 36 weeks), the training frequency (from 3 to 7 sessions/week) and the intensity of exercise (from ~60 to 90 % of maximal HR) varies between the previous studies, and it is thus difficult to compare the results of different studies. However, long duration (12 months) of training may not necessarily lead to greater enhancement in HR variability (Uusitalo *et al.* 2002), as prolonged (12 months) and intense training (from walking to running) may restore these changes in HR variability back to the baseline level (Iwasaki *et al.* 2003). In the most recent study, the long duration (5 years) of regular low-to moderate intensity training did not prevent HR variability from decreasing in older men population (Uusitalo *et al.* 2004).

Despite the rather large body of data concerning aerobic training and HR variability, the enhancing impacts of the volume and duration of aerobic training on cardiac vagal outflow in controlled studies among healthy subjects are not well known.

3 Purpose of the present study

The purpose of the present study was to evaluate the effects of autonomic regulation on HR during physical exercise and during aerobic training interventions. The specific aims were:

1. To examine the effects of dynamic exercise with and without parasympathetic blockade (I) and the effects of aerobic training (III) on the fractal characteristics of HR dynamics.
2. To examine the recovery of cardiovascular autonomic function after prolonged exercise (II).
3. To study the effects of a highly controlled aerobic training program and (III) and the effects of a home-based training program (IV) on the autonomic regulation of HR.
4. To test the hypothesis that individual cardiac autonomic function may predict the response to aerobic training (V).

4 Subjects

The whole study population consisted of 70 volunteer healthy male subjects (age 36±10 years). All were non-smokers without any permanent medication. In this study, we investigated only healthy men, because it may be important to understand the effects of autonomic regulation on HR during physical exercise and during aerobic training interventions in a homogeneous sample of subjects. The protocols were approved by the ethics committee of the Merikoski Rehabilitation and Research Centre, and all subjects gave their written informed consent.

Nine healthy volunteer subjects (table 1) were recruited to participate in a dynamic cross-sectional exercise study (I). The recovery of the autonomic nervous system after prolonged exhaustive exercise was studied in another group of subjects (II). The volunteers (n=10, table 1) in study II had participated regularly in aerobic training at least four times per week for the past three months. One subject was excluded from the final analysis because of technical artefacts in HR variability data, and the final analyses were hence conducted on nine subjects. The subjects for the eight-week aerobic training intervention (III) were recruited by advertising in a newspaper, which attracted 85 replies. The subjects were interviewed with a standardized scheme to ascertain their medical histories and levels of physical activity. The subjects with a high body mass index (BMI>30), the subjects who did regular physical training more than twice a week and those with diabetes mellitus, asthma or cardiovascular disorders were excluded. We invited 60 male subjects to our laboratory for a more specific assessment of physical status and excluded two subjects on account of various relative contraindications for a maximal exercise test. We tested 58 subjects and excluded three from the final analysis because of the number of ectopic beats during the data acquisition. Finally, 55 male subjects were included in the training study. The subjects were randomized into a moderate-volume training group (n=20), a high-volume training group (n=20) and a control group (n=15). One subject in the moderate-volume training group and four subjects in the high-volume group dropped out due to a lack of motivation or because of leg problems. The final analysis included 19 subjects in the moderate-volume, 16 subjects in the high-volume and 11 subjects in the control group (table 1). In the study IV, after the controlled training period, the subjects in the training group were encouraged to continue aerobic training independently, preferably on a daily basis, by following the American College of Sports Medicine recommenda-

tions (ACSM 1998). The final analysis included 18 subjects in the training group and 6 subjects in the control group (table 1). The subjects in study V were the same as those in study III, with the exception of four healthy subjects who had been added to the study population. The final analysis included 20 subjects in the moderate-volume, 19 subjects in the high-volume and 12 subjects in the control group (table 1).

Table 1. Characteristics of study populations.

	I	II	III			IV		V		
			Moderate-volume training	High-volume training	Control	Training	Control	Moderate-volume training	High-volume training	Control
	n=9	n=10	n=19	n=16	n=11	n=18	n=6	n=20	n=19	n=12
Age, years	37±11	36±11	35±10	35±10	36±11	39±10	44±6	36±10	36±8	33±10
Height, cm	178±5	176±6	181±5	180±5	182±7	179±5	178±7	181±6	180±5	183±8
Weight, kg	77±8	75±8	82±11	79±9	81±9	80±10	80±8	83±12	80±9	80±9
Fat, %	13±4	13±4	16±4	17±4	16±3	15±4	17±1	17±3	17±2	16±4
BMI	22±2	22±2	25±3	25±2	25±3	25±2	25±2	25±2	25±2	25±2
SBP, mmHg	134±17	137±17	127±13	126±9	128±10	131±11	133±13	126±12	127±8	128±11
DBP, mmHg	80±6	81±6	78±7	77±8	79±7	87±5	84±5	77±8	79±8	78±7
HR_{max}, bpm	186±13	185±11	189±9	187±8	185±15	188±9	172±13	188±9	186±9	189±15
$\dot{V}o_{2peak}$, $l \cdot min^{-1}$	3.8±0.6	3.8±0.6	3.3±0.4	3.4±0.4	3.4±0.3	3.3±0.4	3.5±0.4	3.2±0.4	3.4±0.4	3.4±0.4
$\dot{V}o_{2peak}$, $ml \cdot kg^{-1} \cdot min^{-1}$	52±5	51±6	41±4	42±5	41±4	42±5	44±5	39±4	43±5	43±4

Abbreviations: The values are means±standard deviation; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; $\dot{V}o_{2peak}$, maximal oxygen consumption; HR_{max}, maximal heart rate; Fat, body fat according to Durnin & Womersley (1974). Blood pressure was measured in a supine position by the auscultatory method.

5 Methods and study protocols

5.1 Methods

Physical examination, HR variability measurements, exercise tests and measurement of oxygen consumption were conducted with standard methods (I-V). Autonomic blockade with atropine sulphate was performed (Hayano *et al.* 1991) in the study I.

5.1.1 Heart rate variability recordings

The R-R intervals were recorded with a real-time microprocessor QRS detector system (Polar Electro Oy, Kempele, Finland) with 1 ms timing accuracy saved in a computer for further analysis of HR variability with the Heart Signal Co software (Heart Signal Co., Oulu, Finland). The R-R intervals were edited manually to exclude all premature beats and noise, which accounted for less than two percent in each case. The details of this analysis technique have been described previously (Huikuri *et al.* 1992, Huikuri *et al.* 1993, Ruha *et al.* 1997). The subjects were not allowed to eat or drink coffee for 3 h before the tests. Vigorous exercise and alcohol were also forbidden for 48 h before the testing day. All the tests were performed between 8:00 AM and 17:00 PM. The repeated tests were always performed at the same time of the day for each subject.

In the study I, the subjects remained in a supine position in a quiet room for 0.5 h before the baseline R-R interval recordings. The baseline recordings were made in a seated position (10-20 min) before the tests. The HR variability analyses were performed for a 3 min period at the baseline and during consecutive steps (3 min) in the maximal exercise tests. During the long-term exercise test HR variability analyses were performed for a 15 min period at the baseline and for 15 min during the exercise. During atropine infusion analyses were performed analogously for a 3 min period at the baseline, at the end of every dose during the atropine infusion (3 min) and, finally, during every load (3 min) in the maximal exercise test.

In the study II, R-R intervals were recorded and analysed 24 hours before the skiing race and continuously for 48 hours afterwards. In addition, the analyses were made between 5 PM and 4 AM (average of 11 hours) before and after the race and separately in one-hour segments. The competitors woke up early on the morning of the race, which started at 9 AM and lasted for about five hours. Therefore, physical activity and sleep-awake status were comparable between the average 11-hour periods starting at 5 PM and between the one-hour segments during the days before and after the race. A period of sleep of 4 hours (midnight to 4 AM) was analysed separately as well, because the level of physical activity is largely standardised during sleep (Pikkujämsä *et al.* 1999). During the study days, the subjects were asked to go to bed by 11 PM.

In the other studies (III-V), 24-hour ambulatory R-R interval recordings were performed before and after the training interventions. The measures of R-R interval dynamics were calculated from the entire 24-h recording and from periods representing circadian variation, the midnight to 6 AM and daytime (9 AM to 6 PM) hours. The subjects were asked to go to bed before midnight and to stay in bed until six AM on the study days. The R-R intervals were recorded during a non-exercise day before and after training interventions. At the end of the training interventions the R-R intervals were recorded after a period of relaxation for 48 hours.

5.1.2 Exercise tests

The subjects performed a graded maximal exercise test on a bicycle ergometer (Ergomedic 818 E, Monark Exercise, Varberg, Sweden) in the studies I and II. The subjects in the studies III-V performed a graded maximal exercise test on a treadmill (Teliney-htymä, Kotka, Finland).

5.1.3 Measurement of oxygen consumption and physical performance

Measurements of $\dot{V}O_2$ (M909 ergospirometer, Medikro, Kuopio, Finland) were made during graded maximal exercise tests (I-V). The $\dot{V}E$ and gas exchange responses were monitored continuously during the study. They were calculated on a breath-by-breath basis, but were reported as mean values for 60 s. The highest value of oxygen uptake measured during the test (1-minute collection) was defined as the maximal ($\dot{V}o_{2max}$, in studies I and II) or peak ($\dot{V}o_{2peak}$, in studies III-V) oxygen consumption. The following criteria were used to document that the $\dot{V}o_{2max}$ level had been attained during each test: 1) a lack of increase in $\dot{V}o_2$ upon an increase in work rate and 2) a respiratory exchange ratio >1.1 (Shephard & Åstrand 1992). The subjects also fulfilled the criteria for $\dot{V}o_{2peak}$ given in the literature (i.e. respiratory exchange ratio >1.1 or maximal HR (HR_{max}) within ±10 beats of the age-appropriate reference value for HR_{max}) (Howley et al. 1995). In addition, in the study IV, the maximal running velocity (km/h) and time to exhaustion (min) were used as

indices of maximal running performance, since the change in Vo_{2peak} alone may not reveal the change in physical fitness (Noakes 1988, Paavolainen *et al.* 1999).

5.1.4 Autonomic blockade

In the study I, fractionated doses (0.2 mg) of atropine sulphate were injected intravenously at 4 min intervals in a seated position until two consecutive doses did not produce a further increase in HR (Hayano *et al.* 1991). Immediately after the last atropine dose, the subjects performed a graded exercise test on a bicycle ergometer until exhaustion.

5.2 Study protocols

5.2.1 Heart rate variability during dynamic exercise (I)

Four different protocols were performed as follows: 1) a graded exercise test until exhaustion; 2) 20 min steady-state low-intensity exercise; 3) 20 min steady-state high-intensity exercise; and 4) incremental parasympathetic blockade by atropine at rest followed by a graded exercise test until exhaustion. The procedures were performed in a weekly stepwise order. The study was performed randomly. The tests were performed at the same time of the day.

The subjects performed a graded maximal exercise test on a bicycle ergometer. Protocol 1 consisted of a 5 min warm-up period at a load of 50 W followed by an incremental protocol with the work rate increasing by 25 W at every 3 minutes until exhaustion. During the 20 min exercise tests (protocol 2), the subjects used the treadmill at steady-state intensities of 4 km/h and 12 km/h. Atropine were injected intravenously at 4 min intervals in a seated position (protocol 3) until two consecutive doses did not produce a further increase in HR (Hayano *et al.* 1991). Immediately after the last atropine dose, the subjects performed a graded exercise test on a bicycle ergometer until exhaustion (protocol 4) using a similar protocol as described above.

5.2.2 Heart rate variability during recovery (II)

The goal of the subjects was to participate in a cross-country skiing competition of 75 km. Long-term (24-hour) HR variability was measured before the 75 km cross-country skiing race and subsequently for 48 hours after the race. Measurements of maximal O_2 consumption were performed on a bicycle ergometer using a similar protocol as in study I.

5.2.3 Heart rate variability after controlled aerobic training (III)

The subjects performed a graded maximal exercise test on a treadmill starting at 4.5 km/h followed by a work rate increase at a rate of 0.5 km/h every two minutes until voluntary exhaustion before and after the aerobic training intervention.

The aerobic training period was eight weeks, including six 30-min sessions a week for the moderate-volume training group and six 60-min sessions a week for the high-volume training group at an intensity of 70-80 % of maximal HR (HR_{max}). The training mode was walking and jogging. All the subjects wore a telemetric HR monitor (Polar Smart Edge, Polar Electro, Kempele, Finland) during the training sessions to reach and stay at the correct training intensity. The subjects also reported the duration and the average training HR of each session. An extra resting day once a week was allowed if they felt exhausted.

5.2.4 Heart rate variability after home-based training (IV)

After the controlled training period, the subjects in the training group were encouraged to continue aerobic training independently, preferably on a daily basis, by following the American College of Sports Medicine recommendations (ACSM 1998) guidelines for ten months at an intensity level of 70-80 % of their individual HR_{max}. The individual HR_{max} was determined by the HR_{max} achieved during the $\dot{V}o_{2peak}$ test after eight weeks of training. The recommended mode of training was jogging, but cross-country skiing, roller-skating and ball games were also allowed if the definition HR and a single training session duration of 30-60 min was achieved. The subjects were recommended to use a HR monitor even during the home-based training program.

After one year, the training status of the past ten months was assessed by means of a modified physical activity questionnaire (Kohl et al. 1988). The original questionnaire was modified in such a way that the subjects also reported their average HR during different sport activities. The mean duration of each episode of physical activity, the mean frequency of physical activity per week, and the mean training intensity were analysed from the original questionnaires.

5.3 Analysis of heart rate variability

5.3.1 Conventional analysis of heart rate variability

The mean HR and the standard deviation of all R-R intervals (SDNN) were used as time domain analysis methods. An autoregressive model was used to estimate the power spectrum densities of HR variability. In study I, the power spectra were quantified by measuring the area under the whole frequency band (total power) and under two frequency

bands: low-frequency power (LF), from 0.04 to 0.15 Hz, and high-frequency power (HF), from 0.15 to 0.4 Hz. In the studies III-V, ultra-low-frequency power (ULF, <0.0033 Hz) and very-low-frequency power (VLF, 0.0033 to 0.04 Hz) were calculated from the entire 24-hour segment. LF and HF power values were calculated from segments of 512 R-R intervals over the 24-hour recording (II-V). LF and HF were also calculated from 1-hour segments of the 24-hour recording (using segments of 512 R-R intervals), and the mean values of these segments were used to detect the day and night HR variability values. The spectral values were expressed as absolute values (II-V) and in normalized units (II and III), which were obtained by dividing the power of each component by total variance from which the VLF component had been subtracted and multiplied this value by 100 (Montano *et al.* 1994). In addition, in study III, the coefficients of component variance (CCV %) were calculated as in the following equation: CCV(%)=100·(power of component)^{½}/(mean R-R interval) (Hayano *et al.* 1991).

Two-dimensional return maps of Poincaré plots were generated by plotting each R-R interval as a function of the preceding R-R interval (II). Two-dimensional vector analysis was used to quantify the shape of the plots as described previously (Huikuri *et al.* 1996b, Tulppo *et al.* 1996). This quantitative method describes separately the instantaneous R-R interval variability (SD1) and the long-term R-R interval variability (SD2) of the plot.

5.3.2 Nonlinear analysis of heart rate variability

Detrended fluctuation analysis (DFA) quantifies the fractal correlation properties of R-R interval data. The root-mean-square fluctuations of integrated and detrended data are measured in observation windows of different sizes and then plotted against the size of the window on a log-log scale. The scaling exponent α represents the slope of this line, which relates (log)fluctuation to (log)window size. In the studies I and III, the short-term (from 4 to 11 beats) scaling exponent (α_1) based on previous experiments (Mäkikallio *et al.* 1997) was used. In the study I, considering a maximal exercise without parasympathetic blockade, we analyzed the α_1 values, at the beginning and at the end of each load to control the effects of increasing data points which accompanied the progressive increase in HR during exercise.

The power-law relationship of R-R interval variability (β) was also calculated (III) from the frequency range 10^{-4} to 10^{-2} by a previously described method to assess the longer-term correlation of HR (Bigger *et al.* 1996).

5.4 Statistical methods

The data were analysed using the SPSS software (SPSS 9.0-11.5, SPSS inc., Chicago, Illinois). Standard statistical methods were used for the calculation of means and standard deviations. The normal Gaussian distribution of the data was verified by the Kolmo-

gorov-Smirnov goodness-of-fit test (Z value < 1.0). The spectral values of 24-hour HR variability were skewed (II-V). Therefore, these data were transformed by using the natural logarithms of the absolute values. Analysis of variance for repeated measurements was used to determine the differences within the groups (I-V) followed by *post hoc* analysis (Student's paired t-test). The differences between the hourly values of HR indices before and after the skiing race were tested for significance using paired t-test with Bonferroni correction (Altman 1991) (II). Pearson's bivariate correlation coefficients were used to analyse the associations between the HR variability parameters, fitness, training and anthropometrics (I-V). In the study IV, the changes in HR variability indices were adjusted for the effects of covariates using a stepwise linear regression analysis procedure for changes in fitness, training and BMI. In the study V, the training response was adjusted for the effects of covariates using a stepwise linear regression procedure for baseline age, together with the HR variability indices. In addition, when the training group was divided into quartiles according to changes in Vo_{2peak} in the study V, analysis of variance was used, followed by *post hoc* analysis of Bonferroni's t-test, to analyse the differences between the groups.

6 Results

6.1 Heart rate variability during dynamic exercise (I)

6.1.1 Changes in heart rate variability during exercise

In the study I, the changes in HR during dynamic exercise are shown in Fig. 1A. Fractal scaling exponent α_1 increased from rest to an exercise intensity level of 40 % of $\dot{V}O_{2max}$ and decreased linearly thereafter until the end of the exercise (Fig. 1B). The LF/HF ratio showed a similar trend as α_1, but the change between the different exercise intensities was not statistically significant (Fig. 1C).

6.1.2 Changes in heart rate variability during steady-state exercise

The mean HR was 57±8 beats/min at the baseline and 79±6 beats/min during the long-term low-intensity steady-state exercise ($P<0.001$) in the study I. The mean HR was 53±9 beats/min at the baseline and 160±10 beats/min during the long-term steady-state high-intensity exercise ($P<0.001$). The α_1 values increased from 1.13±0.18 to 1.36±0.11 ($P<0.01$) during the low-intensity exercise and decreased from 1.19±0.26 to 0.58±0.26 ($P<0.001$) during the high-intensity exercise. The LF/HF ratio changed from 2.34±1.65 to 4.79±2.20 ($P<0.05$) during the low-intensity and from 2.87±1.84 to 3.83±4.47 (P=NS) during the high-intensity exercise.

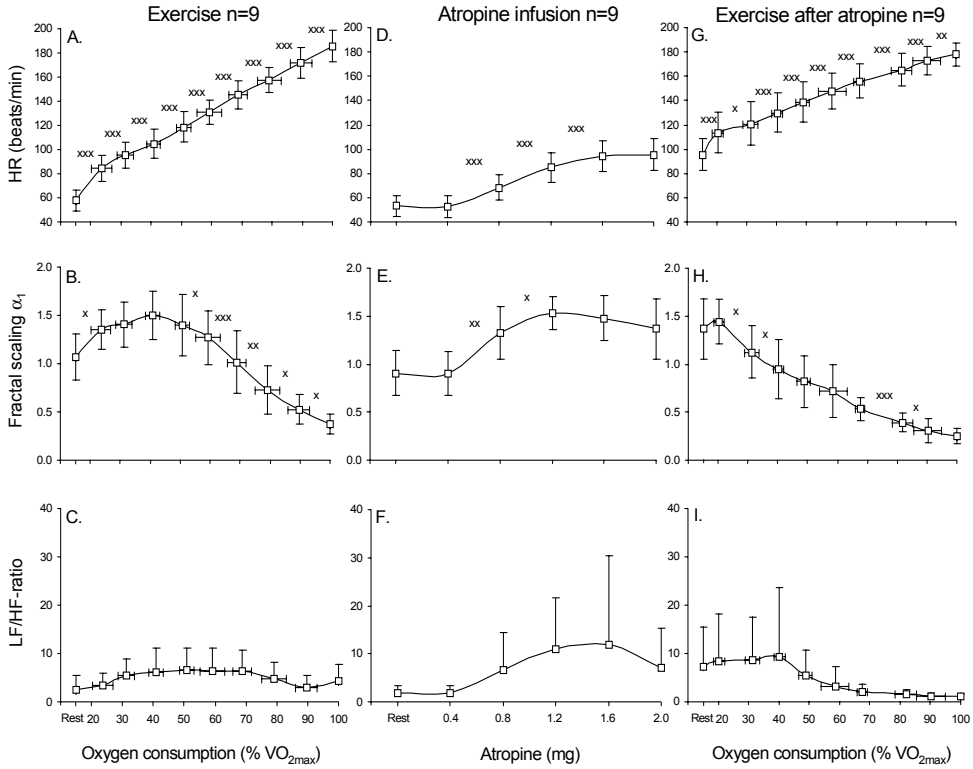

Fig. 1. Changes in heart rate (HR) variability indices during exercise (left panel), during atropine infusion (middle panel) and during exercise after atropine infusion (right panel): Values are means ± SD. The significance levels with paired t-test between two successive measurement periods are as follows: x P<0.05; xx P<0.01; P<0.001.

6.1.3 Changes in heart rate variability during parasympathetic blockade

The mean HR increased linearly during atropine infusion up till the dose of 1.6 mg (Fig. 1D) in the study I. The atropine infusion resulted in an increase in the fractal scaling exponent (Fig. 1E). The LF/HF ratio showed a similar trend as α_1, but the change between the doses was not statistically significant (Fig. 1F). During the exercise following parasympathetic blockade, HR increased progressively until the end of the exercise (Fig. 1G). The fractal scaling exponent α_1 decreased linearly during the exercise after vagal blockade (Fig. 1H). The LF/HF ratio showed a tendency to decrease from the intensity level of 40 % of $\dot{V}O_{2max}$ towards the end of exercise, but the decrease was not statistically significant (Fig. 1I).

6.1.4 Correlations between α_1 and other heart rate variability parameters during exercise

In the study I, the mean value of α_1 correlated strongly with the normalized HF and LF powers (r=-0.88 and r=0.88, respectively, P<0.01 for both) as well as with the LF/HF ratio (r=0.90, P<0.001) at rest without parasympathetic blockade. The correlations showed similar values at rest with parasympathetic blockade, but decreased during the exercise with and without parasympathetic blockade, including the correlation between α_1 and LF/HF ratio during exercise without parasympathetic blockade at the intensity level of 70 % of $\dot{V}O_{2max}$ was –0.48 (P=NS). There was no correlation between the changes in α_1 and the changes in LF/HF ratio from rest to the different intensity levels during the exercise after parasympathetic blockade and during the exercise without parasympathetic blockade. Similarly, during the steady-state high-intensity exercise, there was no correlation between the changes in α_1 and the changes in HR variability indices from rest to exercise.

6.2 Effects of prolonged exercise on the recovery of heart rate variability (II)

The average time needed to ski the 75 km distance was 4 hours and 31 minutes ± 45 min, and the corresponding cardiac strain (161 ± 13 beats/min) was 87 ± 2.8 % of the individual maximal HR (186 ± 13 beats/min). No significant changes were observed in the mean R-R interval one day after the skiing race compared with the mean R-R interval before the race. Two days after the race, the mean R-R interval was longer (P<0.001) compared with the pre-race value and the value recorded one day later. The normalized HF power was lower on the first day from midnight to 2 AM after the exercise as compared to the pre-exercise value, but returned to or even exceeded the pre-race level on the second day from 6 PM to 4 AM (Fig. 2). The normalized LF power was higher at 10 PM and 2 AM on the first day after the exercise compared to the one at the same time of the day preceding the race, and it also returned to the pre-exercise level or even dropped below it between 6 PM and 10 PM and between 2 AM and 4 AM (II, Fig. 2B). The LF/HF ratio behaved analogously to the normalized LF component. Instantaneous R-R interval variability (SD1) showed similar changes as the HF power of HR variability (II, Fig. 2C).

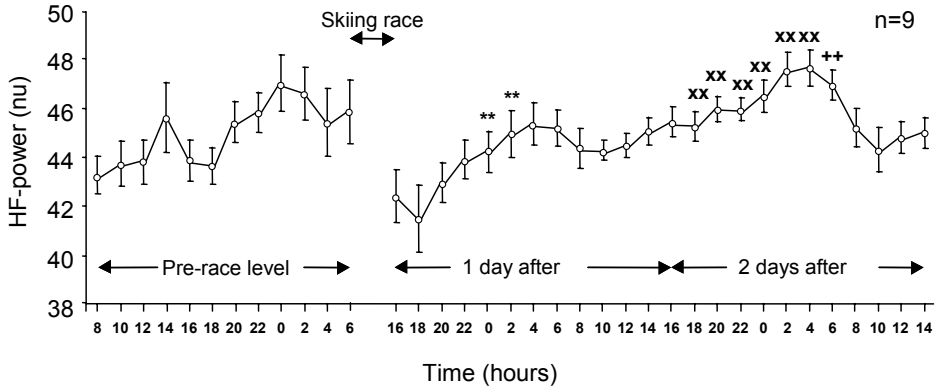

Fig. 2. Changes in the HF power of HR variability in normalized units (nu) in 2-hour periods on the pre-race day and 1 day and 2 days after the skiing race. The values are mean ± SEM. The significance levels with paired t-test with Bonferroni correction between the days are as follows: ** P<0.01 pre-race day vs. 1 day after, ++ P<0.01 pre-race day vs. 2 days after, xx P<0.01 one day after vs. 2 days after.

The mean time in which the normalized HF power was restored to the pre-exercise level was 4.2±4.2 hours. Large inter-individual variation was observed in the recovery time of normalized HF power (ranging from 0 to 12 hours). An inverse correlation was observed between the recovery time of normalized HF and the maximal oxygen consumption analysed from the bicycle exercise test (Fig. 3). Large inter-individual variation was also observed in the recovery time of absolute HF power (ranging from 0 to 14 hours). Analogously, large inter-individual variation was observed in the recovery time of SD1 (ranging from 0 to 22 hours). A correlation emerged between the recovery time of normalized HF power and the recovery time of SD1 ($r=0.75$, $P<0.02$). The recovery time of SD1 also correlated strongly with the recovery time of absolute HF power ($r=0.90$, $P<0.001$).

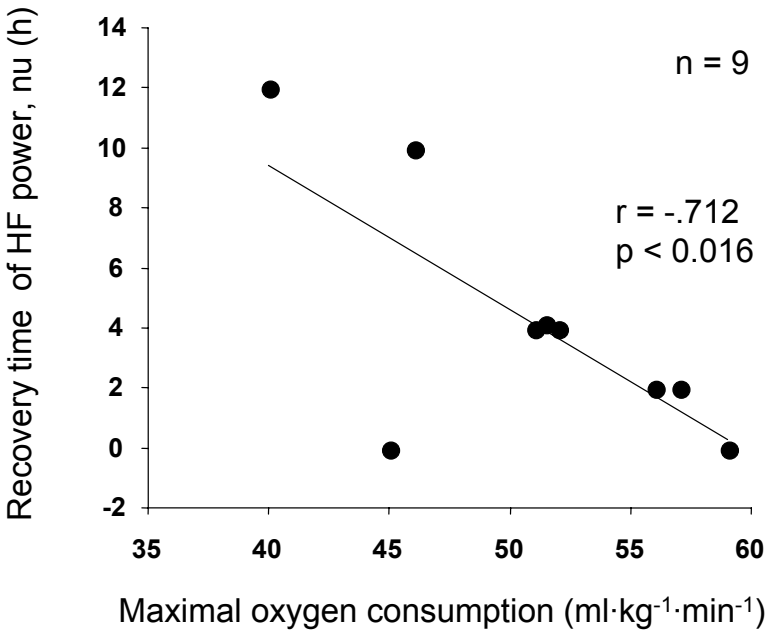

Fig. 3. Correlation coefficient between the recovery time (h) of HF power in normalized units (nu) and maximal oxygen consumption (ml·kg^{-1}·min^{-1}). Spearman's correlation coefficient is given.

6.3 Effects of controlled aerobic training on heart rate variability (III)

The average volume of aerobic training was 5.6±0.4 and 5.7±0.3 sessions a week at an intensity of 76±2 and 75±1 % of maximal HR in the moderate and high-volume groups, respectively. The duration of training was 32±3 and 61±4 min/session in the moderate and high-volume groups, respectively. Eight weeks of both moderate and high-volume training caused an increase in $\dot{V}O_{2peak}$ (10 % in both groups) as well as a reduction in maximal HR. Diastolic blood pressure increased ($P<0.05$) in the high-volume training group (III, Fig. 3). None of the measured variables changed within the control group during the study.

6.3.1 Time and frequency domain analysis of heart rate variability over 24 hours

In the study III, the mean HR decreased significantly in both training groups (Fig. 4). The mean value of SDNN increased in the moderate and high-volume groups ($P<0.01$ and $P<0.05$, respectively). The absolute spectral values of HF, LF and VLF power showed significant ($P<0.001$) and similar increases in both training groups after the interventions. The mean value of ULF power did not change after the training. The HF power corrected by the average R-R interval (CCV%) increased in both training groups after the interventions (Fig. 4), but LF power (CCV%) only increased significantly in the moderate training group ($P<0.01$). The LF/HF ratio decreased in both training groups (Fig. 4). The mean value of normalized HF power increased in both training groups ($P<0.01$). Similarly, the normalized LF power decreased significantly ($P<0.01$). The frequency at the HF spectral peak did not change after the intervention (from 0.25±0.02 to 0.25±0.02 Hz at night and from 0.32±0.03 to 0.31±0.03 Hz in daytime and from 0.23±0.02 to 0.23±0.03 Hz at night and from 0.30±0.03 to 0.30±0.03 Hz in the moderate and high-volume training groups, respectively).

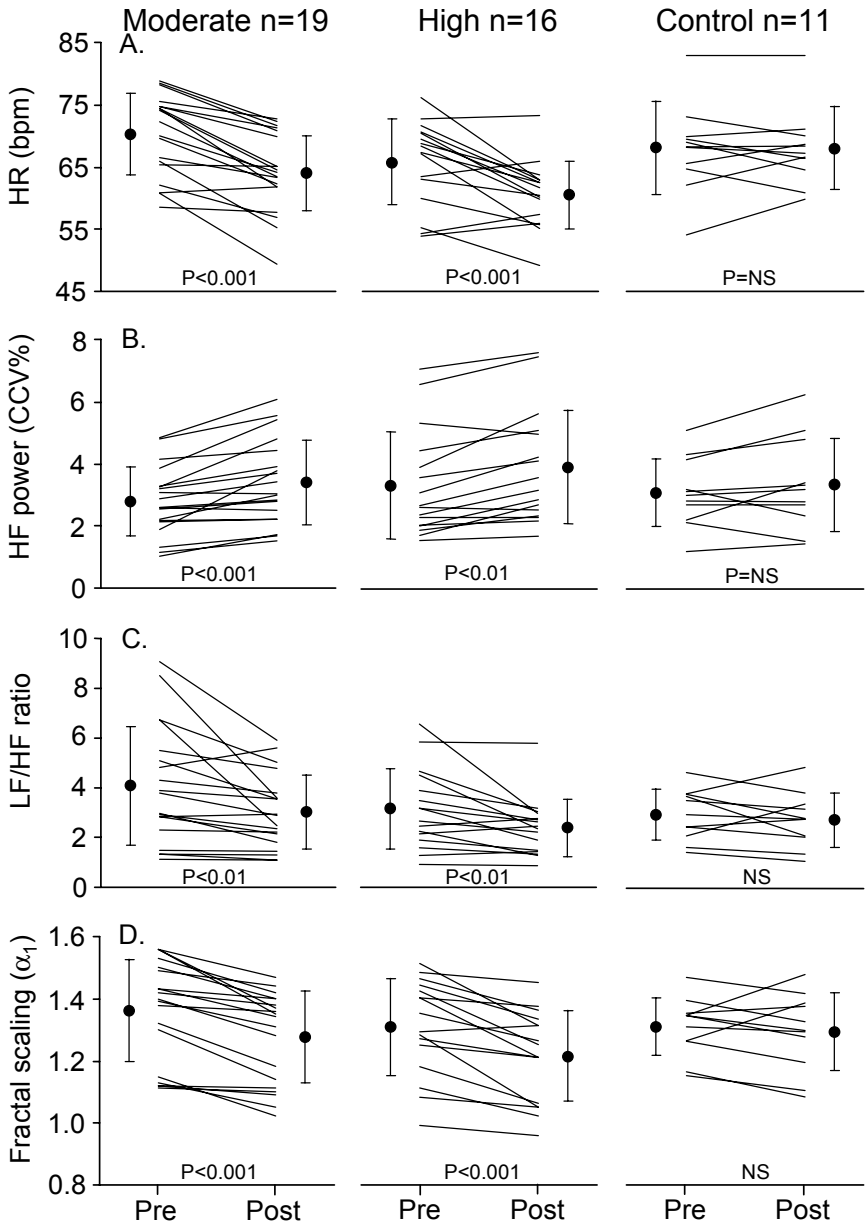

Fig. 4. Mean ± SD and individual values analysed over 24 hours for HR (A), HF power corrected by the average R-R interval (coefficient component variance (CCV%)) (B), LF/HF ratio (C) and α_1 (D). Pre, pre-training; Post; post-training. The differences within the groups after training were evaluated by Student's paired t-test.

6.3.2 Fractal scaling analysis of R-R intervals over 24 hours

In the study III, the mean value of the short-term scaling exponent α_1 decreased in both training groups (Fig. 4). The mean value of β, the power-law scaling exponent, did not change during the intervention.

6.3.3 Correlations between α_1 and other heart rate variability parameters over 24 hours

The change in the scaling exponent α_1 from the baseline to the end of the intervention correlated with the changes in normalized HF power ($r=-0.76$, $P<0.001$), normalized LF power ($r=0.76$, $P<0.001$) and the LF/HF ratio ($r=0.69$, $P<0.001$) as analyzed by the 24-hour recording in the study III. The correlation between the change in α_1 and the change in the LF/HF ratio became weaker during the daytime hours ($r=0.57$, $P<0.001$) as compared to the night-time correlation ($r=0.68$, $P<0.001$).

6.4 Effects of home-based training program on heart rate variability (IV)

The average training frequency was 5.8±0.2 sessions a week during the controlled training period of eight weeks and decreased to 3.3±1.0 sessions a week ($P<0.001$) during the home-based training program. The intensity of training was 75±2 % of maximal HR during controlled training and 76±6 % (P=NS) during home-based training. The mean duration of exercise was 46±15 min/session (P=NS) during controlled training and 54±11 min/session during the home-based program. The mean VO_{2peak} increased by 10 % ($P<0.001$) during the controlled training period and remained 3 % (P=NS) higher than at the baseline after the home-based training program (IV, Fig. 1A). The maximal running performance, expressed as time to exhaustion, increased initially by 16 % ($P<0.001$) and remained 8 % ($P<0.001$) higher than at the baseline after home-based training (IV, Fig. 1B). The mean BMI did not change significantly during the intervention, although a marked individual difference was observed in BMI changes, e.g. a range from -5 % to 6 % during the home-based program (IV, Fig. 1C). None of the measured variables changed in the control group during the intervention.

6.4.1 Time and frequency domain analysis of heart rate variability

In the study IV, the mean HR decreased during the controlled training ($P<0.001$) and remained below the baseline value ($P<0.05$) after the home-based program when analysed over 24 hours. All absolute spectral components increased ($P<0.001$) and LF/HF ratio decreased ($P<0.01$) after controlled training over 24 hours. After home-based training, HF and LF power as well as LF/HF ratio returned to the baseline level, and VLF power remained significantly higher than at the baseline ($P<0.05$).

The mean and individual values of HR dynamics analysed at night are shown in figure 5. HR decreased during the period of controlled training ($P<0.001$) and returned back to the baseline level after the home-based program. The HF and LF components increased ($P<0.001$) after controlled training. After home-based training, HF power remained at a significantly higher level than at the baseline ($P<0.05$), and LF power returned to the baseline level. The mean LF/HF ratio did not change significantly after controlled training and after the home-based program.

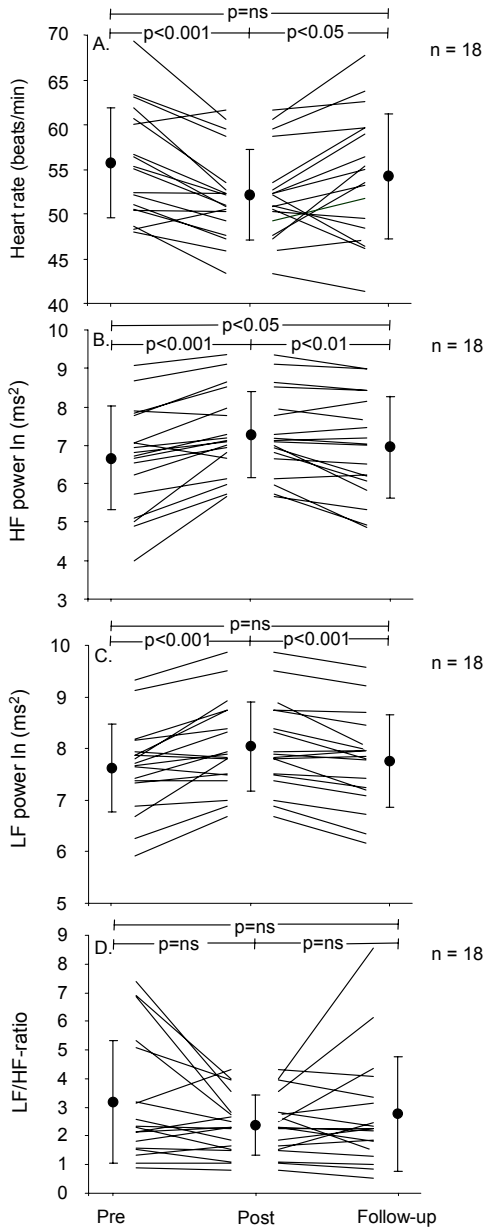

Fig. 5. Mean ± SD and individual values in HR (A), HF power (B), LF power (C) and LF/HF ratio (D) analysed over the night hours (midnight – 6 AM) at baseline (Pre) and after an eight-week controlled aerobic training program (Post) followed by 10 months of home-based training (Follow-up). The differences within the group after training were evaluated by Student's paired t-test.

6.4.2 Correlations between changes in different variables after home-based training

The changes in HF power analysed over 24 hours correlated with the changes in maximal running performance, expressed as time to exhaustion, and with the changes in BMI after the home-based training period (Fig. 6) in the study IV. Similarly, the changes in LF/HF ratio correlated with the changes in running performance ($r=-0.44$, $P<0.05$) and with the changes in BMI ($r=0.48$, $P<0.05$) after home-based training. The changes in the mean duration of a single training session correlated with the changes in all spectrum components ($P<0.05$) after home-based training. When the changes in HR variability parameters were analysed separately over the night hours, the changes in HF power correlated with the changes in running performance ($r=0.41$, $P<0.05$) after the home-based program. After adjustment for changes in fitness, training or BMI, none of these parameters could independently explain the changes in HR variability indices.

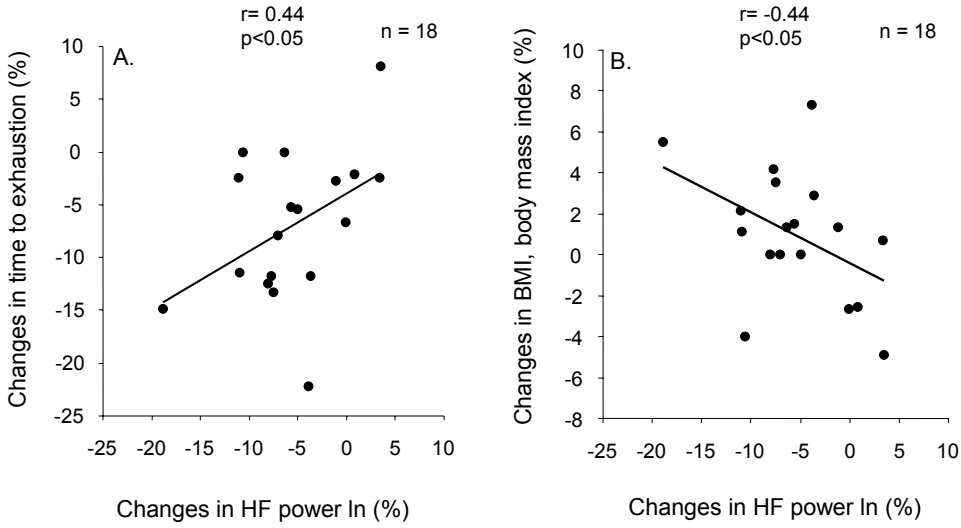

Fig. 6. Pearson's bivariate correlations between the changes in HF power over 24 hours and the changes in maximal running performance, expressed as time to exhaustion (A), and the changes in BMI (B) after the ten-month home-based training program.

6.5 Contribution of heart rate variability to the aerobic training response (V)

The individual training responses after eight weeks of highly controlled training are shown in figure 7. The average increase in $\dot{V}O_{2peak}$ was 11±5 %, range from 2 % to 19 %. The training response did not differ between the moderate and high-volume training groups. None of the measured variables changed within the control group during the study.

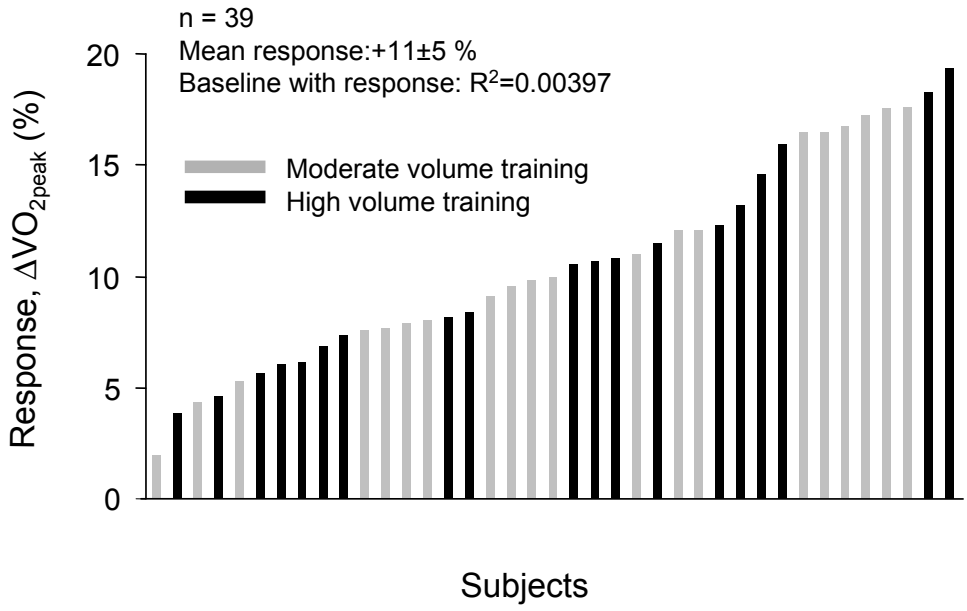

Fig. 7. Heterogeneity of $\dot{V}O_{2peak}$ training responses after eight weeks' controlled aerobic training in healthy males.

The training response correlated with age (r=-0.39, P=0.007), but not with the baseline level of $\dot{V}O_{2peak}$ or BMI (r=-0.06 and -0.12, respectively). Age accounted for 16% of the change as an independent predictor of the aerobic training response, and the corresponding values for $\dot{V}O_{2peak}$ and BMI were 0.4 % and 1.4 %. A significant correlation was observed between the training response and the baseline absolute HF power of R-R intervals analysed over the 24-h recording (r=0.46, P=0.002) and during the night-time (r=0.52, P=0.001) and daytime hours (r=0.35, P=0.028). The absolute LF power during the night hours (r=0.38, p=0.018) and the absolute VLF power over 24 hours (r=0.34, P=0.017) were also related to the training response. After adjustment for age, only the absolute HF power was associated with the training response (V, Fig 2A-C). HF power

during the night hours accounted for 27 % (P=0.001, Fig. 8) of the change as an independent predictor of the aerobic training response. The correlations between the training response and the mean HR, SDNN or ULF power were not significant in any conditions.

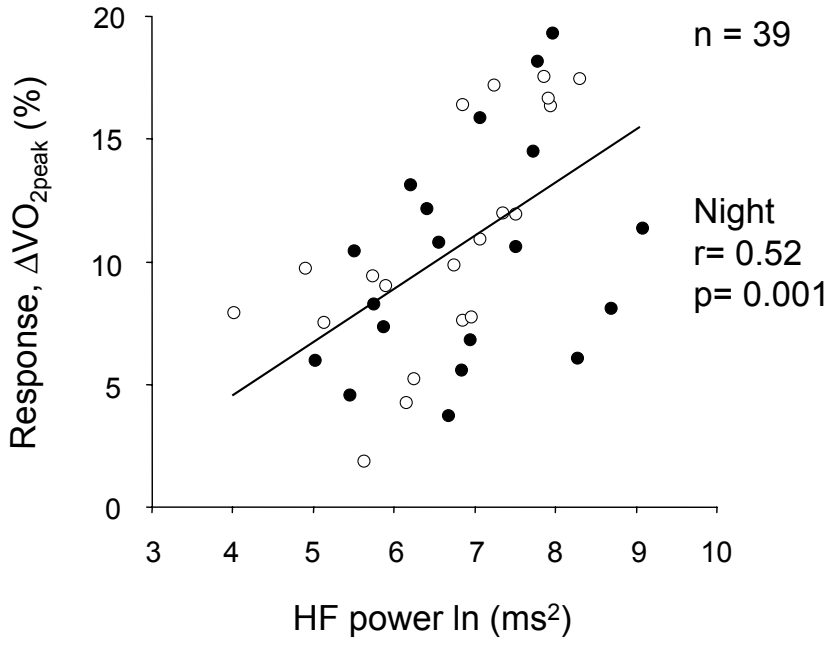

Fig. 8. Correlation coefficients after adjustment for age between the inter-individual training response ($\Delta\dot{V}O_{2peak}$, %) and absolute HF power during the night (midnight to six AM). A stepwise linear regression procedure was used to study the adjustment for age, baseline $\dot{V}O_{2peak}$, BMI and each HR variability parameter. ○ = moderate-volume training group and ● = high-volume training group.

The study group was also divided into quartiles according to the training response (17±1, 11±1, 8±1 and 5±2 % increase in $\dot{V}O_{2peak}$, P<0.001 between the groups). HF power at baseline analyzed over 24 hours or during the night-time hours was higher in the group with the best training response compared to those with lower responses. The groups did not differ from each other in terms of baseline $\dot{V}O_{2peak}$, age and BMI.

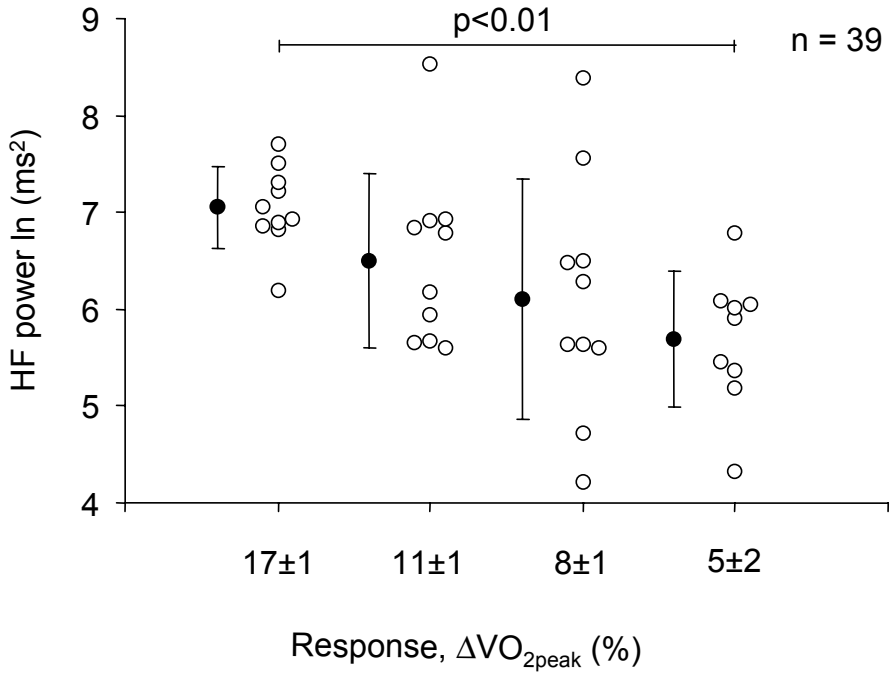

Fig. 9. Individual and mean values of HF power. The subjects were divided into quartiles according to aerobic training as follows: 17±1, 11±1, 8±1 and 5±2 % increase in $\dot{V}O_{2peak}$.

7 Discussion

In the present study, first was discussed the effect of acute dynamic exercise on autonomic regulation of HR. Secondly, sufficient aerobic training volume, frequency and intensity were defined in terms of their effects on autonomic nervous system in healthy subjects. Finally, an interesting association between the aerobic training response and the autonomic nervous system was reported, which opens up a novel insight into the field of exercise physiology.

7.1 Fractal analysis of heart rate variability during exercise (I)

The present data showed that withdrawal of cardiac vagal activity by low-intensity dynamic exercise or atropine administration resulted in a change of the scaling exponent α_1 towards more correlated short-term R-R interval dynamics (α_1~1.5). These results agree with those obtained previously during a passive tilt and steady-state low-intensity exercise (Tulppo *et al*. 2001a). A novel finding was that the mean value of α_1 decreased linearly during dynamic exercise after parasympathetic blockade until the end of exercise. α_1 revealed changes in HR variability at high intensity levels, where conventional HR variability measures failed to detect any changes in HR dynamics. A similar decrease in the α_1 value was observed during exercise without parasympathetic blockade at levels of vigorous exercise. Furthermore, the point of diversion of the scaling exponent from increasing values at low intensity to decreasing values at higher intensity levels represent the intensity level (app. 40 % of $V_{O_{2max}}$) where sympathetic dominance takes over after vagal withdrawal.

Scaling exponent α_1 had a strong correlation with normalised HF and LF spectral components at rest. Consequently, α_1 also correlated with the LF/HF ratio at rest (r=0.90), but the correlation became weaker after the parasympathetic blockade and further declined during the exercise. These correlations also became weaker during steady-state long-term exercise tests confirming that the lack of correlation between these indices during exercise is not due to the duration of data collection. In a previous study, a close relationship between α_1 and LF/HF ratio at rest with a controlled breathing rhythm

and during a passive tilt test was observed (Tulppo *et al.* 2001a). The weak correlation between the conventional and more recent scaling measures during exercise may be partially due to the uncontrolled breathing rate, which emphasizes the applicability of this measure also to detect changes in "free-running" physiological conditions.

7.2 Autonomic regulation of heart rate after prolonged physical exercise (II)

Delayed recovery of HR after maximal or submaximal short-term dynamic exercise is a powerful predictor of overall mortality at the population level (Cole *et al.* 1999, Cole *et al.* 2000, Nissinen *et al.* 2003). Experimental data have shown that vagal activation prevents ischemia-induced ventricular fibrillation after exercise (Schwartz *et al.* 1984, Billman & Hoskins 1989, Vanoli *et al.* 1991), and that exercise training confers anticipatory protection against sudden death by enhancing cardiovascular vagal function (Hull *et al.* 1994). The present results on healthy males demonstrated that HF oscillations of HR analyzed by spectral and Poincaré plot indices of HR variability were attenuated for several hours after strenuous long-term exercise. On the second day after the exercise, however, an accentuated rebound phenomenon was observed with enhanced vagal regulation compared to the pre-exercise level. The changes in cardiovascular autonomic function probably reflect a compensation for altered cardiovascular hemodynamics after exercise. Previous studies have shown that the left ventricular systolic function and the cardiac filling patterns are altered for a long time after prolonged exhaustive exercise (Niemelä *et al.* 1984, Douglas *et al.* 1987). More sympathetic drive may be needed to compensate for the reduced cardiac performance, to maintain the effective cardiac output and to activate sufficient blood flow. After restoration of the hemodynamic and cardiac functions to the normal level, sympathetic drive decreases and vagal dominance returns. It is also evident that these compensatory autonomic responses are protective during the recovery phase of normal cardiovascular homeostasis after prolonged exercise.

Wide interindividual variation was observed in the time of recovery of the reduced vagal outflow: the subjects with better cardiorespiratory fitness showed more rapid recovery of altered autonomic function than those with poor fitness. It is widely accepted that good physical fitness and regular exercise training induce adaptation of the autonomic nervous system, which is most commonly observed in the form of a decrease in the basal HR. It is assumed that cardiac vagal tone increases in well-trained individuals as compared to that of controls (Goldsmith *et al.* 1992, De Meersman 1993, Davy *et al.* 1996, Davy *et al.* 1998, Tulppo *et al.* 1998b, Aubert *et al.* 2001, Ueno *et al.* 2002, Rennie *et al.* 2003). A previous study confirmed that the vagal regulation of HR was more efficient during exercise in subjects with better exercise capacity (Tulppo *et al.* 1998b). The present observations confirm the importance of cardiovascular fitness in cardiovascular autonomic regulation, implying that the rate of recovery of altered autonomic regulation after prolonged exercise is also related to individual fitness.

7.3 Effects of aerobic training on autonomic regulation of heart rate

Poor aerobic fitness and a lack of physical activity have been associated with an increased risk of cardiovascular deaths (Ekelund *et al.* 1988, Blair *et al.* 1989, Kesäniemi *et al.* 2001, Laukkanen *et al.* 2001, Myers *et al.* 2002). Previous studies have also shown that physical fitness is related to cardiac autonomic regulation providing evidence that aerobic training improves cardiovascular autonomic function (Goldsmith *et al.* 1992, De Meersman 1993, Davy *et al.* 1996, Tulppo *et al.* 1998b). Aerobic exercise has been suggested to protect the heart against harmful cardiac events by increasing parasympathetic tone and also by decreasing cardiac sympathetic activity (Billman 2002).

7.3.1 Controlled aerobic training of eight weeks (III)

Bradycardia is a well-known consequence of aerobic training and has been attributed to changes in the autonomic nervous system, i.e. either an increase in vagal activity or a decrease in sympathetic activity, or else to reduced intrinsic HR. The present results confirm the previous findings concerning vagal dominance after aerobic training. The present data showed that moderate-volume training including six 30-min sessions a week at an intensity of 70-80 % of HR_{max}, results in similar changes in the average HR and HR variability indices as high-volume training including six 60-min sessions a week at an intensity of 70-80 % of HR_{max}, in healthy males. HR variability analysed as normalized units by spectral parameters changed towards increased vagal dominance after aerobic training in both the high and the moderate-volume training groups. Similarly, aerobic training changed the scaling exponent α_1 towards less correlated short-term R-R interval dynamics. More specifically, the increased normalized HF power and the decreased normalized LF power as well as the decreased LF/HF ratio showed an alteration in the autonomic regulation of HR towards increased vagal dominance after aerobic training. The LF/HF ratio also decreased during the night hours after the moderate-volume training intervention. This is an important finding because the night hours may reflect a more standardized condition and the subject's behaviour pattern does not disturb the R-R interval recordings. Concurrently, the present data also showed a decreased scaling exponent α_1 in R-R interval dynamics during the "free-running" daytime hours as well as during the night hours after aerobic training. The normalized spectral components remained unaltered during the night hours after a training period, suggesting that the scaling indices may detect subtle alterations in HR more effectively than simple spectral ratios. From the physiological point of view, the altered short-term fractal HR behaviour in various pharmacological and physiological interventions has been previously described (Tulppo *et al.* 2001a, Tulppo et al. 2001b). Briefly, a change in the R-R interval dynamics from fractal behaviour (α_1~1.0) towards a stronger correlation in short-term R-R interval dynamics (α_1~1.5) was observed during a withdrawal of vagal activity by graded atropine infusion and dynamic low-intensity exercise (Tulppo *et al.* 2001a, Tulppo *et al.* 2001b). Similarly, in the present study, the reduced α_1 values correspond to increased vagal activity of the heart after aerobic training.

Although beyond the topic of the present study it was observed that both moderate and high-volume training groups had reductions of 5-7 beats/min in HR_{max}. This may be a consequence of altered autonomic balance, although vagal activity is only elevated at lower HR. Alternatively, a reduction in intrinsic HR and altered sinoatrial node responses to norepinephrine have been identified as potential contributors to reduced HR_{max} following training (Hughson *et al.* 1977). This may suggest that altered autonomic balance and reduced intrinsic HR contribute to the changes we observed in HR after training. Another interesting finding is the increased diastolic blood pressure after the intervention in the high-volume training group. This may be caused by an early stage of over-training, usually referred to as the overreaching syndrome. However, specific and sensitive parameters are not available to identify overreaching syndrome, and we did not focus on that issue in the present study (Kuipers 1998).

7.3.2 Home-based training of ten months (IV)

The present study clearly showed that vagal outflow to the heart increases during eight weeks of highly controlled aerobic training based on the ACSM guidelines. As expected, physical fitness, the volume of training and R-R interval fluctuation all tended to decrease during the home-based training program. However, there were wide inter-individual differences in the changes in these variables after home-based training, resulting in some new interesting findings. First, there was a clear positive correlation between the changes in maximal running performance and the changes in the vagally mediated HF power of R-R intervals. The subjects who were able to maintain their maximal running performance at a high level also maintained their HF power at a high level after home-based training. The subjects whose maximal running performance decreased also had decreased HF power after home-based training. A similar association was observed between the changes in maximal running performance and the changes in LF/HF ratio. On the contrary, the changes in $\dot{V}O_{2peak}$ did not correlate with the changes in any HR variability indices. This is an interesting finding. $\dot{V}O_{2peak}$ is the most widely used index of physical fitness in the field of autonomic nervous system research. Based on the present data, perhaps both indices of physical fitness, i.e. $\dot{V}O_{2peak}$ and maximal performance, should be used in studies on the autonomic nervous system to achieve optimal evaluation of physical fitness, as these indices complement each other.

The physiological background of the association between maximal running performance and autonomic regulation is speculative. The changes in $\dot{V}O_{2peak}$ in healthy subjects are regulated mainly by the changes in peripheral level (blood volume, hemoglobin concentration, etc.), as the changes in maximal running performance also reflect the changes at the central nervous system level, e.g. motivation during the maximal test (Noakes 1988). This could partly explain the association between the changes in the autonomic nervous system, originating at the central nervous system level, and the changes in running performance.

Secondly, there was an association between the changes in BMI and the changes in autonomic regulation after home-based training. The changes in BMI correlated nega-

tively with the changes in HF power and positively with the changes in LF/HF ratio. This is also an interesting finding, since body weight of our subjects were in the normal range (BMI<30), and during the home-based training, even a small change in body weight resulted in a marked change in autonomic regulation. However, the changes in BMI did not explain independently the changes in autonomic regulation. On the contrary, our data showed that the maintenance of "healthy autonomic regulation", meaning high vagally mediated beat-to-beat R-R interval fluctuation and low LF/HF ratio, is not associated with any single physiological parameter, but is rather a result of weight control and good physical fitness due to regular aerobic training.

7.4 Autonomic regulation of heart rate as a determinant of aerobic training response (V)

Individual responses to aerobic training vary from almost 0% to a 40 % increase in aerobic fitness. (Bouchard 1995, Bouchard & Rankinen 2001). The present study showed that baseline HF power during the night hours was the most powerful HR variability index associated with the future training response, accounting for 27 % of the change as an independent predictor of the aerobic training response. This is an important finding because the night hours reflect a more standardized condition and the results are less influenced by the behavioral pattern. This finding supports the concept that cardiac vagal activity is an important physiological determinant of the training response in males.

7.4.1 Individual responses to aerobic training

Marked individual differences in the response to regular aerobic training have been observed after highly standardized exercise programs in healthy subjects (Lortie *et al.* 1984, Kohrt *et al.* 1991, Bouchard & Rankinen 2001). In line with the previous studies, we also found here large variation in the training responses after a controlled aerobic training intervention in healthy males. The HERITAGE Family Study based on 720 healthy subjects summarized the contributions of age, sex, race and baseline fitness level to the response to aerobic training. All these variables together accounted for only 11 % of the variance in the response to 20 weeks of standardized training. The gender was the most powerful predictor of the training response with a contribution of 5.4 %, followed by age with 4 % (Bouchard & Rankinen 2001). In the present male population, age accounted for 16 % of the response as an independent predictor of the aerobic training response. Our training intervention was relatively short (8 weeks) compared to the HERITAGE Family Study (20 weeks), which may emphasize the impact of age on the short-term training response. We also had a relatively large age range (23-52 years) as compared to the training studies performed with older healthy people aged 60-71 years (Kohrt *et al.* 1991) or younger subjects aged 21-29 years (Lortie *et al.* 1984). In agreement with

the previous studies, the baseline fitness level was not significantly associated with the training response in the present study.

7.4.2 Possible mechanisms for individual cardiovascular adaptation

The mechanisms underlying the relationship between the baseline vagal activity and the training response remain speculative. In accordance with the large inter-individual variation in the training response to physical exercise, wide inter-subject variation has also been observed in cardiovascular autonomic regulation in healthy subjects when measured by the HR variability indices (Pikkujämsä *et al.* 2001). Recent studies have shown that genetic factors may determine a large proportion (> 20 %) of the inter-individual variation of R-R interval variability (Singh *et al.* 1999, Singh *et al.* 2002), whereas demographic and other factors, including blood pressure, blood cholesterol, cardiac dimensions, BMI and smoking explain only a small proportion (~10 %) of this variation in autonomic regulation (Pikkujämsä *et al.* 1999). Similarly, it is well known that genetic background causes considerable variation in both the baseline aerobic capacity and the changes in aerobic fitness after exercise training interventions (Bouchard *et al.* 1999, Rankinen *et al.* 2001, Rankinen *et al.* 2002, Perusse *et al.* 2003). Therefore, there might be a common denominator that explains partly both adaptation to aerobic training and HR variability. Genetic factors are the major candidates for this denominator. It is also possible that there is a mechanistic link between the cardiac vagal function and the training response. Subjects with good vagal function may have a better capacity of the cardiovascular system to adapt to various external stimuli, e.g. physical exercise. This adaptation capacity may cause an improvement in overall cardiovascular performance after the regular physical training, thereby also improving aerobic fitness. This hypothesis should be confirmed in future experimental studies.

7.5 Methodological considerations

In this thesis, the respiratory activity during the dynamic exercise tests (I) or during the 24-hour HR variability measurements (II-V) was not controlled. Obviously, control of breathing rate might have given additional information. The transfer function from respiratory activity to the heart changes during the course of exercise and contributes to the changes observed in HR dynamics. However, in the study I, we tested the applicability of the short-term scaling exponent to the detection of relatively well known physiological changes during high-intensity exercise in a normal physiological setting with spontaneous breathing rhythm. Further studies with various designs will be needed to establish the exact physiological background of the scaling exponent during different degrees of sympathetic outflow. Also, regular aerobic training changes respiratory activity. TV increases and breathing frequency is considerably reduced after training interventions, at least at submaximal exercise intensity levels (Jirka & Adamus 1965, Tzankoff *et al.* 1972). Re-

duced breathing frequency is known to increase the spectral values of the HF power of R-R intervals (Penttilä *et al.* 2001). Therefore, changes in the respiratory components of HR variability, reflecting cardiac outflow, may also partly be due to altered respiratory patterns caused by physical training.

8 Conclusions

1. Cardiovascular autonomic function is an important determinant of the individual response to aerobic training among men. High vagal activity at baseline is associated with a superimposed improvement in aerobic fitness caused by aerobic exercise training in healthy subjects as compared with less pronounced vagal activity.
2. The reduction of HR after aerobic training is associated with increased indexes of cardiac vagal outflow in healthy males. Controlled aerobic training intervention of eight weeks, including six 30-min sessions a week at an intensity of 70-80 % of maximal HR, is a sufficient intervention to induce these effects on cardiac autonomic regulation.
3. The vagal dominance reached during controlled aerobic training can be preserved by weight control and by continuing home-based exercise training according the recommendations of the current guidelines.
4. Cardiac vagal outflow is blunted for several hours after prolonged vigorous exercise. The recovery time of reduced vagal outflow depends on individual cardiorespiratory fitness.
5. These results also show that the fractal characteristics of HR dynamics can provide useful information at high exercise intensities even when the conventional HR variability measures fail detect changes in HR behaviour.

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